1 Introduction

Researchers and the pharmaceutical industry are continually searching for new medications with novel or improved actions. Botanicals provide an extensive natural source of new drugs, with various chemical compounds that have been discovered from plant sources, leading to subsequent formulations of potential new drugs. One problem associated with drugs is that most of them are associated with minor and major side effects, so besides new medications, scientists are also interested in finding drugs that have fewer side effects.
effects than the drugs currently in existence. Many different traditional herbal medicine systems exist, for example the systems of Ayurveda, Siddha, Unani, and Hamdard in India and its subcontinent; traditional medicine systems serve the health care needs of more than 80% of the people in Asia and Africa. These traditional herbal medicines are generally regarded to be without major side effects. Approximately 25% of prescribed drugs are from plant sources, and are prescribed for the same purposes as they were used in the different traditional medicine systems[1,2]. Studies on plants used in the traditional medicine systems are investigating their pharmacological and chemical constituents in order to generate ideas for new drugs, as well as to understand the scientific basis of their folkloric uses.

Musa sapientum Lour. (Synonym: Musa sylvestris Colla, Musaceae), a banana species, is a tree-like perennial herb that is widely distributed in tropical and subtropical regions of Asia and Africa. In Bangladesh, it grows all over the country throughout the year, and is locally known as “Bichi Kola” or “Aitta Kola”. It grows from sympodial rhizomes, and reaches 5–9 m in height. Pseudostems are composed of packed leaf sheaths; leaves are spirally arranged, and petiolate. It exhibits a big inflorescence with spirally arranged reddish brown bract, and can be eaten as vegetable. Fruits are juicy and full of seeds. Traditionally banana fruits are used in the treatment of diarrhea, dysentery, and excess menstruation[3]. Flowers are used in the treatment of diabetes, diarrhea, and menorrhagia. Stem juice is used to treat cholera, dysentery, haemoptysis, and otalgia[4]. Different banana species of Musa genus are also used to treat a number of ailments such as diarrhea, dysentery, diabetes, uremia, sprue, gout, intestinal lesions, cardiac diseases, and hypertension[4-5].

A literature survey showed no previous reported pharmacological and chemical investigation on M. seminifera, but such information on other species of Musa genus does exist. Among them, M. sapientum, M. paradisiaca, and M. acuminata have been shown to exhibit various pharmacological activities, and several classes of compounds have been isolated from them. The fruits of M. sapientum have been reported to have significant antiulcerant[6], antibacterial[7], wound-healing[8], and antiallergic activities[9]. The roots of M. paradisiaca have been reported to have antihyperglycemic effect on streptozotocin-induced diabetic male albino rats[9]. Carbohydrates, serotonin, norepinephrine, dopamine[9], indole compounds[10], ascorbic acid, alkaloids, tannin, and flavonoids have been isolated from different banana species[11,12]. Sterols like β-sitosterol, campesterol, and stigmasterol have also been isolated[13,14].

The present study was carried out to investigate antioxidant, analgesic, anti diarrheal, and antibacterial activities of the ethanol fruit extract of M. seminifera as well as to establish scientific basis of folklore uses.

2 Materials and methods

2.1 Collection of plant material

The fruits of M. seminifera including seeds, were collected from Khulna University area in November, 2011. Experts of Bangladesh National Herbarium, Mirpur, Dhaka, identified the plant materials, and a voucher specimen (Accession No. DACB 37523) was submitted there as future reference.

2.2 Drying and grinding

Fresh plant material was sun-dried to remove moisture. Then dried plant materials were crushed into powder using a hammermill. Then powdered plant materials were dried for 2 h in an electrical dryer at 45 °C, and kept in an air-tight container to avoid fungal contamination.

2.3 Extraction

Powdered plant materials were macerated with ethanol for 7 d and subjected to occasional shaking. After maceration, plant debris was separated using cotton plug to obtain a clear solution. Then solvent was evaporated in a rotary evaporator at 50 °C to obtain crude extract. Crude extract was stored at 4 °C in refrigerator. Before experimental use, the crude extract was brought to room temperature to ensure optimum release profile in experimental animals.

