Trop J Nat Prod Res, February 2021; 5(2):281-286

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Investigation of Antidiarrheal, Analgesic, Antidiabetic and Cytotoxic Activities of the Aerial Parts of *Flagellaria indica (Flagellariaceae)*

Utpal K. Karmakar[1]*, Animesh Paul[1], Bishwajit Bokshi[1,2]

[1]*Pharmacy Discipline, Life Science School, Khulna University-9208, Bangladesh*
[2]*Faculty of Science, University of Technology Sydney, Australia*

ARTICLE INFO

Article history:
Received 25 November 2020
Revised 27 December 2020
Accepted 27 January 2021
Published online 01 March 2021

ABSTRACT

Flagellaria indica is a climbing plant traditionally used for the treatment of asthma, shortness of breath and fever. The present study was designed to investigate the phytoconstituents and biological activities (antidiarrheal, analgesic, antidiabetic, and cytotoxic) of the aerial part of *Flagellaria indica* (Flagellariaceae). Phytochemical screening of the extract revealed the presence of alkaloid, flavonoids, saponins, tannins, terpenoids and reducing sugars. The extract showed significant ($p < 0.01$) antidiarrheal activity against castor oil-induced diarrhea in mice in which it decreased the frequency of defecation and increased the mean latent period at the dose of 250 and 500 mg/kg body weight compared to loperamide (3 mg/kg body weight). In acetic acid-induced writhing in mice, the ethanol extract (250 and 500 mg/kg) exhibited significant ($p < 0.01$) inhibition of writhing reflex (43.55% and 60.9%, respectively) compared to standard diclofenac sodium (73.8%). The antidiabetic activity was assessed using oral glucose tolerance test (OGTT). In OGTT, the extract dose-dependently reduced the blood glucose levels in glucose-loaded mice at the dose of 250 and 500 mg/kg body weight in comparison with standard oral antihyperglycemic drug glibenclamide. In the brine shrimp lethality test, the extract showed cytotoxicity with LC_{50} of 71.89 µg/mL using standard drug vincristine (LC_{50} 0.39 µg/mL). All the results tend to justify the traditional uses of the plant.

Key words: *Flagellaria indica*, Phytochemical study, Antidiarrhoeal activity, Analgesic activity, Cytotoxic activity.

Introduction

Flagellaria indica (Family: Flagellariaceae) is a semi-woody climbing plant found in many of the tropical and subtropical regions of the world such as India, Bangladesh, and Australia. It grows often up to 15 m tall, with thick cane-like stems exceeding 15 mm in diameter. The leaves are unusual, they terminate in a coiling tendril that helps the plant to climb. Its leaves, without hairs, are 10 to 40 cm long, and 5 to 20 mm wide. A coiled apex of the leaf forms the holding part of the climbing plant. The flowers are creamy white and occur in large terminal panicles with fragrant bisexual, sessile, and 6-15 cm long panicles. The fruit is an inedible, greenish-red drupe, 5 mm in diameter, usually with only one seed.[1]
Because of its wide distribution, many local common names are used, such as bon chanda, whip vine, hell tail, supplejack, false rattan, and bush cane. The plant is often gathered from the wild for local use, mainly as a source of material for making baskets, etc., but also as food and medicine.[2] The plant has demonstrated antimicrobial activity.[3] It is used traditionally as a diuretic. Several medically active compounds have been found in the plant, including kaempferol 3-glycoside, alkaloids, and cyanogenic glycoside[1]. The root is boiled and the infusion, about 150 mL 3 times daily is taken for 3 days, as health tonic. The fresh stalk is chopped into small pieces in water and the filtrate is drunk in order to relieve stomach aches, dysentery and diarrhea.[3] The plant is used as a contraceptive, and the stem is eaten to cause sterility. A decoction of the leaves is drunk as a treatment for asthma, general shortness of breath and fevers.[4, 5]

*Corresponding author. E mail: ukk146@gmail.com
Tel: +88-01712161906

Citation: Karmakar UK, Paul A, Bokshi B. Investigation of Antidiarrheal, Analgesic, Antidiabetic and Cytotoxic Activities of the Aerial Parts of *Flagellaria indica* (Flagellariaceae). Trop J Nat Prod Res. 2021; 5(2):281-286. doi.org/10.26538/tjnpr/v5i2.11

