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Elucidation of Phytochemical and Pharmacological Nature of Methanolic Extract of *Ixora cuneifolia*

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Authors' contributions

This work was carried out in collaboration between all authors. Author SB designed the study, wrote the protocol and interpreted the data. Author GAH anchored the field study, gathered the initial data and performed preliminary data analysis. Authors Umaychen and MSH managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

This study describes the preliminary phytochemical and pharmacological investigations on *Ixora cuneifolia,* which is an unexplored medicinal plant belonging to the family of Rubiaceae, widely distributed in hilly forests of Bangladesh. The present study protocol was undertaken to evaluate anthelmintic, antidiarrheal, thrombolytic, and antioxidant properties with phytochemical screening of methanolic extract of *I. cuneifolia* for the first time in Bangladesh. Anthelmintic property was tested by using *Perthima posthuma.* Streptokinase enzyme was used as the standard for thrombolytic test, and antioxidant property was calculated as Gallic acid equivalent (GAE). Finally, antidiarrheal activity was tested by following castor oil induced method with Swiss albino mice as experimental animals. The phytochemical screening revealed the presence of alkaloids, glycosides, saponins, carbohydrates, phytosterols, protein, and terpenes in the crude methanolic extract. *I. cuneifolia* showed significant antidiarrheal activity at 200 mg/kg dose following castor oil induced method and anthelminthic assay yielded positive results at 60 mg/ml and 80 mg/ml concentration. In thrombolytic assay, the plant extract showed significant thrombolytic activity (33.03%, $p<0.05$). Total phenolic content determination as an antioxidant activity confirmed that, the plant extract

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possesses moderate antioxidant activity. The current study data confirmed that methanolic extract of *I. cuneifolia* possesses remarkable anthelmintic, thrombolytic, antioxidant, and antidiarrheal activity.

Keywords: Ixora cuneifolia; anthelminthic; thrombolytic; phenolic content; antidiarrheal.

1. INTRODUCTION

The genus *Ixora* is widely found in tropical and subtropical regions of Asia and other parts of the world. Ethnic groups of different region of Asia, Europe and Africa used leaves, flowers, roots, stem and fruits of this plant for different purposes [1-8]. Many chemical compounds were isolated from various plant part of different species with different pharmacological effect [8]. *Ixora cuneifolia* is an unexplored medicinal plant belonging to the family of *Rubiaceae*, which is an evergreen shrub, widely distributed in Chittagong and Sylhet of Bangladesh. It is locally known as kha muchya or kayah museya (Marma). The leaves of this plant are 15-20 cm long, elliptical lanceolate, oblanoceate, shortly acuminate and the flowers are rather small, white, in short peduncled, sub-sessile cymes. This plant is one of the rarest one for studied. Very few studies were carried out on this plant species but many plant of this species is still out of focus [9-10]. A current report was demonstrated on this plant, the leaf of this plant is used in Indo-China to manage fever [11].

Thus, the present study was intended to evaluate anthelmintic, antidiarrheal, thrombolytic, antioxidant properties and also to find the existence of phytochemicals in the methanolic extract of *I. cuneifolia* whole plant for the first time.

2. MATERIALS AND METHODS

2.1 Chemicals

Loperamide, Acetyl salicylic acid, and Lyophilized S-Kinase™ (streptokinase) vial (1500000 IU) was purchased from Popular Pharmaceuticals Ltd., Bangladesh; Batch no: VEH 09. Gallic acid and castor oil were procured from Sigma Chemical Co. Ltd, (St. Louis, MO, USA). Other chemicals required for this present study were provided from pharmacology laboratory of Department of Pharmacy, Noakhali Science and Technology University. All other chemicals and reagents were of analytical grade.

2.2 Collection of Plant

For this present investigation *I. cuneifolia was* collected from hilly forest of Balipara, Thanchi Upazila, Bandarban, Bangladesh in January, 2015. During collection of plant part any type of adulteration was strongly prohibited. The plant was identified by the expert of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh with an accession number of 37790.

2.3 Preparation of Methanolic Extract

About 250 mg of powdered plant material was taken in a clean, flat bottomed glass container (2.5 liters) and emerged in 1500 ml of 99% methanol. The container with its contents was sealed and kept for a period of 15 days accompanying occasional shaking and stirring. After 15 days the solution was filtered by using filter cloth and Whatman's filter paper.

2.4 Phytochemical Screening

Testing of various chemical compounds within the extract represents the preliminary phytochemical studies. Little amount of methanolic extracts of *I. cuneifolia* was subjected to preliminary quantitative phytochemical investigation for detection of phytochemicals like alkaloids, carbohydrates, viscous glycosides, phytosterols, proteins, flavonoids, tannins, saponins, etc. exploiting the quality ways [12-14].

