Natural Resources for Human Health

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Original Research

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Received 03 November 2023
Revised 10 December 2023
Accepted 26 December 2023
Available online 03 January 2024

Edited by Prathapan Ayyappan

KEYWORDS:
Antimicrobial activity
cytotoxic activity
Ipomoea hederifolia
anthelmintic activity
thrombolytic activity

Natr Resour Human Health 2024; 4 (2): 128-135
https://doi.org/10.53365/nrfhh/177626
eISSN: 2583-1194

Evaluation of *in vitro* anthelmintic, cytotoxic, antimicrobial and thrombolytic activities of *Ipomoea hederifolia* stem

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ABSTRACT: *Ipomoea hederifolia*, a member of the convolvulaceae family, is an annual climbing ornamental vine with wide range of pharmacological activities. The methanolic extract of powdered *Ipomoea hederifolia* stem was subjected to evaluate the *in-vitro* anthelmintic, cytotoxic, antibacterial and thrombolytic potentials. The disc-diffusion technique explored the antibacterial activity of *Ipomoea hederifolia* stem extract (IHSE) against six detrimental microorganisms. Anthelmintic and thrombolytic potentials were assessed using earthworms and human erythrocytes as test samples. Moreover, a brine shrimp lethality bioassay procedure was applied to determine cytotoxic activity. The methanolic IHSE demonstrated statistically significant ($p < 0.05$) anthelminthic potential in a dose-dependent fashion. In the brine shrimp lethality assay, IHSE depicted a sharp soar in the death rate of brine shrimp nauplii, and 50% (LC_{50}) of nauplii died at 4.544 μg/mL of IHSE. Moreover, the IHSE revealed moderate clot lysis activity in a dose-dependent manner; the highest clot lysis was 37.167± 2.40% at 20 mg/mL ($p < 0.05$). However, no significant antimicrobial activity was observed for IHSE. Based on the findings, our investigation suggests that the methanol fraction of IHSE possesses substantial anthelmintic, cytotoxic and thrombolytic potentials, and the plant could be deemed as a probable hub for future drug discovery.

1. INTRODUCTION

Traditional medicinal plants, sometimes referred to as herbal medicines, plant medicines, or phytomedicines, are restorative commodities produced from plant roots, stems, leaves, bark, seeds, and consumables that can be used to promote general health and alleviate illnesses (Dewanjee et al., 2023; Mahmud et al., 2023; Tang & Halliwell, 2010). These herbal components might be utilized categorically or processed into various ready-to-use products (Dewanjee et al., 2023; Naznin et al., 2019; Tang & Halliwell, 2010). Plants with medical value are a great source of drug discovery because they contain chemicals that have a physiological effect on the human body (Tang & Halliwell, 2010; M.S. Uddin et al., 2019). These chemicals include alkaloids, essential oils, tannins, proteins, terpenes, carbohydrates, phenolics, glucosides, lignans, curcumines, flavonoids, saponins, resins, steroids and a range of other chemicals (Naher et al., 2019; Shahbaz et al., 2023; Talukder et al., 2022). Additionally, some of the negative outcomes linked to the use of indigenous plants as medicine are mostly the result of over dosage and a lack of information about extra hazardous byproducts found in some plants (Naher et al., 2019).

Ruminant gastrointestinal nematodes are a major source of livestock illness globally, especially in temperate and tropical regions (Oliveira et al., 2017). The continuing rise in resistance of nematodes to commercially available medications like benzimidazoles, and imidazothiazoles (El-Wakil et al., 2023; Štrbac et al., 2022), as well as animal-derived foods, may impair the health of the consumers (Oliveira et al., 2017). Furthermore, the synthetic medications used to heal helminthiasis have certain possible negative effects (El-Wakil et al., 2023). In this situation, repurposing of anthelmintics derived from natural sources may be useful in the treatment of parasitic illnesses. Consequently, herbal anthelmintics are becoming increasingly popular (Kumar et al., 2010).