2.4 Experimental animals

Young Swiss-Albino mice of both sexes (age 4-5 weeks, weight 18-25 g) were used in the present in vivo pharmacological investigations. They were procured from the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B), and kept in standard housing condition maintained at the temperature of (25±1) °C, and relative humidity of 56%-60% with a 12:12 (light: dark) cycle. Animals were fed with ICDDR, B formulated rodent food, and allowed free access to water. All experiments using mice were carried out following the guidelines of the Animal Ethics Committee.

2.5 Test microorganisms

Bacterial strains including both Gram-positive and Gram-negative bacteria were collected from Microbiology Laboratory of ICDDR, B and stored in Microbiology Laboratory of Pharmacy Discipline, Khulna University. Bacterial strains were Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhi, Escherichia coli, Shigella flexneri, Shigella sonnei and Shigella dysenteriae.

2.6 Chemicals and reagents

Ascorbic acid, sodium monobasic phosphate, sodium dibasic phosphate, sodium carbonate, trichloroacetic acid, potassium ferricyanide, ferric chloride, acetic acid, and magnesium sulfate were obtained from Merck, Germany. Folin-Ciocalteu’s reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemical Co. Ltd., (St. Louis, MO, USA). Loba Chemie Pvt Ltd., India provided
Twee-80 and castor oil.

2.7 Reference drugs
Diclofenac sodium and loperamide were purchased from Square Pharmaceuticals Ltd., Bangladesh. Morphine was obtained from Popular Pharmaceuticals Ltd., Bangladesh. Standard ciprofloxacin disk was obtained from Oxoid Ltd., UK.

2.8 In vitro antioxidant assay
The ethanol extract of M. seminifera fruit was assessed for both qualitative and quantitative antioxidant activity\[17,18\]. Qualitative assay was performed through thin layer chromatographic technique followed by DPPH spray and quantitative assay was performed through DPPH free radical-scavenging assay.

2.8.1 Qualitative assay
Thin layer chromatogram of the fruit ethanol extract was developed in polar, medium polar, and non-polar solvent system. After developing, 0.002% DPPH solution in ethanol was sprayed using a spray gun. Compounds having DPPH-scavenging activity showed yellow spot on the purple background of the chromatogram.

2.8.2 Quantitative assay
DPPH-scavenging activity of the M. seminifera fruit ethanol extract was determined according to the method of Sharma et al\[19\]. Sample was prepared by dissolving extract in ethanol at different concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, and 1 mg/L. From each concentration, 1 mL sample was taken in test tubes, and 3 mL of 0.004% DPPH solution in ethanol was added to each test tube. Then it was incubated at room temperature for 30 min. After incubation, absorbance was taken at 517 nm against blank. Ascorbic acid was used as standard. Percent scavenging activity was calculated using the formula: scavenging activity = (A0−A1)/A0 × 100%, where A0 is the absorbance of control, and A1 is the absorbance of sample or standard.

2.9 Determination of total phenolic content
The phenolic content of the M. seminifera fruit ethanol extract was determined using Folin-Ciocalteu’s reagent\[19,20\]. Extract of 0.25 g was extracted with 25 mL of 80% aqueous methanol using ultrasonic bath for 15 min. Then 2 mL of the extract was taken, and centrifuged at 21 006 × g for 5 min to separate insoluble mass. Then 1 mL supernatant was taken into 25 mL volumetric flask. Gallic acid was used as standard, and solution in methanol was prepared at the concentrations of 500, 250, 125, 62.5, 31.25, and 15.62 mg/L. An aliquot of 1 mL of each gallic acid concentration was also taken into each separate volumetric flask of 25 mL. Then 9 mL distilled water was added to each volumetric flask. Folin-Ciocalteu’s reagent of 1 mL was added and shaken vigorously. After a 5 min interval, 7% sodium carbonate solution of 10 mL was added to the mixture. Then final volume was adjusted to 25 mL adding distilled water. After the incubation period of 60 min at room temperature, absorbance was measured at 750 nm against blank. Blank was prepared without addition of sample or standard. Gallic acid standard calibration curve was used to calculate the phenolic content of the extract. Total phenolic content of the extract was expressed as mg gallic acid equivalent (GAE)/100 g of dried fruits extract.

