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# Evaluation of *in-vitro* membrane stabilization and thrombolytic activities of *Morinda angustifolia* Roxb.

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# ABSTRACT

The aim of the present study was to evaluate the in-vitro membrane stabilization and thrombolytic activities of the methanolic extracts of root and leaves of Morinda angustifolia Roxb. Assessment of in-vitro membrane stabilization activity was carried out by hypotonic solution induced hemolysis method in which both root and leaves extracts along with the standard drug acetyl salicylic acid, significantly (p<0.0001) inhibited hemolysis when compared to control. For instance, root and leaves extracts caused maximum inhibition of hemolysis of 40.22% and 21.62% respectively. In in-vitro thrombolytic activity test, 38.26% and 23.66% clots were lysed by root and leaves extract respectively which were also of statistical significance (p<0.0001).

Keywords: Morinda angustifolia Roxb., membrane stabilization activity, thrombolytic activity

# INTRODUCTION

It is well known that screening of medicinal plants with systematic approaches may be a good tool for finding and isolating bioactive lead molecules since these plants have been serving as one of the sources of new drug candidates for decades [1]. Thousands of cures have been discovered for the common ailments over years, nevertheless we are still looking for more potent but less toxic medications which ultimately drive us to conduct the conventional phytochemical research as the preliminary stage of drug discovery.

The plant *M. angustifolia* is from Rubiaceae family [2]; the family which contains plenty of medicinally important plants [3]. *M. angustifolia* is an evergreen shrub, small in size, [2] and widely available in Chittagong Hill Tracts and Cox's Bazar area of Bangladesh [4]. Local people call it Daru haridra and Rong gach [2]. Different parts of the plant have been being used for the treatment of urethritis, abdominal tumours, elephantiasis, urinary diseases, insect bites and fever in traditional medical practice [4]. In *in-vivo* model, the plant showed strong antipyretic activity [5] which vividly verifies its folkloric use. And in chemical group screening, important phytochemicals i.e. reducing sugar, alkaloids, glycoside, flavonoids, tannins, saponins and amides were found to be present [5].

The present study aims to evaluate the *in-vitro* membrane stabilizing and thrombolytic activities of methanolic extracts of root and leaves of *M. angustifolia*.

# MATERIALS AND METHODS

#### Plant materials and chemicals

*M. angustifolia* was collected from Cox's Bazar Hill Tracts, Bangladesh and was subsequently identified by Forest Research Institute (FRI), Chittagong, Bangladesh. To increase the surface area of the plant particles, these were

crushed into coarse powders using crushing mill after shade drying for 10 days. Then these coarse powders of root and leaves parts approximately weighing 120 gm, were placed in separate soxhlet extractor (Quickfit, England) for continuous hot extraction with 700 mL methanol for almost 22 hours at 45°C temperature. After extraction, filtration was carried out and the filtrate was made viscous under ceiling fan. Root and leaves parts rendered yield value of 16.45% and 18.73% respectively. All the chemicals used in the tests were of analytical grade and were purchased from *Merck*, India.

### Assessment of *in-vitro* membrane stabilization activity

The present study was conducted with minor modification of the method described by Shinde [6]. Iso-saline and hypotonic solution were prepared by dissolving 900 mg and 500 mg NaCl in 100 mL water respectively. Particular amount of iso-saline was added to the mixture of fresh human blood and RBC diluting fluid to make 10% RBC suspension. Both the standard drug and the extracts were dissolved in double distilled water. In the different marked test tubes, 1 mL of 10% RBC suspension, 1 mL test substance (e.g. standard drug, extracts) of varying concentrations and 1 mL hypotonic solution were added. In control tubes, in place of test substances, double distilled water was added. Phosphate buffer was used to adjust the pH ( $7.4\pm0.2$ ) of the solution mixture. The tubes were placed in a water bath at a temperature of 56°C for 30 min. Then the reaction mixtures were subjected to centrifugation at 2500 rpm for 5 min and after subsequent filtration the absorbance of the supernatant fluids was spectrophotometrically measured at 556 nm. Three times repetition of the test was done. The percentage inhibition of hemolysis was calculated as follows:

% inhibition =  $(A_{control} - A_{sample}) \times 100/A_{control}$ 

#### Assessment of in-vitro thrombolytic activity

The thrombolytic activity of root and leaves extracts was evaluated following a standard method [7] in which streptokinase (SK) was used as positive control. 5 mL of venous blood was drawn from healthy volunteers, which was distributed in five different previously weighed alpine tubes (0.5 mL/tube) and the tubes were incubated for a period of 45 minutes at 37°C. After 45 minutes when clot formation occurred, the serum portion was removed completely without hampering the clot and each tube was weighed again to determine weight of the clot formed in the alpine tubes (clot weight = weight of clot containing tube – weight of tube alone). The extracts were dissolved in methanol to prepare extract solutions. To the previously marked alpine tubes containing clot of definite weight, 100  $\mu$ L of streptokinase (SK) and methanol were added as positive and negative control to the marked tubes. All the tubes were then incubated for a period of 90 minutes at 37°C and observed for clot lysis. After breakdown of clot. The percentage of clot lysis was estimated from the differences in weights measured before and after clot lysis.

