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Thrombolytic activity of *Lagerstroemia speciosa* Leaves

Md. Arifur Rahman Chowdhury,[1*] Md.Rasadul Islam,[2] Md.Ataul Gani Muktadir[3]

ABSTRACT

Aim: to investigate the thrombolytic activity of *Lagerstroemia speciosa* leaf extract.

Methods: thrombolytic activity assayed by an *in vitro* thrombolytic activity model performed on 10 apparently healthy subjects (both male and female), where the clot lysis ability of methanolic extract of Lagerstroemia speciosa leaves compared with Streptokinase a positive control and saline water as a negative control.

Result: Fraction of methanolic extract of *Lagerstroemia speciosa* exhibited significant percentage (%) of clot lysis of 25.42% compared to positive control streptokinase of 31.06% loss of clot while the negative control saline water showed 3.81% clot lysis ability.

Conclusion: The present investigation revealed that the methanol extracts of the leaves of *Lagerstroemia speciosa* possess potent thrombolytic properties may refer to the treatment of cardiovascular diseases.

Keywords: *Lagerstroemia speciosa (Lf)*, Thrombolytic activity, Fibrinolytic, Streptokinase

*Corresponding Author: Md. Arifur Rahman Chowdhury, Department of Pharmacy, Southeast University, Bangladesh arifur.mpharm@gmail.com

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INTRODUCTION

According to WHO-Cardiovascular diseases (CVDs) are maladies of the heart and blood vessels, including coronary heart disease and cerebrovascular disease and other dysfunctional conditions. Around 17.5 million people, an estimated 31% of all deaths worldwide from cardiovascular diseases (CVDs). In addition, most of the cardiovascular diseases (CVDs) deaths are due to strokes and heart attacks. Cardiovascular diseases are the number 1 cause of death globally more than from any other cause which increasing at an alarming rate in the recent years.[1]

The main reason of cardiovascular disease is thrombus (blood clot) that actually obstructs the blood flow by blocking the blood vessel, therefore depriving tissues of normal blood flow and oxygen that consequences yield necrosis of the tissue in those areas and can lead to acute myocardial infarction and ischemic stroke and leading to death.[2]

Other than surgical mediations to deliver a new blood supply, the only treatment obtainable is the administration of thrombolytic or fibrinolytic drug use to dissolve the blood clot or to dissolve thrombin in acutely occluded coronary arteries, thereby to restore blood supply to ischemic myocardium, to limit necrosis and to improve prognosis.[3,4]

In the management of thrombosis, Thrombolytic/fibrinolytic agents are used to dissolve the clots.[5] As tissue plasminogen activator (t-PA), Urokinase (UK), streptokinase (SK)[6] are the Thrombolytic agents are used all over the world for the treatment[7] However their use is concomitant with hyper risk of haemorrhage[8] anaphylactic reaction and lacks specificity. Remarkable efforts have been made towards the discovery and development of natural constituents from various plant and animal sources which have anti-platelet[9,10] anticoagulant[10] antithrombotic and thrombolytic activity.[11]

From that, point of views we selected *Lagerstroemia speciosa* (popularly called as "Jarul" in West Bengal, India, and in Bangladesh) belongs to the family of Lythraceae for our research interest. It is known as Pride of India, and called Queen's Flowers or Queen Crape Myrtle in English and banabá plant in the Philippines.

In the past 10 years, banaba extracts subjected for chemicals compound isolation as well as pharmacological activity that have attracted significant scientific attention.

Lagerstroemia speciosa are also known to have, diuretic, anti-obesity and anti-gout activity. The leaves extract also used in the Asian subcontinent for the treatment of diabetes and kidney disease.[12,13] previous studies evaluated the cytotoxic, antibacterial and antiviral activity and exhibited prominent activities.[14] Even the roots and flower petals of *Lagerstroemia speciosa* showed hepatoprotective activity.[15]

However, there are no established medicinal claims about the clot lysis activity of this plant. By considering all potent remedial properties, and

[1]Department of Pharmacy, Southeast University, Bangladesh
[2]Department of Pharmacy, Manarat International University, Bangladesh
[3]Department of Biochemistry, National University, Gazipur, Bangladesh.

huge range of potent chemical compounds isolation, we made attempt to investigate the methanolic extract of *Lagerstroemia speciosa* leaves for its thrombolytic ability.

METHODS AND MATERIALS

Collection & Identification

The leaves of *Lagerstroemia speciosa.* (Family: Lythraceae) were collected during the month of November 2016 from the area of Dohar, Nababganj, Dhaka Division, Bangladesh. The plant was mounted on paper and a taxonomist of Bangladesh National Herbarium (BNH), Mirpur, Dhaka taxonomically identified the species. The voucher specimen of the plant has been deposited and preserved in BNH library for further collection and reference and an accession no was provided as DACB -43543.

