

## ANTIBACTERIAL, ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF HELIOTROPIUM INDICUM

### ASBTRACT

Heliotropium indicum has many traditional medicinal uses. The present study is based on this plant to evaluate the antibacterial, antioxidant and cytotoxic activities. *H. indicum* was subjected to bacterial investigation by disc diffusion method against Gram positive and Gram negative bacteria using kanamycin as standard. The methanolic extract of the plant showed good antibacterial activity against *Pseudomonas aeruginosa* with 15 mm zone of inhibition. The extract showed mild to moderate antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus megaterium* with 10 mm, 11mm and 12 mm zone of inhibition. Antioxidant activity of the plant was evaluated spectrophotometrically using DPPH and ascorbic acid as standard. The extract showed significant antioxidant activity with  $IC_{50}$  of 58.30  $\mu\text{g/ml}$  whereas  $IC_{50}$  of ascorbic acid was 45.74  $\mu\text{g/ml}$ . The cytotoxic activity of *H. indicum* was determined by brine shrimp lethality bioassay using vincristine sulfate as standard. The methanolic extract showed significant cytotoxic activity with  $LC_{50}$  of 6.607  $\mu\text{g/ml}$  whereas,  $LC_{50}$  of Vincristine sulphate was 6.026  $\mu\text{g/ml}$ .

**Key words:** Heliotropium indicum, antibacterial, antioxidant and cytotoxic activity.

### 1. INTRODUCTION

Heliotropium indicum (Family: Boraginaceae) is an annual, hirsute plant that is a common weed native to Asia. In Bangladesh, *H. indicum* is known as Hatishur. This plant has great importance in traditional medicine. Traditionally, infusion of the leaves and young shoots are used to treat nettle rash, infusion of the flowers taken in small doses regulates menstruation, where large doses are abortive, juice of the leaves is antiseptic and anti-inflammation and applied to wounds, sores, boils, gum-boils and pimples on the face. Many bioactive constituents were isolated from *H. indicum* as pyrrolizidine alkaloids named indicine, indicine-N-oxide, acetyl-indicine, indicinine, heleurine, heliotrine, supinine, supinidine and lindelofidine, all of them possess hepatotoxic activity<sup>1</sup>. Some aldehydes like phenylacetaldehyde (22.2%), (E)-2-nonenal (8.3%) and (E, Z)-2-nonadienal (6.1%), with a significant quantity of hexahydrofarnesylacetone (8.4%) and another pyrrolizidine alkaloid named as helindicine were identified with moderate antioxidant activity<sup>2, 3</sup>. Most of the alkaloids are hepatotoxic and therefore internal use of these plants is not recommended<sup>4</sup>. Biological and pharmacological activities of *H. indicum* were evaluated and reported time to time. The methanolic extract of this herb showed significant wound healing activity in the wound infection model (with *S. aureus* and *P. aeruginosa*)<sup>5</sup>. Wound healing activity of the ethanolic extract of this plant was studied using excision and incision wound models in rats following topical application and showed better healing activity<sup>6</sup>. The aqueous extract of *H. indicum* leaves was used in ulceration where dose dependent histo-gastroprotective effects were observed<sup>7</sup>. This herb showed significant activity in several experimental tumor systems<sup>8</sup>. This herb produced significant anti-inflammatory effect in both acute and subacute inflammation<sup>9</sup>. The traditional medicinal uses such as relieving abdominal pain, hypertension and impotence and sexual weakness were explained by the receptor activity of *H. indicum*<sup>10</sup>. The essential oil from the aerial parts of the herb showed significant antituberculosis activity<sup>11</sup>. Significant antimicrobial, antioxidant, cytotoxic, thrombolytic and membrane stabilizing activity of this herb were reported<sup>12,13,14</sup>. The aim and objective of the current study was to verify the previous studies and to rationale the folklore use as traditional medicine.

## **2. MATERIALS AND METHODS**

### **2.1. Drugs and chemicals**

DPPH (1, 1-diphenyl-2-picryl hydrazyl) was obtained from Sigma Aldrich USA. Ascorbic acid was obtained from SD Fine Chem. Ltd, Biosar, India. DMSO (dimethylsulfoxide) was purchased from Merck, Germany. Kanamycin was collected from Square Pharmaceuticals Ltd., Bangladesh. Vincristine sulfate was collected from Alfa Aesar Ltd. USA.

### **2.2. Collection and identification of the plant**

*H. indicum* was collected in the month of May 2013 from Jadabpur Union, Dhamrai, Dhaka and a voucher specimen for this collection has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh.

