

# Evaluation of thrombolytic, membrane stabilizing, and antioxidant activities of methanolic extract of *Tabernaemontana recurva* Roxb.



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## ABSTRACT

This study was undertaken to elucidate preliminary phytochemical and pharmacological investigations like thrombolytic, membrane stabilizing activity, and antioxidant property on methanolic extract of *Tabernaemontana recurva*. Thrombolytic test was done by using streptokinase enzyme as the standard, whereas acetyl salicylic acid used as a standard for determination of membrane stabilizing potential and antioxidant property was tabulated as gallic acid equivalent (GAE). The phytochemical screening of crude methanolic

extract of *T. recurva* possesses phenol, alkaloids, glycosides, saponins, carbohydrates, phytosterols, and tannins. Plant methanolic extract showed very negligible clot lysis (4.21%) and membrane stabilizing property (16.12%) at 10 mg/ml dose. Total phenolic content was found to have 5.89 mg of GAE/gm of extractives. We do believe this plants deserve further research, and wish to endeavor every possible source of new medicine, because, to discover cure to disease no stone should be left unturned.

**Keywords:** *Tabernaemontana recurva*, membrane stabilizing, phenolic content, thrombolytic.

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## INTRODUCTION

*Tabernaemontana recurva* Roxb., is an important medicinal plant belongs to the family of Apocynaceae. It is probably native to India and now available throughout South East Asia and the warmer regions of continental Asia and this is an evergreen shrub.<sup>1</sup> This plant is well known and widely used by the ethnobotanics medicine experts (kibiraj) of Chittagong Hill Tracts. They are found to be useful as an antihelmintic, antipyretic, anti-diarrheal, eye tonic, and also good for the teeth, especially in case of dental caries.<sup>2</sup> The plant has found to contain a variety of alkaloids, including tabernaemontanine, coronarine, coronaridine and dregamine.<sup>3</sup>

Modern molecular biological system succeed to developed several effective antioxidant mechanisms to overcome from the harmful effects of free radicals.<sup>4</sup> Antioxidants are naturally confined bioactive molecules that known to inhibit oxidation of biological macromolecules even at relatively small concentrations and thus responsible for diverse physiological roles in the biological systems. Antioxidants have free radical scavenging activity through converting more reactive species to less reactive species and escape biological organelles from oxidative damages.<sup>5</sup> The recent growth in knowledge of antioxidants in biology is producing a great revolution in medical science with a promises

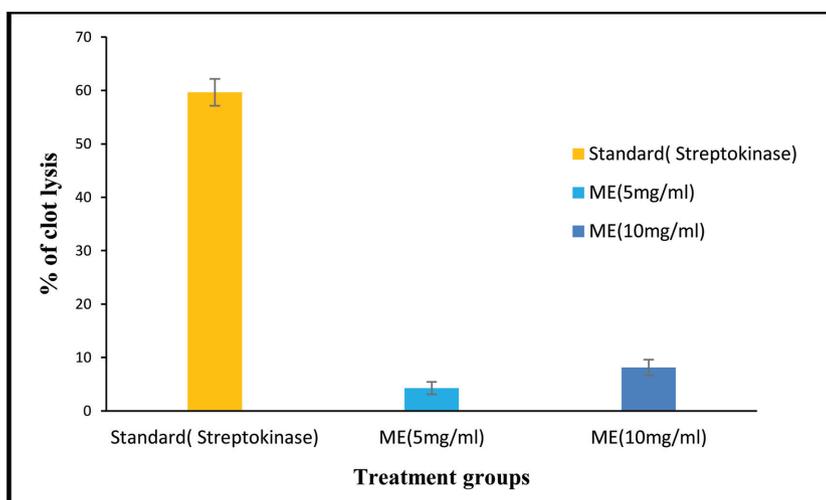
for a new age of health. Reactive oxygen species are increasingly recognized as the responsible for many human diseases, including chronic arterial disease, cancer, and ageing.<sup>6</sup> However, recent synthetic antioxidants has concern with their safety and toxicity profile<sup>7</sup> thus naturally occurring antioxidants, because of lack of toxicity and adverse effects attracted more attention.<sup>8</sup> Phenolic acids are the major phenol compounds in the extract.<sup>9</sup> Phenolic compounds includings, phenolic acids, flavonoids, diterpenes and tannins have taken attention for their high antioxidative activity.<sup>10</sup>

