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INTRODUCTION

Plants represent a rich source of antimicrobial agent1 and natural antioxidants2. Many plant materials used in traditional medicines are readily available in rural areas at relatively cheaper price than modern medicines3. Plants generally produce many secondary metabolites which constitute an important source of microbicides, anti-oxidants. Many natural substances having anti-oxidant and anti-microbial properties have been used in health foods for medicinal and preservative purposes2. Again Drugs which are presently used for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone4. All of these drugs present well known toxic effects. On the contrary many medicines of plant origin had been used since long time without any adverse effects. It is therefore essential that efforts should be made to introduce and develop safer drugs using medicinal plants. *Ipomoea mauritiana* is common in the Indian sub-continent. It has a has wide range of distribution extending up to 6000 feet or even more above sea level in the hills of south-east Asia. All the species of *Ipomoea* are distributed as annuals. The plant has extensive medicinal uses. It is used in skin diseases, in the treatment of anorexia, fever, inflammation and burning sensation5. It is also used to promote breast milk production7. The objective of the current study was to determine the types of compound present in the plant and to investigate the anti-oxidant, anti-microbial and analgesic activity of the leaves and tubers of *Ipomoea mauritiana*.

MATERIALS AND METHODS

Collection of the Plant Sample

The whole plant of *Ipomoea mauritiana* was collected from the Khulna University Campus. The time of collection was October, 2010 at daytime. The fresh whole plants were collected from the healthy host plants. During collection, any type of adulteration was strictly prohibited. The sample was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession No. 35572).

Preparation of Plant Extract

The collected plants were separated from undesirable materials and dried in shade for 18 days. Shade drying ensured that the chemical components in the plant were not degraded. The leaves were grounded into coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place. The leaves were extracted by cold extraction method. 400 gm of grounded tubers’ powder was soaked in 600 ml of ethanol in a glass container for eight days accompanying regular shaking and stirring. The extract was separated from the plant debris by filtration by a piece of clean, white cotton material and it was repeated twice. The filtrate (ethanol extract) was taken into a rotary evaporator and the remaining ethanol was completely evaporated. Then this filtrate was taken into a beaker. The opening of beaker was wrapped by a sheet of aluminum foil. The aluminum foil was perforated for the complete evaporation of any remaining ethanol. The beaker was kept in dry and cool place for several days. It rendered the extract a deep purple color.

Phyto-chemical Screening

Composition of Reagents Used for the Different Chemical Group Tests

The following reagents were used for the different chemical group test8.

Mayer’s Reagent: 1.36 gm mercuric iodide in 60 ml of water was mixed with a solution contains 5 gm of potassium iodide in 20 ml of water.

Dragendorf’s Reagent: 1.7 gm basic bismuth nitrate and 20 gm tartaric acid were dissolved in 80 ml water. This solution was mixed with a solution contains 16 gm potassium iodide and 40 ml water.

Fehling’s Solution A: 34.64 gm copper sulphate was dissolved in a mixture of 0.50 ml of sulfuric acid and sufficient water to produce 500 ml.

Fehling’s Solution B: 17.6 gm of sodium potassium tartarate and 7.7 gm of sodium hydroxide were dissolved in sufficient water to produce 100 ml. Equal volume of above solution were mixed at the time of use.
Benedict's Reagent: 1.73 gm cupric sulphate, 1.73 gm sodium citrate and 10 gm anhydrous sodium carbonate were dissolved in water and the volume was made up to 100 ml with water.

Molish Reagent: 5 gm of pure α-naphthol was dissolved in 50 ml of ethanol.

Tests Procedure for Identifying Different Chemical Groups

The following tests were performed for identifying different chemical groups present in the plant extract.

Tests for Reducing Sugar:

Benedict's test: 0.5 ml of aqueous extract of the plant material was taken in a test tube. 5 ml of Benedict's solution was added to the test tube, boiled for 5 minutes and allowed to cool spontaneously. The formation of a red color precipitate of cuprous oxide would be considered as an indication of the presence of a reducing sugar.

Fehling's Test: 2 ml of an aqueous extract of the plant material was added 1 ml of a mixture of equal volumes of Fehling's solutions A and B and boiled for few minutes. The formation of a red or brick red color precipitate would be considered as an indication of the presence of reducing sugar.

Tests for Tannins:

Ferric Chloride Test: 5 ml solution of the extract was taken in a test tube. Then 1 ml of 5% Ferric chloride solution was added. Greenish black precipitate would indicate the presence of tannins.

Potassium Dichromate Test: 5 ml solution of the extract was taken in a test tube. Then 1 ml of 10% Potassium dichromate solution was added. The formation of a yellow precipitate would indicate the presence of tannins.

Test for Flavonoids:

A few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract of the plant material. Immediate development of a red color would indicate the presence of Flavonoid.

Test for Saponins:

1 ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Formation of one centimeter layer of foam would indicate the presence of saponins.

Test for Gums:

5 ml solution of the extract was taken and molish reagent and sulphuric acid were added. Formations of red violet ring at the junction of two liquids would indicate the presence of gums and carbohydrate.