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Materials and Methods

The fresh aerial parts of *F. indica* were collected from the bank of the river at the Mongla range of Sundarban in July, 2017. During collection, any form of adulteration was strictly avoided. The plant was identified by the expert of the Forestry and Wood Technology Discipline, Khulna University, Bangladesh. The voucher specimen (KUPL-302) was preserved in the Phytochemistry Laboratory, Pharmacy Discipline, Khulna University, Bangladesh. The collected plant parts were cut into small pieces and dried for 21 days without direct contact with sunrays. The dried plant material was finally ground and about 500 g of it was extracted by maceration over 20 days with 1200 mL of 80% ethanol. The extract was filtered off. The solvent was evaporated at room temperature using rotary evaporator.

Animals

Swiss-Albino mice aged 4-5 weeks of both sex (25-30 g body weight) were collected from the animal resources branch of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) and were used for the experiments. The animals were kept in the standard polypropylene cages and provided with standard diets (ICDDR, B formulated). The animals were acclimatized in animal house, Pharmacy Discipline, Khulna University, Khulna under standard Laboratory conditions (relative humidity 55-60%, room temperature 25±2°C and 12 hours light/dark cycle) for a period of 14 days prior to performing the experiments. The ethical approval number of the animals was KUAEC-2020/12/25 provided by the Khulna University Research Cell (KURC).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Chemicals

Sodium molybdate, vincristine and Sodium nitropruside were purchased from Merck, Germany. Ethylene diamine tetraacetic acid (EDTA) and sodium phosphate, sulphanilamide and N (1-napthyl) ethylenediamine dihydrochloride were purchased from BDH, England. The standard drugs Loperamide, Diclofenac sodium and Glibenclamide were collected from Beximco Pharmaceuticals Ltd. Dhaka, Bangladesh.

Phytochemical tests

The crude extract was subjected to preliminary phytochemical screening for the detection of major phytochemical groups.[6]

Determination of antidiarrheal activity

Antidiarrheal activity of the ethanol extract of the aerial parts of *F. indica* was tested using the model of castor oil-induced diarrhea in mice.[7,8] All the mice were screened initially by giving 0.5 mL of castor oil and only those showing diarrhea were selected for the experiment. The test animals were randomly chosen and divided into four groups having five mice in each group. Group I was kept as "control" and received 1% Tween-80 at the dose of 10 mg/kg body weight; Group II was "positive control" and received standard antimotility drug, Loperamide at the dose of 50 mg/kg body weight as oral suspension; Groups III and IV were "test groups" and were treated with suspension of extract of *F. indica* at the oral dose of 250 and 500 mg/kg body weight, respectively. Control vehicle and the extract were administered orally, 30 min. prior to oral administration of castor oil at the dose of 0.5 mL. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhea every hour for five hours. Number of stools or any fluid material that stained the adsorbent paper were counted at each successive hour during the experiment. The latent period of each mouse was also counted. At the beginning of each hour new papers were used to replace the old ones.

Determination of analgesic activity

The analgesic activity of the sample was studied using acetic acid-induced writhing model in mice. Experimental animals were randomly selected and divided into four groups denoted as Control group, Positive control group and Test group I and Test group II consisting of six mice in each group. Control group received orally 1% Tween-80 at the dose of 10 mg/kg body weight and the positive control group received orally diclofenac sodium at the dose of 25 mg/kg body weight. Test group I and Test group II were treated with test sample orally at the dose of 250 and 500 g/kg body weight, respectively. Thirty minutes interval was allowed to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%) was administered intraperitoneally to each of the animals of a group. After an interval of 5 minutes was given for absorption of acetic acid, the number of writhing was counted for 15 minutes. The animals do not always perform full writhing. The incomplete writhing was taken as half-writhing, so two half-writhing were taken as one full writhing. This is why total writhing was halved to convert all writhing to full writhing or real writhing.[9-11]

