Evaluation of Anticancer, Thrombolytic and Antimicrobial Activities of *Heliotropium indicum* (Indian turnsole).

Abstract

This research objective was to investigate some *in vitro* properties of methanol-extracted plant extracts of *Heliotropium indicum* (Indian turnsole). The primary method of assessment for the methanolic extract of *Heliotropium indicum* (MEHI) was investigated for phytochemical screening. It was determined to look into the plant's potential for *in vitro* activities, such as anticancer, thrombolytic and antimicrobial activity due to the pharmaceutical interest in its component parts. Phytochemical screening was performed utilizing a battery of test reagents. Anticancer, thrombolytic and antimicrobial activities were measured at various doses using the Cell Viability Assay, Clot Lysis Assay, the Disk Diffusion Method. The findings of research on MEHI's phytochemical screening showed the presence of a wide range of various phytochemical components. Mild anticancer activity was found in MEHI and at the dose of 1000µg/mL the zone of inhibition was 29.45%, while antibacterial action ranged from 6 mm to 18 mm zones of inhibition. Comparatively, this herb has quite powerful thrombolytic action. Significant antibacterial activity has also been shown by MEHI. To wrap it up, it is clear that this plants phytochemical can be used for wide range of drug discovery field due to its potent pharmacological actions.

Keywords: *Heliotropium indicum*, Anticancer, Thrombolytic, Antimicrobial, Indian turnsole.

1. INTRODUCTION

Medicinal plants play a crucial role in the development of modern healthcare. The natural compounds found in various medicinal plants can pave the way for creating new medications (Thomford et al., 2018). According to the World Health Organisation (WHO), 80% of the world's total population relies directly or indirectly on plant-based medicine for their treatment (Kasilo 2014). About a quarter of all pharmaceuticals on the market today have their origins in plants or chemicals found in plants (Chowdhury et al., 2023). In countries like Bangladesh, medicinal plants are the primary remedy for various health treatments (Rudra et al., 2021). Among these plants, *Heliotropium indicum* deserves particular attention because of its diverse therapeutic properties, such as antimicrobial, anti-inflammatory, anticancer, and thrombolytic properties.

Heliotropium indicum, or Indian heliotrope, is a hairy seasonal herb found in waste areas and settlements (Fayed 2021). Although *H. indicum* is native to Asia, it is found worldwide in tropical regions, making its origin challenging to hypothesize. They usually grow to 1 m but can reach up to 1.5 m (Das et al., 2013). *H. indicum* has been used for centuries to treat verrucae, inflammation, and tumors. In Rodrigues, the entire plant decoction is applied externally to treat herpes, and the fresh plant paste is applied to clean and dress wounds and ulcers. *H. indicum* is used by Tamil Nadu traditional healers to treat skin diseases, poisonous stings, nausea, and neurological disorders (Pahuja et al., 2022)

Although *Heliotropium indicum* is widely used in folk medicine, its therapeutic efficacy remains unexplored. In other words, despite the known traditional uses of *Heliotropium indicum*, its

therapeutic potential remains underexplored in scientifically validated models, particularly in terms of thrombolytic, antimicrobial and anticancer properties. Thrombolytic substances break up the blood clots formed inside deep veins and thus restore normal blood flow. They are vital during any thrombolytic disease, such as a stroke or heart attack (Shomudro et al., 2023). In this paper, we are going to examine the thrombolytic effects of *Heliotropium indicum* thoroghly. Likewise, the antimicrobial qualities of this plant have not been investigated entirely. Antimicrobial agents are essential for fighting against microorganisms, including bacteria, fungi, and viruses (Shomudro et al., 2023). In addition to these properties, *Heliotropium indicum* has traditionally been used to treat a range of ailments, such as skin conditions, inflammation, and pain (Boye et al., 2012). The anticancer aspect of this plant is, however, lacking in scientific research despite its ability to suppress tumorous cells. Thus, developing anticancer drugs would be necessary in light of the increasing cancer rate worldwide and almost all regions.