Cancer treatment options include chemotherapy, radiation therapy and surgery which all aim to expel all malignant cells from the body (Dewan & Das, 2013; Sarwar et al., 2018). Each therapeutic strategy has a number of disadvantages, including medication resistance, toxicity, and a lack of specificity (Dewan & Das, 2013; Hussain et al., 2016). Owing to synthetic chemotherapeutic agent's numerous negative effects, research on innovative chemotherapeutic agents derived from plants has accelerated in recent years (M.S. Hossain et al., 2020).

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Antibiotics are naturally occurring or synthesized chemical substances that, at low doses, inhibit or kill certain microorganisms. Resistance of microorganisms to several medicines has generated a huge clinical concern in the treatment of infectious illnesses (Nagumanthri et al., 2012). Recently, the rise of germs that antibiotics cannot kill has become one of the biggest health threats and a major cause for worry (Rashid et al., 2017). However, it's a matter of glad that currently, some plant extracts are able to prevent the progression of antibiotic-resistant AMPR (*Pseudomonas aeruginosa*) and MRSA (Methicillin-resistant *Staphylococcus aureus*) (Häkkinen et al., 2023). Therefore, researchers are hunting for new antibiotics to resolve antibiotic resistance.

The development of blood clotting inside a vein or blood vessel, restricting the regular flow of blood through the animal body's circulatory system, is referred to as thrombosis. When a blood artery gets damaged, to halt haemorrhaging, the body creates a lot of red blood cells within the vein employing platelets (thrombocytes) as well as fibrin (Labu et al., 2015). Thrombolysis is the pharmacological dissolution (lysis) of blood clots. Injecting tissue plasminogen activator (TPA), the protein which typically activates plasmin stimulates fibrinolysis by plasmin (Wardlaw et al., 2014). Thrombolytic medications are approved for the short-term treatment of heart attack and stroke (Chowdhury et al., 2011). The most often utilized thrombolytic agent is a tissue plasminogen activator (TPA). However other medicines can perform the same function. Thromboembolic illnesses such as deep vein thrombosis, heart attacks, strokes, and pulmonary emboli are the fundamental causes of illness and death in both developed and developing nations (Chowdhury et al., 2011; Umesh et al., 2014). Thrombolytic medicines such as urokinase, alteplase, tissue plasminogen activator (TPA), and streptokinase are routinely used to break apart clots (Moghal et al., 2016). These medicines have specific downsides that can result in significant and often fatal problems, such as bleeding, severe allergic reactions, and a lack of specificity (Hussain et al., 2016). As a result, efforts have been focused on discovering natural chemicals originating from diverse plant sources that exhibit antiplatelet, anticoagulant, antithrombotic, and thrombolytic effects.

With approximately 600 species, the genus Ipomoea is the largest in the family of Convolvulaceae. Numerous species in this genus have been employed in religious rituals, food, medicinal, and aesthetic plants (Srivastava & Rauniyar, 2020). Medicinal applications include anti-psychotic, antioxidant, anticancer, antibacterial, oxytocic, and anti-inflammatory properties (Srivastava & Rauniyar, 2020). Ergoline alkaloids, indolizidine alkaloids, nortropane alkaloids, phenolic compounds, coumarins, norisoprenoids, diterpene, isocoumarin and benzenoids, flavonoids antocianosides, glycolipids, lignan, and triterpenes are the most prominent biologically active components found in plants belonging to the genus Ipomoea (Srivastava & Rauniyar, 2020). However, no ethnopharmacological study has been conducted on *Ipomoea hederifolia*. Hence, the present study was designed to investigate the anthelmintic, cytotoxic, antimicrobial and thrombolytic activities of Ipomoea hederifolia stem.

2. MATERIALS AND METHODS

2.1. Chemicals

Albendazole (Alben DS), Vincristine Sulphate, Dimethyl Sulfoxide, Ciprofloxacin, Ketoconazole and Streptokinase were received from Globe Pharmaceutical Ltd., Bangladesh. The pharmacology laboratory of the department pharmacy, NSTU, Bangladesh provided other reagents for performing this study.