2.5 Anthelmintic Activity

The anthelmintic assay was designed according to the method of Malvankar [15] with slightly modifications [16]. Adult earthworms (*Phertima posthuma*) were subjected to study the anthelmintic activity due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. Due to availability of earthworms in all over the world, they are widely used as effective tools for anthelmintic study [17].

2.6 Thrombolytic Activity

The thrombolytic activity was evaluated by the method [18] by using streptokinase (SK) as positive control with slightly modified [16].

2.7 Total Phenolic Content

The amount of total phenolic content in plant extract was determined by using Folin-Ciocalteau

reagent. Total phenolic content was determined as mg of gallic acid equivalent per gram using the equation obtained from a standard gallic acid calibration curve [19].

2.8 Castor Oil-induced Diarrhea in Swiss Albino Mice

Antidiarrheal effect of this plant part was according to the method described by Teke et al. [20] with some modifications. Animals were fasted for 24 h prior to the experiment, but had free access to water. Mice's were randomly assigned to four groups (n=3). Group-1 received 1 ml of castor oil orally and served as control animals. Group 2 received loperamide (5 mg/kg, p.o) and served as the standard treatment group, whereas group 3 and 4 received orally 100 and 200 mg/kg of methanolic extract of test plant respectively. Immediately after oral administration of treatments, the animals were individually placed in boxes, the floor of which lined with blotting paper for observation of the number and consistency of fecal droppings. The number of both wet and dry droppings was counted every 60 minutes for 4 hours, and the white paper was changed after each evaluation.

2.9 Statistical Analysis

The statistical analysis was performed in this study by using SPSS software package (version 19.0). Calculated values are expressed as mean ± SEM. Data analysis among the groups was compared using one-way ANOVA followed by Dunnett's post Hoc test. P value <0.05 in all cases was measured as statistical significant.

3. RESULTS

3.1 Phytochemical Screening

Phytochemical constituents determined from the methanolic extract of *I. cuneifolia* are presented in Table 1.

3.2 Anthelminthic Activity

The data of anthelmintic activity of methanolic extract of *I. cuneifolia* on earthworms was determined by different concentration of extract compared with standard (Albendazole) and negative control are presented Fig. 1. The paralysis time of earthworms for extract at different concentrations, including 10 mg/mL, 20 mg/mL, 40 mg/mL, 60 mg/mL and 80 mg/mL was 24.67, 19.83, 15.1 and 14.2 min respectively, whereas death time was 70.13, 67.66, 60.83, 60, and 58.7 min respectively compared with albendazole (35.17 min and 71.33 min respectively).

3.3 Thrombolytic Assay

Human blood clot lysis activity of methanolic extract of *I. cuneifolia* showed remarkable clot lysis at 10 mg/dl (32.67%) and 5 mg/dl (15.43%) concentrations when compared to standard streptokinase's clot lysis potentiality (59.6%) activity and the result is represent in Fig. 2.

3.4 Total Phenolic Content (TPC)

The total phenolic contents of the plant extracts of *I. cuneifolia* are presented in Table 2. The result is expressed as the number of equivalents to mg of gallic acid per gram of the plant extract. The plant extract was found to have moderate amount of phenolic content of methanolic extract that was 11.19 mg of GAE/gm.

3.5 Antidiarrheal Activity

The crude methanolic extract of *I. cuneifolia* was subjected to castor-oil induced antidiarrheal activity in Swiss albino mice model and the obtained data are graphically presented in Table 3. The crude extract showed 18.75% and 28.13% of inhibition of diarrhea at two different doses of 100 mg/ml and 200 mg/ml respectively while comparing with standard drug loperamide (59.37% inhibition).

Table 1. Phytochemical screening of the methanolic extract of *I. cuneifolia*

Name of phytochemical	Observation
Alkaloid	+
Carbohydrate	+
Glycoside	+
Saponins	+
Phytosterol	-
Phenol	+
Tannins	-
Flavonoids	+
Proteins and amino acids	-
Diterpenes	+

Here, (+) = presence of constituents; (-) = absence of constituents

Table 2. Total phenolic content (TPC) determination of methanolic extract of *I. cuneifolia*

Extract	Concentration (mg/ml)	Absorbance at 720 nm	Average Absorbance (mean ± SEM)	TPC (mg of GAE/gm)	TPC (mg of GAE/gm of extract)
Methanol extract	12.5	0.737	0.802 ± 0.031 (Y=0.0029x+0.1076, R^2=0.8894)	15.64	11.18 ± 1.56
	25	1.008		11.11	
	50	1.562		8.92	
	100	3.075		9.06	