### **2.3. Extraction of the plant material**

The collected whole plant was sun dried for seven days. The dried plant was ground into small powder by a grinder machine. Then 50 gm of powder of *H. indicum* was extracted by cold extraction process using methanol (300 ml) with daily shaking and stirring for 7 days at room temperature. After 7 days the extract was filtered through cotton followed by filter paper (Double filter paper 102, 11.0 cm). Then the liquid extract was dried at room temperature (37 °C) to obtain a greenish mass.

### **2.4. Microbial strains and culture media**

Antimicrobial activity was carried out against eight Gram negative bacteria such as *Vibrio parahemolyticus*, *Vibrio mimicus*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Shigella boydii*, *Escherichia coli*, *Salmonella paratyphi*, *Aspergillus niger* and five Gram positive bacteria, *Staphylococcus aureus*, *Sarcina lutea*, *Bacillus megaterium*, *Bacillus cereus* and *Bacillus subtilis*. These bacteria were chosen to be studied as they are important pathogens and also due to rapidly developed antibiotic resistance. The microorganisms were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. For bacteria, the culture media was prepared by nutrient agar, reconstituting with distilled water according to specification (2.8% w/v).

### **2.5. Antibacterial screening by disc diffusion method**

Antibacterial activity of *H. indicum* was carried out by the standard disc diffusion method<sup>15</sup>. Solution of known concentration (500 µg/disc) of the test sample was made by dissolving measured amount of the sample (50 mg) in 1 ml of methanol. Then sterile filter paper disc (5 mm diameters) was impregnated with known test substance and dried. The dried disc was placed on plates (Petri dishes, 120 mm diameter) containing a suitable medium (nutrient agar) seeded with the test organisms. Standard disc of kanamycin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control. These plates were kept at low temperature (4 °C) for 24 hours to allow maximum diffusion. The plates were then kept in an incubator (37 °C) for 24 hours to allow the growth of microorganisms. Antibacterial activity of the test sample was observed by growth inhibition of organisms forming clear, distinct zone surrounding the discs. The antibacterial activity was expressed in terms of millimeter by measuring the diameter of the zone of inhibition. The greater zone of inhibition indicates the greater activity of the test material against the test organism.

### **2.6. Cytotoxicity screening by brine shrimp lethality bioassay**

The brine shrimp lethality bioassay was used to evaluate the cytotoxic activity<sup>16,17</sup> of *H. indicum*. The eggs of brine shrimp (*Artemia*

salina) were hatched in a tank in artificial seawater (3.8% NaCl solution) at a temperature around 37<sup>0</sup> C with constant air supply. For the experiment, the samples are prepared by dissolving the extracts in dimethylsulfoxide (DMSO) not more than 50 µl in 5 ml solution and solutions of varying concentrations (20, 40, 60, 80 and 100 µg/ ml) were prepared by the serial dilution process using simulated seawater and a vial containing 50 µl DMSO diluted to 5ml was used as a control. Then 10 live brine shrimp nauplii were added to each of the experimental vial. After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. Vincristine sulfate was used as positive control. From this data, the percent of lethality of the brine shrimp nauplii for each concentration and control was calculated.

### 2.7. Antioxidant activity by DPPH radical scavenging activity

The free radical scavenging activity (antioxidant capacity) of the plant extract on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method of Brand-Williams et al.,<sup>18</sup>. During this experiment the extract of *H. indicum* at different concentrations were mixed with 3.0 ml of DPPH methanol solution. The antioxidant potentiality was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extracts by UV- spectrophotometer (Model NO. 1501PC Shimadzu, Japan) at 517 nm. Ascorbic acid was used as a positive control. Percent scavenging of the DPPH free radical was measured using the following equation-

$$\% \text{ DPPH radical scavenging} = [1 - (As/Ac)] \times 100$$

Here, Ac = absorbance of control, As = absorbance of sample solution.

Then % inhibitions were plotted against respective concentrations used and from the graph IC<sub>50</sub> was calculated. The lower IC<sub>50</sub> indicates higher radical scavenging activity and vice versa.

## 3. RESULTS AND DISCUSSION

### 3.1. Antibacterial activity

The methanolic extract of *H. indicum* was subjected against gram positive and Gram negative bacteria. Most bacteria were sensitive to the extract as shown in (Table 1).