2.2. Collection and extraction of plant materials

Stem of *Ipomoea hederifolia* was collected from the Baroiyadhala National Park, on the east side of the Dhaka-Chittagong highway, Sitakunda, Chittagong, Bangladesh. The collected plant materials were washed with running tap water and allowed to air dry for two weeks. Next, dried stems were powdered into a fine powder and stored in an airtight container. Approximately 350 g of powdered plant materials were placed in a clean, flat-bottomed glass container and soaked in 1800 ml of 98% methanol at room temperature for 3 weeks, with occasional shaking and stirring. Methanol can effectively extract several types of polar compounds like phenolic compounds, flavonoids, steroids, saponins, tannnins, alkaloids, etc. The solution was then filtered by using filter cloth and Whatman filter paper and concentrated with a rotary evaporator. As a result, a greenish-black sticky extract was yielded. The extracts were dried and stored in a sterile container for further use.

2.3. Worm and microbe collection

To investigate anthelmintic activity, adult earthworms *Phertima posthuma* were extracted from moist soil and washed with normal saline to remove refuse and detritus. Specifically, earthworms measuring between 3.5 and 6.2 centimetres in length, 0.2 and 0.3 centimetres in breadth, and weighing between 0.5 and 2.9 grams were chosen for all experimental procedures. These earthworms share morphological and physiological characteristics with parasitic worms found in the human intestines, making them an ideal model for investigating anthelmintic activity. The antibacterial efficacy of IHSE against six distinct microorganisms was evaluated. The Department of Microbiology at NSTU provided microorganism cultures. Fungi (*Aspergillus niger* and *Aspergillus flavus*), gram-positive bacteria (*Enterococcus facealis* and *Staphylococcus aureus*), and gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*) are among the tested microorganisms.

2.4. Anthelmintic activity

The technique used in this work for anthelmintic potential analysis is similar to that described by A. Islam et al. (2017) and T. Islam et al. (2015), with slight adjustments. The earthworm *Pheretima posthuma*, which has anatomical and physiological similarities with human intestinal roundworm parasites, was chosen as the model organism. The earthworms

were divided into five groups. Six different concentrations of IHSE (10, 20, 30, 40, 50, and 60 mg/ml) were prepared in 60 ml solutions using distilled water to prepare the test solutions. Albendazole (15 mg/ml) was used as a reference standard, whereas the control group received saline water. All tests and standard solutions were built from scratch. Prior to the commencement of the investigations, all tests and standard solutions were freshly prepared. The immobility of the worms, absent violent trembling, was regarded as a sign of paralysis. In addition, the worms were observed for lack of movement when immersed in tepid water (50°C), and the corresponding time of mortality was recorded.

2.5. Cytotoxic activity (Brine shrimp lethality bioassay)

Following the method described by M.M. Hossain et al. (2014), a brine prawn lethality screening was carried out to assess the cytotoxic capacity of the extracts (M.M. Hossain et al., 2014). *Artemia salina* leach, also known as brine shrimp eggs, served as the experimental organism for this assessment. The brine shrimp embryos were deposited on one side of a tiny container stuffed with saline, and the container was then capped. Over an interval of 24 hours, shrimp embryos were allowed to mature into Naupli, or shrimp larvae, while a constant supply of oxygen was ensured during the entire procedure. Freshly hatched prawns were attracted to the bulb by perforated mothers. Vincristine sulphate was utilised as the positive control in this study. Vincristine sulphate (VS) was dissolved in dimethyl sulfoxide (DMSO) to generate an initial solution, which was then serially diluted with DMSO to get different concentrations. Likewise, several test solutions (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.18125, 0.390625 μg/ml concentration of IHSE) were produced by adhering to the standard solution preparation procedure. Each solution, including the control and experimental groups, was placed in pre-marked vials containing 10 nauplii of live brine shrimp in 5 ml of simulated seawater. As a control, three labelled transparent vessels having five millilitres of artificial seawater and ten shrimp larvae have been provided with 100 microliters of DMSO. After a full day, we enumerated the number of survivors of nauplii in each container by looking at them under a microscope. Based on these data, the proportion of lethality for brine shrimp nauplii at all concentrations has been determined.