Values are expressed as mean ± SEM

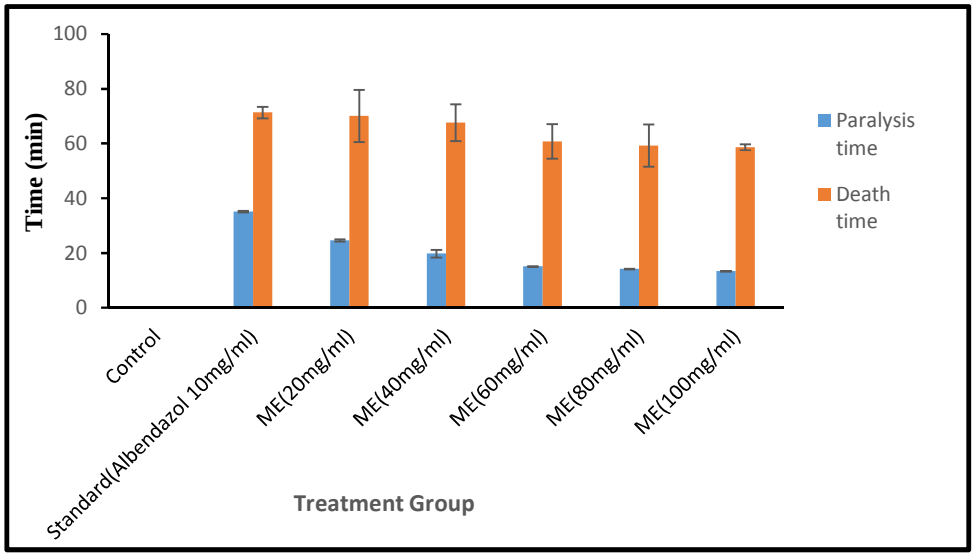

Fig. 1. Anthelminthic activity of crude methanolic extract of *I. cuneifolia* against *Phertima posthuma*

Values are expressed as mean ± SEM (n =3); ME = methanolic extract

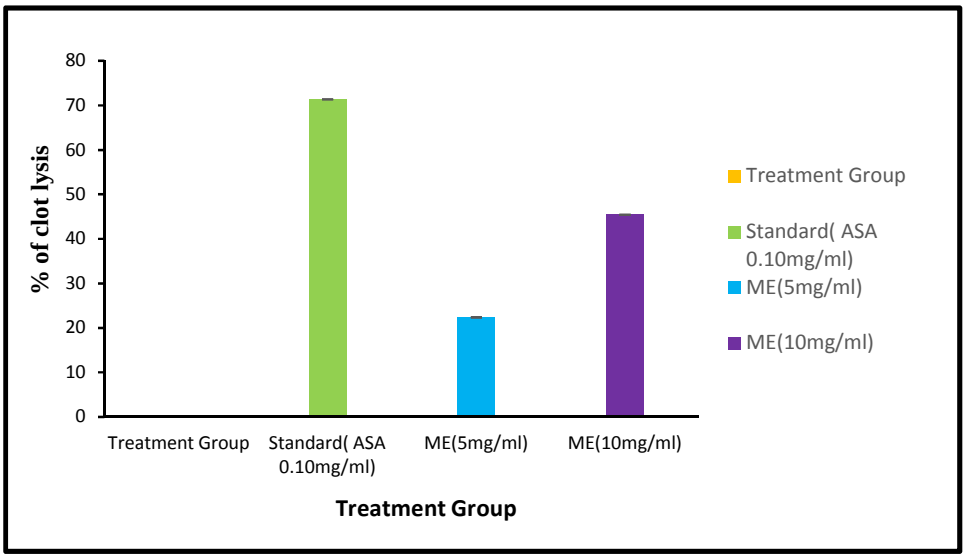

Fig. 2. Thrombolytic activity of crude methanolic extract of *I. cuneifolia*

Values are expressed as mean ± SEM; ME = methanolic extract; ASA = Acetyl Salicylic Acid

Table 3. Effect of methanolic extract on castor oil induced diarrhea in mice model

Treatment	Dose (b.w.)	No. of diarrheal faeces	Inhibition of diarrhea (%)	P value
Control	1 ml	10.67 ± 0.33	-	-
Standard	5 mg/kg	4.33 ± 0.88	59.38*	0.002
ME 1	100 mg/kg	8.67 ± 0.88	18.75	0.101
ME 2	200 mg/kg	7.67 ± 0.88	28.13*	0.032