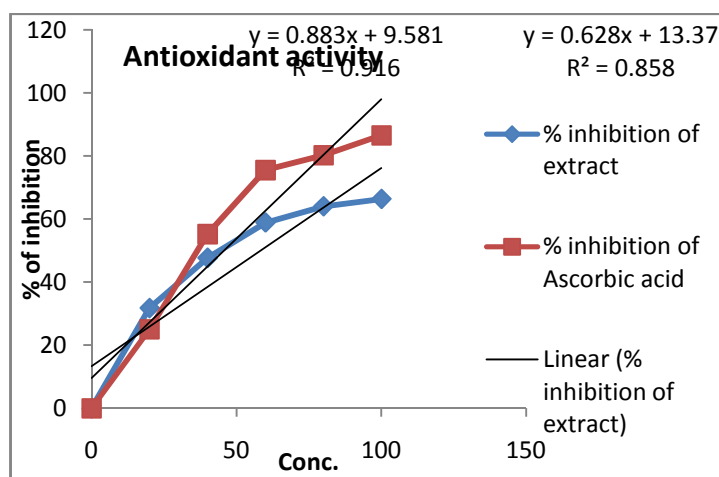
Groups	Name of bacteria	Zone of inhibition in mm of <i>H. indicum</i> (500µg/ disc)	Kanamycin (30 µg/disc)
Gram positive bacteria	<i>B. subtilis</i>	11	29
	<i>B. megaterium</i>	12	28
	<i>B. cereus</i>	7	28
	<i>S. aureus</i>	10	32
	<i>S. lutea</i>	11	25
Gram negative bacteria	<i>S. paratyphi</i>	9	38
	<i>V. mimicus</i>	8	40
	<i>V. parahemolyticus</i>	9	33
	<i>S. boydii</i>	9	26
	<i>S. dysenteriae</i>	9	35
	<i>E. coli</i>	8	34
	<i>P. aeruginosa</i>	15	33
	<i>A. niger</i>	10	36

Table 1: Antibacterial Screening of *H. indicum*.

The extract showed good antibacterial activity against *P. aeruginosa* with 15mm zone of inhibition. This extract showed moderate activity against *B. subtilis*, *S. lutea* and *B. megaterium* with 11 mm, 11 mm and 12 mm zone of inhibition, respectively. It showed mild antibacterial activity against the remaining bacteria.

### 3.2. Antioxidant activity

The antioxidant activity of *H. indicum* was determined spectrophotometrically using DPPH and ascorbic acid as standard. % of inhibitions were plotted against respective concentrations used and from the graph  $IC_{50}$  was calculated (Figure 1).

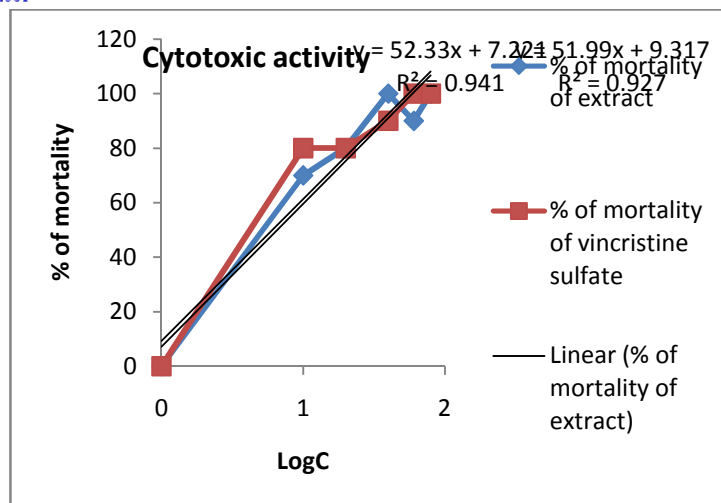


**Figure 1: Determination of  $IC_{50}$  values for standard and extract of *H. indicum* from linear correlation between of concentration versus percentage of scavenging of DPPH.**

The extract showed significant antioxidant activity with  $IC_{50}$  of 58.30  $\mu\text{g/ml}$  as compared with 45.74  $\mu\text{g/ml}$ ,  $IC_{50}$  of ascorbic acid. Previously discussed biological report of the plant complies with this finding.

### 3.3. Cytotoxic activity

The lethality of the extract of *H. indicum* to brine shrimp was determined using vincristine sulfate as standard and the results (% mortality at different concentrations and  $LC_{50}$  values) were shown in (Figure 2). An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality<sup>19</sup> was plotted on the graph paper and the values of  $LC_{50}$  were calculated using Microsoft Excel 2003. The extract showed very good cytotoxic activity with  $LC_{50}$  value at 6.607  $\mu\text{g/ml}$  as compared with the activity of standard with  $LC_{50}$  value at 6.025  $\mu\text{g/ml}$ .



**Figure 2: Determination of LC<sub>50</sub> values for standard and extract of *H. indicum* from linear correlation between logarithms of concentration versus percentage of mortality.**

#### 4. CONCLUSION

*H. indicum* is a potential source of many chemical constituents and widely used for many health problems. This plant also provides many pharmacological properties that have been reported previously. The evaluation of antibacterial, antioxidant and cytotoxic activities of *H. indicum* would play a significant role for the findings of more chemical entities and their bioactivities.

