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Exploration of Anthelmintic, Blood Coagulant, Diuretic and Laxative Activities of Different Solvent Fractions of Flagellaria Indica Leaves

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ABSTRACT

Flagellaria indica (Family: Flagellariaceae) is a common climbing plant found primarily in tropical regions of many countries. The plant has various traditional uses, although most of them lack scientific published reports. The crude ethanolic extract of F. indica leaves was fractionated based on polarity using water, ethyl acetate, and n-hexane. Biological screening was conducted on the anthelmintic, blood coagulation, diuretic, and laxative activities of the water, ethyl acetate, and n-hexane fractions of F. indica leaves. In the anthelmintic test, the n-hexane fraction showed a moderate effect with paralysis times of 16.79 and 13.62 minutes and death times of 27.34 and 21.81 minutes, respectively, at doses of 25 and 50 mg/mL.In the blood coagulant test, only the water fraction showed a notable effect. The clotting times were 4.33, 6.02, 7.68, and 8.32 minutes, respectively, at doses of 200, 100, 50, and 25 mg/mL. Diuretic activity was performed to determine the increase in the volume of excreted urine, and electrolyte analysis of urine was performed to determine pH, density, conductance, and Na+, Cl-, K+ levels, as well as natriuretic, kaliuretic, saluretic, and CAI indexes. The ethyl acetate fraction showed better diuretic activity than the n-hexane fraction, while the water fraction did not reveal a notable diuretic effect. The Na+, Cl-, K+, natriuretic, and saluretic indexes were found satisfactory in the ethyl acetate fraction, and the CAI index was better in the n-hexane fraction. In the laxative test, the n-hexane fraction showed the best laxative properties, with an increase in stool weight of 38% and 54% at doses of 250 and 500 mg/kg, respectively. These results suggest that different fractions of F. indica leaves contain distinct phytochemicals that may be responsible for these biological effects. The isolation of bioactive compounds could help justify its traditional uses in modern medicine.

Keywords: *Flagellaria indica*, Anthelmintic, Blood Coagulant, Diuretic and Laxative activities.

Abbreviations: gm, gram; mg, milligram; kg, kilogram; mg/kg, milligram/kilogram; bw, bodyweight; min, minutes; mm, millimeter; cm, centimeter, L, liter, mL, milliliter; µL, microliter; ICDDRB, International Center for Diarrhoeal Disease Research, Bangladesh; SD, Standard Deviation; ANOVA, Analysis of Variance, WHO, World Health Organization, AEC, Animal Ethics Committee; CAI, Carbonic Anhydrase Inhibitory.

INTRODUCTION

Our universe is the ultimate reservoir of all living creatures. Medicinal plants are the best gift of nature for human survival against different types of physical disorders. Human beings have been solely dependent on medicinal plants since ancient times. Flagellaria indica is a common plant from the Flagellariaceae family (Figure 1). This climbing plant is widely located in many subtropical and tropical areas of Polynesia, South-East Asia, and Australia. The plant is also well-known by many local names, such as rotan tikas, Bon chanda, whip vine, hell tail, supplejack, etc. In Bangladesh, this plant is found in different parts of the Sundarbans. It can often grow up

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to 15 meters tall, with stems that are 15 mm in diameter. Its leaves are 10-40 cm long and 5-20 mm wide, forming a coiled apex that helps the plant climb upward. The white flowers have a pleasant fragrance and are 10-25 cm long. The green fruits are 5 mm in diameter and are inedible. This plant has various local uses by people from different areas of the world. The leaves are used to treat bacterial infectious diseases, coughs, helminthiasis, vomiting, and they also have diuretic effects. The Murut tribe people use its boiled leaves for bathing to treat semi-paralytic disorders. Chopped small stems are used as traditional medicine to treat diarrhea, stomach pain, and cholera. The stem of this plant is also reported to be used as a contraceptive in limited cases. A decoction of the leaves is beneficial for treating asthma, wheezing, and fevers.[1, 2]

Karmakar et al. (2021) reported the presence of various phytochemical groups such as reducing sugars, terpenoids, tannins, saponins, steroids, flavonoids, gums, alkaloids, etc. They also conducted tests for analgesic, antidiarrheal, antihyperglycemic, and cytotoxic effects using its leaves.[2] Gnanaraj et al. (2015) reported on the antioxidant properties of various plant parts [1] Gnanaraj et al. (2016) reported on the hepatoprotective effects of the plant's leaves[3]. After reviewing the traditional uses and reported activities of this plant, our objective was to conduct additional biological tests that had not been previously investigated. To achieve this, we conducted experiments to assess the anthelmintic, diuretic, blood coagulation, and laxative effects of various fractions of F. indica leaves.

Figure 1: *Flagellaria indica* leaves and fruits

MATERIALS AND METHODS

Plant collection and identification

In July 2017, fresh leaves of F. indica were collected from the riverside area of the Mongla range in the Sundarbans, the world's largest mangrove forest. Great care was taken to ensure there were no contaminants. The collected plant material was then identified by experts from Khulna University's Department of Forestry and Wood Technology, and a voucher number (KUPL-302) was assigned for future reference. The harvested leaves were dried in the shade for 45 days, with strict avoidance of direct sunlight exposure. After the drying period, the leaves were ground into a fine powder. A total of 250 grams of powdered material was macerated with 1 liter of 96% ethanol, and this mixture was sealed in a glass bottle for three weeks. During this period, the mixture was periodically agitated using a glass rod to ensure proper mixing. Subsequently, the mixture was filtered to obtain 7.6 grams of crude gum extract. The crude extracts were then fractionated using water, ethyl acetate, and n-hexane, resulting in the isolation of 3.1 grams, 1.9 grams, and 2.3 grams of extract for the water, ethyl acetate, and n-hexane fractions, respectively.