This study aims to address these critical literature gaps by examining the antimicrobial, anticancer, and thrombolytic effects of *Heliotropium indicum* using *in vitro* experimental models. Examining these properties will not only contribute to the current scientific understanding of its bioactive compounds but will also be used to treat conditions such as microbial infections, cancer, and thrombolytic diseases. The results of this work may inspire the development of novel medications based on *Heliotropium indicum,* thus extending its role in modern medicine.

2. MATERIALS and METHODS

2.1 Sample Collection

Leaves of *Heliotropium indicum* were obtained from Narshingdi, Bangladesh. The collecting period was June to July 2024. The plant was subsequently recognized by a representative of the Bangladesh National Herbarium Institute, Mirpur, Dhaka. An accession number was assigned, and a voucher specimen (DACB: 47392) has been placed in the herbarium for future reference.

2.2 Sample Extraction

The leaves and bark of *Heliotropium indicum* were dried at ambient temperature for two weeks. Subsequently, the leaves were amalgamated to produce a fine powder. For extraction, 363 grams of powder were dissolved in 750 mL of methanol in a 2500 mL conical flask for about 15 days. The methanol extract was then filtered through cotton and silk fabric and transferred to a 200 mL beaker. The extract was air-dried for about ten days. Upon complete evaporation of the solvent, the *Heliotropium indicum* extract settled in the beaker (Akter, Nazim, et al., 2024).

2.3 Anticancer Test

2.3.1 Cell viability assay

Cells were grown in their designated medium in 96-well plates until they attained about 70% confluence. Thereafter, the cells were subjected to different concentrations of the extract, along with a control containing the vehicle/DMSO, for roughly 24 hours. Subsequently, the medium was eliminated to purify the cells using phosphate-buffered saline (PBS). A 0.5 mg/mL MTT solution was introduced into each well, and the plate was incubated at 37 °C for 4 hours in darkness. Following incubation, the MTT solution was substituted with 200 µL of DMSO. The plate was stirred at 150 rpm for 5 minutes, and the optical density was assessed at 490 nm using a plate

reader (ELx 800; Biotek, Winooski, VT, USA). The experiment was performed repeatedly to provide precise data for graphing (Ritu et al., 2024).

2.3.2 Morphology study

Cells were cultured in 24-well plates and treated with either DMSO or extract at the IC_{50} concentration for 24 hours. Subsequent to the therapy, the picture was obtained by phase contrast microscopy.

2.4 Thrombolytic Assay

2.4.1 Blood sample

Ten healthy human volunteers, who had never used blood-thinning medications, nicotine, or oral contraceptives, had their venous blood drawn by a medical professional. The Institutional Ethics Council of Stamford University Bangladesh granted ethical clearance for the whole operation. Ten microcentrifuge tubes were then filled with 500 µL of fresh blood.

2.4.2 Affirmation of donors consent

Each donor received a permission document detailing the study goal, project title, and the quantity of blood to be collected. This study examines the consumption of treatment by participants, any discomfort to the piercing location, and the duration for blood collection.

2.4.3 Clot lysis method

Blood was collected from healthy individuals using pre-weighed sterile vials containing 1 mL each, and then distributed into 5 mL vials. At 37°C, blood samples required 45 minutes to coagulate. A new weight measurement was conducted to ascertain the clot weight after the removal of the produced serum from the vials. Subsequently, a 100µL aqueous solution including the plant constituents (2 mg/mL) was introduced into the vials. One hundred microliters of a non-thrombolytic control and thirty thousand units of streptokinase were used as standards. The subsequent stage was transferring the mixture to an incubator maintained at 37°C after 90 minutes. We assessed the altered weights of the vials post-incubation and removed the fluid generated by the clot. The below equation was used to quantify the proportion of clot lysis for assessing thrombolytic activity (Bhuiyan et al., 2023).

$$\% \text{ of Clot lysis} = \frac{A}{B} \times 100$$

where A and B reflect, respectively, the weight of the released clot before treatment and after treatment.