Oral glucose tolerance tests (OGTT) for evaluation of antidiabetic activity

Oral glucose tolerance tests (OGTT) were carried out as per the procedure previously described by Joy and Kuttan, 1999 with minor modifications.[12] The experimental animals were kept in a fasting state (having no food or drink except water for at least 10 hours but not greater than 16 hours). The mice were randomly selected and divided into four groups denoted as group-I, group-II, group-III, and group -IV consisting of 4 mice in each group. Each group received a specific treatment i.e. control, standard and the test sample. Each mouse was weighed properly and the doses of the test sample, reference and control substances were adjusted accordingly. Each group received a particular treatment. Prior to any treatment each mouse was properly weighed and the doses of control and test materials were adjusted properly. At zero-hour, the test sample at the doses of 250 mg/kg and 500 mg/kg, control (1% tween-80 solution in water) and Glibenclamide were administered orally (10 mg/kg bogy weight) by means of a feeding needle. After 30 min, all groups were treated with 10% glucose solution (2 g/kg body weight). After glucose administration about 30, 60, 90, 120 and 150 minutes later, blood glucose loading was determined by collecting blood samples from the tail vein. Finally, blood glucose level was examined using glucometer.[12]

Determination of cytotoxic Activity

The brine shrimp eggs were hatched in a conical flask containing brine shrimp medium (300 mL). The flask was well aerated with the aid of an air pump, and kept in a water bath at 29-30^{o}C. A bright light was left on it. The nauplii hatched within 48 h. The extract was dissolved in brine shrimp medium with addition of few drops of 5% dimethyl sulfoxide (DMSO) to obtain a concentration of 5, 10, 20, 40, 80, 160 and 320 µg/mL. Each preparation was dispensed into clean test tubes in 10 mL volumes and tested in duplicates. For the control, same procedure was followed except for the absence of the test sample. A series of same concentration as for the sample was prepared for the positive control, chloramphenicol. After proper preparation of test tubes, 10 living shrimps were added to each of the test tubes with the aid of a Pasteur pipette. The test tubes containing the sample, control and positive control were then incubated at 29^{o}C for 24 h in a water bath, after which each test tube was examined and the surviving brine shrimp counted and recorded. From this, the percentage of mortality was calculated at each concentration to determine the LC_{50}.[13]

Statistical analysis

Student's t-test was used to determine significant differences between the control group and test group. The statistical significance threshold was set at p value less than 0.05**.**

Results and Discussion

In the preliminary phytochemical screening the extract showed the presence of Reducing sugars, Tannins, Saponins, Gums, Steroids, Alkaloid, Flavonoids and Terpenoids (Table 1). To have preliminary information on the constituents present in the fruit extract, different chemical tests were performed and the result revealed the presence of Reducing sugar, Tannins, Saponins, Gums, Steroids, Alkaloid, Flavonoids and Terpenoids.

Antidiarrheal activity of the ethanol extract of *F indica* was assessed by castor oil-induced diarrhea in mice. The extract caused an increase in latent period (110.4 and 169.6 min) i.e. delayed the onset of diarrheal episode at the dose of 250 and 500 mg/kg body weight as compared to the standard antidiarrhoeal agent Loperamide (3 mg/kg body weight) where the mean latent period was 202 min (Table 2).

Table 1: Result of the phytochemical tests

Constituents	Result
Reducing sugar	+
Combined reducing sugar	+
Tannins	+
Flavonoids	+
Saponins	+
Gums	+
Steroids	+
Alkaloids	+
Glycoside	-
Proteins	-
Terpenoids	+

+ = Presence; - = Absence

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The extract also exhibited the inhibition of defecation 52.5% and 67.5% at the doses of 250 and 500 mg/kg of body weight, respectively while the standard drug Loperamide (3 mg/kg body weight) showed 83.75% inhibition (Table 3 and Figure 1).