2.10 Reducing power assay
Reducing power of the M. seminifera fruit ethanol extract was evaluated according to the method of Oyaizu\[21\]. Sample was prepared at the concentrations of 500, 250, 125, 62.5, 31.25, and 15.62 mg/L by serially diluting the stock solution. An aliquot (1 mL) of the sample at each concentration was mixed with phosphate buffer (200 mmol/L, pH 6.6) of 2.5 mL, and potassium ferricyanide (K3Fe(CN)6, 1%) of 2.5 mL with continuous shaking. The mixture was incubated at 50 °C for 20 min to allow any potential reactions to occur. After the incubation period, trichloroacetic acid (CCl3COOH, 10%) of 2.5 mL was added. Then the mixture was centrifuged for 10 min at 1 006 × g. An aliquot (2.5 mL) of the supernatant was mixed with 0.5 mL of ferric chloride (FeCl3, 0.1%) with continuous shaking. Five minutes later, absorbance was measured at 700 nm against blank. Blank was prepared same as sample without addition of extract or standard. Ascorbic acid was used as standard. Reducing power of the extract was compared with standard ascorbic acid by drawing curve plotting absorbance against concentration.

2.11 Acute toxicity test
The acute toxicity test for M. seminifera fruit ethanol extract was undertaken to assess any possible toxicity using the method of Lorke\[22\]. The extract at the doses of 250, 500, and 1 000 mg/kg body weight was given orally to the respective groups of mice, while control group received distilled water. After a 48-hour observation period, the number of deaths in each group was counted.

2.12 Evaluation of analgesic activity
2.12.1 Writhing test
Experimental mice were divided into four groups of six to carry out the present analgesic activity assessment. M. seminifera fruit ethanol extract was dissolved in distilled water with 1% Twee-80 to make test samples. Test groups were served with the extract at the doses of 250 and 500 mg/kg orally using feeding needle. Diclofenac sodium was the standard drug, and it was fed to the positive control group, at the dose of 25 mg/kg orally. Diclofenac sodium was the standard drug, and it was fed to the positive control group, at the dose of 25 mg/kg orally. Control group received distilled water (10 mL/kg) in oral route. Thirty minutes later each mouse was injected with 0.6% acetic acid intraperitoneally to induce abdominal contraction or writhing.\[23,24\]. After 5 min, number of writhes for each mouse was counted for 10 min\[25\]. Number of writhes of test and standard groups was compared with the control
group, and percent inhibition of writhing was calculated. Percent inhibition was calculated using the formula: inhibition \(= \frac{(W_c - W_i)}{W_c} \times 100\%\), where \(W_c\) is the number of writhes of control group, and \(W_i\) is the number of writhes of test or standard group.

2.12.2 Hot-plate test

Experimental mice were selected based on their susceptibility to this experimental protocol, and divided into four groups consisting of six in each. The *M. seminifera* fruit ethanol extracts (250 and 500 mg/kg, p.o.) were served to the test groups. Morphine (5 mg/kg, i.p.) was the standard drug, and served to the positive control group. Distilled water was used as control, and administered at the dose of 10 mL/kg orally. Each mouse was placed on a hot plate, water was used as control, and administered at the dose of drug, and served to the positive control group. Distilled ethanol extracts (250 and 500 mg/kg, p.o.) were served to the test groups. Morphine (5 mg/kg, i.p.) was the standard drug, was administered at the dose of respective extract, served to the positive control group. Distilled water was used as control, and administered at the dose of 10 mL/kg orally. Each mouse was placed on a hot plate, maintained at the temperature of (55±0.5) °C thermo-statistically. Reaction time was recorded as the amount of time it took for a mouse to withdraw its tail from hot water at the 0, 30, 60, 90, and 120 min after administration. A cut-off period of 20 s was used to avoid accidental paw damage of mice.