Animals

For pharmacological tests, fresh male Swiss albino mice, aged 3-5 weeks and with an average body weight of 25-35 grams, were procured from the Animal Resources Division of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). These mice were accommodated in the animal facility at the Laboratory of Pharmacy, Khulna University, where optimal laboratory conditions were maintained (50-60% relative humidity, 25-30°C, and a 12-hour light/dark cycle). They were provided with rodent food. To ensure their acclimatization to the laboratory environment, the mice were given a period of 14 days before commencing any experiments. All bioassays performed on the mice adhered to the ethical standards outlined by the Khulna University Animal Ethics Committee (AEC), Bangladesh [Reference: KUAEC-2020/12/25].

Chemicals

All laboratory-grade reagents required for conducting the biological experiments, such as ethanol (Merck, India), n-Hexane (Merck, India), Ethyl acetate (Loba, India), and Tween 80 (Merck, India), were used. Albendazole, phytomenadione, and frusemide were procured from Square Pharmaceuticals Limited, Bangladesh, while bisacodyl was obtained from Opsonin Pharmaceuticals Limited, Bangladesh.

Anthelmintic activity Assay

The anthelmintic impact of F. indica was assessed using the method described by Saha et al. (2021) and Akter et al. (2020) to measure its lethal effect on Paramphistomum cervi. Live P. cervi specimens were collected from freshly slaughtered cattle at a local abattoir in Gollamari, Khulna. Ten milliliters of 25 mg/mL and 50 mg/mL concentrations of n-Hexane, ethyl acetate, and water fractions of F. indica were prepared and placed in separate petri dishes. Albendazole was used as the standard drug at a concentration of 15 mg/mL, and 0.1% Tween 80 solutions were also prepared, with 10 mL of each placed in separate petri dishes. In each petri dish, six P. cervi specimens were introduced. Subsequently, the paralysis time (defined as no movement except for slight twitching after vigorous shaking) was recorded using a stopwatch. Additionally, the time of death (defined as no movement after vigorous shaking and immersion in 50º C water) was also recorded.

Blood coagulant Activity Assay

The blood coagulation activity of various fractions of F. indica extract was assessed using the method described by Ikese et al. (2015).[6] Five healthy human volunteers agreed to provide blood for this experiment. Fresh human blood was collected using sterile syringes.

n-Hexane, ethyl acetate, and water fractions of F. indica were prepared at concentrations of 25, 50, 100, and 200 mg/mL. Phytomenadione was used as the standard drug and was prepared at concentrations of 1.25, 2.5, 5, and 10 mg/mL. One milliliter of freshly collected blood was mixed with 100 µL of the above-prepared concentrations of Phytomenadione and different fractions of F. indica in separate labeled test tubes. The test tubes were immediately placed in a water bath containing water at 37º C (normal human body temperature), and the time for clot formation or gel-like substance formation was recorded using a stopwatch. Every 15 seconds, each test tube was tilted to check for the formation of clotting. Finally, the coagulation time was recorded.

Diuretic activity Assay

Diuretic assay was conducted following an established protocol adopted by Mamun *et al.* (2003) and Mekonnen *et al.* (2010).[7, 8] Mice for the study were divided into several groups, each containing 5 mice. The mice were fasted for 18 hours prior to the experiment and were pre-treated with 0.9% NaCl saline solution. In the experiment, the standard group was orally administered furosemide at a dose of 10 mg/kg body weight, while the control group received pre-treated saline solution. The test groups were given oral doses of 250 and 500 mg/kg of the water, ethyl acetate, and n-Hexane fractions of F. indica extract. The solutions were prepared so that each mouse received a 2 mL solution of the respective doses. Subsequently, these mice were placed in metabolic cages according to their respective groups. Urine excreted by the mice was collected and quantified hourly for 6 hours after the experiment was conducted. The collected urine was stored under refrigeration at -20º C for future analysis.

The diuretic effect of F. indica's different fractions was measured using the following equations:

Urinary discharge = (Total urine amount (Vo) / Total fluid administered (Vi)) x 100

The diuretic activity was calculated by dividing the urinary excretion of the test group by the urinary excretion of the control group. The diuretic activity was then determined by comparing the diuretic activity of the test group to that of the control group.

As soon as we collected the urine, it was measured for pH using the D-50 Series Handheld Water Quality Meters by Horiba Scientific. The density of urine was determined by measuring the volume and weight of the urine. Sodium (Na+) and potassium (K+) ion concentrations were measured in mEq/L using a Jenway PFP7 Flame Photometer with a 100 times diluted solution. Chloride (Cl-) ion concentration was assessed through direct titration with a 1% $AgNO_3$ solution using potassium chromate (5%) as an indicator. After measuring the ion concentrations, the natriuretic (Na+/K+), saluretic (Na+/Cl-), carbonic anhydrase inhibitory (CAI) activity [(Cl-/(Na++K+)], and kaliuretic (K+/Na+) values were calculated. Finally, the Na+, K+, Cl-, saluretic, natriuretic, kaliuretic, and CAI indices were determined by comparing the values in the test group with those in the control group[9]

Laxative activity Assay

The laxative effect of the fractions of F. indica extract was evaluated using the method described by Capasso (1986) and Akter et al. (2022).[10,11] The test mice (25-30 gm body weight) were divided into several groups, each consisting of five mice. Prior to the test, the mice were fasted for 12 hours and only water was provided during this period. In the control group, mice were treated with normal saline (0.9% NaCl), and the positive control group received bisacodyl at a dose of 10 mg/kg. In the test groups, mice were administered doses of 250 and 500 mg/kg body weight of n-Hexane, ethyl acetate, and water fractions of F. indica extract. The mice were then placed in metabolic cages according to their respective groups for the next 16 hours, during which no food or water was

provided. After this time, the excreted feces from the mice were collected and weighed.

