Pharmacological Investigations of Different Extracts from the Leaf of *Ipomoea fistulosa* (Family: Convolvulaceae)

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ABSTRACT

The aim of the study was to evaluate the antimicrobial, cytotoxic, anthelmintic, analgesic and neuropharmacological properties of the leaf of *Ipomoea fistulosa*. The extracts of the leaf showed very mild antimicrobial activity compared with the standard drug, amoxycillin. The cytotoxic activity of n-hexane, dichloromethane and methanol extracts from the leaf of *I. fistulosa* were evaluated using brine shrimp lethality bioassay. The results showed very mild cytotoxicity with the LC_{50} value of 33.88, 56.23 and 36.31μg/mL respectively while LC_{50} value of reference drug vincristine sulphate was 0.69 μg/mL. Anthelmintic activity was found to be moderate by the investigation performed on *Pheretima posthuma.* The analgesic activity of the methanol extract of leaf was evaluated by using acetic acid-induced writhing test and tail immersion method on Swiss Albino mice at a dose of 200 and 400 mg/kg body weight. The writhing test displayed 51.53% and 64.96% of inhibition for the extract in acetic acid which is significant to the reference standard drug, diclofenac-Na (72.16%) of inhibitation in acetic acid at a dose of 1 mg/kg body weight). The analgesic activity in tail immersion method was dose dependent. However, the neuropharmacological activity was tested by hole cross and open field test which revealed notable CNS depressant property when compared to the standard drug, diazepam (1 mg/kg b.w.). Over all, the extracts showed notable anthelmintic, analgesic and neuropharmacological activities

Keywords: Ipomoea fistulosa, Convolvulaceae, Antimicrobial activity, Anthelmintic activity, Cytotoxicity, Analgesic activity, Neuropharmacological property.

1. INTRODUCTION

Ipomoea fistulosa (Family: Convolvulaceae) is popularly known as Dhol-kalmi in Bangladesh and Morning glory in English. It is a large diffuse shrub with milky juice. The flowers are pale rose, pink or light violet in lax, dichotomously branched axillary and terminal, pedunculate cymes; fruits have a glabrous capsule; seed is silky[(1)]. It is quite a common flower in the rural areas of Bangladesh as well as the roadside.

The leaves of *I. fistulosa* contain 1-3 flavonol glycosides and Ergine (D-Lysergic acid amide). Polyhydroxylated alkaloids were isolated from the leaves,flowers and seeds. Chromatographic separation of the leaf extract resulted in the isolation of swainsonine, 2-epi-lentiginosine, calystegines B (1), B (2), B (3) and C(1) and N-methyl-trans-4-hydroxy-l-proline and betasitosterol[(2)]. The plant part is used as aphrodisiac, purgative and cathartic[(3)]. The plant had immense potential as an anti-Inflammatory, antioxidant, antidiabetic, antimicrobial Activity, wound healing,

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immunomodulatory, cardiovascular, antifungal, hepatoprotective activities, embryotoxic effect and anxiolytic properties[4].

MATERIALS AND METHODS

Chemicals

Drugs and chemicals used in the study include diclofenac-Na, vincristine sulphate (Gedeon Richter), albendazole, amoxycillin, acetic acid (Merck, Germany), methanol (Merck, Germany), DMSO (Merck, Germany), DMF (Merck, Germany).

Plant Materials And Extraction

The leaves of *I. fistulosa* were collected from Sirajganj, Bangladesh in October, 2012. The plant was identified by a scientist of Bangladesh National Herbarium Institute, Mirpur, Dhaka. An accession number was given from there and a voucher specimen (DACB: 37934) has been deposited in the herbarium for future reference. The shade dried and powdered plant material was separately extracted to exhaustion in a soxhlet apparatus with n-hexane, dicholoromethane and methanol respectively. The extractives were separately filtered through fresh cotton bed and finally with Whatman filter paper number 1 and then concentrated by using a rotary evaporator at low temperature (40-50^{0}C) and reduced pressure.

Animals

For the experiment, 3 to 4 weeks old male Swiss Albino mice weighing between 20 to 25 g were collected from the Animal Resources Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR,B). Soft wood shavings were used as bedding of cages. Animals were maintained under standard environmental conditions: temperature (24.0±1.0°C), relative humidity (55-65% and 12 h light /12 h dark cycle). Husk and excreta were removed from the cages every day. For anthelmintic activity, adult earthworm *Pheretima posthuma* were collected (due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being) from moist soil. All the worms were washed with normal saline to remove all fecal matters were used for the anthelmintic study. In brine shrimp lethality bioassay, brine shrimp nauplii is used as a favourable monitor for screening[5].