2.12.3 Tail-flick test

Experimental mice were divided into four groups consisting of six in each, and used in the assessment of analgesic activity of *M. seminifera* fruit ethanol extract according to the method described by Aydin et al\(^\text{[27]}\). They were held in position in a suitable restrainer with the tail marked the area of 2-3 cm, and immersed in the water-bath maintained at the temperature of (51±0.5) °C thermo-statistically. Reaction time was recorded as the amount of time it took for a mouse to withdraw its tail from hot water at the 0, 30, 60, 90, and 120 min after administration. A cut-off period of 20 s was used to avoid accidental damage of tail of mice. Extract was provided at the doses of 250 and 500 mg/kg orally. Morphine (5 mg/kg, i.p.) was used as the reference drug. Distilled water was used as control (10 mL/kg, p.o.).

2.13 Evaluation of antidiarrheal activity

2.13.1 Castor oil-induced diarrhea

Experimental mice of either sex susceptible to castor oil-induced diarrhea model were divided into four groups consisting of six in each. *M. seminifera* fruit ethanol extracts (250 and 500 mg/kg, p.o.) were served to the test groups using feeding needle. Mice of control group received distilled water (10 mL/kg, p.o.). Loperamide, used as the reference drug, was administered at the dose of 3 mg/kg in oral route. Sixty minutes later, all mice across the four groups received 0.5 mL castor oil orally\(^\text{[28]}\). Then each mouse was housed in a separate cage with white blotting paper at the base to count the number of faces. Observation period was 4 h, and blotting paper was changed each hour. Latent period of defecation for each group was also recorded. Percent inhibition of defecation was calculated using the formula: inhibition \(= (D_0 - D_i)/D_0 \times 100\%\), where \(D_0\) is the number of defecation of control group, and \(D_i\) is the number of defecation of test or standard group.

2.13.2 Magnesium sulfate-induced diarrhea

Experimental mice of both sexes were divided into four groups of six to evaluate antidiarrheal activity of the *M. seminifera* fruit ethanol extract in magnesium sulfate-induced diarrhea model according to the method described by Doherty\(^\text{[29]}\). Magnesium sulfate (2 g/kg, p.o.) was administered to all experimental groups after the interval of 30 min of the administration of respective treatment. Test groups were treated with the extracts (250 and 500 mg/kg, p.o.). Loperamide (3 mg/kg, p.o.) was the reference drug. Control group was treated with distilled water (10 mL/kg, p.o.). All the treatments were administered using a feeding needle. Each mouse was placed in a separated transparent cage with white blotting paper at the base. Latent period and frequency of defecation of all groups were recorded throughout the observation period of 4 h. Percent inhibition of defecation was calculated using the same formula mentioned in castor oil-induced diarrhea model.

2.13.3 Gastrointestinal motility test

Experimental mice were divided into control, positive control, and test groups with six in each for the gastrointestinal motility test according to the method of Abdullahi et al\(^\text{[28]}\) with slight modification. Distilled water was given to the control group at the dose of 10 mL/kg in oral route. Antimotility drug loperamide (3 mg/kg, p.o.) was used as the reference drug and provided to the positive control group. The *M. seminifera* fruit ethanol extracts (250 and 500 mg/kg, p.o.) were provided to the test groups. After a 30-min interval, all mice in the four groups were fed 1 mL of charcoal meal which was prepared with 3% suspension of deactivated charcoal in 0.5% aqueous methyl cellulose. Thirty minutes later, each mouse was sacrificed and dissected to separate intestines. Then length of the intestine (pyloric sphincter to caecum) and distance travelled by charcoal meal as a fraction of the length of intestine were measured for each mouse. Percent distance travelled by charcoal in test and standard groups was compared to the control group.