2.6. Antimicrobial test

The antibacterial activity of methanolic IHSE was evaluated using a modified Kirby-disc Bauer's diffusion method (Rashid et al., 2017). The antifungal efficacy of IHSE on Potato dextrose agar (PDA) growing medium against *Aspergillus niger* and *Aspergillus flavus* was evaluated by disc diffusion (Thangavelu et al., 2013). Their concentration was determined by dissolving a predetermined amount of test materials in solvents. Six-millimetre discs of dried, sterile filter paper were contacted with test substances at predetermined concentrations using a micropipette. The IHSE discs were placed on a bacterial-contaminated Muller-Hinton agar substrate to combat microorganisms. The standard was ciprofloxacin-containing discs, while the controls were solvent-socketed blank discs. IHSE discs were placed on PDA medium contaminated with the microorganisms whose antifungal activity was being evaluated. At 4 °C, agar plates were incubated for two hours. The test components disassemble and disseminate as the disc absorbs water. The standard was discs containing ketoconazole, while the control was solvent-socketed blanks. To maximize growth, agar plates were incubated at 37 °C for 24 hours. The zone of inhibition was used to quantify the antibacterial efficacy of IHSE. Plates were then incubated at 28 °C. After observing the zone of inhibition for 48 hours, antifungal efficacy was determined.

2.7. Thrombolytic activity

To examine the *in vitro* thrombolytic activity of IHSE, Sarker et al and Uddin et al methods were used with slight modification (Sarker et al., 2016; M. Uddin et al., 2020). Streptokinase (SK) (lyophilized) 15,000,000 I.U. in a stac vial serves as positive control (standard) in this assy. After mixing 5 ml of sterile distilled water into the container, a suspension was formed. The stock solution was used to prepare 100 μl of suspension for in vitro thrombolytic activity assays. Five healthy subjects donate five milliliters of blood to five sterile microcentrifuge tubes (1 mL each). These five tubes were kept for 45 minutes in a 37°C incubator. After clot formation, all serum was withdrawn from the tubes without compromising clotting. The weight of the clot was calculated by recalculating each clot-containing tube mass. Each per-weighted clot-containing centrifuge tube was received 100 μl of IHSE at 2.5, 5, 10, or 20 mg/mL. We monitored outcomes using SK (100 μl) and sterile distilled water (100 μl) as positive and negative controls respectively. All micro centrifuging tubes were then incubated at 37°C for 90 minutes to check for clot lysis. Finally, the % difference in weight before and after clot lysis was determined by applying the following equation.

$$\text{of clot lysis} = \frac{Weight\ of\ total\ clot - weight\ of\ lysed\ clot}{Weight\ of\ total\ clot}$$

$$= \frac{WTC - WLC}{WTC} \times 100$$

Where WTC represents the clot weight after 45 min incubation period and WLC represents the remaining lysed clot weight after 90 min incubation.

2.8. Statistical analysis

SPSS version 26 was used to conduct statistical analysis of the experiment data. Results were shown as means and standard deviations (mean ± SD) with corresponding p values. To evaluate differences in the parameters of interest between the experimental sample and the control or standard group, a one-way analysis of variance (ANOVA) was performed using Dunnett's t-test.

3. RESULTS

3.1. Anthelmintic Test

The outcomes of anthelmintic activity are presented in Table 1. The paralysis time for IHSE extract at various

Table 1
Anthelmintic activity of methanolic extracts of IHSE and standard.

Treatment	Concentration (mg/ml)	Paralysis time (min) Mean ± SD	Death time (min) Mean ± SD
Distilled water	0	-	-
Standard (Albendazole)	15	55.67 ± 0.58	63.34 ± 0.50
IHSE1	60	12.502 ± 0.233*	25.23 ± 0.233*
IHSE2	50	19.29± 0.39*	29.364 ± 0.35*
IHSE3	40	23.69± 0.23*	48.02 ± 0.12*
IHSE4	30	43.71± 0.41*	63.01 ± 0.30*
IHSE5	20	50.99± 0.29*	72.89 ± 0.40*
IHSE6	10	84.71± 0.79*	106.97 ± 1.77*