Antimicrobial Activity

The disc diffusion method[6] was used to test antimicrobial activity against two Gram positive (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) and three Gram negative bacteria (*Escherichia coli*, *Salmonella paratyphi* and *Klebsiella pneunoniae*) (Table 1). Solutions of known concentration (500 mg/mL) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs (impregnated with solvents) were used as positive and negative control. These plates were then kept at low temperature (4^{o}C) for 24 h to allow maximum diffusion. There is a gradual change of test materials concentration in the media surrounding the discs. The plates were then incubated at 37^{o}C for 24 h to allow maximum growth of the organisms. The test material having antimicrobial activity, inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment is carried out three times and the mean of the readings is required[6]. Standard disc of amoxycillin (30 μg/disc) was used for comparison purpose.

Brine Shrimp Lethality Bioassay

Brine shrimp lethality bioassay technique was applied for the determination of general toxic property of the plant extractives. Brine shrimp eggs collected from pet shops was used as the test organism. Seawater was taken

in the small tank. Shrimp eggs were added to one side of the tank, and then this side was covered. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was provided throughout the hatching time. The hatched shrimps were attracted to the lamp through the perforated dam and with the help of a pasteur pipette 10 living shrimps were added to each of the vials containing 5ml of seawater[7].

Preparation of positive control group: Vincristine sulphate was used as the positive control. Measured amount of vincristine sulphate was dissolved in DMSO to get an initial concentration of 40 μg/mL from which serial dilutions were made using DMSO to get 20, 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.0781 μg/mL. Then the solutions were added to the premarked vials containing ten live brine shrimp nauplii in 5 mL simulated sea water.

Preparation of negative control group: 100 μL of DMSO was added to each of three pre-marked glass vials containing 5 mL of simulated sea water and 10 shrimp nauplii. If the brine shrimps in these vials show a rapid mortality, then the test is considered as invalid as the nauplii died due to some reasons other than the cytotoxicity of the compounds.

Preparation of test groups: 4 mg of sample was dissolved in 100 μL of DMSO. From that test solution different volumes were added to premarked glass vials or test tubes containing 5 mL of seawater and 10 shrimp nauplii, so as to make the final concentration of samples in the vials or test tubes 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, and 0.781 μg/mL, respectively.

Counting of nauplii: After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration. The median lethal concentration (LC_{50}) of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration.

Anthelmintic Activity

Fresh juice extract of the leaves of *I. fistulosa* was dissolved in minimum amount of DMF and the volume was adjusted to 10 mL with saline water. All drugs and extract solutions were freshly prepared before starting the experiment. In each case, 6 earthworms released into 10 mL of desired formulations as follows: vehicle (5% DMF in normal saline), albendazole (25, 50 and 100 mg/mL) and fresh juice extract (25, 50 and 100 mg/mL) of the leaf of *I. fistulosa* in normal saline solution containing 5% DMF. Observation was made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in saline solution[8].

Analgesic Activity

The study of analgesic activity of the *I. fistulosa* was performed in animal models for both central and peripheral mechanism of pain. For the screening of analgesic activity against peripheral mechanism of pain acetic acid-induced writhing test was considered. On the other hand, to evaluate the analgesic activity against centrally mediated pain, tail immersion test was used.

Acetic acid-induced writhing Test: The acetic acid induced writhing method is an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in mice. 5 mL test sample and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% (5 mL) acetic acid but diclofenac-Na was administered 15 min before injection of acetic acid after an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min[9].

Tail immersion test: The procedure is based on the observation that morphine like drugs selectively prolong the reaction time of the typical tail withdrawal reflex in mice. The animals of the control, positive control and test groups were treated with water (administered orally at a dose of 0.1 mL/kg body weight), diclophenac-Na (administered intraperitoneally at a dose of 1.0 mg/kg body weight) and test samples at the doses of 200 and 400 mg/kg body weight respectively. 1 to 2 cm of the tail

of mice was immersed in warm water kept constant at 55^0C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 20 s was defined as complete analgesia and the measurement was stopped when the latency period exceeded to avoid injury to mice. The latent period of the tail-flick response was taken as the index of antinociception and was determined at 0, 30, 60 and 90 min after the administration of the test drugs and standard[5].

