Introduction

Medicinal plants have faithfulness in use for the alleviation of various ailments and human sufferings since ancient era. They have been used in traditional medicine for hundreds of years with reputation as efficacious remedies although these may lack of sufficient scientific data for the evidence of their efficacy. Medicinal plants are the prime sources of bioactive compounds and serve as raw materials for new drug discovery[1]. Alkaloids, tannins, glycosides, flavonoids, volatile oils, etc., are synthesized from medicinal plants containing a great potential for pharmacological activity and can be used for therapeutic purposes[2].

Medicinal plants play a significant role in developing modern medicine. With the advancement of human civilization...
and knowledge, therapeutic uses of the medicinal plants have been increased in considerable extent. Researchers always take an attempt to perform pharmacological tests to identify and isolate the bioactive compound(s). Modern medicine is developed gradually in this way.

Alocasia indica Schott (synonym: Alocasia macrorrhizos (L.) G. Don, Araceae) is an evergreen robust herb growing up to 1.8 m under favorable conditions and distributed widely in Asia, and cultivated in many Pacific islands. It grows all over in Bangladesh all the year round and locally known as Mankachu. It is commonly known as Elephant Ear Taro or Gaint Taro in English.

Traditionally, A. indica is used in inflammation and abdominal diseases. The leaf juice of the plant is used as anthelmintic, digestive, astringent, and laxative. The whole plant is used in Kavirajes medicine in different districts of Bangladesh as a remedy of tiger bite, rheumatoid arthritis, and itching. The plant contains ascorbic acid, malic acid, gallic acid, oxalic acid, amino acids, succinic acid, flavonoids, and glycosides. The different parts of the plant are eaten as vegetable all over in Bangladesh.

A literature survey demonstrated no precedent reported pharmacological investigation on A. indica tuber. But few studies were performed on leaves and rootstocks of A. indica. The leaves have been reported to have significant antioxidant, antinociceptive, anti-inflammatory, anti-diabetic, and hypolipidemic properties. It has also been reported with antifungal and hepatoprotective properties. The rootstocks have been reported to have anthelmintic activity.

In the present investigation, the ethanol extract of the tuber of A. indica was pharmacologically tested to assess the antioxidant, antidiarrheal, cytotoxic, and antibacterial activities.

2 Materials and methods

2.1 Collection of plant material

The tubers of A. indica were collected from the district of Tangail, Bangladesh in November, 2012. Any type of adulteration was strictly avoided during collection. Botanical identification was performed by the experts of Bangladesh National Herbarium, Mirpur, Dhaka, where a voucher specimen (Accession No.: DCAB 37916) has been deposited for further reference.

2.2 Drying and grinding

The rootstocks were cut into small pieces with the sharp knife. Then plant parts were subjected to shade drying to remove moisture. The plant materials were grinded into coarse powder using a mechanical grinder hammermill. The powder was kept in an airtight container to avoid any possible fungal attack and then stored in a dark, cool and dry place until the extraction was started.

2.3 Extraction

Powdered plant material was subjected to cold extraction technique for the extraction. The powder was soaked in 95% ethanol for 7 d with regular shaking and stirring. The extract was filtered off to separate the plant debris. Clean cotton plug and filter paper were used to get clear solution. Then filtrate was evaporated at 50 °C with the help of rotary vacuum evaporator to yield crude extract. Then the crude extract was stored in a refrigerator at 4 °C (yield 1.73%).

2.4 Experimental animals

In vivo pharmacological investigation was conducted on young Swiss albino mice of both sexes (age 4-5 weeks, weight 18-25 g). They were collected from Animal Resources Department of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). The animals were housed under standard laboratory conditions (room temperature of (25±1) °C, and relative humidity of 56%-60% with a 12-h light:12-h dark cycle) and had free access to ICDDR, B-formulated rodent food and water. They were allowed to acclimatize to the environment for 7 d prior to the pharmacological experiments.