2.14 Evaluation of antibacterial activity

Disk diffusion assay was used to assess antibacterial activity of the *M. seminifera* fruit ethanol extract against some Gram-positive and Gram-negative bacterial strains\(^\text{[30,31]}\). Second generation subculture of each bacterial strain was prepared. Using sterile loop, a small portion of each subculture was transferred into vials containing nutrient broth medium, and incubated at 37 °C for 2-4 h to reach log phase of bacterial growth. Then standard inoculum suspension was mixed with nutrient agar medium. After mixing, seeded nutrient agar medium was poured into
Petri-dishes, and allowed to solidify at room temperature in aseptic condition. Discs (Oxoid Ltd., UK) impregnated with the fruits extracts (250 and 500 µg/disk), standard antibiotic disc (Ciprofloxacin 5 µg/disk, Oxoid Ltd., UK) and blank discs containing ethanol (control) were placed on the Petri-dishes with sterile forceps. Then Petri-dishes were incubated at 37 °C for 18 h. After the incubation period, zone of inhibition was measured using digital slide calipers in millimeters.

2.15 Statistical analysis

The results were expressed as mean ± standard error of mean and statistical significance was assessed by one-way analysis of variance followed by Dunnett’s test. Statistical analysis was conducted in Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA). Results were considered statistically significant when \( P<0.05 \).

3 Results

3.1 DPPH-scavenging assay

The ethanol extract of \( M. \) seminifera fruit showed 50% inhibitory concentration (IC\(_{50}\)) value of 12.85 mg/L, while the standard ascorbic acid showed IC\(_{50}\) value of 6.76 mg/L (Figure 1). Activity was increased linearly in a concentration-dependent manner at lower concentration, and saturation was reached at higher concentration for both the extract and standard.

3.2 Determination of total phenolic content

The total phenolic content of the ethanol extract of \( M. \) seminifera fruit was found to be 589.83 mg GAE per 100 g of dried fruit extract (Figure 2).

3.3 Reducing power assay

Reducing power of the ethanol extract of \( M. \) seminifera fruit was increased in a concentration-dependent manner, and was highly comparable with the standard ascorbic acid (Figure 3).

3.4 Acute toxicity test

After the observation period of 48 h, no mortality was observed even at the maximum dose of 1 000 mg/kg body-weight. So we can estimate, the minimum lethal dose of the extract is more than 1 000 mg/kg.

3.5 Evaluation of analgesic activity

3.5.1 Writhing test

The ethanol extract of \( M. \) seminifera fruit exhibited significant (\( P<0.01 \)) inhibition of writhing at both dose levels in comparison to the control. The extract showed 48.14% and 65.92% inhibition of writhing at the doses of 250 and 500 mg/kg, respectively. Standard diclofenac sodium showed 77.04% inhibition of writhing at the dose of 25 mg/kg. So the analgesic activity of the extract was highly comparable with the standard diclofenac sodium (Table 1).

3.5.2 Hot-plate test

The reaction time of the \( M. \) seminifera fruit ethanol extract-treated groups was significantly increased (\( P<0.01 \)). The maximum reaction time was 6.00 and 7.32 s at 90 min at the doses of 250 and 500 mg/kg, respectively. Standard diclofenac sodium showed 77.04% inhibition of writhing at the dose of 25 mg/kg. So the analgesic activity of the extract was highly comparable with the standard diclofenac sodium (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Number of writhes</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>22.50±0.42</td>
<td>—</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>6</td>
<td>5.17±0.30</td>
<td>77.04</td>
</tr>
<tr>
<td>Extract (250 mg/kg)</td>
<td>6</td>
<td>11.67±0.33</td>
<td>48.14</td>
</tr>
<tr>
<td>Extract (500 mg/kg)</td>
<td>6</td>
<td>7.67±0.33</td>
<td>65.92</td>
</tr>
</tbody>
</table>

**\( P<0.01 \), vs control group.**
morphine showed the maximum reaction time of 8.71 s at 90 min at the dose of 5 mg/kg. Both the extract and standard morphine increased reaction time in comparison to the control, which supports the potential analgesic activity of *M. seminifera* fruit (Table 2).