% of clot lysis = (wt of released clot /clot wt)  $\times$  100

#### Statistical analysis

The results obtained from the tests were expressed as the mean  $\pm$  SEM and data were analyzed using one-way ANOVA followed by using Dunnett's t-test. Windows version of SPSS software 20.0 was used to carry out the statistical analysis. A difference was considered significant at p<0.0001.

#### **RESULTS AND DISCUSSION**

#### In-vitro membrane stabilization activity

In *in-vitro* membrane stabilization activity test as shown in **Table 1**, root extract at concentrations of 500  $\mu$ g/mL, 250  $\mu$ g/mL and 125  $\mu$ g/mL showed mean inhibition of hemolysis of 40.22%, 29.84% and 19.57% respectively. In contrast, Leaves extract showed mean inhibition of hemolysis of 21.62%, 17.19% and 9.73% at concentrations of 500  $\mu$ g/mL, 250  $\mu$ g/mL and 125  $\mu$ g/mL respectively while in the same concentration range acetyl salicylic acid showed mean inhibition of hemolysis of 73.30%, 69.41% and 60.43%.

The membrane stabilizing activity of the plant extracts actually accentuates their possible anti-inflammatory potentials. A variety of disorders are initiated by the lysosomal enzymes which are found to be released during inflammation. It is believed that there is a relation between the extracellular activity of these enzymes and acute or chronic inflammation. The anti-inflammatory agents show their actions through the inhibition of cyclooxygenase enzyme which induces the conversion of arachidonic acid into prostaglandins (PG) [8]. The non-steroidal drugs (NSAIDs) show their activity either by restraining these lysosomal enzymes or causing stabilization of the lysosomal membranes [9]. Since human red blood cell (HRBC) membrane is found to be similar to that of lysosomal

membrane components, the prevention of hypotonicity induced HRBC membrane lysis by any substance to a particular extent is taken as an estimation of anti-inflammatory activity of that substance [10].

Another probable delineation of the membrane stabilizing activity of plant extracts maybe due to an increase in the surface area/volume ratio of the erythrocytes which could be brought about by an expansion of the membrane or shrinkage of the cell and an interaction with membrane proteins. The findings of the present study suggest that the membrane stabilizing activity of *M. angustifolia* may be playing a significant role in its anti-inflammatory activity [11].

Test group(s)	Concentrations	%MIHLs		
Control (DDW)		0		
	500 µg/mL	$73.30 \pm 0.08^{a}$		
Positive control (ASA)	250 µg/mL	$69.41 \pm 0.04^{a}$		
	125 µg/mL	$60.43 \pm 0.07^{a}$		
Root Extract	500 µg/mL	$40.22 \pm 0.88^{a}$		
	250 µg/mL	$29.84 \pm 0.43^{a}$		
	125 µg/mL	$19.57 \pm 0.55^{a}$		
	500 µg/mL	$21.62 \pm 0.18^{a}$		
Leaves Extract	250 µg/mL	$17.19 \pm 0.35^{a}$		
	125 µg/mL	$09.73 \pm 0.47^{a}$		
Values are expressed as mean $\pm$ SEM, <sup>a</sup> p<0.0001, MIHLs = mean inhibition of hemolysis, ASA=Acetyl Salicylic Acid				

1 able 1: <i>in-vitro</i> membrane stabilization activity of test groups	Table 1: in-	<i>vitro</i> membrane	stabilization	activity of	test groups
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#### **Treatment groups**



#### In-vitro thrombolytic activity test

In case of *in-vitro* thrombolytic activity test as shown in **Table 2**, positive control Streptokinase (100  $\mu$ L) and negative control methanol (100  $\mu$ L) caused clot lysis of 81.54% and 1.94% respectively. Root and leaves extracts both at a concentration of 500  $\mu$ g/100 $\mu$ L caused clot lysis of 38.26% and 23.66% respectively.

Thrombosis meaning the blood clot formation inside a blood vessel, obstructs the blood flow through the circulatory system. As a response of vascular injury, the body forms blood clot using thrombocytes and fibrin for the prevention of blood loss [12] but ultimately results in complications such as myocardial infarction, pulmonary embolism and deep vein thrombosis [13]. Thrombolytic medications such as streptokinase also known as fibrinolytics [14] are said to produce extra plasmin in blood, which breaks down fibrin, thereby lysing clots [15]. The plant extracts under investigation might have caused clot lysis by following this very mechanism.

Test groups	% clot lysis		
Streptokinase (100 µL)	$81.54\pm3.70^{\mathrm{a}}$		
Methanol (100 µL)	$01.94\pm0.32^{\rm a}$		
Root extract (500 µg/100µL)	$38.26\pm0.65^a$		
leaves extract (500 µg/100µL)	$23.66 \pm 0.99^{a}$		
Values are expressed as mean $\pm$ SEM, <sup>a</sup> p<0.0001			

Table 2: in-vitro	Thrombolytic activity	of test groups
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Figure 2: Graphical representation of thrombolytic activity

#### CONCLUSION

No matter how effective the conventional medications are, they are not completely free of adverse effects and these undesired effects are yet to be minimized along with achieving maximum efficacy. So we should work on synthesizing more compatible alternatives obviously considering patients' safety. The methanolic extracts of root and leaves of the plant *M. angustifolia* exhibited some promising activities in the above mentioned tests. However further researches should be conducted to isolate the active compounds and to carry out subsequent clinical trials.

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