Each value is represented as mean ± SD (n=5). Here, IHSE: *Ipomoea hederifolia* stem extract. The results were analyzed by using one-way ANOVA Dunnet's t-test in SPSS version 26. *$p < 0.05$ denotes a statistically significant difference as compared to standard Albendazole.

concentrations (10, 20, 30, 40, 50 and 60 mg/mL) was 84.71 ± 0.79, 50.83 ± 0.95, 43.71 ± 0.41, 23.69 ± 0.36, 19.29 ± 0.39, 12.50 ± 0.23 minutes, and the death time was 107.36 ± 1.08, 72.93 ± 0.31, 63.09 ± 0.7, 48.02 ± 0.77, 29.36 ± 0.35, and 25.23 ± 0.233 minutes, respectively. These results imply that IHSE extract demonstrates anthelmintic action in a dose-dependent fashion. At a concentration of 15 mg/mL, the death and paralysis time for Albendazole were 63.34 ± 0.5 and 55.67 ± 0.58 minutes. It was observed that the paralysis times of IHSE extract at various concentrations were significantly higher as compared to the standard group ($P < 0.05$). However, no paralysis and death time were noticed for the control group.

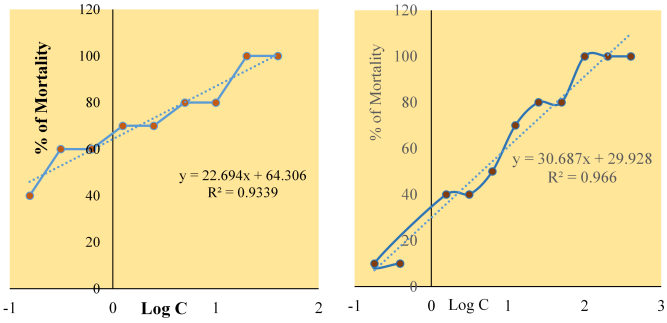

Figure 1. A: Cytotoxic activity of Vincristine sulfate. The figuredenotes that Vincristine Sulfate exhibits cytotoxicity based on dose dependence.The death of 100% naupli occurred when Vincristine Sulfate concentration is ≥20 μg/mL or Log C ≥ 1.3. **B**: Cytotoxic activity of methanolic IHSE. The figuredenotes that IHSE exhibits cytotoxicity based on a dose-dependent manner. The death of 100% naupli occurred when IHSE concentration is ≥ 100 μg/mL or Log C ≥2.

3.2. Cytotoxic activity (Brine shrimp lethality bioassay)

The cytotoxic effects of Vincristine Sulfate and IHSE on brine shrimp nauplii are shown in Figure 1A and Figure 1B respectively. As the concentration of IHSE climbed, it was shown that the mortality rate of brine shrimp nauplii increased, and 100% of mortality was observed for 100 μg/ml concentration of IHSE calculated from the dose-response curve of brine shrimp mortality. IHSE was found to be effective, having an IC_{50} value of 4.544 μg/mL for 50% nauplii mortality. Vincristine sulfate, an anticancer drug, was utilized as the standard, and its IC_{50} value was 0.865 μg/mL (Figure 2).

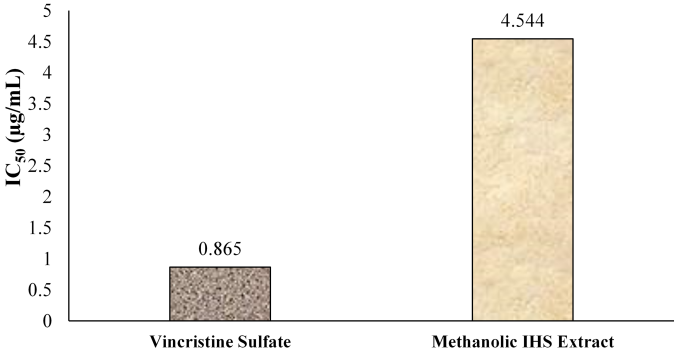

Figure 2. Comparative IC_{50} values of Vincristine Sulfate andmethanolic IHSE. The figure shows that the Vincristine Sulfate reveals an almost5 times higher mortality rate for 50% naupli death as compared to methanolic IHSE.