Test for Steroids:

Sulphuric Acid Test: 1 ml solution of chloroform extract was taken and added to 1 ml Sulphuric acid. Presence of red color would indicate the presence of steroid.

Test for Alkaloids:

Mayer's Test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube and 1 ml of Mayer's reagent was added. Formation of yellow color precipitate would indicate the presence of alkaloids.

Dragendorff's Test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube and 1 ml of Dragendorff's reagent was added. Formation of orange brown precipitate would indicate the presence of alkaloids.

Tests for Glycosides:

General Test: A small amount of an alcoholic extract of the fresh or dried plant material was taken in 1 ml of water and a few drops of aqueous sodium hydroxide were added. A yellow color was considered as an indication for the presence of glycosides.

Fehling's solution test: A small amount of an alcoholic extract of the plant material was taken in water and alcohol and boiled with Fehling's solution. Brick-red precipitate was considered as an indication for the presence of glycosides.

Evaluation of anti-oxidant activity

Brand-Williams method or DPPH assay\(^9\) was used to estimate free radical scavenging activities of the ethanolic extract of *Ipomoea mauritiana*. 2.0 mg of the extracts was dissolved in methanol for the experiment. Solution of different concentrations such as 500 \(\mu\)g/ml, 250 \(\mu\)g/ml, 125 \(\mu\)g/ml, 62.50 \(\mu\)g/ml, 31.25 \(\mu\)g/ml, 15.62 \(\mu\)g/ml, 7.8125 \(\mu\)g/ml, 3.91 \(\mu\)g/ml, 1.95 \(\mu\)g/ml and 0.98 \(\mu\)g/ml were obtained by serial dilution technique. 50 \(\mu\)l of methanol solution of the extract of each concentration was mixed with 5 ml of a DPPH-methanol solution (40 \(\mu\)g/ml). The reaction mixture was vortexes thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm by spectophotometric method and corresponding percentage of inhibitions were calculated by using the following equation:

\[
\% \text{ inhibition} = \left[1 - \left(\frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right)\right] \times 100\% 
\]

Where \(\text{Abs}_{\text{sample}}\) is the absorbance of the sample material and \(\text{Abs}_{\text{control}}\) is the absorbance of the control reaction (containing all reagents except the test material). Then percent inhibitions were plotted against respective concentrations. IC\(_{50}\) values were calculated as the concentration of each sample required to give 50% DPPH radical scavenging activity from the graph. Ascorbic acid was used as positive control.

Evaluation of anti-microbial activity

Antimicrobial screening was performed using disc-diffusion method\(^9\). 8 mg of samples from different extracts was dissolved in methanol to obtain desired concentration in aseptic condition. Sterilized filter paper discs were taken in a blank Petridish under laminar hood. Then discs were soaked with solutions of test samples and dried. Standard Azithromycin (30 \(\mu\)g/disc) discs were used as positive control and blank discs were used as negative control. The sample discs, standard antibiotic discs and control discs were placed gently on marked zones in the agar plate’s pre-inoculated with test bacteria. The plates were then kept in a refrigerator at 4 °C for about 24 hours to allow sufficient diffusion of materials from discs to surrounding agar medium. The plates were then inverted and kept in an incubator at 37 °C for 24 hours. Both gram positive and gram-negative organisms were taken for the test and they are listed in Table 1.

Evaluation of analgesic activity

The peripheral analgesic activity of tubers of *Ipomoea mauritiana* was measured by the acetic acid induced writhing test\(^1\). Briefly, the inhibition of writhing produced by the plant extract was determined by comparing with the inhibition produced by the control group. Diclofenac at oral dose of 100 mg/kg was used as standard analgesic agent. Intraperitoneal injection of acetic acid (0.7%) at a dose of 0.1 ml/10g of body weight was used to create pain sensation. The number of writhings was calculated for 10 min, 5 min after the application of acetic acid.
TABLE 1: LIST OF MICRO-ORGANISMS USED FOR THE ANTI-MICROBIAL SCREENING

<table>
<thead>
<tr>
<th>Gram negative</th>
<th>Gram positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Escherichia coli</td>
<td>1. Staphylococcus aureus</td>
</tr>
<tr>
<td>2. Shigella dysenteriae</td>
<td>2. Staphylococcus pyogenes</td>
</tr>
<tr>
<td>3. Shigella sonnei</td>
<td></td>
</tr>
<tr>
<td>4. Shigella flexneri</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2: RESULTS OF THE DIFFERENT CHEMICAL TESTS PERFORMED

