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Phytochemical Screenining and *in-vitro* Thrombolytic Activity of Methanolic Leaf Extract of *Zanthoxylum rhetsa.*

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Abstract:

Atherothrombosis is a major cause of global life threatening heart and cerebral diseases. Considering this, present study was designed to investigate thrombolytic activity of methanolic extract of Zanthoxylum *rhetsa* leaves. The methanolic extract was found to have significant thrombolytic activity **(25.23 ± 0.04 %)** compared to the effect of Streptokinase (**66.98 ± 0.11 %)** used as a positive control and water (**3.14 ± 0.3 1%)** used as a negative control. Preliminary phytochemical screening of the extract showed the presence of flavonoids, terpenoids and tannin were present in leaves extract of Zanthoxylum *rhetsa* one of which has thrombolytic properties.

Key Words:

Atherothrombosis, Thrombolytic activity, Streptokinase, Phytochemical screening, *Zanthoxylum rhetsa*

INTRODUCTION:

Formation of blood clot is thrombus and process is thrombosis that obstructs the flow of blood through circulatory system. Body uses platelets and fibrin to form blood clot as first step of repairing process after injury.[1] There are many drug that are used to dissolve a clot and to treat heart attack, stroke, deep vein thrombosis and occlusion of peripheral artery such as streptokinase, S-Kinase etc.[2] Circulatory platelets are aggregated to the site of injury and become the major component for thrombus development. Thrombosis is a critical stage for arterial disease associated with myocardial infarction and stroke responsible for considerable morbidity and mortality. Moreover, for cancer patients, venous thrombosis is the second leading cause of death.[3] For treatment of these disease, thrombolytic agents like tissue plasminogen activator (t-PA), Urokinase (UK), Streptokinase (SK) are used. In India among the thrombolytic agent, UK and SK are widely used.[4][5] They have high risk of hemorrhage[6]and severe anaphylactic reactions. Moreover, various treatment with SK is restricted due to immunogenicity.[7] Developing of improved recombinant variants of these drugs is disturbing due to unavailability of thrombolytic drugs.[8-13] Plants are the wide source of bioactive principles and medicine and traditional medicine is one of the primary health care system in many developing countries.[14][15]

In myocardial infarction (heart attack)[16] and pulmonary embolism, SK is used as thrombolysis medication.[17] Streptokinase belongs to fibrinolytics medication and it has three domains such as α (residues 1-150), β (residues 151-287) and γ (residues 288-414). Though each of them can't activate plasminogen but binds with plasminogen.[18] Zanthoxylum *rhetsa* (roxb.) is a deciduous tree of about 12 m tall from the Rutaceae family native to warm temperate subtropical areas worldwide. It is used to treat stomach pain, chest pain, cholera, asthma, rheumatism, etc. [19-21] Chemical constituents of Z. rhetsa include volatile oils, terpenenes (sabinene).[22]

MATERIALS AND METHODS

Plant material:

The plant *Zanthoxylum rhetsa* (roxb.) was collected from Chittagong, Bangladesh and identified by the experts at Bangladesh National Herbarium, Dhaka.

Preparation of the crude extract:

The leaves were shade dried and then ground into coarse powder with the help of a suitable grinder. The powder was taken in a clean, flat-bottomed amber glass container and soaked in methanol. The container with its contents was sealed and kept for a period of 10 days accompanying occasional stirring. The whole mixture then underwent cotton filtration followed by filtration with whatman filter paper. The filtrate was kept in an open space to evaporate the solvent thus crude extract was obtained.

Phytochemical Screening:

Phytochemical studied was carried out for identification of chemical groups present as described. [23-25]

Drugs and chemicals: Streptokinase was purchased from local market made by popular pharmaceuticals Ltd, Bangladesh.

Thrombolytic activity Test:[26]

The blood was drawn from healthy human volunteers (n=3) without a history of oral contraceptive or anticoagulant therapy and 1.0 ml of venous blood was transferred to the previously weighed micro centrifuge tubes and incubated at

37° C for 45 min and was allowed to clot. The thrombolytic activity of extract was evaluated by using streptokinase (SK) as the standard substance. The extract (100 mg) from each plant was suspended in 10 ml of distilled water and was kept overnight. Then the soluble supernatant was decanted and filtered through a 0.22 micron syringe filter. After clot formation, the serum was completely removed without disturbing the clot and each tube containing the clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). The ethical clearance for the experiment was obtained from the institutional ethical review committee and was performed by following the safe animal handling protocol. To each micro centrifuge tube with the pre-weighed clot, 100 µl aqueous solution of crude extract was added separately. Then, 100 µl of streptokinase (30,000 IU) and 100 µl of distilled water were separately added to the positive and negative control tubes, respectively. All tubes were then incubated at 37° C for 90 min and observed for lysis of clot, if any. After incubation, the released fluid was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis as shown below:

% of clot lysis, = (wt of clot after release of fluid/clot wt) × 100

RESULT AND DISCUSSION:

Results of Phytochemical Screening:

Table 1: Results of Phytochemical Screening of *Z. rhetsa*

Tested Groups	Methanolic Extract of *Z. rhetsa*
Terpenoids	+
Glycoside	+
Flavonoids	+
Tannin	-
Alkaloids	-

(Note: (+) = Indicates the presence and (−) = Indicates the absence. The tests identify the presence of Terpenoids, Flavonoids and Glycoside in methanolic extract of *Z. rhetsa*

Thrombolytic activity test:

Table 2 : Thrombolytic activity (in terms of % clot lysis) of. *Z. rhetsa*

Sample	% of clot lysis
Blank	3.4±0.31
SK	66.98±0.11***
ZR extract	25.23±0.04***

(SK = Streptokinase, ZR= *Zanthoxylum rhetsa*, Blank= Water) Data are expressed as Percentage ± SEM and ANOVA statistical significance indicates ****P*<0.001

DISCUSSION:

Addition of 100 µl SK, a positive control (30,000 IU), to the clots and subsequent incubation for 90 minutes at 37 °C, showed 66.98±0.11% lysis of clot. On the other hand, distilled water was treated as negative control which exhibited a negligible percentage of lysis of clot 3.14±0.31%. The mean difference of in percentage of clot lysis between positive and negative control was found to be statistically significant. In this study *Zanthoxylum hetsa(Roxb.)DC.* displayed highest thrombolytic activity (25.23 ± 0.04%).

CONCLUSION:

In the context of the above result and discussion it can be said that the methanolic extract of *Z. hetsa* possesses mild thrombolytic activity compared to standard streptokinase. In conclusion, further study is needed to investigate the in vivo thrombolytic activity, and the causative component(s), and mechanism for clot lysis by *Z. hetsa*

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