Antidiarrheal activity of the ethanol extract of *F indica* was tested by castor oil-induced diarrhea in mice. Castor oil mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinoleic acid remains in the intestine and produces its anti-absorptive or antisecretory effect.[14] The ricinoleic acid thus liberated readily forms ricinoleate salts with sodium and potassium in the lumen of the intestine. The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. Most agreed view is that ricinoleate salts stimulate the intestinal epithelial cell's adenyl cyclase or release prostaglandins, which results in an increase in the net secretion of water and electrolytes in the small intestine.[15,16] The ethanol extract of *F. indica* significantly and dose-dependently inhibited and delayed the onset of diarrhea in mice. The maximum effect was found at 500 mg/kg body weight. On the basis of this result, it can be concluded that the ethanol extract of *F. indica* might possess antidiarrhoeal activity.

Analgesic activity of the ethanol extract of *F. indica* was tested by acetic acid-induced writhing model in mice. The extract produced 43.55% ($p < 0.05$) and 60.9% ($p < 0.01$) acetic acid-induced writhing inhibition in mice at the dose of 250 and 500 mg/kg body weight, which was comparable to diclofenac sodium 73.8% ($p < 0.01$) at the dose of 25 mg/kg body weight (Table 2).

Analgesic activity of the ethanol extract of *F. indica* was examined by acetic acid-induced writhing model in mice. Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings.[17] Increased levels of PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid.[18] The ethanol extract of *F. indica* exhibited significant writhing inhibition comparable to the standard drug diclofenac sodium. On the basis of this result, it can be concluded that the ethanol extract of *F. indica* possesses analgesic activity.

The antidiabetic activity of the ethanol extract of *F. indica* was examined using OGTT. The extract dose-dependently reduced the blood glucose level 5.80 and 4.98 mM/L after 150 min at the dose of 250 and 500 mg/kg body weight compared to the standard drug Glibenclamide (5 mg/kg body weight) 3.80 mM/L (Tables 5 and 6).

The oral glucose tolerance test (OGTT) is the most sensitive test for detecting borderline diabetes mellitus. Glucose tolerance means ability of the body to utilize glucose in the circulation. Thus "glucose tolerance test" is a valuable diagnostic aid in the diagnosis of diabetes mellitus, insulin resistance, impaired beta-cell function and sometimes reactive hypoglycemia and acromegaly. The *F. indica* extract dose-dependently decreased blood glucose level compared to standard Glibenclamide. After 120 and 150 min, the extract-treated mice showed blood sugar level of 5.83 and 4.98 m mol/L at the dose of 500 mg/kg body weight whereas Glibenclamide exhibited 3.83 and 3.80 m mol/L.

Brine shrimp lethality bioassay indicates cytotoxicity of extract. The extract was found to show lethal activity against brine shrimp nauplii and the LC_{50} was found to be 71.89 µg/mL for extract and the LC_{90} value was 0.39 µg/mL for standard vincristine (Figures 2 and 3, Tables 7 and 8).

Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor, etc. of the compound.[19, 20] The ethanol extract of *F. indica* was found to show mild activity against the brine shrimp nauplii; LC_{50} was found to be 71.89 µg/mL. However, further investigations using carcinoma cell line are necessary to isolate the active compounds responsible for the activity.

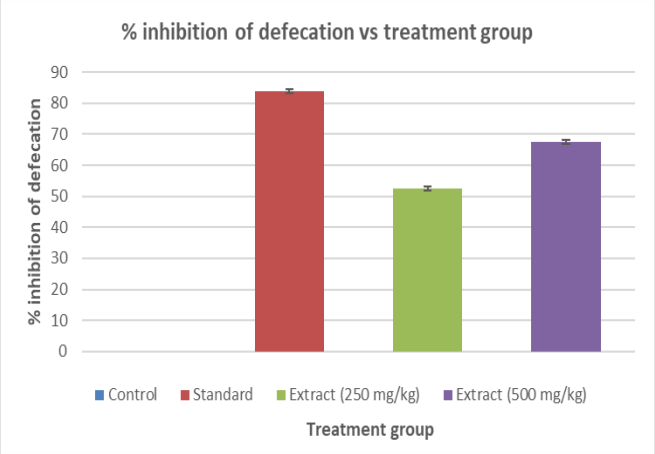

Figure 1: Effect of extract of *F. indica* on the basis of percentage inhibition of defecation in castor oil-induced diarrheal episode in mice