2.5 Test microorganisms

The antibacterial assessment was performed by using both Gram-positive and Gram-negative bacteria supplied by the Microbiology Laboratory of ICDDR, B. Test organisms were Staphylococcus aureus, Salmonella typhi, Staphylococcus epidermidis, Escherichia coli, Shigella flexneri, Shigella dysenteriae, and Shigella sonnei.

2.6 Chemicals and reagents

Ascorbic acid, sodium carbonate, gallic acid, ferric chloride, potassium ferricyanide, magnesium sulfate, dimethyl sulfoxide (DMSO) and ferric chloride were purchased from Merck, Germany. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and Folin-Ciocalteu’s reagent were obtained from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). Castor oil was supplied by Loba Chemie Pvt Ltd., India.

2.7 Reference drugs

Loperamide was purchased from Square Pharmaceuticals Ltd., Bangladesh. Standard ciprofloxacin disk was obtained from Oxoid Ltd., UK.

2.8 In vitro antioxidant assay

The antioxidant activity of the ethanol extract of the tuber was evaluated by both qualitative and quantitative assay. Thin-layer chromatographic (TLC) technique was applied for qualitative assay, and DPPH-scavenging technique was used for quantitative assay.

2.9 Qualitative assay

When TLC plates were developed, solvent systems of different polarities were used to resolve non-polar, medium polar and polar compounds. Then the plates were subjected to 0.002% DPPH solution in ethanol sprayed by using a spray gun. After 30 min, bleaching of DPPH (yellow on purple background) radial was observed and noted.
2.10 Quantitative assay
This experiment was carried out according to the method of Sharma et al.\cite{13} for the assessment of DPPH radical-scavenging activity. For quantitative assessment, stock solution of the plant extract was prepared in ethanol. The sample was serially diluted in ethanol to obtain the desired final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, and 1 µg/mL. Then 1 mL of each diluted sample was mixed with 3 mL of 0.004% DPPH solution and allowed to stand for 30 min in dark place to complete reactions. Then the absorbance was taken at 517 nm against blank. Control was prepared without addition of any extract or standard as sample preparation. Ascorbic acid was used as standard. Percent scavenging activity was calculated using the following formula:

Percent scavenging activity = \((1 - A_s/A_c) \times 100\%\),
where \(A_s\) is the absorbance of control, and \(A_c\) is the absorbance of sample or standard.

2.11 Determination of total phenolic content
The total phenolic content of the ethanol extract of the tuber was determined by using Folin-Ciocalteu’s reagent\cite{15-17}. Dried 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 sonicated extract was taken and centrifuged at 21,913 × g for 5 min. Then 1 mL liquid from the supernatant was transferred into a 25-mL volumetric flask. Gallic acid (used as standard) solution in MeOH was prepared at the concentrations of 512, 256, 128, 64, 32, and 16 µg/mL. From each gallic acid concentrations, as well as from the extract, an aliquot of 1 mL was 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 (diluted in ten times) was added to each volumetric flask with continuous shaking. After the interval of 5 min, 7% Na₂CO₃ solution (10 mL) was added to the mixture. Then distilled water was added to make the final volume of 25 mL. After an incubation period of 60 min at room temperature, absorbance was measured at 700 nm against the blank. Blank was prepared same as sample without addition of extract or standard. Phenolic content of the extract was calculated using gallic acid standard calibration curve. Total phenolic content of the extract was expressed as mg gallic acid equivalent (GAE) per 100 g of dried tuber extract.