### 3.5.3 Tail-flick test

In the tail-flick test, the groups treated with the ethanol extract and morphine both significantly raised pain threshold level in comparison to the control (*P*<0.01). The maximum reaction time of the extract-treated groups were 8.65 and 9.55 s at 90 min at the doses of 250 and 500 mg/kg, respectively. The morphine-treated group (5 mg/kg) showed the maximum reaction time of 11.26 s at 90 min. The extract showed considerable analgesic activity, which was highly comparable with the standard drug morphine (Table 3).

### 3.6 Evaluation of antidiarrheal activity

#### 3.6.1 Castor oil-induced diarrhea

In the castor oil-induced diarrheal model, the ethanol extract of *M. seminifera* fruit significantly delayed the onset of diarrhea (*P*<0.01), and decreased the frequency of defecation in comparison to the control. The extract showed 56.29% and 71.67% inhibition of defecation at the doses of 250 and 500 mg/kg, respectively, while loperamide showed 87.40% inhibition at the dose of 3 mg/kg (Table 4).

#### 3.6.2 Magnesium sulfate-induced diarrhea

In the magnesium sulfate-induced diarrhea model, the ethanol fruit extract of *M. seminifera* and loperamide significantly delayed the onset of diarrhea (*P*<0.01) as well as decreased the frequency of defecation (Table 5). The extract showed 40.72% and 64.99% inhibition of defecation at the doses of 250 and 500 mg/kg, respectively, in comparison to the control. Loperamide showed 82.13% inhibition of defecation at the dose of 3 mg/kg.

#### 3.6.3 Gastrointestinal motility test

In the gastrointestinal motility test, the ethanol extract of *M. seminifera* fruit and standard antimotility drug loperamide significantly retarded the gastrointestinal transit of charcoal meal in comparison to control (*P*<0.01) (Table 6).

### 3.7 Evaluation of antibacterial activity

The ethanol extract of *M. seminifera* fruit exhibited a

### Table 2 Effects of *M. seminifera* fruit in hot-plate test in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Reaction time (s)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>3.27±0.05</td>
<td>3.44±0.05</td>
<td>3.37±0.08</td>
<td>3.33±0.08</td>
<td>3.54±0.06</td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td>6</td>
<td>3.58±0.07</td>
<td>6.08±0.12**</td>
<td>7.10±0.14**</td>
<td>8.71±0.18**</td>
<td>7.30±0.13**</td>
<td></td>
</tr>
<tr>
<td>Extract (250 mg/kg)</td>
<td>6</td>
<td>3.35±0.06</td>
<td>4.86±0.15**</td>
<td>5.20±0.08**</td>
<td>6.00±0.12**</td>
<td>4.75±0.15**</td>
<td></td>
</tr>
<tr>
<td>Extract (500 mg/kg)</td>
<td>6</td>
<td>3.40±0.05</td>
<td>5.09±0.13**</td>
<td>6.51±0.09**</td>
<td>7.32±0.12**</td>
<td>6.05±0.09**</td>
<td></td>
</tr>
</tbody>
</table>

**P*<0.01, vs control group.

### Table 3 Effects of *M. seminifera* fruit in tail-flick test in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Reaction time (s)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>4.00±0.03</td>
<td>4.03±0.04</td>
<td>4.06±0.05</td>
<td>4.05±0.09</td>
<td>4.10±0.06</td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td>6</td>
<td>4.27±0.05</td>
<td>6.29±0.14**</td>
<td>9.22±0.13**</td>
<td>11.26±0.10**</td>
<td>10.24±0.13**</td>
<td></td>
</tr>
<tr>
<td>Extract (250 mg/kg)</td>
<td>6</td>
<td>4.23±0.04</td>
<td>5.40±0.11**</td>
<td>6.71±0.07**</td>
<td>8.65±0.10**</td>
<td>7.54±0.09**</td>
<td></td>
</tr>
<tr>
<td>Extract (500 mg/kg)</td>
<td>6</td>
<td>4.25±0.04</td>
<td>6.56±0.11**</td>
<td>7.58±0.10**</td>
<td>9.55±0.12**</td>
<td>8.60±0.12**</td>
<td></td>
</tr>
</tbody>
</table>

