

Research Article



Evaluation of *In Vitro* Thrombolytic Activity of *Bougainvillea Spectabilis* Leaf Extract

Sikandar Khan Sherwani^{1*}, Muhammad Mumtaz Khan², Arif Zubair³, Muhammad Ajmal Shah⁴, Shahana Urooj Kazmi⁵

¹Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan

²Department of Biotechnology, University of Karachi, Karachi, Pakistan

³Department of Environmental Sciences, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan

⁴Department of Pharmacognosy, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan

⁵Department of Microbiology, University of Karachi, Karachi, Pakistan

*Corresponding author's E-mail: sikander_biology@hotmail.com

Accepted on: 07-04-2013; Finalized on: 30-06-2013.

ABSTRACT

Thrombotic disorders like myocardial and cerebral infarction are fatal blood clotting related diseases. Synthetic therapeutics used in such disorders have serious adverse effects, so there is a need to investigate some more safe natural thrombolytic agents. Present study is a preliminary work towards such endeavors. During this study analysis of thrombolytic activity of *Bougainvillea spectabilis* leaf extract using a simple and quick in-vitro clot lysis assay was performed which exhibited maximum 84.24% clot lysis at 800 µg/ml concentration in 72 hrs of incubation. Various concentrations of leaf extract i.e. 200µg/ml, 400µg/ml, 600µg/ml and 800µg/ml were tested at various time intervals including; 24hrs, 48hrs and 72hrs duration of incubation at 37^o C for observing maximum clot lysis. The result findings indicated that concentrations of leaf extract enhanced the percentage of clot lysis in dose dependent manner along with the incubation time factor. However; streptokinase SK a reference standard and water were used as a positive and negative control showed clot lysis maximum 96.63% and 41.32% in 72 hrs of incubation respectively.

Keywords: Thrombolytic activity, *Bougainvillea spectabilis*, Thrombotic disorders.

INTRODUCTION

Thrombus (blood clot) can develop in blood circulatory system because of homeostasis which causes vascular blockage that can be responsible to health threatening results. Atherothrombotic diseases like cerebral or acute myocardial infarction can also even lead to death.¹ Thrombolysis is a complex mechanism which interacts with clot components and surrounding plasma. In this interaction, plasmin, plasminogen, plasminogen activator and fibrin are involved.² The thrombolytic activity of plasma is physiologically very important.^{3,4} Usually, thrombolytic agents are anistreplase, streptokinase, alteplase, TPA (tissue plasminogen activator) and urokinase.^{1,5} All available thrombolytic agents still have significant shortcomings, including the need for large doses to be maximally effective, limited fibrin specificity and bleeding tendency. Due to shortcoming of accessible thrombolytic drugs, studies are underway to design new improved recombinants variants regimen.⁶ Aspirin and Heparin are significantly effective for activation of lysis and prevention of reocclusion.⁷ The selective antiplatelet agents and thrombin inhibitor are most potent though safety is yet a main concern. The new studies and investigations in this area will give new imminent that encourage the advancement of the ideal thrombolytic therapy.¹ The use of herbal for treatment of disease has been in practice since ancient times. Herbal medicines are considered safer due to their natural activity.⁸ It has been reported from studies that herbal products showing their thrombolytic activity significantly.⁹ With the advancement

in phytochemistry and identification of new plants compounds having significant efficacy against certain diseases, it has been proved by research conducted on herbal medicines. The anti thrombotic activity of herbs and their natural compound has reported previously.¹⁰⁻¹¹ Nyctaginaceae family of plants has significant importance regarding herbal medicines. Three species of genus *Bougainvillea* are important e.g. *B. peruviana*, *B. glabra* and *B. spectabilis* from horticultural point of view.¹² It has been reported that leaf extracts of *Bougainvillea spectabilis* have medicinal properties e.g. antiviral, anti inflammatory, antibacterial, anti-diabetic, anti-fertility potential and larvicidal.¹³⁻¹⁹ The significant effective constituent 3-o-methylchiroinositol (D-pinitol) isolated from leaves of *B. spectabilis* that has biological activity. In Northern part of Nigeria crude extract of *B. spectabilis* is still used as remedy of multiple diseases.²⁰⁻²¹ The purpose of the undertaken research study was to evaluate thrombolytic activity of *Bougainvillea spectabilis* leaves extract.

