



Evaluation of Anti-Inflammatory Activity through Assaying the Stability of Erythrocytes' Membrane by Different Organic Extracts of *Heliotropium Indicum* (Boraginacea)

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ABSTRACT

In this study, different organic soluble extracts i.e. methanol, n-hexane, carbon tetrachloride and chloroform along with aqueous soluble fraction of both roots & leaves of *Heliotropium indicum* were designed to investigate for cell membrane stabilizing activity. An in vitro study of different organic soluble extracts stabilized erythrocytes' (RBC) membrane significantly. The chloroform soluble fraction of roots revealed 31.59 % and 64.7 % whereas n-hexane soluble fraction of leaves demonstrated 29.27 % and 56.9 % inhibition of hemolysis of RBC caused by heat and hypotonic solution respectively. The assay result was compared with standard acetyl salicylic acid (0.10 mg/ml) which inhibited 35.26 % and 73.56% hemolysis of RBC caused by heat and hypotonic solution respectively.

Keywords: *Heliotropium indicum*, membrane stabilizing activity, Acetyl salicylic acid.

INTRODUCTION

Heliotropium indicum (Bengali-Hatisur, Family: Boraginaceae) is an annual herb with branched stem and strong taproot having usually 1 meter height but sometimes it may grow as tall as 1.5 meter. Stem deeply grooved and covered with large and coarse white hairs. Leaves are opposite or alternate, 3-15 cm long, 2-10 cm wide, ovate to oblong-ovate with dense and long white hairs on both surfaces [1].

Different plant parts of *H. indicum* have folk medicinal properties and local uses in different parts of the world. Different research works showed that it has been used locally to treat against ulcers, sores, wounds, skin infections, rheumatism, eye diseases, fever, urticaria etc. It has been also reported that roots and flowers of *H. indicum* has been used as stomachic and in abortion [2]. The plant has been widely used for centuries to treat warts, inflammations and tumors. Throughout tropical Africa it has been used as an analgesic to lessen rheumatic pain, as diuretic agents and anti-ulcerants [3].

The erythrocyte (RBC) can be simulated with lysosome or lysosome like organelle which membrane contain different hydrolytic enzymes virtually responsible for breaking down of the all kinds of biomolecules. So RBC membrane stabilizers may interfere the action of different enzymatic functions to moderate the release of histamine, serotonin or prostaglandins which regulate the cells functions. [4,5].

METHODS AND MATERIALS

Plant materials

The leaves and roots of *H. indicum* were collected from Gazipur area, Dhaka and Voucher specimen (DACB 39159) for the plant sample was kept in Bangladesh National Herbarium for future reference. The leaves and roots were sun dried for several days and then oven dried at low temperature (less than 40°C) for better grinding. The powdered

materials of *H. indicum* (150 gm each) were soaked with occasional stirring in 1L (for each) of methanol, chloroform, carbon tetra chloride, n-hexane and pure water respectively for 7 days. After maceration of plant samples then it was filtered using cotton plug and finally by Whatman filter paper number 1 for clear solution of extracts. The filtrates were concentrated at low temperature (40-45 °C) using a rotary evaporator under reduced pressure. The concentrated organic soluble extracts *i. e.* methanol, chloroform, carbon tetra chloride, n-hexane and aqueous soluble fraction were subjected for assay of cell membrane stabilizing activity.

Preparation of erythrocyte suspension

Healthy human volunteers were selected to collect whole blood under standard conditions. After collection of blood, sodium EDTA was used to prevent clotting and finally washed several times by isotonic solution (0.9% NaCl solution). After washing, a 40% (v/v) mixture of isotonic and buffer solution was added to maintain the blood pH consistently. Afterward, the mixture of blood sample was centrifuged at 3000 rpm for 10 minutes to collect the RBC suspension as stock erythrocytes for further analysis.

Evaluation of hemolysis caused by hypotonic solution

The RBC membrane stabilizing activity of different organic soluble extractives was assayed by analyzing hemolysis of erythrocytes' membrane by hypotonic solution [6]. The experiment was performed by preparing a test sample which contained stock erythrocyte (RBC) suspension (0.50 ml) with 5 ml 40% (v/v) mixture of hypotonic and sodium phosphate buffer solution (pH 7.4) and different organic soluble extracts (2.0 mg/ml) of *H. indicum*. Standard acetyl salicylic acid (0.10 mg/ml) was as positive control. The mixtures containing plant samples, RBC suspension and standard (positive control) were incubated for 10 minutes at 37°C temperature and then centrifuged for 10 minutes at 3000 rpm to get the supernatant. Finally absorbance of the supernatant was measured at 540 nm using UV spectrophotometer.