3.3. Antimicrobial test

At different concentrations of IHSE, the sample extract's antimicrobial activity was evaluated against two fungi and four bacteria. For the purposes of comparison, ciprofloxacin (30 μg/disc) was utilized as the reference antibacterial disc and Ketoconazole (30 μg/disc) was employed as the reference antifungal disc. Table 2 depicts the outcomes of the antibacterial action. Both *Staphylococcus aureus* and *Enterococcus faces* have shown a zone of inhibition of 0.5cm for IHSE1, whereas it was 5.5cm and 7cm for ciprofloxacin, respectively. However, no zone of inhibition was noticed for IHSE2 and IHSE3 discs. In the case of gram-negative bacteria, the zone of inhibition was 0.25cm for each bacterium in IHSE1 only. However, no zone of inhibition was found for fungi. Based on the evidence, it is obvious that plant extract of IHSE produces a very small inhibitory zone in bacterial petri plates at high concentrations where standards produced a clear zone of inhibition.

3.4. Thrombolytic effect

The clot lysis activity of 30,000 I.U. of streptokinase in 100μl, used as a positive control or standard, was 40.130 ± 2.401% (Table 3). However, when employed as a negative control, distilled water only showed a 5.493 ± 1.759% clot lysis. At different dosages including 2.5, 5, 10, and 20 mg/ml, the plant extract's capacity to dissolve clots was weak to moderate, with the value of percentage clot lysis 13.284 ± 1.106%, 20.787 ± 1.953%, 28.001± 1.535%, and 37.167 ± 2.401% respectively (Table 3). The maximum clot lysis effect, 37.168 ± 2.4%, at 20 mg/ml concentration of IHSE. The results are summarized in shown in Table 3 and Figure 3. It was

Table 2
Antimicrobial activity of IHSE on different microorganisms determined by disc diffusion assay.

Microorganisms	Zone of Inhibition (cm)				
	Standard (Ciprofloxacin) (30 μg/disc)	IHSE1 (80μg/disc)	IHSE2 (40μg/disc)	IHSE3 (20μg/disc)	Control (0μg/disc)
Gram positive					
Staphylococcus aureus	5.5	0.5	0	0	0
Enterococcus facealis	7	0.5	0	0	0
Gram Negative					
Escherichia coli	3.5	0.25	0	0	0
Klebsiella pneumoniae	5	0.25	0	0	0
	Standard (ketoconazole) (30 μg/disc)	IHSE1 (80μg/disc)	IHSE2 (40μg/disc)	IHSE3 (20μg/disc)	Control (0μg/disc)
Fungi					
Aspergillus niger	2	0	0	0	0
Aspergillus flavus	2.5	0	0	0	0

IHSE: *Ipomoea hederifolia* stem extract. Antimicrobial activity of IHSE with different concentrations (20,40 and 60 μg/disc) was assessed against gram-positive, gram-negative and fungi microbe.

Table 3
Effect of different concentrations of the methanolic extract of IHSE and the controls on thrombolytic activity.

Concentration (mg/ml)	% of clot lysis
Extract (20 mg/ml)	37.167 ± 2.401*
Extract (10 mg/ml)	28.001± 1.535*
Extract (5 mg/ml)	20.787 ± 1.953*
Extract (2.5 mg/ml)	13.284 ± 1.106*
SK (30,000 I.U.)	40.130± 0.390*
Control(0 mg/ml)	5.493 ± 1.759

Each table value is represented as mean ± SD (n=5). The results were analyzed using one-way ANOVA Dunnet's t-test in SPSS version 26. *$p < 0.05$ denotes a statistically significant difference as compared to standard Streptokinase.

discovered that the clot dissolution was substantially significant ($P < 0.05$) when compared to the control group. Additionally, it was observed that the IHSE extract exhibits thrombolytic potential in a dose-dependent fashion.