<table>
<thead>
<tr>
<th>Chemical Group test</th>
<th>Specific test</th>
<th>Observation</th>
<th>Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test for Alkaloids</td>
<td>a) Mayer's test</td>
<td>Positive</td>
<td>Presence of Alkaloids</td>
</tr>
<tr>
<td></td>
<td>b) Dragendorff's test</td>
<td>Positive</td>
<td>Presence of Alkaloids</td>
</tr>
<tr>
<td>Test for Steroid</td>
<td>a) Sulphuric acid test</td>
<td>Positive</td>
<td>Presence of steroid</td>
</tr>
<tr>
<td>Test for Flavonoids</td>
<td>-</td>
<td>Negative</td>
<td>Absence of Flavonoids</td>
</tr>
<tr>
<td>Test for Saponins</td>
<td>-</td>
<td>Negative</td>
<td>Absence of saponins</td>
</tr>
<tr>
<td>Test for Tannins</td>
<td>a) Feric Chloride Test</td>
<td>Positive</td>
<td>Presence of Tannins</td>
</tr>
<tr>
<td></td>
<td>b) Potassium dichromate test</td>
<td>Positive</td>
<td>Presence of Tannins</td>
</tr>
<tr>
<td>Test for Gums</td>
<td>-</td>
<td>Positive</td>
<td>Presence of gums</td>
</tr>
<tr>
<td>Test for Reducing Sugars</td>
<td>a) Benedict's Test</td>
<td>Positive</td>
<td>Presence of reducing sugar</td>
</tr>
<tr>
<td></td>
<td>b) Fehling's Test</td>
<td>Positive</td>
<td>Presence of reducing sugar</td>
</tr>
<tr>
<td>Test for Glycosides</td>
<td>a) General Test</td>
<td>Positive</td>
<td>Presence of glycosides</td>
</tr>
<tr>
<td></td>
<td>b) Fehling's solution test</td>
<td>Positive</td>
<td>Presence of glycosides</td>
</tr>
</tbody>
</table>

TABLE 3: IN VITRO ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Bacterial Strains</th>
<th>Type of Bacterial Strains</th>
<th>Blank</th>
<th>Azithromycin (30 µg/disc)</th>
<th>Crude Ethanol Extract (500 µg/disc)</th>
<th>Crude Ethanol Extract (500 µg/disc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Escherichia coli</td>
<td>Gram(-)</td>
<td>-</td>
<td>22</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Shigella dysenteriae</td>
<td>Gram(-)</td>
<td>-</td>
<td>14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Shigella sonnei</td>
<td>Gram(-)</td>
<td>-</td>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Shigella flexneri</td>
<td>Gram(-)</td>
<td>-</td>
<td>21</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Staphylococcus aureus</td>
<td>Gram(+)</td>
<td>-</td>
<td>21</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Staphylococcus pyogenes</td>
<td>Gram(+)</td>
<td>-</td>
<td>27</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

TABLE 4: % INHIBITION OF WRITHING OF THE DIFFERENT TEST SAMPLES

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Writhing (Mean ± SEM)</th>
<th>% of Inhibition of Writhing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Control</td>
<td>39.00±4.74</td>
<td>-</td>
</tr>
<tr>
<td>2) Standard</td>
<td>6±4.58*</td>
<td>86.64</td>
</tr>
<tr>
<td>3) Ethanol extract of tuber (500 mg/kg)</td>
<td>7.50±1.94</td>
<td>80.77</td>
</tr>
<tr>
<td>4) Ethanol extract of tuber (250 mg/kg)</td>
<td>12±4.42*</td>
<td>71.15</td>
</tr>
</tbody>
</table>

Probability values (calculated as compared to control using one way-ANOVA followed by Dunnet’s Test): *P<0.05,
All values are means of individual data obtained from five mice (n = 5).

RESULTS
Results of Phytochemical Screening
The chemical group tests were performed and the results are mentioned in the table 2. Results indicated the presence of alkaloids, tannins, steroids, gums, glycosides, carbohydrates and saponins in the crude ethanolic extract.

In Vitro Antioxidant Activity
The antioxidant activity of the crude ethanolic extract was measured on the basis of its DPPH scavenging activity. The concentration of the crude ethanolic extract needed for 50% scavenging (IC50) of DPPH was found to be 164 µg/ml which is mild comparable to that of ascorbic acid (IC50 = 12.5 µg/ml), a well-known standard antioxidant.

Antimicrobial activities
In vitro antimicrobial screening of the ethanolic extract of the tubers of Ipomoea mauritiana was evaluated. Azithromycin was used as a standard. The crude extract did not show any significant anti-microbial activity. The results of the antimicrobial screening are shown in table 3.

Analgescic activity
The ethanolic extract of the tubers of I. mauritiana exhibited significant analgesic effect in acetic acid induced writhing of white albino mice (Swiss-webstar strain). The extract produced 71.15% and 80.77% writhing inhibition (p < 0.001) at doses of 250 and 500 mg/kg-body weight respectively. The results are shown in table 4.

DISCUSSION
The present study confirms the use of the tubers of Ipomoea mauritiana as an analgesic agent. NSAIDS offer relief from inflammatory pain by suppressing the formation of pain substances in the peripheral tissues, where prostaglandins and bradykinin were suggested to play an important role in the pain process. Therefore, it is likely that the extract might suppress the formation of these substances or antagonize the action of these substances and thus exert its analgesic activity.

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REFERENCES

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