Statistical data analysis

One-way ANOVA analysis was conducted using Dunnett's t-test ($p < 0.05$, versus control). Pairwise comparisons were carried out using the Post-hoc Tukey test ($p < 0.05$, versus standard/extract). To analyze the data, the IBM SPSS program (version 25.0) from IBM Corporation, New York, USA, was utilized.[12]

RESULTS

Anthelmintic activity Assay

In evaluating the anthelmintic assay of different fractions of F. indica, significant paralysis and death times of P. cervi were observed (Table 1). Among the three fractions of F. indica, the n-Hexane fraction showed the best anthelmintic activity, causing the least time for paralysis and death of the nematodes. Conversely, the water fraction showed comparatively poor anthelmintic activity across the tested doses. The results obtained demonstrated a dose-dependent effect.

Table 1: Representation of anthelmintic effects of different fractions of *F. indica* extract

Tested group	Dose (mg/mL)	Mean paralysis time (min)	Mean death time (min)
Negative control (0.9% NaCl)	------	------	------
Standard (Albendazole)	15	6.95 ± 0.44^{abcdef}	14.71 ± 0.75^{abcdef}
n-Hexane fraction	25	$16.79 \pm 0.99^{\theta bcdef}$	$27.34 \pm 0.76^{\theta bcef}$
n-Hexane fraction	50	$13.62 \pm 0.76^{\theta acdef}$	$21.81 \pm 1.13^{\theta acdef}$
Ethyl Acetate fraction	25	$20.99 \pm 1.13^{\theta abde}$	$30.48 \pm 0.7^{\theta abd}$
Ethyl Acetate fraction	50	$18.70 \pm 0.79^{\theta abcef}$	$28.17 \pm 0.78^{\theta bcef}$
Water fraction	25	$22.17 \pm 1.18^{\theta abcdf}$	$31.43 \pm 0.87^{\theta abdf}$
Water fraction	50	$20.26 \pm 1.18^{\theta abde}$	$30.33 \pm 1.21^{\theta abde}$

Data are plotted as average of six (06) replicates ± SD (standard deviation);
$^{\theta}$ $p < 0.05$ vs. Albendazole 15 mg/mL; ^{a} $p < 0.05$ vs. *n*-Hexane fraction 25 mg/mL; ^{b} $p < 0.05$ vs. *n*-Hexane fraction 50 mg/mL; ^{c} $p < 0.05$ vs. Ethyl Acetate fraction 25 mg/mL; ^{d} $p < 0.05$ vs. Ethyl Acetate fraction 50 mg/mL; ^{e} $p < 0.05$ vs. Water fraction 25 mg/mL; ^{f} $p < 0.05$ vs. Water fraction 50 mg/mL; (pair-wise comparison by Post Hoc Tukey test)

Blood coagulation activity Assay

In the blood coagulation test, various concentrations of the standard (Phytomenadione) and test groups (n-Hexane, ethyl acetate, and water fractions of F. indica extract) were utilized. The average blood coagulation times for the different tested samples are listed in Table 2. Among the tested groups, only the water fraction showed some blood coagulation effect, while the ethyl acetate and n-hexane fractions did not exhibit any notable blood coagulant effect.

Table 2: Representation of Blood Coagulation Effect of different fractions of *F. indica* extract

Tested group	Dose (mg/mL)	Average coagulation time
Negative control	------	8.87 ± 0.00 [1 2 3 4 a e f g i j k l]
Standard (Phytomenadione)	10	2.52 ± 0.09* [2 3 4 a b c d e f g h i j k l]
Standard (Phytomenadione)	5	4.8 ± 0.23 [*1 3 4 a b c d e f g h I j k l]
Standard (Phytomenadione)	2.5	5.73 ± 0.14* [1 2 4 a b c d e f g h I k l]
Standard (Phytomenadione)	1.25	7.77 ± 0.15 [*1 2 3 a b c d e f h i j l]
n-Hexane fraction	200	8.39 ± 0.2* [1 2 3 4 b e f i j k]
n-Hexane fraction	100	8.98 ± 0.14 [1 2 3 4 a e f h i j k l]
n-Hexane fraction	50	8.82 ± 0.22 [1 2 3 4 e f g i j k l]
n-Hexane fraction	25	8.59 ± 0.57 [1 2 3 4 e f g i j k]
Ethyl acetate fraction	200	6.78 ± 0.14* [1 2 3 4 a b c d g h i j k l]
Ethyl acetate fraction	100	7.22 ± 0.19* [1 2 3 4 a b c d g h i j k l]
Ethyl acetate fraction	50	8.12 ± 0.09 [*1 2 3 b c d e f h i j]
Ethyl acetate fraction	25	8.74 ± 0.09 [1 2 3 4 e f g i j k]
Water fraction of *F. indica*	200	4.33 ± 0.16 [*1 2 3 4 a b c d e f g h j k l]
Water fraction of *F. indica*	100	6.02 ± 0.13 [*1 2 3 a b c d e f g h i k l]
Water fraction of *F. indica*	50	7.68 ± 0.16 * [1 2 3 4 a b c d e f h i j l]
Water fraction of *F. indica*	25	8.32 ± 0.08 [*1 2 3 4 b c e f i j k]

