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Characterization of phytoconstituents and potential bioactivity of *Annona reticulata* L. leaf extract

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Abstract
Annona reticulata (Annonaceae) is known as atafol in Bangla and custard apple in English is an important medicinal plant. It has been used traditionally in diarrhea, dysentery, cold, as abortifacient, insecticidal drug etc. In this present study, the leaf extracts of *Annona reticulata* were subjected to evaluation of the thrombolytic activity was assessed by using human erythrocyte and the results were compared with standard streptokinase (SK). The chloroform extract showed 36.82% clot lysis as compared to 93.79% clot lysis produced by standard streptokinase. The leaf extracts of *Annona reticulata* 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 chloroform extract inhibited 75.15% haemolysis of RBCs as compared to 72.99% produced by acetyl salicylic acid (ASA) and during heat induced condition different organic soluble materials of *Annona reticulata* demonstrated methanol, ethanol and chloroform extract showed 68.81%, 72.06% and 78.17% inhibition of RBC hemolysis respectively whereas standard acetylsalicylic acid showed 70.87% inhibition of RBC haemolysis.

Keywords: *Annona reticulate*, leaf extract, phytoconstituents, Membrane stabilizing, Thrombolytic activity.

Introduction
Annona reticulata is commonly called custard apple (Atafol) in Bangladesh. It belongs to the family Annonaceae. *A. reticulata* is commonly found throughout Bangladesh. Plant parts of *A. reticulata* are used in the folk medicinal system of Bangladesh for treatment of epilepsy, toothache, tumor, fever and dysentery [1]. Its leaves are medicinally used as a vermifuge, as paste on boils, abscesses and ulcers. The leaves have insecticidal and antifeedant properties [2]. The phytochemical and pharmacological activities of *A. reticulata* components suggest a wide range of clinical application in lieu of cancer chemotherapy [3]. Most important phytochemicals are alkaloids, tannins, flavanoids and phenolic compounds [4]. Scientific investigations have shown that the crude extract possesses anxiolytic [5], miticidal [6], antifeedant [7] and antidiabetic [8, 9] activities. In spite of the numerous medicinal uses attributed to this plant, pharmacognostic information about this plant has not been published. Hence the present investigation is an attempt to study the thrombolytic and membrane stabilizing activities of the leaf extracts of *A. reticulate*.

Materials and Methods
Collection and Processing of Plant Samples: Plant sample of *Annona reticulata* was collected from University of Dhaka in July, 2014 and a plant sample was submitted to the Bangladesh National Herbarium for identification. Leaves were sun dried for seven days. The dried leaves 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 (20 gm) were successively extracted in a soxhlet extractor at elevated temperature using 400 ml of distilled methanol (40-60) °C

which was followed by ethanol, and chloroform. After extraction all extracts kept in refrigerator at 4 °C for future investigation with their necessary markings for identification.

Photochemical Screening: Different extracts were screened for the presence of phenols, flavonoids, tannin, saponin, alkaloids, glycosides, phytosterols and carbohydrate by using standard protocols [10].

Streptokinase (SK): Commercially available lyophilized alteplase (Streptokinase) vial (Popular pharmaceutical Ltd.) of 15, 00,000 I.U, was collected and 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for *in vitro* thrombolytic activity evaluation.

Blood Sample: Blood (n=6) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and 1ml of blood was transferred to the previously weighed micro centrifuge tubes and was allowed to form clots.

Thrombolytic Activity: The thrombolytic activity of all extracts of the plants was evaluated by the method developed by Prasad *et al*., (2006) [11] with slight modification [12], streptokinase (SK) as the standard.

Membrane Stabilizing Activity: 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 [12]. The membrane stabilizing activity of the extractives was assessed by using hypotonic solution and heat induced methods [13]. To prepare the erythrocyte suspension, whole blood was obtained from healthy human volunteer and was taken in syringes containing anticoagulant EDTA (3.1% Na-Citrate). The blood was centrifuged and blood cells were washed three times with solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4) through centrifugation for 10 min at 3000 g.

Hypotonic Solution-Induced Haemolysis: The test sample consisted of stock erythrocyte (RBC) suspension (0.50 mL) mixed with 5 mL of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing either the extracts (1.0 mg/mL) or acetyl salicylic acid (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 10 min at room temperature, centrifuged for 10 min at 3000 g and the absorbance of the supernatant was measured at 540 nm using UV-VIS spectrophotometer. The percentage inhibition of either haemolysis or membrane stabilization was calculated using the following equation:

% inhibition of haemolysis = 100 x (OD_1-OD_2/OD_1).