MATERIALS AND METHODS

Collection and authentication of plant material

Fresh green leaves of the plant were collected in sufficient quantity from the road city in of Karachi, Pakistan during the month of May 2012. Some of the leaves were kept in sterile plastic bag duly sealed and was sent to Museum of Natural History, Islamabad for the sake of authentication as a species of *Bougainvillea* i.e. *B. spectabilis*.



Preparation of extract

The collected leaves of *Bougainvillea spectabilis* after authentication were brought to the laboratory of Federal Urdu University of Arts, Science and Technology (FUUAST), Karachi, Pakistan. The extract was prepared in 5% concentration (5 grams of weighted *Bougainvillea spectabilis* leaves added in 100 ml of distilled water). The aqueous extract was prepared by boiling method of tea in water bath by constant agitation of for 15 minutes. After extract preparation, coarse suspended particles of tea were removed by passing through strainer and later by passing via 0.22µm filter.²² The extract was stored in refrigerator in small vials as aliquots for further use.

In vitro Thrombolytic analysis

The thrombolytic activity of *Bougainvillea spectabilis* was done by following the methods of using streptokinase (SK) as a standard reference.²³⁻²⁴

Streptokinase (SK) Solution Preparation

Commercially available lyophilized (Streptokinase) vial of 15, 00,000 I.U., was collected and then 5 ml of sterile distilled water was added and mixed properly. This suspension served as a stock from which 100µl (30,000 I.U) was used for *in vitro* thrombolysis.²⁵

Blood Collection

Whole blood (3 ml) was drawn from healthy human volunteers (n = 10) by phlebotomist without a history of oral contraceptive or anticoagulant therapy.²⁶ 500 µl of blood was transferred to each of the ten previously weighed alpine tubes to form clots.

BIOASSAY

Effect of crude leaf extract on clot lysis

Blood sample (500µl) was distributed in pre weighed sterile micro centrifuge tubes and incubated at 37°C for 90min for clot formation. After clot formation, the serum was finely and completely aspirated without disturbing the clot and the tubes were again weighed to determine the clot weight.²⁷

(Clot weight = Weight of the tube containing clot – Weight of the empty tube).

Each eppendorf tube containing clot was properly labeled and 100 µl of plant extract was added to the tubes. On the other hand, as a positive control, 100 µl of SK and as a negative non thrombolytic control, 100 µl of distilled water were separately added to the numbered control tubes. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, fluid obtained was removed and tubes were again weighed to observe the difference in weight after clot disruption.²⁷ Difference obtained in weight taken before and after clot lysis was denoted as percentage of clot lysis. The test was repeated thrice.

% of clot lysis = (wt of released clot /clot wt) × 100

Maximum clot lysis observation with respect to concentration and incubation of time

Various concentrations of leaf crude extract extracts i.e. 200µg/ml, 400µg/ml, 600ug/ml and 800µg/ml were tested at various time intervals including; 24hrs, 48hrs and 72hrs duration of incubation at 37°C for maximum clot lysis.²⁸

Table 1: *In vitro* - thrombolytic activity of control (water) and standard (Streptokinase):

	Incubation time	Clot lysis
Standard	24hrs	79.32%
	48hrs	92.22%
	72hrs	96.63%
Control	24hrs	8.85%
	48hrs	27.65%
	72hrs	41.32%

Table 2: *In vitro* - thrombolytic activity of crude extract of *Bougainvillea spectabilis*:

Concentrations of crude leaf extract	Incubation time	Clot lysis
200 µg/ml	24hrs	5.36%
	48hrs	19.64%
	72hrs	31.12%
400 µg/ml	24hrs	8.54%
	48hrs	23.77%
	72hrs	42.57%
600 µg/ml	24hrs	12.34%
	48hrs	35.67%
	72hrs	65.74%
800 µg/ml	24hrs	17.73%
	48hrs	44.69%
	72hrs	84.24%

RESULTS AND DISCUSSION

Atherothrombotic diseases like myocardial or cerebral infarction (Lee, 1995) occur owing to the development of thrombus that causes hindrance in the passage of vessels.²⁹ Even, sometimes in critical conditions, patients die due to embolism.³⁰⁻³¹ A number of earlier conducted researches indicated that herbs and natural products possessed thrombolytic activity.³² Thrombolytic agents that include tissue plasminogen activator (t-PA), Urokinase (UK), streptokinase (SK) etc. are used globally for the treatment of these diseases.³³ Currently, various thrombolytic agents in practice are being used to dissolve the clots that have already formed in the blood vessels; but these drugs have certain limitations and can lead to potential fatal consequences in some cases.³⁴