2.12 Determination of total flavonoid content
Total flavonoid content of the ethanol extract of the tuber was evaluated by aluminum chloride colorimetric assay\cite{16,17}. Extract (1 mL) or standard MeOH solution of quercetin (512, 256, 128, 64, 32, 16, 8, 4, 2, and 1 µg/mL) was taken into a 10-mL volumetric flask and then 4 mL of distilled water was added. After that, 5% NaNO₂ (0.3 mL) was added to the mixture. After 5 min, 10% AlCl₃ (0.3 mL) was added to the mixture. Then 1 mol/L NaOH (2 mL) was added to the mixture and the final volume was adjusted to 10 mL with distilled water, and shaken vigorously. Then absorbance was measured at 510 nm against the blank. Quercetin standard calibration curve was developed by plotting absorbance against concentration. Total flavonoid content of the extract was expressed as mg quercetin equivalent (QE)/g of dried tuber extract.

2.13 Reducing power assay
Reducing power of the ethanol extract of the tuber was evaluated according to the method described by Oyaizu\cite{18}. Sample was prepared, serially diluting the stock solution, at different concentrations of 512, 256, 128, 64, 32, and 16 µg/mL. An aliquot (1 mL) of the sample, at each concentration, was mixed with 2.5 mL of phosphate buffer (200 mmol/L, pH 6.6) and 2.5 mL of 1% potassium ferricyanide \([K₃Fe(CN)₆]_{0.1}\) with vigorous shaking. Then the mixture was incubated for 30 min at 50 °C to complete the possible reactions. After that, 10% trichloroacetic acid (CCl₃COOH, 2.5 mL) was added to the mixture. Then the mixture was centrifuged for 10 min at 1,006 × g. The supernatant of 2.5 mL was then mixed with 0.5 mL of ferric chloride (FeCl₃, 0.1%) with vigorous shaking. After 5 min, absorbance was measured at 700 nm against the blank. Blank was prepared same as sample without addition of extract or standard. Ascorbic acid was used as standard in reducing power assay. Reducing ability of the extract was measured by comparing the result with the standard ascorbic acid.

2.14 Acute toxicity test
The acute toxicity test was conducted for the extract to determine any possible toxicity and safe doses, using the method illustrated by Lorke\cite{19}. The extract was given orally to the respective group of mice at the doses of 250, 500, and 1,000 mg/kg body weight, while distilled water was given to the control group. After observing 48 h, number of deaths in each group was counted.

2.15 Evaluation of antidiarrheal activity
2.15.1 Castor oil-induced diarrhea
The experiment was performed on Swiss albino mice of either sex based on their sensitivity to castor oil-induced diarrheal model and divided into four groups containing six mice in each. Ethanol extract of the tuber was administered orally to the mice of test groups at doses of 250 and 500 mg/kg by using a feeding needle. Distilled water (10 mL/kg, p.o.) was served to the control group while loperamide (reference drug) was administered to the positive control group at the dose of 3 mg/kg in oral route. After an interval of 60 min, each mouse of all the groups was administered with 0.5 mL of castor oil in oral route\cite{20}. Individual animal was then kept separately in transparent cage having white blotting paper at the beneath to count the number of faeces\cite{15,21,22}. Blotting paper was changed every hour and the severity of diarrhea was observed.
hourly within a period of 4 h. Latent period of faecal drops for each group was also recorded. Latent period of faecal drops and percent inhibition of defecation of each group were measured and compared. By using the following formula percent inhibition of defecation was calculated:

\[
\text{Percent inhibition} = \left(1 - \frac{D_1}{D_0}\right) \times 100\% ,
\]

where \( D_0 \) is the number of defecation of the control group, and \( D_1 \) is the number of defecation of the test or standard group.

### 2.15.2 Magnesium sulfate-induced diarrhea

The experiment was carried out according to the method described by Doherty\(^{[23]}\). To evaluate antidiarrheal activity, experimental mice were divided into four groups consisting of six mice of both sexes. All the experimental groups were administered with magnesium sulfate (2 g/kg, p.o.) after a period of 30 min of the administration of individual treatment. Plant extract (250 and 500 mg/kg, p.o.) was administered to the test groups. The reference drug was loperamide (3 mg/kg, p.o.). Distilled water was supplied to the control group (10 mL/kg, p.o.). Feeding needle was used for all oral administration. Each animal was then kept in individual perforated cage with white blotting paper at the bottom of the cage. Latent period of defecation of all groups was noted for 4 h\(^{[24]}\). For the assessment of antidiarrheal activity, percentage of inhibition was calculated using the formula that was discussed previously in castor oil-induced diarrheal model.

