



Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

***In vitro* membrane stabilizing activity of erythrina variegata bark**

**Mohammad Shahriar^{1*}, Nishat Zareen Khair², Rumana Akhter¹
and Sayeeda Fahmee Chowdhury¹**

¹Phytochemistry Research Laboratory, University of Asia Pacific, Dhaka, Bangladesh

²Department of Pharmacy, BRAC University, Dhaka, Bangladesh

ABSTRACT

In this present study, the bark extracts of Erythrina variegata were subjected to evaluation of the membrane stabilizing activity by using human erythrocyte and the results were compared with standard anti-inflammatory drug, acetyl salicylic acid (ASA). In vitro membrane stabilizing activity for hypotonic solution induced haemolysis, the ethanol extract inhibited 78.92% haemolysis of RBCs as compared to 85.42% produced by acetyl salicylic acid (ASA) and during heat induced condition different organic soluble materials of Erythrina variegata demonstrated 75.68%, 77.19% and 73.83% inhibition of haemolysis of RBCs respectively whereas ASA inhibited 79.04%.

Key words: *Erythrina variegata*, membrane stabilizing activity, human erythrocyte, acetyl salicylic acid

INTRODUCTION

Medicinal plants are widely used in the traditional system for the treatment of several diseases that contain chemical compounds which act as precursor for the synthesis of useful drugs [1]. Many plants have been listed in the ancient literatures for their medicinal values and their formulation has been found to be effective for the treatment of various diseases. Different parts of the plants are used in the traditional treatment system without any scientific validation. They play significant role in providing primary health care services to rural people as the synthetic drugs are costly and sometimes they are unavailable. Besides their role as therapeutic agents, they also act as a major source of income by exporting medicinal plants to other countries [2].

Erythrina variegata belongs to the Fabaceae family and *Erythrina* genus which includes about 110 species of trees and shrubs. The plants under the *Erythrina* genus are collectively known as “coral tree”. These plants are native to the Old World tropics, especially from India to Malaysia, but are native of ancient westward to Zanzibar and eastward to eastern Polynesia (the Marquesas). It is typically found on sandy soil in littoral forest, and sometimes in coastal forest up to 250m (800ft) in elevation. The coral tree is cultivated particularly as an ornamental tree and as a shade and soil improvement tree (it fixes nitrogen) for other tree crops such as coffee and cacao [3].

The plant parts from *Erythrina variegata* are widely used for the treatment of different diseases and complications. The leaves are used for their stomachic, anthelmintic, laxative, diuretic, gatactagogue and emmenagogue properties; sometimes also applied externally for dispersing venereal buboes, relieve pain of the joints and inflammations; juice is poured in to the ear to relief earache and is used as an anodyne in toothache. The bark is astringent, febrifuge anti-bilious and anthelmintic; useful in dysentery and as a collyrium in ophthalmia. The roots are emmenagogue [4]. The objective of the present study was to explore the membrane stabilizing activity of the bark extract of *Erythrina variegata*.

EXPERIMENTAL SECTION

Collection, identification and processing of plant sample

The bark extracts of *Erythrina variegata* was collected from Botanical garden, Curzon Hall at the University of Dhaka in June 2014 and was taxonomically identified with the help of the National Herbarium of Bangladesh, Mirpur-1, Dhaka (DACB; Accession Number- 36148). Bark was sun dried for seven days. The dried bark were then ground in coarse powder using high capacity grinding machine which was then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation.

Extraction procedure

The powdered plant parts (22 gm) were successively extracted in a soxhlet extractor at elevated temperature using 250 ml of distilled Methanol (40-60)°C which was followed by ethanol, and chloroform. After extraction all extracts kept in refrigerator 4°C for future investigation with their necessary markings for identification.

Blood sample

Blood (n=6) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and 5 ml of blood was transferred to the previously weighed micro centrifuge tubes and was allowed to form clots.

