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Evaluation of bioactivities of *Heliotropium indicum*, a medicinal plant of Bangladesh

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The crude methanol extract of whole plant of *Heliotropium indicum* L. as well as its organic and aqueous soluble partitionates were subjected to screening for antioxidant, cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities. In free radical scavenging activity assay, the carbon tetrachloride soluble fraction revealed the highest free radical scavenging activity (IC_{50}= 37.88±0.51 µg/ml) which could be correlated to its total phenolic content (40.35±0.17 mg of GAE/g of extractives). In brine shrimp lethality bioassay, the crude methanol extract (LC_{50}= 2.57±0.22 µg/ml) revealed the presence of the highest amount of considerable bioactive principles. During assay for thrombolytic activity, the carbon tetrachloride soluble materials revealed 36.90±0.75 % of clot lysis as comparable to 66.77% by standard streptokinase. In hypotonic solution and heat induced conditions, the carbon tetrachloride soluble fraction inhibited haemolysis of human erythrocyte by 41.47±1.12 % and 37.97±0.14 %, respectively. The carbon tetrachloride soluble materials demonstrated activity against microbial growth with zone of inhibition ranging from 7.0 to 20.0 mm.

Keyword: *Heliotropium indicum* L., Total Phenolic Content, Free Radical Scavenging Activity, Cytotoxicity, Thrombolytic Activity, Membrane Stabilizing Activity, Zone Of Inhibition.

1. Introduction

Heliotropium indicum L. (Synonyms: *Heliophytum indicum* L. DC., *Eliopia riparia* Raf., *Eliopia serrata* Raf.; Bengali name: Hatishur) is an annual, erect, branched herb belonging to Boraginaceae family. The plant is a native to Asia and is a common weed in waste places and settled areas[1]. The plant is astringent, emollient, vulnerary and diuretic. The plant is useful in ulcers, sores, wounds, gum boils, skin affections, stings of insects and rheumatism. Leaf juice is used in eye disease and decoction is used in fevers and urticaria. Root is aphrodisiac and used for the cure of night blindness. Decoction of the root is used in coughs and fevers. Seeds are stomachic. The flowers are considered emmenagogue in small doses and abortifacient in large doses.[2]

As part of our ongoing investigations on medicinal plants of Bangladesh[3,4,5,6,7,8,9], the methanol extract of whole plant of *H. indicum* growing in Bangladesh as well as its organic and aqueous soluble fractions were studied for the antioxidant potential in terms of total phenolic content and free radical scavenging property; cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities for the first time and we, here in, report the results of our preliminary investigations.

2. Materials and Methods

2.1 Plant Materials

The whole plant of *H. indicum* was collected from Dhaka, Bangladesh, in May 2012. A voucher specimen for this collection has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh for future reference.

The sun dried and powdered whole plant (800 g) was macerated in 2.5 liters of methanol for 7 days. The extract was filtered through fresh cotton bed and finally with Whatman filter paper number 1 and concentrated with a rotary evaporator at reduced temperature and pressure. An aliquot (5 g) of the concentrated methanol extract was fractionated by modified Kupchan partition protocol[10] and the resultant partitionates were evaporated to dryness with rotary evaporator to yield hexane (HXSF, 1.5 g), carbon tetrachloride (CTCSF, 1.5 g), chloroform (CSF, 1 g) and aqueous (AQSF, 0.5 g) soluble materials. The residues were then stored in a refrigerator until further use.

Chemicals and drugs: Gallic acid, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), ascorbic acid and vincristine sulphate were supplied by Beximco Pharmaceuticals Ltd., Dhaka, Bangladesh. Streptokinase (Altepase) from Beacon pharmaceutical Ltd., Dhaka, Bangladsh and acetyl salicylic acid from Beximco Pharmaceuticals Ltd., Dhaka, Bangladsh were used in thrombolytic and membrane stabilizing activity assays, respectively. Dimethyl sulphoxide (DMSO, Merck Chemicals Ltd., Germany) was used as solvent. Sterile normal saline solution (0.9 % NaCl) from Beximco Infusion Ltd., Dhaka, Bangladesh; was used as vehicle. Other reagents used were from Merck Chemical Ltd., Germany.

2.2 Total Phenolic Content:

The total phenolic content of the extractives was determined with Folin-Ciocalteau reagent by using the method developed by Harbertson and Spayd (2006) [11].

2.3 DPPH Free Radical Scavenging Assay:

Following the method developed by Brand-Williams *et al*. (1995)[12], the antioxidant activity of the test samples was assessed by evaluating the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical by using synthetic antioxidants, butylated hydroxytoluene (BHT) and ascorbic acid as positive controls.

2.4 Brine Shrimp Lethality Bioassay:

This technique was applied for the determination of general toxic properties of the DMSO solutions of plant extractives against *Artemia salina* in a single day *in vivo* assay[13]. Vincristine sulphate was used as positive control.

2.5 Thrombolytic Activity:

The thombolytic activity was evaluated by the method developed by Prasad *et al.* (2006)[14] by using streptokinase as positive control.

2.6 Membrane Stabilizing Activity:

The membrane stabilizing activity of the extractives was assessed by evaluating their ability to inhibit hypotonic solution and heat induced haemolysis of human erythrocytes following the method developed by Omale *et al*. (2008)[15].

2.7 Antimicrobial screening:

Antimicrobial activity was determined by disc diffusion method[16].

3. Statistical Analysis:

For all bioassays, three replicates of each sample were used for statistical analysis and the values are reported as mean ± SD.

4. Results and Discussion

The present study was undertaken to evaluate the antioxidant potential in terms of total phenolic content and free radical scavenging property; cytotoxic, thrombolytic and membrane stabilizing and antimicrobial activities of different organic and aqueous soluble materials of the crude methanol extract of *H. indicum*.