Heat- induced Haemolysis: Isotonic buffer containing aliquots (5 ml) of the different extracts 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 test tube and mixed properly. One pair of the tubes was incubated at 54 ^{0}C for 20 min in a water bath, while the other pair was maintained 0 ^{0}C to 5 ^{0}C in an ice bath. The reaction mixture was centrifuged for 3 min at 1500g and the absorbance of the supernatant was measured at 560 nm using UV-VIS spectrophotometer. The percentage inhibition or acceleration of hemolysis in tests was calculated according to the equation:

% Inhibition of hemolysis = 100 x [1- (OD_2-OD_1/ OD_3-OD_1)]

Where, OD_1 = optical density of unheated test sample
OD_2 = optical density of heated test sample
OD_3 = optical density of heated control sample

Results and Discussion

Phytochemical Screening: The leaves of *Annona reticulata* showed either presence or absence of different phytochemicals. The results are listed below in the table 1.

Table 1: Results of chemical group test of various leaf extracts of *Annona reticulata*

Name of test	Name of the extracts		
	Methanol	Ethanol	Chloroform
Alkaloids	-	+	+
Flavonoid	-	-	-
Carbohydrate	+	+	+
Glycoside	-	-	+
Tannin	+	+	+
Steroid	+	+	+
Phenol	-	+	+
Saponin	+	+	+

[+ = presence in bioactive compound, - = absence]

***In Vitro* Thrombolytic Effect:** Investigation of the thrombolytic activity of the *Annona reticulata* leaf extracts were carried out using a simple and rapid *in vitro* clot lysis model. On the basis of the result obtained in the present study we can say that the *Annona reticulata* leaf extracts have some thrombolytic activity compared with the standard. Streptokinase used as a standard showed 93.79% clot lysis whereas methanol, ethanol and chloroform extracts showed 30.01%, 32.91% and 36.82% of clot analysis respectively (figure 1).

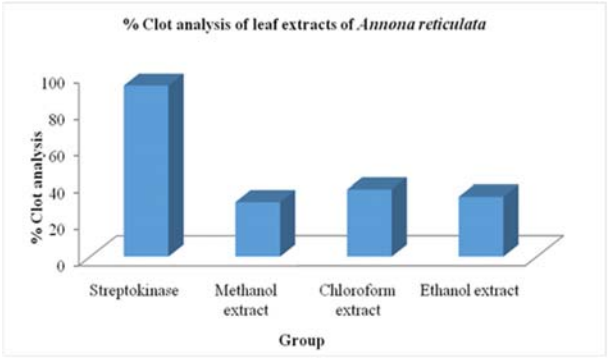

Fig 1: Percentage of clot analysis of leaf extracts of *Annona reticulata*

Membrane Stabilizing Activity: In hypotonic solution induced haemolysis, and standard Acetylsalicylic Acid (0.1 mg/ml) showed 72.99% inhibition of RBC haemolysis whereas the methanol extract, ethanol extract and chloroform extract, at a concentration of 1 mg/ml, showed 65.39%, 67.41% and 75.15% inhibition of RBC hemolysis respectively (figure 2). The results revealed that although all

the leaf extracts have very good potential of membrane stabilizing activity, chloroform extract showed higher % of inhibition of haemolysis. The mode of action of the extract and standard anti-inflammatory drugs could be connected with binding to the erythrocyte membrane with the subsequent alternation of the surface changes of the cells. This might have prevented physical interaction with aggregation agents on promote dispersal by mutual repulsion of like charges which are involved in the haemolysis of red blood cell. It has been reported that certain flavonoids excreted profound stabilizing effect on lysosomal membrane both *in vivo* and *in vitro* while tannins and saponins posses stabilizing erythrocyte membrane and other biological macromolecules [14]. It was noted that ethanol extracts showed the highest membrane stabilities effect due to the presence of flavonoid. The lowest membrane stabilizing activities observed with chloroform extract due to the presence of other phytochemical constituents which mask the action of membrane stabilizing activities by other phytoconstituents. On the basis of these results, it could be inferred that the extracts of *Annona reticulata* capable of stabilizing bovine red blood cells membranes against hypotonic induced lyses. The plant therefore could be regarded as a natural source of membrane stabilizers and was capable of providing an alternative remedy for the management and treatment of inflammatory related disorders and diseases. On the other hand, in heat induced haemolysis, methanol extract, ethanol extract and chloroform extract showed 68.81%, 72.06% and 78.17% inhibition of RBC hemolysis respectively whereas standard acetylsalicylic acid showed 70.87% inhibition of RBC haemolysis. Ethanol and chloroform extracts showed better potential in heat induced haemolysis that is even higher than the standard (figure 3).