Membrane stabilizing activity

The membrane stabilization by hypotonic solution and heat-induced haemolysis method was used to assess anti-inflammatory activity of the plant extracts by following standard protocol [5]. Since the erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane [6]. The membrane stabilizing activity of the extractives was assessed by using hypotonic solution-induced and heat-induced human erythrocyte haemolysis. To prepare the erythrocyte suspension, whole blood was obtained from healthy human volunteer and was taken in syringes (containing anticoagulant 3.1% Na-citrate). The blood was centrifuged and blood cells were washed three times with solution (154mM NaCl) in 10mM sodium phosphate buffer (pH 7.4) through centrifugation for 10min at 3000g [7-8].

Hypotonic solution-induced haemolysis

The test sample consisted of stock erythrocyte (RBC) suspension (0.5mL) mixed with 5mL of hypotonic solution (50mM NaCl) in 10mM sodium phosphate buffered saline (pH 7.4) containing either the extract (1.0 mg/mL) or acetyl salicylic acid (ASA) (0.1 mg/mL). The control sample consisted of 0.5 mL of RBCs mixed with hypotonic-buffered saline alone. The mixture was incubated for 10min at room temperature, centrifuged for 10min at 3000g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of either haemolysis or membrane stabilization was calculated using the following equation:

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

Where, OD₁= optical density of hypotonic-buffered saline solution alone (control)

OD₂= optical density of test sample in hypotonic solution

Heat-induced haemolysis

Isotonic buffer containing aliquots (5 mL) of the different extractives were put into two duplicate sets of centrifuge tubes. The vehicle, in the same amount, was added to another tube as control. Erythrocyte suspension was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 56°C for 30 min in a water bath, while the other pair was maintained at (0-5)°C in an ice bath. The reaction mixture was centrifuged for 5min at 2500g and the absorbance of the supernatant was measured at 560 nm. The percentage inhibition or acceleration of hemolysis in tests and was calculated according to the equation:

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

Where, OD₁= optical density of unheated test sample

OD₂= optical density of heated test sample

OD₃= optical density of heated control sample

RESULTS AND DISCUSSION

Membrane stabilizing activity

In the study of membrane stabilizing activity, the bark extracts of *Erythrina variegata* at concentration of 1.0mg/mL were tested against the lysis of human erythrocyte membrane induced by hypotonic solution as well as heat, and compared with the standard acetyl salicylic acid (ASA) (table 1). For hypotonic solution induced haemolysis, at a concentration of 1.0mg/ mL, the ethanol extract inhibited 78.92% haemolysis of RBCs as compared to 85.42% produced by acetyl salicylic acid (0.10mg/mL). The methanol and chloroform soluble extractives also revealed good inhibition of haemolysis of RBCs. On the other hand, during heat induced condition different organic soluble materials of *Erythrina variegata* demonstrated 75.68%, 77.19% and 73.83% inhibition of haemolysis of RBCs, respectively whereas ASA inhibited 79.04%. To confirm the membrane stabilizing activity of *Erythrina variegata* of the above mentioned model, experiments were performed on the erythrocyte membrane. A possible explanation of the stabilizing activity of different extractives due to an increase in the surface area/volume ratio of the cells which could be brought about by an expansion of the membrane or shrinkage of the cell and an interaction with membrane proteins. The present investigation suggests that the membrane stabilizing activity of *Erythrina variegata* may be playing a significant role in its anti-inflammatory activity [8].

Table 1: Effect of extractives of *Erythrina variegata* on hypotonic solution and heat induced haemolysis of erythrocyte

Samples	Concentration (mg/mL)	% Inhibition of Haemolysis	
		Hypotonic solution	Heat induced
Control	50mM	-	-
Acetyl salicylic acid (ASA)	0.1	85.42±0.025	79.04±0.033
Methanol extract	1	72.47±0.018	75.68±0.041
Ethanol extract	1	78.92±0.023	77.19±0.065
Chloroform extract	1	63.97±0.004	73.83±0.052

Values are mean ± SEM (n=6)

CONCLUSION

In the light of the results of the present study, it can be concluded that the plant extract of *E. variegata* possesses moderate membrane stabilizing. This finding justifies the use of *E. variegata* in folk medicine to treat inflammation. However, chemical studies are required to isolate the bioactive compounds and elucidate the precise molecular mechanisms responsible for the pharmacological activities of the plant.

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