Data are plotted as average of five replicates ± SD (standard deviation); * $p < 0.05$ vs. Control (Dunnett's t test); [1] $p < 0.05$ vs. Phytomenadione 10 mg/mL; [2] $p < 0.05$ vs. Phytomenadione 5 mg/mL; [3] $p < 0.05$ vs. Phytomenadione 2.5 mg/mL; [4] $p < 0.05$ vs. Phytomenadione 1.25 mg/mL; [a] $p < 0.05$ vs *n*-Hexane fraction 200 mg/mL; [b] $p < 0.05$ vs. *n*-Hexane fraction 100 mg/mL; [c] $p < 0.05$ vs. *n*-Hexane fraction 50 mg/mL; [d] $p < 0.05$ vs. *n*-Hexane fraction 25 mg/mL; [e] $p < 0.05$ vs. Ethyl Acetate fraction 200 mg/mL; [f] $p < 0.05$ vs. Ethyl Acetate fraction 100 mg/mL; [g] $p < 0.05$ vs. Ethyl Acetate fraction 50 mg/mL; [h] $p < 0.05$ vs. Ethyl Acetate fraction 25 mg/mL; [i] $p < 0.05$ vs. Water fraction 200 mg/mL; [j] $p < 0.05$ vs. Water fraction 100 mg/mL; [k] $p < 0.05$ vs. Water fraction 50 mg/mL; [l] $p < 0.05$ vs. Water fraction 25 mg/mL (Pair-wise comparison by Post Hoc Tukey test)

Diuretic activity Assay

In the diuretic test, the urinary volume, urinary excretion, diuretic activity, and diuretic effects are depicted in Figures 2, 3, 4, and 5, respectively. The pH, density, conductivity, urinary volume, and diuretic index of mice in different groups are listed in Table 3. Tables 4 and 5 show the effects of various fractions of F. indica on electrolyte excretion in mouse urine after 6 hours of treatment.

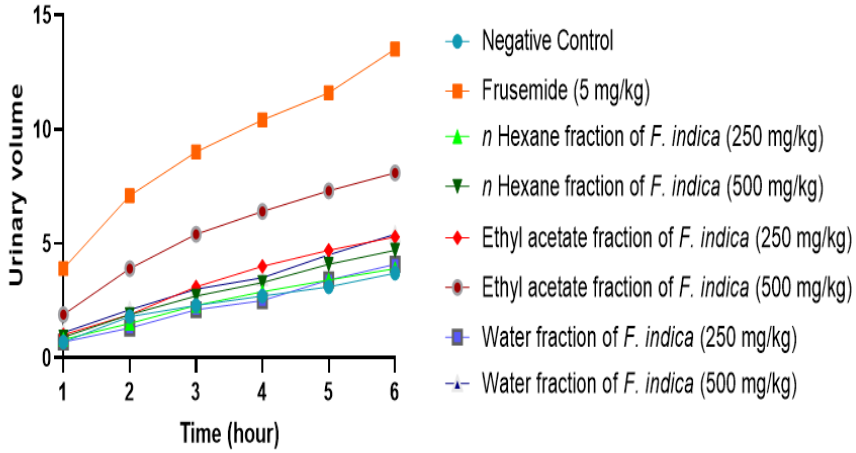

Figure 2: Urinary volume of frusemide and different fractions of *F. indica* leaves

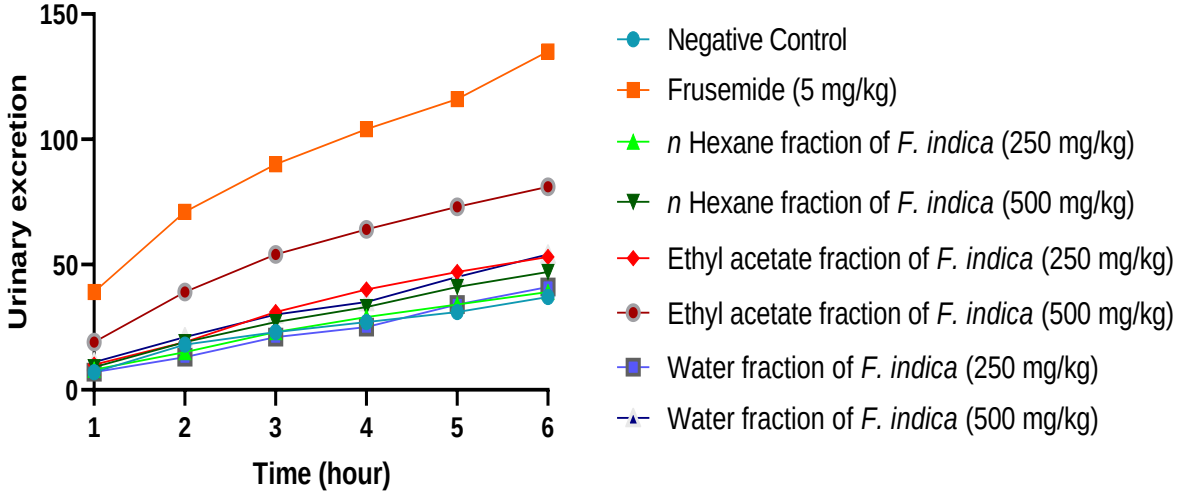

Figure 3: Urinary excretion of frusemide and different fractions of *F. indica* leaves