### 2.15.3 Gastrointestinal motility test

The gastrointestinal motility test was performed for the present study according to the method of Saha et al\(^{[25]}\) with slight modification. Test animals were divided into control, positive control, and test groups containing six in each. The control group received distilled water (10 mL/kg) in oral route. The positive control group was administered with the loperamide (3 mg/kg, p.o.), which was used as the standard. Ethanol extract of the tuber was administered to the test groups in oral route at the doses of 250 and 500 mg/kg. After 30-minute interval, 1 mL of charcoal meal (used as a marker diet) was provided to the individual mouse of each group that was prepared with deactivated charcoal suspension (3%) in aqueous methyl cellulose (0.5%). After 30 min, individual animal of all groups was dichotomized to separate intestine. Length of the intestine from pyloric sphincter to caecum and distance moved by charcoal meal to separate intestine. Length of the intestine from pyloric sphincter to caecum and distance moved by charcoal meal were measured for 4 h. For the assessment of antidiarrheal activity, percentage of inhibition was calculated using the formula that was discussed previously in castor oil-induced diarrheal model.

### 2.16 Evaluation of antibacterial activity by disk diffusion assay

Antibacterial activity of the ethanol extract of the tuber, against a number of Gram-positive and Gram-negative bacterial strains, was evaluated by disk diffusion assay\(^{[26,27]}\). Subculture of each bacterial strain (second generation) was prepared. Diminutive portion of each subculture was migrated, using sterile loop, into the vial that contains nutrient broth medium. To reach log phase of bacterial growth, the vial was incubated at 37 °C for 2-4 h. Then standard inoculum suspension was placed on the Petri-dishes with sterile forceps. Then Petri-dishes were placed into incubator at 37 °C temperature for 18 h. After incubation period, digital slide calipers were used to measure the zone of inhibition.

### 2.17 Brine shrimp lethality assay for general toxicity

Brine shrimp lethality assay was carried out for testing general toxicity of the extract\(^{[28]}\). A total of 20 g of NaCl and 18 g of table salt was dissolved in 1 L of distilled water in order to make artificial sea water. Then the solution was filtered off to get clear solution. By using a porous separator, a rectangular tank was divided into two unequal compartments. The larger compartment was darkened whereas the smaller one was enlightened. The eggs of *Artemia salina* were hatched at 25-30 °C (room temperature) for 24-48 h. The light attracted the larvae (nauplii) and they were moved to the smaller compartment through holes. A pipette was used to collect them. Sample was dissolved in DMSO and transferred into test tubes in such a way that each tube contains 5 mL artificial sea water and ten nauplii with sample concentrations of 320, 160, 80, 40, 20, 10 and 5 μg/mL. At the concentrations of 5, 2.5, 1.25, 0.625 and 0.312 μg/mL, anticancer drug vincristine sulphate was used as positive control. DMSO concentration did not exceed 0.01% in any of the test tubes. Using a magnifying glass, the number of viable nauplii was counted after the incubation period of 24 h at room temperature (25-30 °C).

### 2.18 Statistical analysis

All experimental data was expressed as mean ± standard error of mean. Dunnett’s test was used to assess statistical significance by one-way analysis of variance. Statistical analysis was executed in Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA). Results of the present study were considered as statistically significant when \( P<0.05 \).