Neuropharmacological activity

Neuropharmacological effect of methanolic extract of the leaves of *I. fistulosa* was performed on mice in a peripheral model of CNS activity test.

Hole cross test

The method was adopted as described by following the method of Takagi et al, 1971[10]. A steel partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The number of passage of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60 and 90 min after oral administration of the test drugs at the dose of 200 and 400 mg/kg body weight respectively and the intraperitoneal administration of standard drug Diazepam at a dose of 1 mg/kg body weight.

Open field test

This experiment was carried out as described by Gupta et al., 197[11]. The animals were divided into control and test groups containing five mice each. The test group received *I. fistulosa* extract at the doses of 200 and 400 mg/kg body weight orally whereas the control group received vehicle (DMSO+water) and the positive control group received diazepam at a dose of 1 mg/kg body weight intraperitoneally .The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 min at 0, 30, 60 and 90 min after oral administration of the test drugs.

RESULTS AND DISCUSSION

Antimicrobial Activity

The n-hexane, dichloromethane and methanol extract of the leaves of *I. fistulosa* (500 μg/disc) were screened against two Gram positive and three Gram negative bacteria which exhibited very mild antimicrobial activity against most of the test organisms. (Table 1). The maximum to minimum zone of inhibition produced by n-hexane, dichloromethane and methanol extracts of the leaf were found to be 7-8 mm wheraeas, to that produced by the standard amoxycillin was found to be 7-19 mm, respectively.

Table 1. Antimicrobial activity of *I. fistulosa* leaf extract

Test Microorganisms	**Diameter of Zone of Inhibition (mm)**			
	n-hexane (500 μg/disc)	**Dichloromethane** (500 μg/disc)	**Methanol** (500 μg/disc)	**Amoxycillin** (30 μg/disc)
Gram positive bacteria				
Pseudomonas aeruginosa	7	8	8	19
Staphylococcus aureus	7	8	7	7
Gram negative bacteria				
Escherichia coli	7	8	7	7
Salmonella paratyphi	7	8	7	13
Klebsiella pneunoniae	7	7	8	8

Brine Shrimp Lethality Bioassay of *I. fistulosa*

Following the procedure of Meyer et al, 1982[7], the lethality of n-hexane, dichloromethane and methanol leaf extracts of *I. fistulosa* to brine shrimp were determined and the regression analysis data is given in Table 2.

Table 2. Regression analysis data for vincristine sulphate and plant extracts of *I. fistulosa*

Sample	LC_{50} (μg/mL)	Regression equation	R^2
Vincristine sulphate (positive control)	0.69	y = 32.01x + 55.08	0.980
n-hexane extract	33.88	y = 25.16x + 11.60	0.984
Dichloromethane extract	56.23	y = 25.97x + 4.597	0.967
Methanol **e**xtract	36.31	y = 25.77x + 9.848	0.962

All the plant extracts of *I. fistulosa* showed very mild cytotoxic activity against brine shrimp nauplli with LC_{50} value of 33.88, 56.23, 36.31 μg/mL respectively when compared with the standard vincristine sulphate (0.69 μg/mL) (Table 2).

Anthelmintic Activity

Helminthiasis is a macroparasitic disease of humans and animals in which a part of the body is infested with parasitic worms such as pinworm, roundworm, or tapeworm. Anthelmintics or antihelminthics are the drugs or the agents that destroy or cause the expulsion of such parasitic intestinal worms and helps to treat helminthiasis, one of the most common infections in humans and cattle. Resistant worms accumulate and finally treatment failure occurs. To overcome the resistance, plant derived drugs can serve as prototype to develop more effective and less toxic medicines[12].

The results of anthelmintic activity of *I. fistulosa* are given in Table 3.

Table 3. *In vitro* anthelmintic activity of *I. fistulosa* leaf extract

Test Samples	Conc. (mg/mL)	Time Taken for Paralysis (minutes)	Time Taken for Death (minutes)
Fresh juice extract of leaf	25	12 min 45 s	25 min 35 s
	50	11 min 50 s	22 min 50 s
	100	9 min 10 s	20 min 30 s
Albendazole	25	6 min 30 s	10 min 10 s
	50	5 min 10 s	8 min 30 s
	100	4 min 30 s	7 min 10 s

The fresh juice extracts of the leaf of *I. fistulosa* showed moderate anthelmintic activity when compared with the standard drug, albendazole (Table 3).