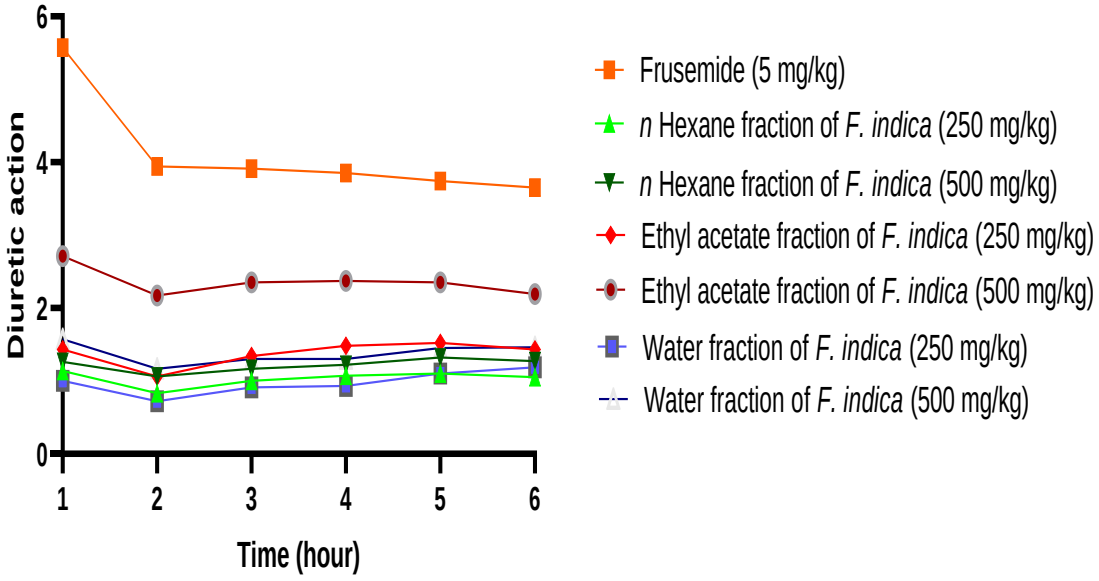

Figure 4: Diuretic action of frusemide and different fractions of *F. indica* leaves

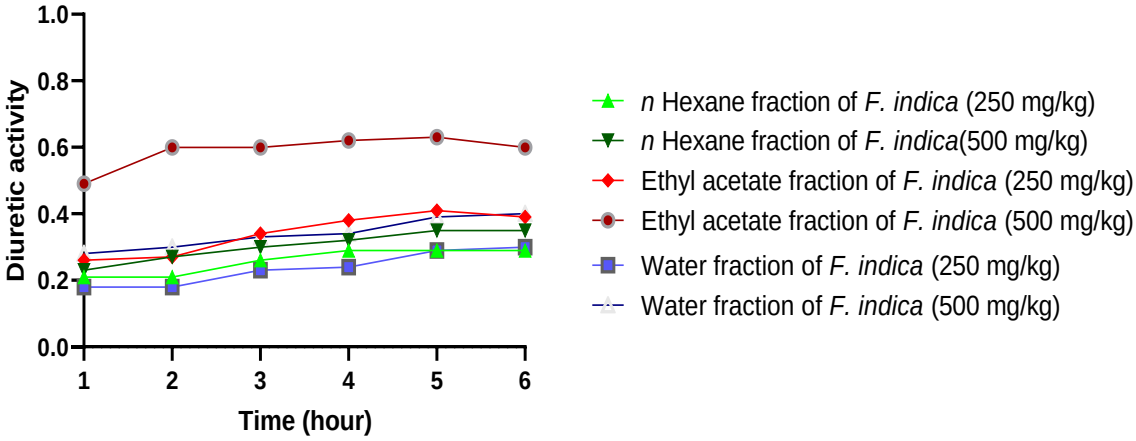

Figure 5: Diuretic activity of different fractions of *F. indica* leaves

Table 3: Impact of various fractions of *F. indica* on pH, density, conductivity, urinary volume and diuretic index of mice urine after 6 hours of medication in the diuretic assay

Tested group	pH	Density (gm/mL)	Conductivity (mS/cm)	Urine Volume (mL/6h)	Diuretic index
Control	7.12 ± 0.02	0.93 ± 0.01	13.33 ± 0.04	3.7	1
Frusemide (5 mg/kg)	7.42 ± 0.03	0.98 ± 0.02	12.32 ± 0.07	13.5	3.65
n-hexane (250 mg/kg)	7.88 ± 0.02	0.89 ± 0.03	14.43 ± 0.08	3.9	1.05
n-hexane (500 mg/kg)	7.78 ± 0.03	0.91 ± 0.02	18.86 ± 0.33	4.7	1.27
Ethyl acetate (250 mg/kg)	7.68 ± 0.02	0.84 ± 0.03	17.54 ± 0.41	5.3	1.43
Ethyl acetate (500 mg/kg)	7.63 ± 0.03	0.89 ± 0.11	23.32 ± 0.22	8.1	2.19
Water (250 mg/kg)	7.56 ± 0.03	0.93 ± 0.05	14.52 ± 0.53	4.1	1.11
Water (500 mg/kg)	7.52 ± 0.02	0.88 ± 0.10	15.11 ± 0.22	5.4	1.46

Table 4: Effect of different fractions of *F. indica* on electrolyte excretion of mice urine after 6 hours of medication in diuretic assay

Tested group	Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)	Cumulative Saluretic (Na^++Cl^-)	Natriuretic (Na^+/K^+)	Kaliuretic (K^+/Na^+)	CAI [Cl^-/ (Na^+ +K^+)]
Control	83.91 ± 0.00	71.90 ± 0.00	85 ± 2.5	168.91	1.17	0.86	0.55
Frusemide (5 mg/kg)	149.22 ± 0.00	141.49 ± 0.00	120 ± 2.5	269.22	1.05	0.95	0.41
n-hexane (250 mg/kg)	67.57 ± 16.34	89.29 ± 5.8	81.25 ± 1.25	148.82	0.76	1.32	0.52
n-hexane (500 mg/kg)	116.61 ± 0.00	106.72 ± 0.00	91.25 ± 1.25	207.86	1.09	0.92	0.41
Ethyl acetate (250 mg/kg)	100.26 ± 16.35	112.52 ± 5.8	105 ± 2.5	205.26	0.89	1.12	0.49
Ethyl acetate (500 mg/kg)	165.57 ± 16.35	147.31 ± 17.41	118.75 ± 1.25	284.32	1.12	0.89	0.38
Water (250 mg/kg)	83.91 ± 0.00	95.08 ± 0.00	98.75 ± 1.25	182.66	0.88	1.13	0.55
Water (500 mg/kg)	100.26 ± 16.35	141.49 ± 11.59	106.25 ± 1.25	206.51	0.71	1.41	0.44

Table 5: Effect of different fractions of *F. indica* on electrolyte excretion of mice urine after 6 hours of medication in diuretic assay (Cont.)

