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Research Article

PHYTOCHEMICAL SCREENING AND THE EVALUATION OF THE ANTIOXIDANT, ANTIMICROBIAL AND ANALGESIC PROPERTIES OF THE PLANT *IPOMOEA MAURITANA* (FAMILY: CONVOVULACEAE)

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ABSTRACT

Ipomoea mauritiana is an important medicinal plant having widespread application in a variety of disorders. The aim of this study was the phytochemical investigation and evaluation of the anti-oxidant, anti-microbial and analgesic activities of the whole plant *Ipomoea mauritiana*. Phytochemical screening of the ethanolic extract of *Ipomoea mauritiana* ensured the presence of Alkaloids, Tannins, Steroids, Gums, Glycosides, Carbohydrate and Saponins. The anti-oxidant activity was measured by DPPH free radical scavenging activity (IC_{50} = 164 µg/ml). The crude ethanolic extract did not reveal any significant anti-microbial activity. The crude ethanolic extract of the tubers of *Ipomoea mauritiana* exhibited significant analgesic activity at a dose of 250 mg/kg and 500 mg/kg with 71.15% and 80.77 % inhibition of writhing respectively.

KEYWORDS: *Ipomoea mauritiana*, phytochemical screening, DPPH, Disc-diffusion method, writhing inhibition.

INTRODUCTION

Plants represent a rich source of antimicrobial agent[1] and natural antioxidants[2]. Many plant materials used in traditional medicines are readily available in rural areas at relatively cheaper price than modern medicines[3]. 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 purposes[4]. 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. hydrocortisone[5]. 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 sensation[6]. It is also used to promote breast milk production[7]. 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 grinded 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 test[8].

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.

Dragendroff's Reagent: 1.7 gm basic bismuth nitrate and 20 gm tartaric acid ware 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.

Benedicts 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. 5ml 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: 2ml of an aqueous extract of the plant material was added 1ml 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 to1ml 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.

Dragendroff'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 Dragendroff'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 1ml 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 µg/ml, 250 µg/ml, 125 µg/ml, 62.50 µg/ml, 31.25 µg/ml, 15.62 µg/ml, 7.8125 µg/ml, 3.91 µg/ml, 1.95 µg/ml and 0.98 µg/ml were obtained by serial dilution technique. 50 µl of methanol solution of the extract of each concentration was mixed with 5 ml of a DPPH-methanol solution (40µg/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

$$\% \text{ inhibition} = [1 - (Abs_{sample} / Abs_{control})] \times 100\ \%$$

Where Abs_{sample} is the absorbance of the sample material and $Abs_{control}$ 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[10]. 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 µg/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[11]. 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

Gram negative	Gram positive
1. *Escherichia coli* 2. *Shigella dysenteriae* 3. *Shigella sonnei* 4. *Shigella flexneri*	1. *Staphylococcus aureus* 2. *Staphylococcus pyogenes*

TABLE 2: RESULTS OF THE DIFFERENT CHEMICAL TESTS PERFORMED

Chemical Group test	Specific test	Observation	Interference
Test for Alkaloids	a)Mayer's test b)Dragendroff's test	positive positive	Presence of Alkaloids
Test for Steroid	a) Sulphuric acid test	Positive	Presence of steroid
Test for Flavonoids	-	Negative	Absence of Flavonoids
Test for Saponins	-	Negative	Absence of saponins
Test for Tannins	a)Ferric Chloride Test b)Potassium dichromate test	Positive Positive	Presence of Tannins
Test for Gums	-	Positive	Presence of gums
Test for Reducing Sugars	a) Benedict's Test b) Fehling's Test	Positive Positive	Presence of reducing sugar
Test for glycosides	a) General Test b) Fehling's solution test	Positive Positive	Presence of glycosides

TABLE 3: IN VITRO ANTIMICOBIAL ACTIVITY OF ETHANOLIC EXTRACT

Serial No.	Bacterial Strains	Type of Bacterial Strains	Diameter of Zone of Inhibition (mm)			
			Blank	Azithromycin (30 µg/disc)	Crude Ethanol Extract (500µg/disc)	Crude Ethanol Extract (500µg/disc)
1	*Escherichia coli*	Gram(-)	-	22	-	-
2	*Shigella dysenteriae*	Gram(-)	-	14	-	-
3	*Shigella sonnei*	Gram(-)	-	20	-	-
4	*Shigella flexneri*	Gram(-)	-	21	-	-
5	*Staphylococcus aureus*	Gram(+)	-	21	-	-
6	*Staphylococcus pyogenes*	Gram(+)	-	27	-	-

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

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

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 ethanol 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 (IC_{50}) of DPPH was found to be 164 µg/ml which is mild comparable to that of ascorbic acid (IC_{50} = 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 anti-microbial screening are shown in table 3.

Analgesic 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[12]. 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

1. Mahesh B, Satish S. Antimicrobial activity of some important medicinal plants against plant and human pathogens. World Journal of Agricultural Science. 2008; 4: 839-843.
2. Halliwell B, Aeschbach R, Liger J. The characterization of antioxidant. Food Chemistry and Toxicology. 1995; 33: 601-617.
3. Mann A, Banso A, Clifford L. An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides*. Tanzania Journal of Health Research. 2008; 10(1): 34-38.
4. Reynolds T. The compounds in Aloi leaf exudates. Botanical Journal Linn Society. 1985; 90: 157.
5. Ahmad F, Khan RA, Rasheed S. Journal of Islamic Academy of Sciences. 1992; 5(2):111-114.
6. Mishra SS, Datta KC. A preliminary pharmacological study of Ipomoea digitata Linn. Indian J Med Res. 1962 Jan; 50:43-45.
7. Matin MA, Tewari JP, Kalani DK. Pharmacological investigations of Ipomoea digitata Linn. Indian J Med Sci. 1969 Sep; 23(9):479-82.

8. Trease GE, Evans WC. Trease and Evans Pharmacognosy 16nth Edition.(Harcourt Publishers), 2009, Australia.
9. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity, LWT – Food Sci and Tech. 1995; 28 (1): 25–30.
10. Rizvi SMD, Biswas D, Arif JM, *et al. In-vitro* antibacterial and antioxidant potential of leaf and flower extracts of *Vernonia cinerea* and their phytochemical constituents. Int J Pharm Sci Rev and Res. 2011; 9(2): 164-169.
11. Saha A, Masud MA, Bachar SC, Kundu JK, Datta BK, Nahar L and Sarker SD . The Analgesic and Anti-Inflammatory Activities of the Extracts of *Phyllanthus reticulatus* in Mice Model. Pharm Biol. 2007; 45(5): 355-359.
12. Hirose K, Jyoyama H, Kojima Y, Eigyo M, Hatakeyama Hl. Pharmacological properties of 2-[44-(2-triazolyloxy)-phenyl [propionic acid (480156-5)], a new non-steroidalanti- inflammatory agent. Arzeim Forsch/Drug Research, 1984; 34:280-6.

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