2.5 Antimicrobial test

2.5.1 Test Microorganisms

5 bacteria named *Bacillus cereus, Klebsiella pneumoniae, Vibrio cholerae, Escherichia coli, Streptococcus aureus* and 4 fungi named *Penicillium chrysogenum, Aspergillus niger, Yeast budding* and *Mucor hiemalis* was collected from laboratory of microbiology, University of Asia Pacific.

2.5.2 Antimicrobial susceptibility test

Microorganisms were inoculated onto Mueller Hinton Agar (MHA) in petri dishes, followed by the insertion of sterilized discs (6 mm in diameter) into the agar plates as part of the disc diffusion method (Shomudro et al., 2023). MEHI was dissolved in suitable solvent quantities to create solutions with defined concentrations of 300, 500, and 700 µg/mL. The petri dishes were thereafter incubated at 4 °C for 2 hours to facilitate the diffusion of the extracts into the agar. The inhibition zones around the discs were measured during a 24-hour incubation of the petri dishes at 37 °C. The zone of inhibition was measured in centimeters after 24 hours.

3. STATISTICAL ANALYSIS

The bioassay readings were performed twice, and the mean value is represented in the tabular data. Microsoft Excel was employed to conduct the statistical analyses.

4. RESULT and DISCUSSION

4.1 Anticancer activity

The alcoholic extract (MEHI) of the plant materials was standardized using established methods, and its potential as an anticancer agent was evaluated on IMR-32 cell lines. The methanolic extract from the *Heliotropium indicum were* plant showed promising results, as indicated in Table 1.

Table 1. Anticancer activity of MEHI

Concentration (µm/mL)	Survival of the cell (%)	% of Inhibition
125	85.69	14.36
250	76.94	24.06
500	69.63	19.37
1000	60.84	29.16

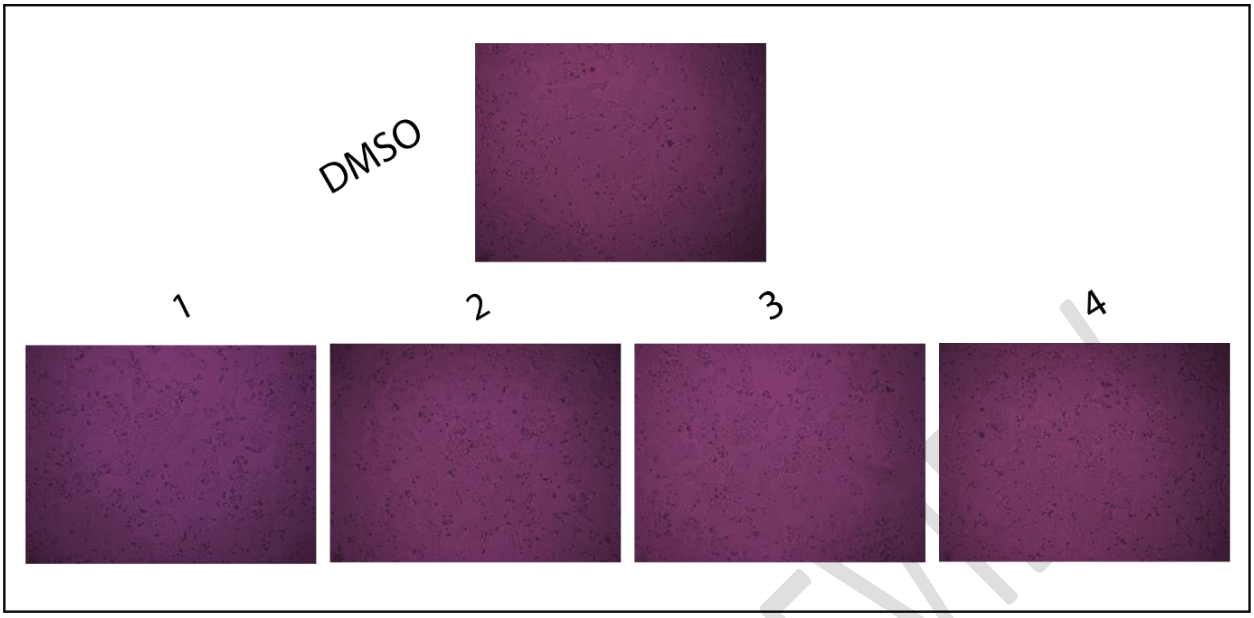

Figure 1: Morphological changes under phase contrast microscopy at varying MEHI concentrations