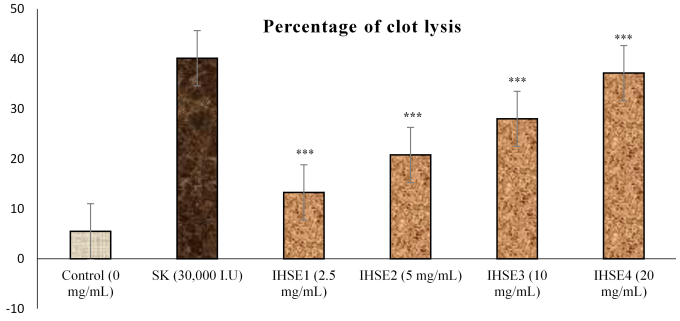

Figure 3. Percentage of clot lysis activity of methanolic IHS extract. Here, SK: Streptokinase; IHSE: *Ipomoea hederifolia* stem extract. Each value is represented as mean ± SD (n=5). Theresults were analyzed by using one-way ANOVA Dunnet's t-test in SPSS version26. *$p < 0.05$ denotes a statistically significant difference as compared to standard Streptokinase.

4. DISCUSSION

A conventional strategy is to employ medicinal plants as an unprocessed product or as refined compounds to treat different illnesses ranging from infectious to non-infectious (Roy et al., 2023). In a study for anthelminthic action, IHSE extract paralyses and kills earthworms in a dose-dependent manner. The study showed that earthworm paralysis (PT) and death time (DT) were negatively correlated with IHSE concentrations. The paralysis periods of IHSE at different doses were found to be substantially longer than those of the control group ($P < 0.05$). Extract concentrations of 10, 20, 30, 40, 50, and 60 mg/ml for death time demonstrated extremely significant differences from the standard (Figure 3). When compared to standard, the methanolic extract of IHSE showed a dose-dependent reduction in paralysis and death times (Figure 3). Numerous investigations have shown that alkaloids, phenolic, flavonoid, and tannin compounds are what give plants their anthelmintic effects (Athanasiadou et al., 2001; Aziz et al., 2014; Mazumder et al., 2023). By attaching to free host animal proteins or glycoprotein on the parasite's cuticle in the gastrointestinal tract (GIT), tannins may cause death (Marzan et al., 2023). Tannins might kill worms by decoupling oxidative phosphorylation, which would interfere with their ability to produce energy (Aziz et al., 2014). Alkaloids, on the other hand, operate on the central nervous system of worms to paralyse them (Aziz et al., 2014). Alkaloids, flavonoids, tannins, and phenolic compounds were discovered during the IHSE extract phytochemical screening (M.M. Hossain et al., 2022). As a result, we may anticipate the IHSE extract may serve as a different source of anthelmintic medication.

The safest and most effective drugs for the treatment of cancer or tumours have been the focal point of several research investigations. We searched for any chemical that may be in the IHSE extract and be effective against cancer or tumours using cytotoxic testing. In this study, exposure to varied dosage levels of test samples resulted in varying degrees of mortality.

As test sample concentrations grow, it was shown that the amount of lethality is directly proportional to the gradient of concentrations, suggesting that the risk of death rises steadily, and 100% mortality was observed at 100 μg/ml (Figure 1A and B). In case of Vincristine Sulfate the 100% mortality was found at 20 μg/ml (Figure 1A and B). IHSE had an IC_{50} value of 4.544 μg/ml whereas it was 0.865 μg/ml for Vincristine Sulfate (Figure 2). As a result, the IHSE showed a significant toxicity to brine prawns (Rahman et al., 2016). The negative control groups' lack of mortality demonstrates the test's validity. The cytotoxic action of plant extracts is caused by the presence of phytochemical components, including flavonoids, glycosides, tannins, saponins, and alkaloids, as described in various investigations (Campos et al., 2023; Musa, 2012; Sikam et al., 2022). Saponins cause cells to die in a controlled manner via a process called apoptosis (Musa, 2012). Another research found that saponins prevent the cellular mutation that causes cancer (Alam et al., 2017; Prasad et al., 2006). According to a theory put up by researchers, tannin causes cytotoxicity by preventing the activation of an enzyme necessary for the development of cancer cell lines (Musa, 2012). Once again, flavonoids exhibit cytotoxicity by increasing intracellular ROS production while alkaloids inhibit the development of a variety of cancer cells (Lamchouri et al., 2013). These phytochemicals were found throughout our investigation's IHSE extract phytochemical screening (M.M. Hossain et al., 2022). We may thus conclude that the plants may contain cytotoxic compounds that might be exploited to create medications based on the encouraging findings of our study.