### 3 Results

#### 3.1 DPPH-scavenging assay

The ethanol extract of the tuber exhibited the 50% inhibitory concentration (IC\(_{50}\)) value of 42.66 μg/mL, while the standard ascorbic acid showed the IC\(_{50}\) value of 12.58 μg/mL (Figure 1). At the lower concentration, both the extract and ascorbic acid showed gradual increases of scavenging activity but
at the higher concentration a plateau was achieved.

3.2 Determination of total phenolic content

The total phenolic content of the ethanol extract of the tuber was found to be 542.26 mg GAE per 100 g of dried tuber extract (Figure 2).

3.3 Determination of total flavonoid content

The total flavonoid content of the ethanol extract of the tuber was found to be 4.30 mg QE/g of dried plant material (Figure 3).

3.4 Reducing power assay

The ethanol extract of the tuber showed an increase in reducing power with the increase in concentration. The reducing ability was highly comparable to the standard ascorbic acid (Figure 4).

3.5 Acute toxicity test

No mortality was found even at the maximum dose of 1 000 mg/kg body weight after an observation period of 48 h. So it can be estimated that the minimum lethal dose of the extract is more than 1 000 mg/kg.

3.6 Evaluation of antidiarrheal activity

3.6.1 Castor oil-induced diarrhea

The ethanol extract produced a significant ($P<0.01$) and dose-dependent prolongation in onset of diarrhea and reduction in frequency of defecation in comparison to the control. The extract revealed 38.90% and 56.34% inhibition of defecation at the doses of 250 and 500 mg/kg, respectively, whereas the reference drug, loperamide, exhibited 85.71% inhibition at the dose of 3 mg/kg (Table 1).

3.6.2 Magnesium sulfate-induced diarrhea

In the magnesium sulfate-induced diarrheal model, the ethanol extract of the tubers produced a significant ($P<0.01$) and dose-dependent prolongation in onset of diarrhea and reduction in frequency of defecation in comparison to the control. The extract revealed 37.49% and 59.60% inhibition of defecation at the doses of 250 and 500 mg/kg, respectively, whereas the reference drug, loperamide, exhibited 84.56% inhibition at the dose of 3 mg/kg (Table 2).

3.6.3 Gastrointestinal motility test

In the gastrointestinal motility test, the ethanol extract of the tuber and loperamide delayed the gastrointestinal transit of charcoal meal as compared with the control in a statistically significant manner ($P <0.01$). The extract showed 25.06% and 35.63% inhibition of intestinal transit at the doses of 250 and 500 mg/kg, respectively, while the standard drug, loperamide, exhibited 49.91% inhibition at the dose of 3 mg/kg (Table 3).
3.7 Evaluation of antibacterial activity by disk diffusion assay

The ethanol extract of the tubers showed moderate zone of inhibition against all the tested Gram-positive and Gram-negative bacterial strains. Zone of inhibition ranged between 5.80 to 9.86 mm and 12.10 to 18 mm, at the doses of 250 and 500 μg/disc, respectively (Table 4).

3.8 Brine shrimp lethality bioassay

In brine shrimp lethality bioassay, 50% lethal concentration (LC₅₀) was calculated using Ldp line probit analysis software, USA. The LC₅₀ for ethanol extract of A. indica tuber was found to be 81.09 μg/mL whereas that of vincristine sulphate was 0.47 μg/mL.

Table 1 Effects of A. indica tubers on castor oil-induced diarrhea in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Onset of diarrhea (mean ± SEM, min)</th>
<th>Number of stools after 4 h (mean ± SEM)</th>
<th>Inhibition of defecation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>35.33±2.33</td>
<td>21.00±1.86</td>
<td>—</td>
</tr>
<tr>
<td>Loperamide (3 mg/kg)</td>
<td>6</td>
<td>177.70±3.29**</td>
<td>3.00±0.57**</td>
<td>85.71</td>
</tr>
<tr>
<td>A. indica tuber extract (250 mg/kg)</td>
<td>6</td>
<td>66.17±2.83**</td>
<td>12.83±0.94**</td>
<td>38.90</td>
</tr>
<tr>
<td>A. indica tuber extract (500 mg/kg)</td>
<td>6</td>
<td>144.30±2.99**</td>
<td>9.17±0.47**</td>
<td>56.34</td>
</tr>
</tbody>
</table>