Graphical Presentation

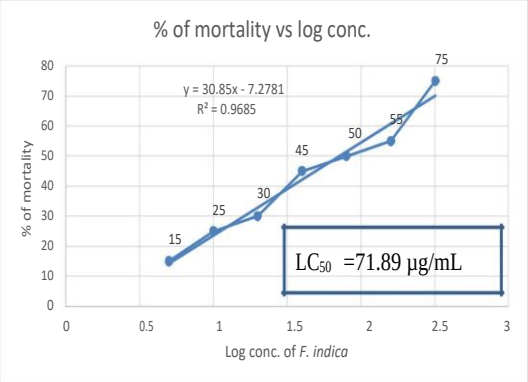

Figure 2: Graphical representation of brine shrimp lethality bioassay and LC_{50} for the extract of *F. indica*

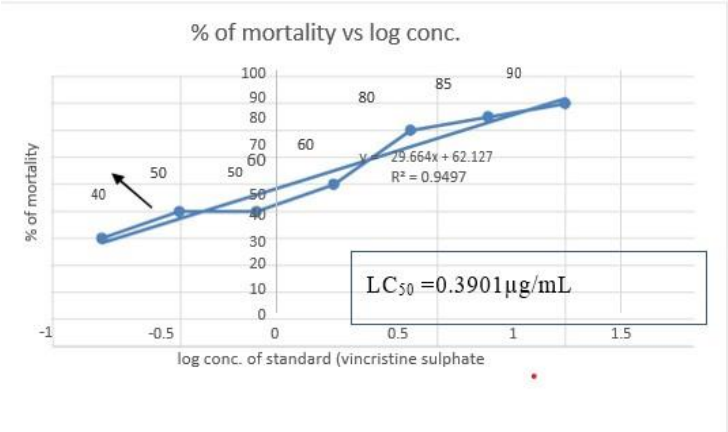

Figure 3: Graphical representation of brine shrimp lethality bioassay and LC_{50} for the standard (vincristine sulfate)

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Effect of *F. indica* on prolongation of the latent period of castor oil-induced diarrheal episode in mice

Animal Group	Dose (p.o)	Latent Period (min)
Group I (Control) 1% Tween-80	10 mL/kg	72 ± 6.81
Group II (Positive Control) Loperamide	3 mg/kg	202 ± 8.32*
Group III (Test Group) Et. Extract	250 mg/kg	110.4 ± 4.71**
Group IV (Test Group) Et. Extract	500 mg/kg	169.6 ± 5.12*

Values are expressed as mean ± SEM (n = 5); *: $p < 0.01$, **$p < 0.01$ vs. control, p.o: per oral

Table 3: Data presentation and statistical evaluation of effect of *F. indica* extract on decrease in stool count of castor oil-induced diarrheal episode in mice

Animal Group	Dose (p.o)	% inhibition of defecation
Group I (Control) 1% Tween-80	10 mL/kg	-
Group II (Positive Control) Loperamide	3 mg/kg	83.75
Group III (Test Group) Et. Extract	250 mg/kg	52.5
Group IV (Test Group) Et. Extract	500 mg/kg	67.5

Table 4: Effect of *F. indica* on acetic acid-induced writhing in mice

Animal Group	Treatment	Writhing Count (%Writhing)	%Writhing Inhibition
Control (n = 5)	1% tween-80 solution in water	24.0 ± 1.0 (100)	0
Positive Control (n = 5)	Diclofenac sodium (25 mg/kg)	6.5 ± 0.42* (26.2)	73.8
Test group I (n = 5)	Et. Extract (250 mg/kg)	14 ± 0.96** (56.45)	43.55
Test group II (n = 5)	Et. Extract (500 mg/kg)	97 ± 0.66* (39.1)	60.9

Values are expressed as mean ± SEM, SEM = Standard error of Mean, n = No. of mice, Et. = Ethanol, *: $p < 0.01$; **: $p < 0.05$ vs. control

Table 5: Oral Glucose Tolerance Test result of extract of aerial part of *F. indica*