A process of maintaining the integrity of biological membranes such as erythrocytes (RBCs) and lysosomal membranes against osmotic and heat induced lyses are widely known as membrane stabilization.<sup>11-12</sup> It is established that stabilization of lysosomal membrane limiting the inflammatory response through inhibiting the release of lysosomal constituents such as bactericidal enzymes and proteases which cause further tissue inflammation and damage upon extracellular release.<sup>13</sup> It is evidence that RBC membrane represents the lysosomal membrane. So, if the drugs effect on the stabilization of erythrocyte membrane could be resembled to the stabilization of lysosomal membranes.<sup>14</sup> Anti-inflammatory agent causes the RBCs membrane stabilization, subjected to

**Table 1** Features of various phytochemical groups present in methanolic extract of *T. recurva*

Phytochemicals	Test	Observation
Alkaloid	Hager's test	+
	Meyers test	+
Phenol	Ferric chloride test	+
Carbohydrate	Molisch's Test	-
	Benedict's Test	-
Saponines	Froth test	+
	Foam test	+
Tannin	Gelatin test	++
Flavonoid	Alkaline reagent test	+
Glycosides	Legals test	-
Phytosterols	Liebermann-Burchards test	+
Terpenes	Copper acetate test	+
Fats and Fixed oils	Saponification test	-

“++” indicating strongly present, “+” indicating weakly present and “-” indicating absent

**Figure 1** Thrombolytic activity of methanolic extract of *T. recurva*

hypotonic stress, through the release of hemoglobin from RBCs. Therefore, the stabilization of RBCs hypotonic solution induced condition represent useful technique for the assessing the anti-inflammatory activity of various plant extractives.<sup>15</sup>

Like other developing countries, in Bangladesh thromboembolic disorders are become hold the major cause of death.<sup>16</sup> Most of the drugs available for treating thromboembolic disorder are seems to have some shortcomings such as, needs of large doses of prescribed drug, limited fibrin specificity, and tendency for bleeding. The test model was done by using a current, sensitive, widely used, reliable and validated technique<sup>17</sup> that was performed through limited facilities available.

Since this plants phytochemical screening showed presence of many water soluble

phytochemicals, it is very much possible that methanolic extract of this plant may have thrombolytic activity, membrane stabilizing and antioxidant activity. Thus, present study was undertaken.

## MATERIALS AND METHODS

### Chemicals

Standard for membrane stabilizing activity (acetyl salicylic acid) and for thrombolytic activity (streptokinase, 1500000 IU) was purchased from Popular Pharmaceuticals Ltd., Bangladesh. Gallic acid were obtained from Sigma Chemical Co. Ltd, (St. Louis, MO, USA). Other chemicals required for this present study were provided by pharmacology laboratory of department of Pharmacy of Noakhali Science and Technology University. All the chemical purchased for this study was in analytical grade.

### Blood specimen

Venous blood samples were drawn from 5 male healthy volunteers (age 22-24 years) without any recent history of oral contraceptive, anticoagulant therapy, anti-inflammatory therapy for membrane stabilization and thrombolytic assay. A volunteer consent form and Ethics Committee approval letter were filed up for each volunteer for future reference.

### Plant collection and extraction

Whole plant was collected from hilly forest of Balipara, Thanchi Upazila, Bandarban, Bangladesh in January, 2015 and voucher specimens for this collections (DACB 37789) were deposited in Bangladesh National Herbarium for future utility. The plants were first cleaned, cut into small pieces and air-dried for several days. The air-dried plant then powdered and soaked in 99% methanol for 15 days at room temperature with occasional shaking and stirring. It was then filtered, and the filtrate after 15 days the solution was filtered by using filter cloth and Whitman's filter paper. Then methanol was evaporated for reducing volume by using a rotary evaporator at low temperature and pressure.

### Phytochemical screening

Testing of various chemical compounds within the extract, represents the preliminary phytochemical studies. Little amount of methanolic extracts of *T. recurva* was subjected to preliminary quantitative phytochemical investigation for detection of phytochemicals like alkaloids, carbohydrates, viscous glycosides, phytosterols, proteins, flavonoids, tannins, saponins, etc. exploiting the quality ways.<sup>18</sup>

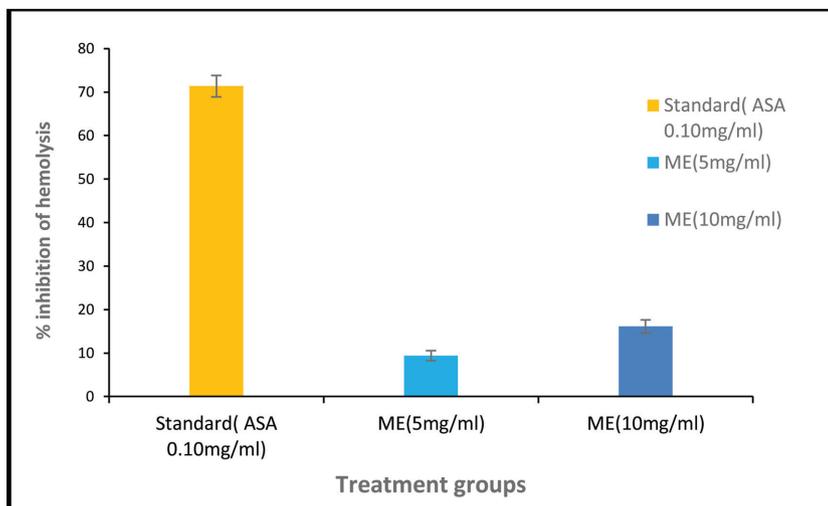
### Thrombolytic activity

The thrombolytic activity was evaluated by the method developed by Prasad et al. by using