Figure 1 displays a phase contrast image that reveals notable and distinct morphological alterations. The serial 1, 2, 3, 4 indicates the concentration of MEHI ranging from 125 to 1000 µm/mL, as presented in Table 1.

Heliotropium indicum, recognized for its medicinal properties, demonstrates notable anticancer activity attributed to its diverse phytochemical composition, especially the presence of pyrrolizidine alkaloids (PAs). The alkaloids, such as heliotrine and indicine N-oxide, have been associated with the induction of apoptosis and cell cycle arrest in multiple cancer cell lines (Lanchhana et al., 2023). So, the experimented plant, *H. indicum*, may contain pyrrolizidine alkaloids which are likely responsible for its anticancer properties. The mechanism of action entails the metabolic conversion of PAs into reactive intermediates, capable of alkylating DNA and generating DNA adducts, ultimately resulting in DNA damage and cellular apoptosis (Li, et al., 2011). Furthermore, PAs have demonstrated the ability to inhibit topoisomerase II, a critical enzyme involved in DNA replication and transcription, thereby enhancing their anticancer efficacy (Schoental, 1970). It is crucial to recognize that PAs possess hepatotoxic properties, which can lead to significant liver damage, thereby constraining their clinical application (Fu, Lin, & Xia, 2004). Consequently, additional investigations are essential to formulate approaches that reduce the toxicity of PAs while maintaining their anticancer efficacy.

4.2 Thrombolytic Activity

The efficacy of plant extracts in promoting the dissolution of blood clots is assessed through an *in vitro* thrombolysis assay, conducted within a regulated laboratory environment. Upon sustaining an injury, the physiological response involves the aggregation of blood cells and proteins, resulting in the formation of a clot, which serves to inhibit additional haemorrhaging. Excessive clotting can lead to serious health

complications, including myocardial infarctions and cerebrovascular accidents, which may be life-threatening. The subsequent table illustrates that the percentage of clot lysis achieved by MEHI is markedly significant when compared to the standard streptokinase.

Table 2. Mean value of percent of clot lysis (N=10)

Sample	% of clot lysis
Negative control	7.296
Streptokinase	91.304
MEHI	77.27

In the *in vitro* thrombolysis assay, a clot is typically incubated within a test tube containing a botanical extract, and the extent of clot dissolution over a specified duration is subsequently quantified. The mass of the thrombus or the extent of fibrinolytic activity generated by the degradation of the thrombus can serve as indicators for assessing the level of thrombolysis (Takey et al., 2024). The capacity of plant extracts to stimulate the body's fibrinolytic system is thought to contribute to their mechanism of action in the dissolution of clots. The fibrinolytic system represents a complex interplay of enzymes and proteins that function collaboratively to degrade clots and inhibit further coagulation. Plant extracts have the potential to enhance the activity of fibrinolytic enzymes, thereby accelerating the process of clot disintegration. Additionally, specific plant extracts may inhibit the process of platelet aggregation, which leads to the adhesion of blood cells and proteins, thereby preventing the formation of clots (Chowdhury et al., 2023). The *in vitro* thrombolysis assay of plant extract, in conclusion, serves as an effective method for assessing the therapeutic potential of phototherapeutics in the management of thrombotic conditions. The enhancement of fibrinolytic activity alongside the suppression of platelet aggregation constitutes essential elements of the complex and multifaceted mechanism through which plant extracts facilitate thrombolysis (Akter, Shomudro, et al., 2024). Further research is essential to develop effective therapeutics for thrombotic disorders and to gain a deeper understanding of the mechanisms of action of these extracts.