#### 5. ACKNOWLEDGEMENT

The author wish to acknowledge the Phytochemical research laboratory of Southeast University of Bangladesh and Bangladesh National Herbarium, Dhaka, Bangladesh.

#### 6. REFERENCES

1. Prota 11(1): Medicinal plants/Plantes médicinales1,Record display
2. Ogunbinu AO, Flamini G, Cioni PL, Adebayo MA, Ogunwande IA. Constituents of *Cajanus cajan* (L.) Millsp., *Moringa oleifera* Lam., *Heliotropium indicum* L. and *Bidens pilosa* L. from Nigeria. *Natural Product Communications* 2009; 4(4):573-578.
3. J. Braz. Journal of the Brazilian Chemical Society 2005; 16.
4. G. K. Dash and M. S. Abdullah. A review on *Heliotropium indicum* L (Boraginaceae). *IJPSR* 2013; 4(4): 1253-1258.
5. G. K. Dash and P. N. Murthy. Studies on Wound Healing Activity of *Heliotropium indicum* Linn. Leaves on Rats. *ISRN Pharmacol* 2011; 2011: 847980.
6. J.Suresh Reddy, P.Rajeswara Rao, Mada S Red. Wound healing effects of *Heliotropium indicum*, *Plumbago zeylanicum* and *Acalypha indica* in rats. *J. of Ethnopharmacology* 2002; 79: 249-251.
7. Akinlolu Abdulazeez Adelaja, M. D. Ayoola, J. O. Otulana, O. B. Akinola, Abimbola Olayiwola and A. B. Ejiwunmi. Evaluation of the Histo - Gastroprotective and Antimicrobial Activities of *Heliotropium Indicum* Linn (Boraginaceae). *Malays J Med Sci.* 2008; 15(3): 22-30.
8. Kugelman M, Liu WC, Axelrod M, McBride TJ, Rao KV , Lloydia. Journal Article, Research Support, U.S. Gov't, P.H.S. 1976; 39(2-3):125-128.

9. Srinivas, K. Anti-inflammatory Activity of *Heliotropium indicum* Linn. and *Leucas aspera* Spreng. in albino rats. *Indian Journal Pharmacology* 2000; 32: 37-8.
10. George Asumeng Koffuor , Alex Boye , Patrick Amoateng , Elvis O. Ameyaw and Alfred K. Abaitey, Investigating the Site of Action of an Aqueous Extract of *Heliotropium indicum* Linn (Boraginaceae) on smooth muscles. *Research Journal of Pharmacology* 2012; 6(1):12-19.
11. Osungunna, M. O. and Adedeji, K. A. Phytochemical and antimicrobial screening of methanol extract of *Heliotropium indicum* leaf. *Journal of Microbiology and Antimicrobials* 2011; 3(8): 213-216.
12. P. R. Rao, S. Nammi, A. D. V. Raju. Studies on the Antimicrobial Activity of *Heliotropium indicum* Linn. *Journal of Natural Remedies*.
13. Shoge Mansurat Oluwatoyin, Ndukwe George Illeogbulamand ,Amupitan Joseph. Phytochemical and antimicrobial studies on the aerial parts of *Heliotropium indicum*.*Scholars Research Library* 2011; 2 (2) :129-136.
14. Nusrat Ahmed Mourin, Tasnuva Sharmin,Sharmin Reza Chowdhury, Farhana Islam, Mohammad S. Rahman, MohammadA. Rashid. Evaluation of bioactivities of *Heliotropium indicum*, a medicinal plant of Bangladesh. *The pharma innovation journal* 2013;2 (2).
15. Bauer, A.W., Kirby, W.M.M., Sherris and Tuck M. Antibiotic susceptibility testing by a standardized disc diffusion method. *American Journal of Clinical Pathology* 1966; 45:493-49.
16. Meyer B.N, Ferrigni N.R, Putnam J.E, Jacobsen L.B, Nichols D.E and McLaughlin J.L. Brine shrimp: A convenient general bioassay for active plant constituents 1982. *Planta Medica*; 45: 31-4.
17. McLaughlin J.L and Rogers L.L. The use of biological assays to evaluate botanicals. *Drug Information Journal* 1999 ; 32:513.
18. Brand-Williams W, Cuvelier M. E and Berset C. (). Use of free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft und Technologie*. 1995; 28:25-30.
19. Persoone G, Sorgeloos P, Roels O and Jaspers E. Proceedings of the international symposium on the brine shrimp *Artemia salina*, 1979 & 1980; Aug 20-23, Texas, USA. Belgium: Universa Press.

**Yesmin Begum**

Lecturer, Department of Pharmacy,  
Southeast University, Banani,  
Dhaka