Acetic acid-induced writhing test

The analgesic effect of the methanol extract of *I. fistulosa* leaf on acetic acid-induced writhing in mice was determined. The extract significantly inhibited writhing response induced by acetic acid in a dose dependent manner. The result was statistically significant and was comparable to the reference drug, diclofenac- Na.

Table 4. Effect of *I. fistulosa* leaf extract on acetic acid-induced writhing test on mice

Test group	Doses (mg/kg b.w.) and routes of administration	Number of writhing	% Inhibition
Control (DMSO+water)	0.1 mL/mice, oral	32.33±1.78	0.00
Diclofenac-Na	1.0, intraperitoneal	9.00±2.55	72.16
Group-I	200, oral	15.67±1.47	51.53
Group-II	400, oral	11.33±1.08	64.96

Values are expressed as mean±SEM, where n=5, $p<0.05$ compared with the control group (Dunnett's test), Control: DMSO+water (0.1 mL/Mouse), Positive control: Diclofenac-Na (1.0 mg/kg), Group I = *I. fistulosa* (200 mg/kg), Group II = *I. fistulosa* (400 mg/kg).

I. fistulosa leaf extract caused dose dependent anti-nociception against chemical induced which exhibit significant writhing response inhibitory effect (Table 4).

Tail Immersion Test

The analgesic activity tests were carried out in the laboratory on five groups of mice by tail-flick method. Time interval for the test was 30 min. The tail withdrawal reflex time after administration of the *I. fistulosa* was found to increase with increasing dose of the extrac**t.**

Table 5. Effect of the *I. fistulosa* on tail withdrawal reflex in mice

Test group	Doses (mg/kg b.w.) and routes of administration	Number of movements			
		0 min	**30 min**	**60 min**	**90 min**
Control (DMSO+water)	0.1 mL/mice, oral	1.37±0.13	1.94±0.04	2.09±0.22	1.92±0.26
Diclofenc-Na	1.0, intraperitoneal	2.83±0.54	5.37±0.36	7.43±0.51	6.93±0.28
Group-I	200, oral	3.65±0.88	11.30±4.44	13.19±5.19	10.36±6.00
Group-II	400, oral	6.20±1.76	15.26±5.39	11.00±4.84	8.77±1.01

Values are expressed as mean±SEM, where n=5**,** $p<0.05$ compared with the control group (Dunnett's test), Control: DMSO+water (0.1 mL/Mouse), Positive control: Diclofenac-Na (1.0 mg/kg), Group I = *I. fistulosa* (200 mg/kg), Group II = *I. fistulosa* (400 mg/kg).

The tail immersion tests are widely used for assessing analgesic activities. In our experiments, *I. fistulosa* exhibited significant analgesic activity in tail immersion test. It seems possible that the higher doses of the extract have potent analgesic effect (Table 5).

Hole cross test

The number of hole crossed from one chamber to another by mice of the control group is increased from 30 min to 90 min (Table 6). Hole cross test of *I. fistulosa* 200 and 400 mg/kg dose showed significant decrease of movement from its initial value at 0 to 90 min.

Table 6. Effect methanol extract of the leaf of *I. fistulosa* on hole cross test in mice

Test group	Doses (mg/kg b.w.) and routes of administration	Number of movements			
		0 min	30 min	60 min	90 min
Control	0.1 mL/mice, oral	21±2.55	12±1.41	11.40±0.75	7±1.41
Diazepam	1.0, intraperitoneal	9.67±1.08	6.67±1.47	3.67±0.8	2.33±0.82
Group-I	200, oral	6.67±4.71	5.33±3.89	8±5.34	5±3.94
Group-II	400, oral	5.67±2.86	2.67±1.48	1.33±1.08	3±3.08

Values are expressed as mean±SEM, where n=5**,** p<0.05 compared with the control group (Dunnett's test), Control: DMSO+water (0.1 mL/Mouse), Positive control: Diazepam (1.0 mg/kg), Group I = *I. fistulosa* (200 mg/kg), Group II = *I. fistulosa* (400 mg/kg).

Open field test

Open field test of *I. fistulosa* 200 and 400 mg/kg dose showed significant decrease of movement from its initial value at zero minute to 90 min (Table 7). Initially it was observed that the number of movements of the control group overall increased from 30 min to 90 min.