The percentage of inhibition of RBC membrane hemolysis was calculated using the following equation:

$$\% \text{ inhibition of hemolysis} = 100 \times \{(\text{OD}_1 - \text{OD}_2) / \text{OD}_1\}$$

Where, OD_1 = Absorbance of hypotonic-buffered solution

OD_2 = Absorbance of test sample in hypotonic solution

Evaluation of hemolysis caused by heat

Methanol, chloroform, n-hexane, carbon tetrachloride and aqueous soluble fractions (2.0 mg/ml) were taken into two duplicate sets of centrifuge tubes containing 5 ml isotonic–buffer solution (40% v/v). Then 30 µl of erythrocyte suspension was added each tubes and mixed gentle. After proper mixing one set of centrifuge tubes were kept on ice bath at 0-5°C temperature and other set was incubated at 54°C for 20 minutes for both cases [7]. After incubation the mixture of sample were centrifuged for 3 minutes at 1300 rpm to get the supernatant. Finally absorbance of the supernatant was measured at 540 nm using UV spectrophotometer.

The percentage inhibition of hemolysis of the tests was calculated according to the equation:

$$\% \text{ Inhibition of hemolysis} = 100 \times [1 - (\text{OD}_2 - \text{OD}_1) / (\text{OD}_3 - \text{OD}_1)]$$

Where, OD_1 = Absorbance of test sample unheated

OD_2 = Absorbance of test sample heated

OD_3 = Absorbance of control sample heated

Statistical analysis

Each experiment was performed three times for statistical analysis and the values are reported as mean ± SD.

RESULT AND DISCUSSION

Different organic soluble fractions of *H. indicum* at concentration 2.0 mg/ml significantly stabilized the breaking down of erythrocyte membrane under heat and hypotonic solution experimental model, which was compared with standard, acetyl salicylic acid (0.10 mg/ml).

The n-hexane soluble fraction of leaves and chloroform soluble fraction of roots of *H. indicum* produced 56.9 % and 64.7% inhibition of hemolysis of RBC respectively, caused by hypotonic solution where acetyl salicylic acid (0.10 mg/ml) revealed 73.56% inhibition of hemolysis. Additionally methanol, chloroform soluble fractions of leaves and methanol, n-hexane soluble fractions also protected hemolysis significantly (Table2).

In case of heat induced hemolysis, n-hexane soluble fraction of leaves and chloroform soluble fraction of roots of *H. indicum* produced 29.27 % and 31.59% inhibition of hemolysis of RBC respectively where acetyl salicylic acid

(0.10 mg/ml) revealed 35.26% inhibition of hemolysis. Besides, chloroform soluble fraction of leaves and n-hexane soluble fraction of roots also produced significant inhibition of hemolysis (Table 1).

Table 1: Effect of different extractives of *Heliotropium indicum* under heat-induced experimental model

SI	Plant samples	Code	Concentration (mg/ml)	Heat induced haemolysis (%inhibition)	
				Leaves	Roots
1	Methanol soluble fraction	MSF	2.0	17.40 ± 0.87	21.31 ± 0.60
2	Aqueous soluble fraction	ASF	2.0	12.68 ± 0.75	8.04 ± 0.95
3	Chloroform soluble fraction	CSF	2.0	19.45 ± 0.62	31.59 ± 0.88
4	Carbon tetra chloride soluble fraction	CTSF	2.0	7.33 ± 0.55	11.99 ± 1.26
5	n-Hexane soluble fraction	NHSF	2.0	29.27 ± 0.66	23.58 ± 0.35
6	Acetyl Salicylic Acid (Standard)	ASA	0.1	35.26 ± 0.81	35.26 ± 0.55

Mean ± S.D. (standard deviation)