**P<0.01, vs control group. SEM: standard error of mean.

Table 2 Effects of A. indica tuber on magnesium sulfate-induced diarrhea in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Onset of diarrhea (mean ± SEM, min)</th>
<th>Number of stools after 4 h (mean ± SEM)</th>
<th>Inhibition of defecation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>34.33±1.50</td>
<td>22.67±1.05</td>
<td>—</td>
</tr>
<tr>
<td>Loperamide (3 mg/kg)</td>
<td>6</td>
<td>185.2±3.07**</td>
<td>3.50±0.67**</td>
<td>84.56</td>
</tr>
<tr>
<td>A. indica tuber extract (250 mg/kg)</td>
<td>6</td>
<td>72.33±1.47**</td>
<td>14.17±0.87**</td>
<td>37.49</td>
</tr>
<tr>
<td>A. indica tuber extract (500 mg/kg)</td>
<td>6</td>
<td>137.2±1.60**</td>
<td>9.16±0.47**</td>
<td>59.60</td>
</tr>
</tbody>
</table>

**P<0.01, vs control group. SEM: standard error of mean.

Table 3 Effects of A. indica tuber on charcoal meal-stimulated gastrointestinal transit in normal mice

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean intestinal length (mean ± SEM, cm)</th>
<th>Mean distance traveled by charcoal (mean ± SEM, cm)</th>
<th>Gastrointestinal transit (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>63.43±2.04</td>
<td>44.37±2.28</td>
<td>69.95</td>
<td>—</td>
</tr>
<tr>
<td>Loperamide (3 mg/kg)</td>
<td>6</td>
<td>58.72±1.08**</td>
<td>20.58±1.36**</td>
<td>35.04</td>
<td>49.91</td>
</tr>
<tr>
<td>A. indica tuber extract (250 mg/kg)</td>
<td>6</td>
<td>62.72±1.57**</td>
<td>32.88±1.24**</td>
<td>52.42</td>
<td>25.06</td>
</tr>
<tr>
<td>A. indica tuber extract (500 mg/kg)</td>
<td>6</td>
<td>57.56±1.23**</td>
<td>25.92±1.52**</td>
<td>45.03</td>
<td>35.63</td>
</tr>
</tbody>
</table>

**P<0.01, vs control group. SEM: standard error of mean.

Table 4 Results of the disk diffusion assay of A. indica tuber

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. indica (250 μg/disc)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>5.80</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>6.46</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>7.38</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>7.00</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>8.56</td>
</tr>
<tr>
<td>Shigella sonnei</td>
<td>9.00</td>
</tr>
<tr>
<td>Shigella dysentery</td>
<td>9.86</td>
</tr>
</tbody>
</table>
4 Discussion

Antioxidant potential of the plants is a startling capability to remove free radical intermediates from the body that are responsible for some life-threatening diseases like cancer, diabetes, stroke, etc. Presence of free radicals in the body can accelerate the chain reactions resulting cell damage. By removing the free radical molecules, antioxidant plays a significant role in blocking these chain reactions.

DPPH free radical assay was conducted for the assessment of antioxidant potential of the ethanol extract of the tuber of A. indica. In this assay, compound like phenols and flavonoids of the plant extract, converts DPPH (highly reactive free radical) into stable non-reactive DPPH-H form by donating electron or hydrogen radical. Potential DPPH-scavenging activity was exhibited by the plant extract which was strongly comparable to the standard antioxidant ascorbic acid.