In DPPH free radical scavenging assay, all the fractions demonstrated free radical scavenging potential with IC_{50} values ranging from 37.88 µg/ml to 193.03 µg/ml. The highest free radical scavenging activity was demonstrated by the carbon tetrachloride soluble fraction (IC_{50}= 37.88±0.51 µg/ml) which could be correlated to its phenolic content 40.35±0.17 mg of GAE / g of extractives (Table 1).

Table 1: Total phenolic content, free radical scavenging and cytotoxic activities of *H. indicum*

Samples/ Standards	Total phenolic content (mg of GAE/ g of dried extract)	Free radical scavenging activity IC_{50} (µg/ml)	Brine shrimp lethality bioassay LC_{50} (µg/ml)
ME	6.01±0.23	71.19±0.32	2.57±0.22
HXSF	1.69±0.48	103.58±0.44	3.91±0.53
CTCSF	40.35±0.17	37.88±0.51	9.69±0.82
CSF	6.94±0.82	93.31±0.11	31.44±0.45
AQSF	4.34±0.37	193.03±0.52	24.98±0.15
VS	-	-	0.45±0.04
BHT	-	27.52±0.54	-
Ascorbic acid	-	5.80±0.21	-

ME= Methanolic crude extract; HXSF= Hexane soluble fraction; CTCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction; AQSF= Aqueous soluble fraction; BHT= Butylated hydroxytolune; VS= Vincristine sulfate

In case of brine shrimp lethality bioassay, all the fractions demonstrated significant cytotoxic potential against *A. salina* with LC_{50} values ranging from 2.57 to 31.44 µg/ml. The crude methanol extract revealed the highest cytotoxic activity with LC50 value of 2.57±0.22 µg/ml as compared to 0.45 µg/ml for Vincristine sulphate (Table 1).

The extractives of *H. indicum* demonstrated mild to moderate thrombolytic activity. The carbon tetrachloride soluble fraction showed 36.90±0.75 % of clot lysis as compared to 66.77% clot lysis by standard streptokinase (Table 2).

Table 2: Thrombolytic activity of *H. indicum*

Samples	**% of lysis of RBC**
ME	25.85±0.03
HXSF	6.57±0.44
CTCSF	36.90±0.75
CSF	17.84±0.94
AQSF	7.13±0.55
Water	3.79±0.55
Streptokinase	66.77±0.36

ME = Methanolic crude extract; HXSF= Hexane soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF = Chloroform soluble fraction; AQSF = Aqueous soluble fraction

At concentration 1.0 mg/ml, the extractives of *H. indicum* protected the haemolysis of RBCs induced by hypotonic solution and heat as compared to the standard acetyl salicylic acid (0.10 mg/ml). The carbon tetrachloride soluble fraction inhibited 41.47±1.12 % and 37.97±0.14 % of haemolysis of RBCs induced by hypotonic solution and heat as compared to 71.92 % and 42.12 % by acetyl salicylic acid, respectively (Table 3).

The antimicrobial activity of *H. indicum* test samples was evaluated against five gram positive and eight gram negative bacteria and three fungi and the results were compared with standard antibiotic, ciprofloxacin. Among the test samples of *H. indicum*, only the carbon tetrachloride soluble fraction revealed antimicrobial activity with zone of inhibition ranging from 7.0 to 20.0 mm. The highest zone of inhibition (20.0 mm) was showed by the carbon tetrachloride soluble fraction against *Bacillus cereus* (Table 4).

Table 3: Effect of different extractives of leaf of *H. indicum* on heat and hypotonic solution-induced haemolysis of erythrocyte membrane

Sample/Standard	% Inhibition of haemolysis	
	Heat induced	Hypotonic solution induced
Hypotonic medium	--	--
ME	29.68±0.72	30.43±0.15
HXSF	32.77±0.22	35.64±0.23
CTCSF	37.97±0.14	41.47±1.12
AQSF	11.21±0.55	23.03±0.34
CSF	21.71±0.63	32.24±0.11
ASA	42.12±-0.38	71.92±0.78

ME = Methanolic crude extract; HXSF= Hexane soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF = Chloroform soluble fraction; AQSF = Aqueous soluble fraction; ASA= Acetyl salicylic acid.

Table 4: Antimicrobial activity of *H. indicum*

Test microorganisms	Diameter of zone of inhibition (mm)	
	CTCSF	Ciprofloxacin
Bacillus cereus	20.0±0.03	45.0±2.01
B. megaterium	-	42.0±1.17
B. subtilis	-	42.0±0.73
Sarcina lutea	8.0±0.18	42.0±0.23
Staphylococcus aureus	-	42.0±0.56
Escherichia coli	-	42.0±0.43
Pseudomonas aeruginosa	8.0±0.24	42.0±1.11
Salmonella typhi	7.0±0.73	45.0±0.73
S. paratyphi	8.0±0.49	47.0±2.33
Shigella boydii	10.0±0.33	34.0±0.58
S. dysenteriae	11.0±0.72	42.0±0.22
Vibrio mimicus	-	40.0±0.45
V. parahaemolyticus	-	35.0±0.44
Candida albicans	-	38.0±0.49
Aspergillus niger	-	37.0±0.33
Sacharomyces cerevacae	-	38.0±0.11

CTCSF= Carbon tetrachloride soluble fraction

5. Conclusion

It is clearly evident from the above findings that the test samples of *H. indicum* possess different types of bioactivities. Therefore, the plant is a good candidate for further systematic, chemical and biological studies to isolate the active principles.

6. Acknowledgement

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