Treatment group	Na^+ index	K^+ index	Cl^- index	Saluretic Index	Natriuretic Index	Kaliuretic Index	CAI Index
Control	1	1	1	1	1	1	1
Frusemide (5 mg/kg)	1.78	1.97	1.41	1.59	0.89	1.1	0.74
n-hexane (250 mg/kg)	0.8	1.24	0.95	0.88	0.65	1.53	0.94
n-hexane (500 mg/kg)	1.39	1.48	1.07	1.23	0.93	1.07	0.74
Ethyl acetate (250 mg/kg)	1.19	1.56	1.23	1.21	0.76	1.3	0.89
Ethyl acetate (500 mg/kg)	1.97	2.04	1.39	1.68	0.96	1.03	0.69
Water (250 mg/kg)	1	1.32	1.16	1.08	0.75	1.31	1
Water (500 mg/kg)	1.19	1.96	1.25	1.22	0.61	1.64	0.8

Laxative activity Assay

After conducting the laxative test, the reference drug (Bisacodyl 10 mg/kg) increased the stool weight by 80.17% after 16 hours. Similarly, all fractions of F. indica also increased the stool weight. Among these three fractions, the n-Hexane fraction showed the highest increase in stool weight, while the water fraction exhibited the lowest laxative activity (Table 6).

Table 6: Representation of laxative effect of different fractions of *F. indica* extract

Tested group	Dose (mg/kg)	Average weight of stool	% Increase in stool weight
Negative control	------	$0.522 \pm .044^{\theta\,a\,b\,c\,d\,f}$	------
Standard (Bisacodyl)	10	$0.94 \pm 0.04^{*\,a\,b\,c\,d\,e\,f}$	$80.172 \pm 7.721^{*\,a\,b\,c\,d\,e\,f}$
n-Hexane fraction	250	$0.721 \pm 0.056^{*\,\theta\,b\,c\,e\,f}$	$38.22 \pm 10.701^{*\,\theta\,b\,c\,e\,f}$
n-Hexane fraction	500	$0.802 \pm 0.017^{*\,\theta\,a\,c\,d\,e\,f}$	$53.735 \pm 3.386^{*\,\theta\,a\,c\,d\,e\,f}$
Ethyl acetate fraction	250	$0.599 \pm 0.012^{*\,\theta\,a\,b}$	$14.856 \pm 2.302^{*\,\theta\,a\,b}$
Ethyl acetate fraction	500	$0.655 \pm 0.011^{*\,\theta\,b\,e}$	$25.478 \pm 2.167^{*\,\theta\,b\,e}$
Water fraction	250	$0.573 \pm 0.018^{\theta\,a\,b\,d}$	$9.77 \pm 3.522^{*\,a\,b\,d}$
Water fraction	500	$0.623 \pm 0.003^{*\,\theta\,a\,b}$	$19.348 \pm 0.541^{*\,\theta\,a\,b}$

Data are plotted as average of five replicates ± SD (standard deviation);

* $p < 0.05$ vs. Control (Dunnett's t test); $^{\theta}$ $p < 0.05$ vs. Bisacodyl 10 mg/kg; ^{a} $p < 0.05$ vs *n*-Hexane fraction 250 mg/kg; ^{b} $p < 0.05$ vs. *n*-Hexane fraction 500 mg/kg; ^{c} $p < 0.05$ vs. Ethyl Acetate fraction 500 mg/kg; ^{d} $p < 0.05$ vs. Ethyl Acetate fraction 500 mg/kg; ^{e} $p < 0.05$ vs. Water fraction 500 mg/kg; ^{f} $p < 0.05$ vs. Water fraction 250 mg/kg; (pair-wise comparison by Post Hoc Tukey test)

DISCUSSION

The plant kingdom is perhaps one of the most important gifts from nature to humans and other animals. Each plant is a powerhouse of numerous phytochemicals. Phytoconstituents are mainly classified into secondary and primary metabolites. Secondary metabolites are not specifically involved in typical growth and reproduction; instead, they often protect the plant from various diseases. Secondary metabolites are primarily responsible for various biological activities in both the plant and animal kingdoms. Modern medical science heavily relies on various plant-derived drugs. Many important drugs for cardiovascular health, antidiabetic treatment, anticancer therapies, hepatoprotection, antidiarrheal remedies, laxatives, antimicrobial agents, and blood purifiers are directly obtained from various types of natural medicinal plants.[12,13,14] In the primary phytochemical assay of the F. indica extract, Karmakar et al. (2021) reported the presence of saponins, reducing sugars, gums, tannins, flavonoids, steroids, terpenoids, and alkaloids.[2] These phytochemicals might be beneficial for use in various therapeutic purposes, and their bioactivities are already well-documented.