The disc-diffusion method was used to test two gram-negative bacteria, two gram-positive bacteria, and two fungi for the antibacterial activity of IHSE extract (Table 2). Numerous studies have shown the antimicrobial properties of alkaloid, tannins and phenolic compounds. Since a variety of phytoconstituents, including flavonoids, tannins, saponins, and alkaloids, can function alone or in combination to reveal a potential defense mechanism against bacteria (Millat et al., 2019; Roy et al., 2023). We discovered these chemicals in the metanolic extract of IHSE during our phytochemical screening (M.M. Hossain et al., 2022). We thus hypothesised that the IHSE's methanolic extract might possess antibacterial qualities. However, the extract's impact in the high concentration IHSE (80 micro g/disc) was very weak (Table 2). Therefore, more investigation may be required to determine the antimicrobial potential of IHSE.

Platelets, tissue factor, and fibrin deposition in thrombosis, or blood clot formation, block endothelial cell surface and blood vessel damage (Furie & Furie, 2008). The activated platelets make connections with other active platelets to start the formation thrombotic process. When leucocytes and activated platelets bind to one another, a complex process of plaque formation and development is triggered (Das et al., 2013). The fibrinogen and fibrin contained in a clot are broken apart by thrombolytic medications in order to dissolve it. Blood clots are often dislodged with a medication called streptokinase. It functions by converting surplus plasminogen to plasmin (Ramjan et al., 2014). Many studies have been undertaken by many researchers in an effort to find new sources of herbs and natural foods and their supplements that have antithrombotic effects with minimum side effects (Dewan & Das, 2013). We also investigated the potential for clot lysis by IHSE methanolic extract as part of that investigation. There was very minimal clot disintegration when adding water to the clot, as we found when contrasting the positive control (SK) with the negative control (distilled water). This unexpected result prompted us to test four more samples against the negative control, and we found that all of them had significant thrombolytic activity (Table 3 and Figure 3). When compared to the control group, it was shown that the clot lysis of the IHSE extract was statistically significant (Table 3 and Figure 3) ($P < 0.05$). The presence of tannins, alkaloids, and saponins in extract has been revealing the thrombolytic activity in several studies (Bhowmick et al., 2014; Ramjan et al., 2014). The thrombolytic activity of the plant may be explained by the saponins, tanins, and alkaloids found in the stem extract of *Ipomoea hederifolia* (IHSE) (M.M. Hossain et al., 2022).

5. CONCLUSION

In conclusion, the plant extract demonstrated statistically significant action against helminths and thrombolysis and exhibited cytotoxicity in brine shrimp lethality bioassay. Further study is necessary to distinguish and characterize the chemicals that oversee these pharmacological effects. Finally, a rigorous investigation is needed for future drug development to counteract diseases like helminth infection, blood clotting and cancers using the stem of *Ipomoea hederifolia*.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

ACKNOWLEDGMENTS

The authors would like to express their heartiest gratefulness to Globe Pharmaceutical Ltd., Bangladesh. The authors are also thankful to all the teachers and staffs of the Pharmacology Laboratory, Department of Pharmacy, Noakhali Science & Technology University for their cordial co-operation to carry out the research work.

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FUNDING

This project was partially funded by Research Cell, Noakhali Science and Technology University.

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AUTHOR CONTRIBUTIONS

We declare that this study was conducted by the authors named in this article: MSU, MF, and MSM designed the study. MMH carried out the laboratory work and analysed the data and writing of the manuscript. MSU helped to supervise the work and collaborated in the data analysis while MSM and MSI revised and corrected the manuscript. All authors read and approved the final manuscript.

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