**P*<0.01, vs control group.

### Table 4 Effects of *M. seminifera* fruit on castor oil-induced diarrhea in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Onset of diarrhea (Mean ± standard error of mean, min)</th>
<th>Number of stools after 4 h (Mean ± standard error of mean)</th>
<th>Inhibition of defecation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>31.33±1.92</td>
<td>25.17±0.42</td>
<td>—</td>
</tr>
<tr>
<td>Loperamide</td>
<td>6</td>
<td>191.20±1.86**</td>
<td>3.17±0.30**</td>
<td>87.40</td>
</tr>
<tr>
<td>Extract (250 mg/kg)</td>
<td>6</td>
<td>97.00±2.58**</td>
<td>11.00±0.36**</td>
<td>56.29</td>
</tr>
<tr>
<td>Extract (500 mg/kg)</td>
<td>6</td>
<td>123.3±5.72**</td>
<td>7.13±0.30**</td>
<td>71.67</td>
</tr>
</tbody>
</table>

**P*<0.01, vs control group.
**Table 5** Effects of *M. seminifera* fruit on magnesium sulfate-induced diarrhea in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Onset of diarrhea (Mean ± standard error of mean, min)</th>
<th>Number of stools after 4 h (Mean ± standard error of mean)</th>
<th>Inhibition of defecation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>38.50±1.61</td>
<td>23.33±0.61</td>
<td>—</td>
</tr>
<tr>
<td>Loperamide</td>
<td>6</td>
<td>194.20±3.31**</td>
<td>4.17±0.47**</td>
<td>82.13</td>
</tr>
<tr>
<td>Extract (250 mg/kg)</td>
<td>6</td>
<td>70.67±1.88**</td>
<td>13.83±0.47**</td>
<td>40.72</td>
</tr>
<tr>
<td>Extract (500 mg/kg)</td>
<td>6</td>
<td>107.00±1.50**</td>
<td>8.17±0.30**</td>
<td>64.99**</td>
</tr>
</tbody>
</table>

**P<0.01, vs control group.**

**Table 6** Effects of *M. seminifera* fruit on charcoal meal-stimulated gastrointestinal transit in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean intestinal length (Mean ± standard error of mean, cm)</th>
<th>Mean distance traveled by charcoal (Mean ± standard error of mean, cm)</th>
<th>Gastrointestinal transit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>51.13±1.34</td>
<td>45.67±1.98</td>
<td>89.32</td>
</tr>
<tr>
<td>Loperamide</td>
<td>6</td>
<td>56.67±1.27**</td>
<td>22.78±1.86**</td>
<td>40.19</td>
</tr>
<tr>
<td>Extract (250 mg/kg)</td>
<td>6</td>
<td>57.12±1.47**</td>
<td>35.13±1.12**</td>
<td>61.50</td>
</tr>
<tr>
<td>Extract (500 mg/kg)</td>
<td>6</td>
<td>55.25±1.13**</td>
<td>27.39±1.42**</td>
<td>49.57**</td>
</tr>
</tbody>
</table>

**P<0.01, vs control group.**

**Table 7** Results of the disc diffusion assay of *M. seminifera* fruit

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Diameter of zone of inhibition (mm)</th>
<th>M. seminifera (250 μg/disc)</th>
<th>M. seminifera (500 μg/disc)</th>
<th>Ciprofloxacin (5 μg/disc)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>7</td>
<td>16</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>8</td>
<td>15</td>
<td>15</td>
<td>26</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>9</td>
<td>17</td>
<td>17</td>
<td>27</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>7</td>
<td>15</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td><em>Shigella flexneri</em></td>
<td>9</td>
<td>14</td>
<td>14</td>
<td>26</td>
</tr>
<tr>
<td><em>Shigella sonnei</em></td>
<td>10</td>
<td>16</td>
<td>16</td>
<td>25</td>
</tr>
<tr>
<td><em>Shigella dysenteri</em></td>
<td>9</td>
<td>15</td>
<td>15</td>
<td>28</td>
</tr>
</tbody>
</table>

A significant zone of inhibition against all the tested Gram-positive and Gram-negative bacterial strains. The diameter of zone of inhibition ranged between 7 to 17 mm (Table 7).