Administered dose to mice Group	No. of mice	Weight (g) of mice	Blood Glucose Level (m Mol/L)					
			0 min	30 min	60 min	90 min	120 min	150 min
	1	26	4.6	17.1	10.7	9.5	6.9	6.9
	2	25	5.1	21.0	15.5	12.8	7.8	6.2
Control	3	25	4.8	19.7	14.8	12.3	10.3	7.9
	4	26	6.5	18.9	11.7	11.0	9.2	9.3
	Average		5.25	19.18	13.18	11.4	8.55	7.58
	1	27	3.9	13.2	6.9	5.7	2.9	3.7
Positive control	2	26	3.2	12.8	7.9	4.8	3.6	3.8
Glibenclamide (5 mg/kg)	3	25	4.7	15.6	6.8	6.0	4.7	3.7
	4	25	5.1	15.9	6.7	4.1	4.1	4.1
	Average		4.23	14.38	7.08	5.15	3.83	3.80
	1	26	4.1	13.3	10.5	7.9	6.2	5.8
Extract of.	2	25	3.6	14.6	12.1	8.1	5.9	5.3
F indica (250 mg/kg)	3	26	4.5	14.8	11.3	10.7	7.1	6.0
	4	25	3.9	11.9	10.3	9.7	7.7	6.1
	Average		4.03	13.65	11.05	9.1	6.73	5.80
	1	26	4.1	13.4	10.3	7.1	5.1	4.7
Extract of.	2	27	3.6	12.9	10.7	9.0	7.5	5.9
F indica (500 mg/kg)	3	25	4.3	14.4	12.0	8.2	5.9	5.1
	4	26	3.2	10	7.6	5.8	4.8	4.2
	Average		3.8	12.68	10.15	7.53	5.83	4.98

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 6: Oral Glucose Tolerance Test result of *F. indica* extract

Animal group		Blood Glucose Level (m Mol/L)					
		0 min	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**
	Average ± SD	5.25 ± 0.86	19.18 ± 1.63	13.18 ± 2.33	11.40 ± 1.48	8.55 ± 1.50	7.58 ± 1.34
Control	SEM	0.429	0.816	1.167	0.738	0.751	0.673
Positive control	Average ± SD	4.23 ± 0.85	14.38 ± 1.16	7.08 ± 0.56	5.15 ± 0.87	3.83 ± 0.76	3.80 ± 0.19
Glibenclamide	SEM	0.423	0.800	0.278	0.433	0.382	0.095
(5 mg/kg)	*p* value	0.1399	0.0057	0.0023	0.0003	0.0014	0.0015
Extract of.	Average ± SD	4.03 ± 0.38	13.65 ± 1.34	11.05 ± 0.82	9.1 ± 1.34	6.73 ± 0.83	5.8 ± 0.36
F indica (250 mg/kg)	SEM	0.189	0.671	0.411	0.668	0.413	0.178
	p value	0.0400	0.0020	0.1368	0.0603	0.0773	0.0434
Extract of.	Average ± SD	3.80 ± 0.50	12.68 ± 1.89	10.15 ± 1.85	7.53 ± 1.39	5.83 ± 1.21	4.98 ± 0.72
F indica (500 mg/kg)	SEM	0.248	0.945	0.924	0.694	0.605	0.359
	p value	0.0265	0.0020	0.0884	0.0087	0.0301	0.0143

Table 7: Result of brine shrimp lethality bioassay of aerial part *of F. indica* extract

Conc. (µg/mL)	Log conc.	No. of live shrimp in Test-1	No. of live shrimp in Test-2	Avg. no. of live shrimp (Sample)	Avg. no. of live Shrimp (Control)	Mortality (%)
5	0.699	8	9	8.5		15
10	1	7	8	7.5		25
20	1.301	7	7	7		30
40	1.602	6	5	5.5	10	45
80	1.903	5	5	5		50
160	2.204	5	4	4.5		55
320	2.505	2	3	2.5		75

Table 8: Result of brine shrimp lethality bioassay of standard (vincristine sulphate)

Conc. (µg/mL)	Log conc.	No. of live shrimp in Test-1	No. of live shrimp in Test-2	Avg. no. of live shrimp (Standard)	Avg. no. of live Shrimp (Control)	Mortality (%)
0.1562	-0.806	7	5	6		40
0.3125	-0.505	4	6	5		50
0.625	-0.204	6	4	5		50
1.25	0.097	3	5	4	10	60
2.5	0.397	2	2	2		80
5	0.6989	1	2	1.5		85
10	1	1	1	1		90