4.3 Antimicrobial Activity

The antibacterial and antifungal activity of various dosages of plant extract was assessed by quantifying the zone of growth inhibition in mm against both gram-positive and gram-negative bacteria, in addition to four different fungal species. The findings indicated that an increase in the concentration of the plant extract corresponded with an expansion of the inhibitory zone (Table 3, 4). The zone of inhibition for antibacterial activity ranged from 09 to 18 mm, while the zone of inhibition for fungi measured between 06 and 15 mm. The results indicate that MEHI exhibits the most significant antibacterial activity against *Escherichia coli* and *Bacillus cereus*, which are responsible for serious conditions such

as urinary tract infections, respiratory illnesses, pneumonia, gastrointestinal disorders, and diarrhoea.

Table 3. Inhibition zone of MEHI against different bacteria

Diameter of Zone of Inhibition (mm)				
Test organisms	MEHI (300 µg/disc)	MEHI (500 µg/disc)	MEHI (700 µg/disc)	Ciprofloxacin (50 µg/disc)
Gram Positive Bacteria				
Bacillus cereus	14	15	18	25
Staphylococcus aureus	12	14	15	26
Gram Negative Bacteria				
Escherichia coli	13	16	18	25
Vibrio cholerae	12	13	14	27
Klebsiella pneumonia	09	10	12	24

Table 4. Inhibition zone of MEHI against different fungi

Diameter of Zone of Inhibition (mm)				
Test organisms	MEHI (300 µg/disc)	MEHI (500 µg/disc)	MEHI (700 µg/disc)	Griseofulvin (50 µg/disk)
Fungi				
Penicillium chrysogenum	06	10	15	19
Aspergillus niger	08	09	10	20
Yeast budding	10	11	15	21
Mucor hiemalis	07	10	13	21

Evaluating the effectiveness of antimicrobial agents necessitates the implementation of antibacterial and antifungal assays. Antibacterial assays are conducted to assess the capacity of a compound to impede bacterial proliferation, while antifungal assays are

utilized to investigate the efficacy of a compound in inhibiting fungal growth. The agar diffusion method, commonly referred to as the Kirby-Bauer method, is one of the prevalent techniques employed to assess antibacterial activity. In this assay, microorganisms are cultivated on an agar medium, and disks infused with the antimicrobial compound are positioned on the agar surface (Shaira et al., 2023). The diameter of the inhibition zone surrounding each disk serves as a quantitative measure of the extent to which bacterial proliferation is suppressed. The diameter of the inhibition zone is quantified and analysed against a standard reference chart to assess the antimicrobial susceptibility of the bacterial strain (Khaliq et al., 2023).

Antifungal assays are conducted utilizing the agar diffusion technique, akin to the Kirby-Bauer method employed for bacterial evaluations. The agar diffusion method involves cultivating a fungal strain on an agar medium, followed by the placement of disks infused with the antifungal agent onto the agar surface. The measurement of the zone of inhibition surrounding each disk is conducted to assess the antifungal efficacy of the compound (Lee et al., 2022).

In conclusion, the evaluation of antibacterial and antifungal activities is essential for determining the effectiveness of antimicrobial compounds. The agar diffusion and broth dilution techniques are frequently employed to assess the extent of inhibition or lethality of various agents against bacterial and fungal organisms. These assessments are crucial for the advancement and assessment of novel antimicrobial compounds, as well as for the identification of suitable agents in the management of bacterial and fungal infections.

5. CONCLUSION

This study sought to assess the anticancer, thrombolytic, and antimicrobial properties of the methanolic extract derived from *Heliotropium indicum.* The findings indicated notable anticancer efficacy against the IMR-32 cell line, implying its potential as a viable candidate for anticancer therapy. The extract demonstrated significant thrombolytic activity, suggesting its potential utility in the prevention and management of thrombotic disorders. Furthermore, the extract exhibited antimicrobial properties against various pathogenic microorganisms. Nonetheless, additional investigations are necessary to isolate and characterize the specific bioactive compounds that contribute to these activities, as well as to assess their safety and efficacy in clinical environments.

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