Plant Extract preparation

The collected leaves washed with fresh water to avoid undesirable dirt and dried under shades for a week then powdered into a coarse powder with the help of a suitable grinder .By using soxhlet apparatus about 287 gm/each of leaves powder was refluxed with methanol. The final extract collected and successively filtered through filter paper and finally got 22.88 gm of evaporated greenish extract as yield value 7.97%.

Instruments & Chemicals

Soxhlet apparatus, Eppendorf tubes, Syringe, Methanol (Analytical grade) Streptokinase (15, 00,000 I.U) - Beacon pharmaceutical Ltd, Bangladesh .Additional required chemicals (analytical grade) for this present study were provided by laboratory of department of Pharmacy.

Blood specimen collection

3ml of Blood was drawn from healthy human volunteers (n = 10 both Male & Female; Age=23-25) without a history of oral contraceptive or anticoagulant therapy for 2 weeks.

Statement on informed consent of the donors

The title of the research project, purpose of the research as well as name and detail contact of investigators was provided to the volunteer donors (students of the departments) as a consent form. Probable discomforts, damage/injuries, or difficulty associated with donors in this study was added as informed consent declaration.

Discretion statement was included in the consent form in the way that "confidentiality will be respected and no evidence that discloses the individuality of the participant will be released or published without permission. For further query about this study, detail contact of investigators was provided to the donors. The consent form was accomplished with major questions on above disclosures in Yes/NO form followed by the signature (with date) of the donor.[16]

Preparation of Positive Control (Standard) and Negative Control:

Streptokinase (15, 00,000 I.U) used as a standard whttps://lyricsbanglasong.wordpress.com/hich was collected from Beacon pharmaceutical Ltd, Bangladesh. 5mL normal saline was added to streptokinase vial and was mixed properly and marked as positive control.[17] As a negative control Saline water used in thrombolytic assay.

Preparation of Extract Solution for Thrombolytic Test

10 mg of the extract was suspended in 10ml distilled water and shaken vigorously on a vortex mixer. Then the suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a filter paper .The solution, was then ready for *in vitro* evaluation of clot lysis activity.

Statistical Analysis

All the obtained data from in vivo experiments were analyzed using SPSS Software (SPSS for Windows, Version 20.0, IBM Corporation, USA) and Microsoft Office Excel 2007. All values are expressed as mean ± SEM. P value evaluated by One-way ANOVA. Data are expressed as mean ± standard deviation. The mean difference between positive and negative control was considered significant at $p < 0.05$.

Test Procedure for *In vitro* thrombolytic activity test:

For the healing of acute myocardial infarction (heart attack), pulmonary embolism, and arterial thrombo-embolism, streptokinase performed as a thrombolytic agent. Streptokinase is an enzyme originated from Gram-positive bacteria -*Streptococcus.* It forms a complex with plasminogen, which then converts plasminogen to plasmin. Plasmin breaks down clots as well as fibrinogen and other plasma proteins.[18]

The thrombolytic activity of the extract was evaluated by comparing with streptokinase (Plasmin activator) as the standard. Empty Eppendorf tubes were weighed as W_1. From the collected blood sample 500µL of fresh blood were transferred to the pre weighed Eppendorf tubes (500µL/tube) and incubated at 37°C for 45 minutes for the development of clot, the pale yellow plasma fluid or serum was completely removed by the syringe, without the disruption of

clot. The tube with the removed serum having the clot was again weighed as W_2.

Clot weight W_C = Weight of clot containing tube W_2 – Weight of tube alone W_1

The clot containing eppendorf tubes was appropriately labeled. 100µL of the methanolic extract solution as experimental solution, 100µL of streptokinase (1500000 I.U/ml) as positive control, and as negative control 100µL of saline were added to the labeled tubes.

After incubation for 90 minutes at 37°C, observed for the lysis of clot. The released fluid were withdrawn from each tubes and weighed as W_{RC}" to see 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 = (Weight of released clot R_C /Clot weight W_C) × 100

Or

Percentage clot lysis = (weight of the clot after lysis by sample and removal of serum/weight of the clot before lysis by sample) ×100.

Result of Thrombolytic Assay

To evaluate Thrombolytic effect addition of 100µl Streptokinase, (as positive control) saline water (negative control) and *Lagerstroemia speciosa* (Lf_1) to the clots along with 90 minutes of incubation at 37°C, showed (31.08 ± 1.85)%, (25.42 ± 2.98)% and (3.80±1.07)% clot lysis activity respectively.

Which is tabulated in (Table: 1 and Figure: 1)

The mean differences between positive control, negative control and sample ware considered statistically significant at $p < 0.05$ level.