Plant extracts exhibit antioxidant potential due to the presence of phenolics that are secondary metabolites of plant origin. Phenolic compounds have the ability to donate electron that results the conversion of highly reactive free radicals to nonreactive stable molecules. Phenolic compounds not only neutralize lipid free radicals but also prevent the decomposition of highly reactive species. Significant amount of total phenolic content was found in the extract that strengthened the evidence of its antioxidant activity.

Flavonoids are another important functional groups that are responsible for the antioxidant potential in plant source. Flavonoids have a significant role in scavenging different reactive oxygen species like hydrogen peroxide, hydroxyl, peroxyl, superoxide anion, etc. The extract exhibited good amount of flavonoid contents that strengthen the logic for radical-scavenging activity showed in DPPH assay.

Reducing power is an established method for the assessment of antioxidant potential. Reducing power of the extract of tuber was measured, by evaluating the ability of the extract to reduce ferricyanide [Fe(CN)₆]³⁻ into ferrocyanide [Fe(CN)₆]⁴⁻ through redox mechanism. After reduction, formation of Prussian blue (Fe⁵⁺)[Fe⁷⁺(CN)₆]₃ was observed as a resultant product, because of adding free Fe³⁺ ions from FeCl₃. Then absorbance was taken at 700 nm. Potential reducing power was exhibited by the extract in a concentration-dependent manner and the result was highly comparable to the standard ascorbic acid.

Diarrhea refers to an increased frequency or decreased consistency of bowel movements resulting in excess passage of watery stools. Antidiarrheal activity of the plant extract was assessed by castor oil- and magnesium sulfate-induced diarrheal models and gastrointestinal motility test in mice. Castor oil induces diarrhea by releasing its active metabolite ricinoleic acid that stimulates the peristaltic movement of small intestine and secretion of intestinal content due to the release of prostaglandins. Magnesium sulfate enhances intestinal content by inhibiting reabsorption of fluids and electrolytes that causes diarrhea. It has been proved that magnesium sulfate has the ability to release cholystokinin from the duodenal mucosa that increases intestinal motility. So the plant extract might have the ability to elevate the reabsorption of watery materials and electrolytes. The extract showed remarkable antimotility activity by decreasing gastrointestinal motility significantly (P<0.01) in mice. It demonstrated that the extract might have considerable antimotility activity.

One of the most popular, inexpensive, and easy methods for determining antibacterial activity against pathogenic bacterial strains is disk diffusion assay. Considerable zone of inhibition was exhibited by the plant extract against all the tested pathogenic bacterial strains. Although disk diffusion assay is simple but it has some limitations. As the agar media is prepared in water, non-polar compounds do not diffuse in water. So it is not possible to determine the antibacterial activity of non-polar compounds correctly through disk diffusion assay.

The brine shrimp lethality bioassay is a rapid, simple and convenient technique for identifying biological active compounds having cytotoxic activity in the crude extract. This method is well known for identifying important pharmacological activities like enzyme inhibition, ion channel interference and cytotoxic activity. A significant correlation between the brine shrimp lethality bioassay and in vitro growth inhibition of rapidly growing human tumor cell lines is authenticated by the National Cancer Institute, USA. In the present evaluation, both extract and vincristine sulfate (an anticancer drug) showed increase amount of mortality rate in a concentration-dependent manner. The LC₅₀ obtained for the extract was low, indicating that the extract is quite potent.

5 Conclusion

From the present investigation, it can be proclaimed that the ethanol extract of A. indica tuber showed notable antioxidant, cytotoxic, antidiarrheal, and antibacterial potentials that strengthen the logic for its use in traditional medicine. Results demand further investigations to isolate bioactive compounds responsible for these bioactivities.

6 Acknowledgements

The authors are grateful to the authorities of Phytochemistry and Pharmacology Laboratory, Pharmacy Discipline, Life Science School, Khulna University, Bangladesh for providing...
necessary instrumental and financial support and also to the ICCDR, B for providing experimental mice.

7 Competing interests

We authors declare that we have no competing interests.

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