**Table 2** Total phenolic content (TPC) determination of methanolic extract of *T. recurva*

Extract	Concentration	Absorbance at 720 nm	Average Absorbance	TPC (mg of GAE/mg)	TPC (mg of GAE / gm)
Methanol Extract	12.5	0.324	0.970 ± 0.031 (Y=0.0029x+0.1076, R <sup>2</sup> =0.8894)	5.60	5.88 ± 0.29
	25	0.641		6.65	
	50	1.081		5.99	
	100	1.833		5.27	

Values are expressed as mean ± SEM

**Figure 2** Membrane stabilizing activity methanolic extract of *T. recurva*

streptokinase (SK) as positive control with slight modification.<sup>19</sup>

### Membrane stabilizing activity

The membrane stabilizing activity of the extractives was assessed by evaluating their ability to inhibit hypotonic hemolysis of human erythrocytes following the method developed by Omale et al.<sup>15</sup>

### Total phenolic content

The total phenolic content of the extractives was determined with Folin-Ciocalteu reagent by using the method developed by Harbertson and Spayd.<sup>20</sup>

### Statistical analysis

All the results obtained by *in vitro* and *in vivo* experiments are expressed as mean ± SD. P-value were evaluated by one-way ANOVA using SPSS software, version 22.0 (IBM Corporation, New York, NY, USA). Where P<0.05 was considered as statistically significant.

## RESULTS

### Phytochemical screenings

Phytochemical constituents elucidated from the methanolic extract of *T. recurva* are presented in the Table 1.

### Thrombolytic assay

Human whole blood clot lysis activity of methanolic extract of *T. recurva* showed moderate clot lysis activity at 10 mg/dl (8.1%) and 5 mg/dl (4.27%) when compared to standard streptokinase's clot lysis potentiality (59.6%) (Figure 1).

### Membrane stabilizing activity test

The plant methanolic extract inhibited hypotonic solution induced hemolysis of RBC at 10 mg/dl (16.12%), and 5 mg/dl (9.4%) dose as comparable with the standard (71.35%) (Figure 2).

### Total phenolic content assay

Table-2 showed the antioxidant capacity of *T. recurva* methanolic extract. The result is expressed as the number of equivalents to mg of gallic acid per gram of the plant extract. The plant extract was found to have moderate amount of phenolic content that was 5.89 ± 0.29 mg GAE/gm of methanolic extract.

## DISCUSSION

The present study was carried out to investigate the thrombolytic activity of *T. recurva* in contrast with positive and negative control clearly confirmed that this plant extract may have moderate clot lysis activity. Several studies supports that plant extract possesses tannin, alkaloid, and saponin content should have thrombolytic activity.<sup>21-22</sup> As phytochemical screening of our plant methanolic extract shown to have all of this phytochemical constituent that is why our plant extract possess moderate thrombolytic activity. *T. recurva* methanolic extract inhibited hypotonic solution induced hemolysis of RBC to varying percentage that was comparable with membrane stabilizing activity shown by standard acetyl salicylic acid. As through the standard anti-inflammatory drug showed higher stabilization activity than the experimental plant methanolic extract, but our plant extract will be the existing source of anti-inflammatory activity with fewer or no side effects. The moderate membrane stabilizing activity shown by our plant methanolic extract may

be due to the presence of flavonoid contents. It has been established by many experimental study that plants with flavonoids shown profound stabilizing effects on lysosomes both *in vitro* and *in vivo* laboratory conditions.<sup>23</sup> The estimated total phenolic content of *T. recurva* methanolic extract was to have  $5.88 \pm 0.29$  mg/gm total phenol expressed as GA. Phenolic compounds in plant extracts are recognized to have antioxidant activity<sup>24</sup> and the anti-oxidant potential of plants have been attributed to their phenolic components.<sup>25</sup>

## CONCLUSION

This is an important finding, which may have importance in cardiovascular and inflammatory disease. We do believe this plants deserve further research, and wish to endeavor every possible source of new medicine, because, to discover cure to disease no stone should be left unturned.

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## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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