Parasites are organisms that live in or on a living creature of another species (its host) and benefit by stealing vital essential nutrients from the host's body. Many types of parasites live in mammals. In humans, numerous helminths reside in our gastrointestinal tract, where they steal vital nutrients and other essential components from our stomach. As a result, patients suffer from various complications, weight loss, morbidity, etc.[4] These are the causative agents of many diseases such as gastrointestinal upset, dyspepsia, colon and rectal cancer, etc. These problems are mainly found in tropical and subtropical regions worldwide, leading to increased human mortality and causing serious economic losses in the livestock farming business.[15,16,17] High costs, anthelmintic drug resistance, and serious adverse effects have prompted the evaluation of medicinal plants as an alternative source of anthelmintics. Medicinal plants have been used for many decades to treat gastrointestinal helminthiasis, and there is currently a growing demand for plant-derived anthelmintic compounds due to their potency, efficacy, and reduced side effects.[11,5,18] In the anthelmintic assay, we observed that the fractions of F. indica exhibited moderate anthelmintic properties. The different solvent fractions caused both the loosening of motion and death of the P. cervi nematodes in a dose-dependent manner compared to the standard albendazole. Among the three fractions, the n-Hexane fraction showed moderate anthelmintic properties compared to the other two fractions. Therefore, it can be assumed that there may be one or more non-polar anthelmintic compounds present in F. indica. Phytoconstituents in the plant, such as flavonoids, tannins, alkaloids, saponins, and polyphenols, might be responsible for the anthelmintic effect, as their properties have already been reported.[19]

In humans, blood is the most critical fluid that carries vital oxygen and nutrients to the cells while also returning carbon dioxide and harmful substances to be excreted from the body. Blood coagulation (clotting) is the process by which blood changes from a liquid to a gel, thereby stopping bleeding. It is extremely important to stop bleeding in emergencies because excessive blood loss can lead to death. Blood coagulation involves twelve factors that work together to arrest bleeding. The mechanism of coagulation includes the activation, attachment, and accumulation of platelets, along with the deposition and formation of fibrin.[20] Primary hemostasis involves vasoconstriction at the damaged site, preventing blood loss and reducing the diameter of vessels and capillaries. The platelets are activated and then attracted to the site of injury. They also adhere to each other to create a temporary platelet plug over the bleeding area, sealing the injured surface.

On the other hand, secondary hemostasis results in the formation of fibrin over the temporary platelet plug formed during primary hemostasis. This secondary stage involves

the coagulation cascade, which consists of three distinct but interconnected pathways: the intrinsic, extrinsic, and common pathways.[21] So, a decrease in prothrombin time indicates better blood coagulant properties. In the blood coagulation test, we observed that the fractions of F. indica did not exhibit profound coagulant activities. Only the water fraction showed a mild blood coagulant effect. This may be due to the presence of a few polar blood coagulants in the water fraction of F. indica. Phytoconstituents such as saponins, tannins, flavonoids, and steroids might be responsible for this blood coagulant effect, as they are well-documented for having this property.[22]

Constipation is a very common gastrointestinal disorder in which stool becomes dry, hard, and difficult to expel. Other symptoms may include painful spasmodic bowel movements, dyspepsia, acute stomach pain, and discomfort, among others. These factors can contribute to various colorectal problems such as hemorrhoids and colorectal malignancy. Globally, constipation affects 8-15% of the population. However, constipation is often a result of various underlying issues rather than a single disorder on its own. Potential causes include digestive disorders, metabolic and endocrine disorders, and neurological problems. Additionally, many medications can produce constipation as a side effect, including anticholinergics, antidepressants, iron supplements, and aluminum-containing compounds.[23] In the treatment of constipation, plant-derived laxatives have been used for centuries. Laxatives can work by retaining water inside the bowel lumen through osmotic effects or by stimulating intestinal secretion or motility, which increases the amount of intestinal bulk. This, in turn, increases stool weight and makes the stool softer and easier to expel. However, due to a lack of efficacy, the treatment of constipation with available drugs is often insufficient for reducing bloating and other associated symptoms.[23] Acetylcholine plays a significant role as the principal excitatory neurotransmitter in the enteric neurological network. Hence, the existence of cholinomimetic components within the plant may clarify the value of the laxative effect. Besides, previous experiments have revealed that the laxative properties of plants depend on the presence of phytochemicals like flavonoids, tannins, terpenoids, alkaloids, sterols, and phenolic compounds.[11,24]

In the laxative test, we found that the F. indica n-Hexane fraction exhibited the best laxative effect. At doses of 250 and 500 mg/kg, the stool weight increased by up to 38% and 54%, respectively, while bisacodyl increased the stool weight by 80% at a dose of 10 mg/kg. However, the other two fractions did not show such an increase in stool weight as the n-hexane fraction. It is already reported that phytoconstituents such as saponins, flavonoids, tannins, sterols, alkaloids, terpenoids, and phenolic components in various fractions of plant extracts are found to be responsible for laxative, stimulant, and alimentary peristaltic properties.[11,24] So, we may assume that this F. indica plant might also possess these types of compounds, especially in the n-Hexane fraction.