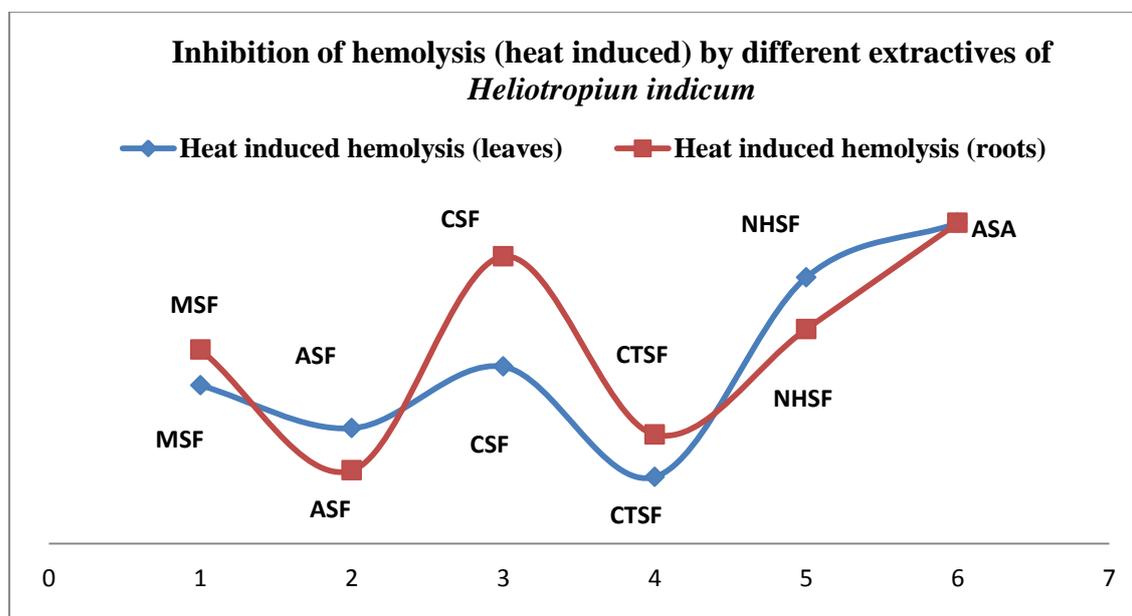


Figure 1: Comparison of % of inhibition of different fractions of *Heliotropium indicum* (leaves & roots) under heat induced model

Table 2: Effect of different extractives of *Heliotropium indicum* (leaves & roots) under hypotonic solution induced experimental model

SI	Plant samples	Code	Concentration (mg/ml)	Hypotonic solution induced haemolysis (%inhibition)	
				Leaves	Roots
1	Methanol soluble fraction	MSF	2.0	37.85 ± 0.75	40.35 ± 0.55
2	Aqueous soluble fraction	ASF	2.0	36.7 ± 1.04	17.52 ± 0.42
3	Chloroform soluble fraction	CSF	2.0	45.6 ± 0.29	64.7 ± 0.45
4	Carbon tetra chloride soluble fraction	CTSF	2.0	28.24 ± 0.47	27.10 ± 1.05
5	n-Hexane soluble fraction	NHSF	2.0	56.9 ± 0.81	42.5 ± 0.65
6	Acetyl Salicylic Acid (Standard)	ASA	0.1	73.56 ± 0.55	73.56 ± 0.55

Mean± S.D. (standard deviation)

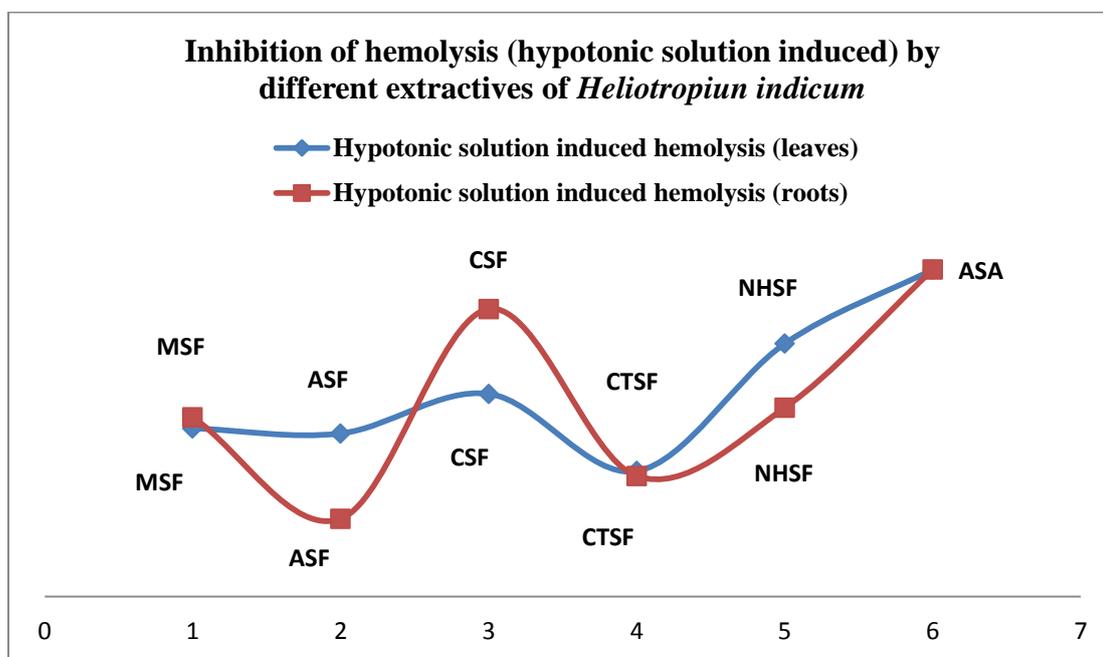


Figure 2: Comparison of % of inhibition of different fractions of *Heliotropium indicum* (leaves & roots) under hypotonic solution induced model

CONCLUSION

Different organic soluble partitionates both leaves & roots of *H. indicum* were subjected for screening of percent inhibition of hemolysis of RBC membrane at two experimental conditions *i.e.* heat and hypotonic solution. Among all the fractions n-hexane and chloroform soluble extract of both leaves and roots prevented hemolysis of RBC significantly.

Significant stabilization of RBC membrane by different extractives can be explained by possible interaction with membrane protein and different hydrolytic enzymes which mediate the lysis of all kinds of biomolecules [8]. So it can be concluded that *H. indicum* contains important secondary metabolites that may play important role as anti-inflammatory agents in therapeutic application.

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