**4 Discussion**

DPPH radical-scavenging activity assessment allows us to determine the intrinsic ability of chemical substances to donate a hydrogen atom, donate an electron to reactive species, or to convert them into stable form, in a homogeneous system. In this method, compounds such as phenols and flavonoids, present in the extract, converted reactive DPPH free radicals into nonreactive stable DPPH-H form\(^{32}\). The extract showed considerable free radical-scavenging activity in the DPPH assay.

Phenolics or polyphenols are secondary metabolites of plant origin, and usually show high antioxidant activities\(^{33}\). Phenolic compounds show antioxidant activity by redox mechanism. Phenolic compounds also neutralize lipid free radicals as well as prevent decomposition of reactive species\(^{34,35}\). This study found considerable amounts of phenolic compounds in the total phenolic content determination using Folin-Ciocalteu’s reagent.

Reducing power of the ethanol extract of *M. seminifera* fruit was determined, by assessing the ability of the extract to reduce ferricyanide \([Fe(CN)₆]^{3-}\) into ferrocyanide \([Fe(CN)₆]^{4-}\) by donating an electron. After reduction, the end product was visualized by adding free \(Fe^{3+}\) ions from \(FeCl₃\) which leads to the formation of Prussian blue \((Fe^{3+})(Fe^{2+}(CN)₆)_{6}\) and it was quantitatively assessed by taking absorbance at 700 nm\(^{36}\). The extract showed considerable reducing power in a concentration-dependent manner, and the activity was highly comparable with the standard ascorbic acid.

The extract showed potential peripherally and centrally acting analgesic activity in all the tested experimental models, in a significant and dose-dependent manner \((P<0.01)\). Abdominal contraction or writhing was produced by acetic...
acid (0.6% v/v) which leads to the release of pain mediators like prostaglandin E₂ and prostaglandin E₃. Hot-plate and tail-flick tests are promising methods of assessing the analgesic activity at the spinal cord level[37,38]. The extract significantly increased pain threshold, in both tests, in comparison to the control (P<0.01).

The extract showed significant anti-diarrheal activity (P<0.01) in castor oil- and magnesium sulfate-induced diarrhea models as well as retarded gastrointestinal transit of charcoal meal in mice. Castor oil causes diarrhea by liberating ricinolic acid, which releases prostaglandins by irritating intestinal mucosa, and the released prostaglandins stimulate intestinal motility and secretion of intestinal content[39]. Magnesium sulfate causes diarrhea by inhibiting reabsorption of fluids and watery materials. It also releases cholecystokinin from the duodenal mucosa which stimulates secretion and intestinal motility, and ultimately reabsorption of fluids and electrolytes is inhibited[40]. So the extract may increase reabsorption of fluids and electrolytes. The extract also decreased gastrointestinal motility significantly in mice (P<0.01), which revealed potential antimotility activity. Given the above, the extract may have considerable antisecretory and antimotility activity.

Disk diffusion assay is the most flexible, inexpensive, and simple method to assess antibacterial activity against pathogenic bacteria. The extract showed a considerable zone of inhibition against all the tested bacterial strains. But disk diffusion assay has some limitations, because polarity of the compound affects the diffusion of natural products in culture medium. Less polar compounds diffuse more slowly and poorly than polar compounds, because agar media is prepared in water[41]. So it is not possible to accurately measure the antibacterial activity of the non-polar compounds in disk diffusion assay.

5 Conclusion

This study demonstrates that the ethanol extract of M. seminifera fruit has potential antioxidant, analgesic, antidiarrheal, and antibacterial activities. Results are quite promising and further work is needed to better understand the biological activities of M. seminifera fruit.

6 Acknowledgements

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7 Competing interests

The authors declare that they have no competing of interests.

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