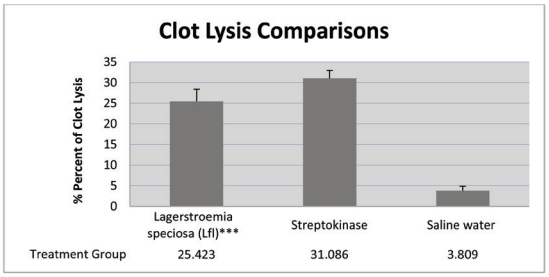

Figure 1 Comparative thrombolytic (as Clot lysis percentage (%)) effect of *Lagerstroemia speciosa* (Lf_1): 25.42%, streptokinase: 31.086% and Saline water: 3.809%

Table 1 **ANOVA done in SPSS Version 20.0 followed by Dunnet T Test; ***indicate P < 0.05; values are expressed as Mean ± SD (N=10)**

	Lagerstroemia speciosa (Lf_1)				Streptokinase				Saline Water			
SL No	W_C	W_{RC}	R_C	% of Clot lysis = R_C/ W_C ×100	W_C	W_{RC}	R_C	% of Clot lysis=R_C/ W_C ×100	W_C	W_{RC}	R_C	% of Clot lysis = R_C/ W_C ×100
1	0.306	0.221	0.085	27.78	0.352	0.238	0.114	32.38	0.523	0.511	0.012	2.29
2	0.458	0.341	0.117	25.55	0.472	0.329	0.143	30.29	0.486	0.471	0.015	3.08
3	0.446	0.338	0.108	24.22	0.533	0.377	0.156	29.27	0.477	0.463	0.014	2.94
4	0.523	0.418	0.105	20.01	0.387	0.265	0.122	31.52	0.382	0.361	0.021	5.49
5	0.378	0.265	0.113	29.89	0.365	0.243	0.122	33.42	0.411	0.394	0.017	4.13
6	0.413	0.324	0.089	21.55	0.438	0.318	0.120	27.40	0.499	0.484	0.015	3.01
7	0.467	0.354	0.113	24.20	0.331	0.223	0.108	32.63	0.365	0.351	0.014	3.68
8	0.344	0.251	0.093	27.03	0.298	0.201	0.097	32.55	0.299	0.283	0.016	5.35
9	0.399	0.292	0.107	26.82	0.378	0.259	0.119	31.48	0.320	0.305	0.015	4.68
10	0.298	0.217	0.081	27.18	0.361	0.253	0.108	29.92	0.378	0.365	0.013	3.44

			95% Confidence	
	Mean ±SD	SEM	Lower Bound	Upper Bound
Lagerstroemia speciosa (Lf_1)	25.42 ± 2.98***	0.945	23.28	27.56
Streptokinase	31.08±1.85	0.585	29.76	32.41
Saline Water	3.80±1.07	0.340	3.03	4.57

Notes: W_C= Wt of Clot; W_{RC}= Wt of clot after incubation with applied sample; R_C = Wt loss after administration. *** = Level of significance; sample ***Lagerstroemia speciosa*** (Lf_1) leaves showed significant clot lysis ability in compared with positive control (Streptokinase) and negative control (Saline Water)

DISCUSSION

Plants derived chemical compounds used for mitigating various diseases from the ancient era. Today's pharma industry invests millions for drug development purpose, whereas many drugs have origins to plant sources. A distinct number of plant-originated compounds having anticoagulant, antiplatelet and fibrinolytic activity and leads to prevention of coronary events and stroke.

Thrombo embolic disorders such as pulmonary emboli, deep vein thrombosis, strokes and heart attacks are the main causes of morbidity and mortality in developed countries. All available thrombolytic agents still have a significant shortcoming, which actually indicate the necessity of safest remedy like Plant-based drug. Current phyto-pharmacological studies propose remedial estimations of these natural preparations, including glowering of blood pressure and lipids, thrombolytic activity and the promotion of microcirculation.[19,20]

The previous randomized research[21,22-25] claimed that *Lagerstroemia speciosa* leaves have distinct pharmacological properties like antidiabetic, analgesic, antimicrobial, antibiotic, hepatoprotective etc. The present investigation intended to assess the thrombolytic and/or fibrinolytic effect (s) of methanolic extract of chosen plants leaves.

In our *in vitro* thrombolytic assay, the comparison of positive control with negative control clearly established that clot dissolution does not enhance when saline water was added to the clot. When Lagerstroemia speciosa (Lfl) leaves extract compared with the clot lysis percentage obtained through SK and saline water, a significant (p value < 0.05) thrombolytic activity was observed.

CONCLUSION

This work has demonstrated that the leaves of *L. speciosa* possess significant ($P < 0.05$) thrombolytic potentiality, that may establish a new source of medicine for coronary heart disease and cerebrovascular treatment. However, we do believe this plant deserve further scientific approaches to authenticate to understand the exact mechanisms of such actions. Through In silico analysis, each isolated bioactive unit of *L. speciosa* leaves can be counted for precise evaluation.

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

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