Hypertension, the elevation of blood pressure from normal values, is considered one of the dominant health problems and a significant well-being challenge worldwide. The World Health Organization (WHO) reports that one out of every eight deaths is caused by hypertension globally. In a recent survey, it was observed that approximately one-third of the adult world population is suffering from hypertension (31.1%, 1.39 billion), and this issue is more prominent in low- and middle-income countries. Chronic smoking, alcohol consumption, a diet rich in fat, obesity, mental stress, sedentary lifestyle, hypercholesterolemia, and hyperglycemia are the main reasons for hypertension. Hypertension also contributes to the development of other serious cardiovascular diseases.[25]

Diuretics are the first-line drugs used to treat hypertension and related complications. Different types of diuretics work by reducing the reabsorption of water and electrolytes in the nephrons. They are medically significant in the treatment of clinical conditions such as nephrotic problems, hypertension, and cardiovascular disorders.[9]

Various medicinal plants are traditionally used to treat hypertensive complications because they possess numerous secondary metabolites that are useful in this regard. In the diuretic test, we observed that different fractions of F. indica leaves increased urinary output as well as diuretic activity over time. These observations indicated diuretic-like effects in the mice, which were further confirmed by electrolyte analysis. Controlling the levels of K+, Na+, and Cl- in the blood is exceptionally significant for regulating blood volume, blood pressure, cardiac output, acid-base balance, and maintaining the functionality of heart muscles. In our test, we used frusemide as the standard drug. Frusemide is a recognized loop diuretic that acts in the ascending part of the loop of Henle. It inhibits sodium reabsorption, leading to significant urinary sodium and chloride excretion.[26] After conducting the diuretic test, we also performed an electrolyte analysis of the urine. Consequently, we calculated the natriuretic activity, kaliuretic activity, and saluretic activity for different fractions of F. indica. Subsequently, we measured CAI activity. In our diuretic and urinary electrolyte analysis test, we found that the F. indica ethyl acetate fraction at 500 mg/kg doses exhibited the best diuretic activity, and a diuretic index exceeding 1.5 indicates a good diuretic effect.[27] On the other hand, the ethyl acetate fraction at 500 mg/kg doses also exhibited the best Na+, K+, Cl-, saluretic, and natriuretic indexes. The carbonic anhydrase enzyme is responsible for the production of H_2CO_3 and ultimately HCO_3- in the nephron. Because of this enzymatic activity, HCO_3- ions can be readily reabsorbed from proximal tubules. So, the inhibition of carbonic anhydrase may indicate good diuretic properties, and a CAI (carbonic anhydrase inhibition) index lower than 0.8 indicates the best diuretic effect.[9] Based on this information, once again, the ethyl acetate fraction of F. indica extract at a dose of 500 mg/kg showed the best diuretic effect. Considering these overall diuretic effects, we can assume that the ethyl acetate fraction of F. indica contains both loop diuretics and carbonic anhydrase inhibitors. Phytochemicals like flavonoids, organic acids, saponins, polyphenolic compounds, alkaloids, and steroids might be responsible for this diuretic effect, as their diuretic activity has already been reported.[28]

CONCLUSION

Medicinal plants have been used for centuries for numerous therapeutic purposes. From our observations, we have found that different fractions of F. indica leaves can be used as anthelmintic, blood coagulant, diuretic, and laxative agents. These preliminary results might be helpful for natural product researchers to isolate pure bioactive compound(s) from this plant in the future, and this could lead to the development of new drugs derived from this plant.

Data availability

All reported data are preserved by the authors and will be made accessible upon request for clarification.

Conflict of interest

The authors hereby declare that there is no conflict of interest among them, and they were all informed about this matter before submitting this article.

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استكشاف الأنشطة المضادة للديدان، ومخثر الدم، ومدر للبول، وملين لأجزاء المذيبات المختلفة لأوراق فلاجيلاريا إنديكا

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ملخص

فلاجيلاريا إنديكا (الأسرة: فلاجيلارياسيا) نبات قشور شائع يوجد بشكل رئيسي في المناطق الاستوائية في العديد من البلدان. للنبات بعض الاستخدامات التقليدية ولكن معظمها لا يحتوي على تقارير علمية منشورة. تم تجزئة المستخلص الإيثانولي الخام لأوراق F. Indica اعتمادًا على القطبية باستخدام الماء، وخلات الإيثيل، و n-Hexane. تم إجراء فحص بيولوجي للأنشطة المضادة للديدان، وتخثر الدم، ومدر للبول، وملين باستخدام الماء، وخلات الإيثيل، و n-Hexane من أوراق F. في اختبار طارد الديدان، أظهر جزء n-Hexane تأثيرًا معتدلًا مع فترات الشلل كانت 16.79 و 13.62 دقيقة وأوقات الوفاة كانت 27.34 و 21.81 دقيقة على التوالي بجرعة 25 و 50 مجم / مل. في اختبار تخثر الدم، أظهر جزء الماء تأثيرًا ملحوظًا (كانت أوقات التخثر 4.33 و 6.02 و 7.68 و 8.32 دقيقة على التوالي بجرعة 200 و 100 و 50 و 25 مجم / مل). تم إجراء زيادة حجم البول المفرز وتحليل الكهارل في البول لتحديد الرقم الهيدروجيني والكثافة والتوصيل و Na + وCl- وK + وnatriuretic و kaliuretic saluretic و CAI index. أظهر جزء أسيتات الإيثيل نشاط مدر للبول أفضل من جزء الهكسان n بينما لم يكشف جزء الماء عن تأثير مدر للبول ملحوظ. تم العثور على فهارس Na +وCl- وK + وnatriuretic و saluretic مرضية في جزء خلات الإيثيل وتم العثور على مؤشر CAI بشكل أفضل في جزء n-Hexane. في اختبار الملين، أظهر جزء n-Hexane أفضل خصائص ملين بينما تم العثور على الزيادة في وزن البراز بنسبة 38٪ و 54٪ عند جرعات 250 و 500 مجم / كجم على التوالي. تشير هذه النتائج إلى أن الأجزاء المختلفة من أوراق F. Indica لها مواد كيميائية نباتية مميزة قد تكون مسؤولة عن مثل هذه التأثيرات البيولوجية. قد يكون عزل المركبات النشطة بيولوجيًا مبررًا لاستخداماته التقليدية في الطب الحديث.

الكلمات الدالة: فلاجيلاريا إنديكا، طارد للديدان، مخثر الدم، مدر للبول وأنشطة ملين.

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