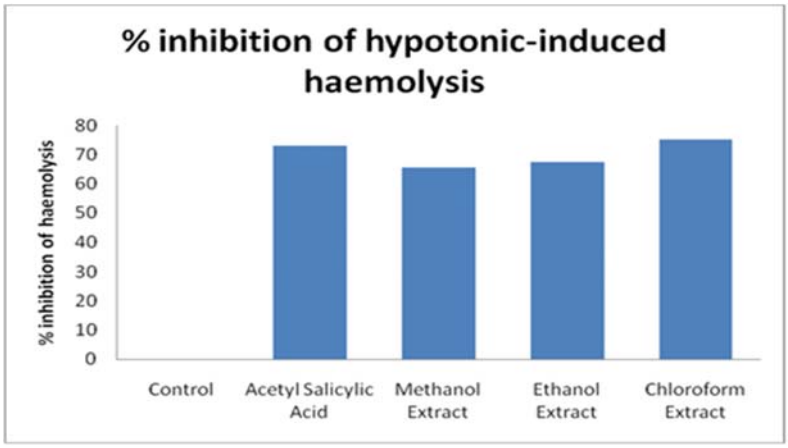

Fig 2: Percent inhibition of hypotonic induced haemolysis

As lysosomal membrane stabilization contributes to protect cells from inflammation, the present investigation suggests that the membrane stabilizing activity of *Annona reticulata* leaf extracts may play a very significant role in development of anti-inflammatory drugs.

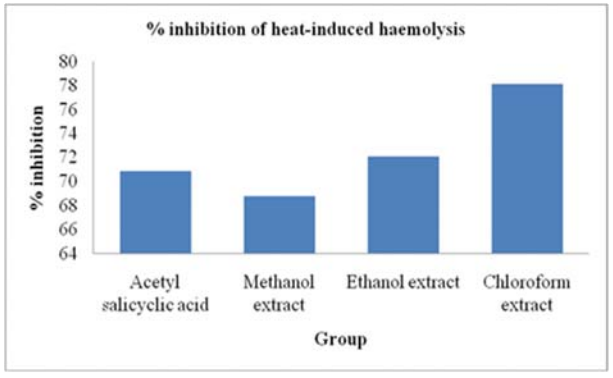

Fig 3: Percent inhibition of heat induced RBC haemolysis of *Annona reticulata* leaf

Conclusion

From this study it can be concluded that the leaf extracts of *A. reticulata* L. possess thrombolytic and membrane stabilizing activity. From the literature as well as the current study revealing the great potential of plants for therapeutic treatment. Therefore, more studies need to be conducted to search for new compounds. Additional *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential of this *A. reticulata* L. plant.

References

1. Mizanur RSK, Islam MR, Rahman S, Mosaiab T, Ahmed R, Khatun F *et al.*, Antihyperglycemic Studies with Methanol Extract of *Annona reticulata* L. (Annonaceae) and *Carissa carandas* L. (Apocynaceae) Leaves in Swiss Albino Mice. Advances in Natural and Applied Sciences 2011; 5(2):218-222, 2011.
2. Tyagi BK. Alternative control strategies for mosquito vectors of human diseases in India. In: Perspectives in Entomology Research. Agrawal OP, Ed, Scientific Publishers, Jodhpur, 1994, 359-372.
3. Suresh HM, Shivakumar B, Hemalatha K, Heroor SS, Hugar DS, Rao KRSS. *In vitro* anti-proliferative activity of *Annona reticulata* roots on human cancer cell lines. Pharmacognosy Research 2011; 3(1):9-12.
4. Krishnadev N, Meleth AD. Current opinion in ophthalmology 2010; 21(3):184-9.
5. Agrawal MN. Anxiolytic activity of Annona squamosa leaf extracts in Mice. Indian Journal of Pharma. Educ. Res. 2009; 43(1):99-104.
6. Medicinal and aromatic plants abstracts, National institute of science communication and resources, CSIR, New Delhi, India 1993; 15:41, 73, 571.
7. Medicinal and aromatic plants abstracts, National institute of science communication and resources, CSIR, New Delhi, India 2000; 22:333.

8. Medicinal and aromatic plants abstracts, National institute of science communication and resources, CSIR, New Delhi India 1999; 21:537.
9. Medicinal and aromatic plants abstracts, National institute of science communication and resources, CSIR, New Delhi India 1998; 20:532.
10. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. International Pharmaceutica Sciencia 2011; 1:103-104.
11. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Development of an *in vitro* model to study clot lysis activity of thrombolytic drugs. Thrombosis Journal 2006; 4:14.
12. Sharif S, Ahmed T, Haque MA, Bhuiyan MA, Shahriar M. Phytochemical screenings, thrombolytic activity, membrane stabilizing activity and cytotoxic properties of *Polygonum hydropiper*. Research Journal of Medicinal Plant. 2014; 8(2):92-98.
13. Shahriar M, Ahmed T, Sharif S, Akhter R, Haque MA, Bhuiyan MA. Phytochemical screenings, membrane stabilizing activity, thrombolytic activity and cytotoxic properties of leaf extracts of *Mimosa pudica*. International Journal of Pharmacy. 2014; 4(2):155-158.
14. Mannan MMM, Akhter R, Shahriar M, Bhuiyan MA. Evaluation of *In vitro* membrane stabilizing activity and thrombolytic activity of *Averrhoa bilimbi* leaf extracts. International Journal of Pharmacy. 2015; 5(2):357-360.