Conclusion

F. indica contains important chemical constituents that confer upon it as a medicinal agent. It was revealed that the fruit extract contains Reducing Sugar, tannins, saponins, gums, steroids, alkaloids, and terpenoids which have potential role in its antidiarrhoeal, analgesic, antidiabetic, and cytotoxic activity.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors are grateful to the authority of International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) for providing the experimental mice. The authors are also grateful to the

authority of Beximco Pharmaceuticals Ltd. for providing Diclofenac sodium, and Loperamide. The authors are also grateful to the authority of Khulna University Research Cell for financial support of this research.

References

1. Medicinal Plants in the South Pacific, WHO Regional Publications, Manilla. 1998.
2. Medicinal Plants in Papua New Guinea, WHO 2009.
3. Facciola S. Cornucopia II, Kampong, California, 1998.
4. Gnanaraji C, Shahi MD, Haque ATME, Iqbal M. Phytochemical Screening, antioxidant properties in various extracts from the leaves of *Flagellaria indica* L. from Sabah Malaysia. Int J Pharm and Pharmaceutical Sci. 2015; 7(9):510-512.
5. Hesse L, Wagner ST, Neinhuis C. Biomechanics and functional morphology of a climbing monocot. AoB Plants. 2016; 8:1-16.
6. Evans WC. Trease and Evan's Textbook of Pharmacognosy. 1989. 13:546 p.
7. Chatterjee TK. Handbook of laboratory Mice and Rats. 1st Ed. Jadavpur University, India. 1993; 133-139 p.
8. Karmakar UK, Rahman MA, Roy DN, Sadhu SK, Ali ME. Chemical and Biological Investigations of *Coriandrum sativum* Linn. Int J Pharm Sci Res. 2011; 2(4):999-1006.
9. Whittle BA. The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics. Br J Pharmacol Chemother. 1964; 22:246-253.
10. Ahmed F, Selim MST, Das AK, Choudhuri MSK. Anti-inflammatory and antinociceptive activities of *Lippia nodiflora* Linn. Pharmazie. 2004; 59:329-333.
11. Asadujjaman M, Mishuk AU, Hossain MA, Karmakar UK. Medicinal potential of *Passiflora foetida* L. plant extracts: biological and pharmacological activities. J Integr Med. 2014; 12(2):121-126.
12. Joy KL and Kuttan R. Anti-diabetic activity of *Picrorrhiza kurroa* extract. J Ethnopharmacol. 1999; 67(2):143-148.
13. Mayer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, Mclaughlin JL. Brine shrimp: a convenient bioassay for active plant constituents. Planta Med. 1982; 45:31-34.
14. Tripathi KD. Essentials of Medical Pharmacology. Jaypee Brothers. 2001. 4:432 p.
15. Racusen LC and Binder HJ. Ricinoleic acid stimulation of active anion secretion in colonic mucosa of the rat. J Clin Invest. 1979; 63:743-749.
16. Beubler E and Juan H. Effect of Ricinoleic acid and other Laxatives in Net Water Flux and Prostaglandin E release by the Rat Colon. J Pharm Pharmacol. 1979; 31:681-685.
17. Taesotikul T, Panthong A, Kanjanapothi D, Verpoorte R, Scheffer JJC. Anti-inflammatory, antipyretic and antinociceptive activities of *Tabernaemontana pandacaqui* Poir. J Ethnopharmacol. 2003; 84:31-35.
18. Derardt R, Jougney S, Delevalcee F, Falhout M. Release of prostaglandins E and F in an allogenic reaction and its inhibition. Eur J Pharmacol. 1980; 51:17-24.
19. Meyer S. Phytochemical methods (a guide to modern techniques to plant analysis). Champan and Hall, USA. 1982. 3:335-337 p.
20. McLaughlin JL, Anderson JE, Chang CJ. Bioactive components of *Allamanda schottii*. J Nat Prod. 1988; 51(2):307-308.