Table 7. Effect of methanol extract of the leaf of *I. fistulosa* on open field test in mice

Test group	Doses (mg/kg b.w.) and routes of administration	Number of movements			
		0 min	30 min	60 min	90 min
Control	0.1 mL/mice, oral	109±4.64	104.33±2.16	93±2.12	85.33±2.27
Diazepam	1.0, intraperitoneal	76±5.61	31.67±3.34	24.67±1.78	6±4.8
Group-I	200, oral	67.33±15.59	36.33±11.43	37.67±8.2	9.33±2.4
Group-II	200, oral	24.33±16.07	12.67±11.23	30.67±12.17	21.67±25.31

Values are expressed as mean±SEM, where n=5**,** p<0.05 compared with the control group (Dunnett's test), Control: DMSO+water (0.1 mL/Mouse), Positive control: Diazepam (1.0 mg/kg), Group I = *I. fistulosa* (200 mg/kg), Group II = *I. fistulosa* (400 mg/kg).

The most important step in evaluating drug action on CNS is to observe its effect on locomotors activity of the animal. The activity is a measure of the level of excitability of the CNS and this decrease may be closely related to sedation resulting from depression of the central nervous system. The extracts significantly decreased the locomotor activity as shown by the results of the open field and hole cross tests.

Statistical Analysis

All experiments were performed thrice and the averaged data were expressed as mean± SD.

CONCLUSION

Based on the result of the present studies, it can be concluded that the crude leaf extract of *I. fistulosa* possess anthelmintic activity, analgesic potential and neuropharmacological effects. At higher dose, simple analgesic activity was observed from tail-flick and acetic acid-induced writhing test. Significant activity can also be suggested from the results of neuropharmacological test. Dose dependant activity was observed in all the performed pharmacological investigations. Hence, further investigation is required to explore their activities against various diseases and to isolate bioactive principles.

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فحص التأثيرات الدوائية لعدة مستخلصات من أوراق إيبوميا فستيولوزا (عائلة: كونفولفيوليسه)

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ملخص

تهدف هذه الدراسة إلى فحص الخصائص الدوائية لعدة مستخلصات من أوراق الاويبوميا فستيولوزا في الفئران، كالتأثيرات المضادة للجراثيم، السامة على الخلايا، المضادة للديدان، والتأثيرات على الجهاز العصبي.

لقد أظهرت المستخلصات تأثيراً "فيفا كمضادات للجراثيم بالمقارنة مع الدواء المعياري" amoxycillin". لقد تم فحص التأثير السام على الخلايا للمستخلصات المحضرة بـ n-hexane dichloromethane and methanol باستخدام فحص "brine shrimp lethality bioassay". أظهرت النتائج تاثيراً سميا خفيفا على الخلايا لهذه المستخلصات وكانت قيم ال LC50 وعلى التوالي: 33.88، 36.31، 56.23 ميكرو غرام/مل. بينما كانت قيم ال LC50 للدواء المعياري "vincristine sulphate" هي 0.69 ميكرو غرام/مل. لقد تم دراسة التأثير المضاد للديدان لهذه المستخلصات على "pheretima posthuma" وقد كان هذا التأثير متوسط. أما عن تأثير المستخلص كمسكن للألم، فقد تم فحص تأثير مستخلص الميثانول باستخدام فحص "acetic acid-induced writhing test" وفحص "tail immersion method" على فئران السويس البينو باستخدام جرعة 200 ملغم/كغم وجرعة 400 ملغم/كغم. لقد تسبب مستخلص الأوراق المعياري دايكلوفيناك الصوديوم (72.16%). أما عن تأثير المستخلص كمسكن للألم فقد كان معتمداً على الجرعة المعطاة. أما بالنسبة للتأثير على الأعصاب، فقد تم فحصه باستخدام فحص "hole cross" وفحص "open field test"، وقد تم ملاحظة تثبيط للجهاز العصبي بالمقارنة مع الدواء المعياري دايازيبام (1 مغم/كغم). بالمحصلة، فقد أظهرت نتائج هذه الدراسة تأثيرات ملحوظة لهذه البتة كمضادة للديدان، مسكنة للألم ومثبطة للجهاز العصبي في الفئران.

الكلمات الدالة: إيبوميا فستيولوزا، كونغولفيسوليسه، التأثيرات المضادة للجراثيم.

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