*Values are expressed as Mean ± SEM (n=3). *P< 0.05 compared to control (One way ANOVA followed by Dunnett's 't'-test); ME = methanolic extract*

4. DISCUSSION

From the very beginning of civilization, human are very much depends on plants for the recovery from illness, nowadays ethno and phyto-pharmacological investigation has generated a new area to discovery plant derived drugs, which are already proved to effective in treatment of many diseases. It is found that about 30% of the pharmaceuticals are invented from plants derivatives [21,22]. The phytochemical screening of methanolic extracts of *I. cuneifolia* shows the presence of alkaloids, glycosides, saponins, carbohydrates, phytosterols, protein, terpenes, those are may be basis in the management of various disease in harmony with other plant studies [23,24]. From the beginning of life helminthic infections are the most frequently occurring infections in man, affecting a very large proportion of the world's population [7]. Parasitic helminthes causing considerable hardship and stunted growth of animals and man, it is become one of the major concern in field of medical science for centuries that is why lot of drugs have been developed by scientists to treat this problem. This study suggests that this plant extract possess very significant importance in the field of anthelmintic. The extract of *I. cuneifolia* shows considerable activity nearly adequate to the activity shown by albendazole solution. Albendazole, an anthelmintic drug, acting by disturbing fibre bundle transmission in worm could also be by acting like GABA. This allows host to simply rescue them out the harmful organisms. Former study data suggested that presence of alkaloid, phenol, tannin and terphnoids may be responsible for anthelmintic activity [25,26]. In accordance with these studies, those compounds are present in the plant extract and may be responsible for anthelmintic activity. The present study was carried out to investigate thrombolytic activity of *I. cuneifolia*. Where, Streptokinase (SK) was used as a positive control [18] and purified water, was designated as a negative control. The data generated by positive control with negative control clearly revealed that clot dissolution did not happened when water was added to the clot. By correlating result obtained from both positive and negative control, a significant thrombolytic activity was observed after treating the clots with the plant extract. It was reported that the presence of phytochemicals like saponin, tannin and alkaloids in the plant extract are the probable reason for demonstrating the thrombolytic activity [16,27]. In present study, the extract of *I. cuneifolia* possess thrombolytic activity may be present of responsible phytochemicals except tannin.

Phenolic compounds are an unrivalled category of phytochemicals particularly in terms of their grandiose health-benefiting properties. They have diverse biological effects and also act as antioxidants by prohibiting the oxidation of low density lipoproteins (LDL), platelet aggregation and destroy of RBC. These chemical constituents (secondary metabolites) present in plant vary following to their age and ripeness. The antioxidant activity of phenolic compounds were immensely correlative with their total phenolic contents and phenolic diterpenes, which can rending an important role in absorption and neutralization of free radicals, extinguishing singlet and triplet oxygen or putrefaction of peroxides. Our plant methanolic extract showed moderate presence of phenolic content, so our plant extract might have antioxidant activity. The abnormally frequent defecation of feces of low consistency which may be due to a disturbance in the transport of water and electrolytes in the intestines is known as diarrhea. Following etiologies are available to describe diarrhea, (i) secretory diarrhea -increased electrolytes secretion, (ii) osmotic diarrhea-increased luminal osmolarity, (iii) deranged intestinal motility causing a decreased transit time, and (iv) decreased electrolytes absorption may be responsible for pathophysiology [28,29]. Recent study suggests that nitric oxide in castor oil is very much responsible for the diarrheal effect. However, it is evidenced that ricinoleic acid is also responsible for diarrhea via an over secretory response which is the most active

component of castor oil [30,31]. Usually castor oil is chemically modified into ricinoleic acid in the gut, which manifested to irritation and inflammation in the intestinal mucosa, resulting in the release of inflammatory mediators (e.g., prostaglandins and histamine). The released prostaglandins cause mucus secretion, vasodilatation, and smooth muscle contraction in the small intestines. For both animals and human being prostaglandins E series are considered to be the agent responsible for diarrhea [32]. Our present study suggests that this plant methanolic extract showed moderate anti-diarrheal activity while comparing with standard drug loperamide. It was supported that flavonoids and polyphenols were responsible for the antidiarrheal activity properties [33], our plant methanolic extract contain both of this chemical constituent may be that is why our plant extract showed antidiarrheal effect through blocking prostaglandin pathway.

5. CONCLUSION

The findings of the present study provide convincing evidence that methanolic extract of *I. cuneifolia* possesses remarkable anthelmintic, thrombolytic, antioxidant, and antidiarrheal activity. However, further biochemical studies are required to isolate and determination of the bioactive compounds and determine the precise mechanisms responsible for the noticed biological activities of this plant.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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