In our result findings, noticeably, concentrations of leaf extract enhanced in the clot lysis in dose dependent manner along with the incubation time factor. In another similar nature of study conducted in other species of *Bougainvillea glabra* also showed clot lysis ability; the process believed to be a complex and not known absolutely even today.²⁸

In this study, investigation of thrombolytic activity of *Bougainvillea spectabilis* leaf extract was carried out using a simple and rapid in-vitro clot lysis model. The results indicated maximum 84.24% clot lysis at 800 ug/ml concentration in 72 hrs of incubation as mentioned in Table No-2. The results indicated clearly that concentrations of leaf extract enhanced the percentage of clot lysis. However; streptokinase SK a reference standard and water were used as a positive and negative control that showed clot lysis maximum 96.63% and 41.32% in 72 hrs of incubation respectively. Some studies indicate that thrombolytic activity probably due to the diverse composition of plant extracts like phytoconstituents including rich sources of alkaloids, flavonoids, tannins and terpenoids.³⁵ Apart from natural plant product, there is also a report that marine algae having a product called Seanol (phlorotannin – active compound), possessing the ability in promotion of dissolution of intravascular blood clot via antiplasmin inhibition.³⁶ In this study, a simple model was used for figuring out the potential of our *Bougainvillea spectabilis* aqueous extract by the above mentioned method' nonetheless, there are a number of other sophisticated and modern technology available for finding out the mechanism of clot lysis by applying any agents. Among some are widely practiced like, radiolabelling of factors that are actively involved in clot lysis mechanism, Magnetic resonance imaging technique (MRI), a latest technique and ultrasound frequency.^{31, 37, 38}

CONCLUSION

This is however; a preliminary study yet indeed a valuable addition in the list of natural plant products that have been screened lately for thrombolytic activity. As, presently the entire world is in search of and designing of such molecules that could have therapeutic implication in the atherothrombotic diseases like myocardial or cerebral infarction. However; further studies ought to be conducted later to isolate and characterize the molecule that have clot lysis potential.

Acknowledgement: The authors are highly thankful to Dr. Syed Aneel Gillani, Senior Curator – Pakistan Natural History Museum for the identification of plant material.

REFERENCES

- Collen D. Coronary thrombolysis: streptokinase or recombinant tissue-type plasminogen activator, *Annals of Internal Medicine*, 112, 1990, 529–538.
- Alkjaersig N, Fletcher AP, Sherry S, The mechanism of clot dissolution by plasmin, *Journal of Clinical Investigation*, 38, 1959, 1086.
- Astrup T, Fibrinolysis in the organism, *Blood*, 11, 1956, 781–806.
- Sherry S, Fletcher AP, Alkjaersig N, Fibrinolysis and fibrinolytic activity in man, *Physiological Reviews*, 39, 1959, 343–382.
- Gesler WM. Therapeutic landscapes: medical issues in light of the new cultural geography, *Social Science & Medicine*, 34, 1992, 735–746.
- Marder VJ, Recombinant streptokinase – opportunity for an improved agent, *Blood Coagulation and Fibrinolysis*, 4, 1993, 1039–1040.
- Shannon MB, Jeffrey IW, Prevention of activation of blood coagulation during acute coronary ischemic syndromes: beyond aspirin and heparin, *Cardiovascular Research*, 41, 1999, 418–432.
- Demrow HS, Slane PR, Folts JD, Administration of wine and grape juice inhibits in vivo platelet activity and thrombosis in stenosed canine coronary arteries, *Circulation*, 91, 1995, 1182–1188.
- Giuseppina B, Cristiana L, Guido L, Piero C, Antonio LA, Daniele R, Therapeutic effect of diagnostic ultrasound on enzymatic thrombolysis. An in vitro study on blood of normal subjects and patients with coronary artery disease, *Journal of Thrombosis and Haemostasis*, 91, 2004, 1078–1083.
- Yamamoto J, Yamada K, Naemura A, Yamashita T, Arai R, Testing various herbs for antithrombotic effect, *Nutrition*, 21, 2005, 580–587.
- Elumalai A, Eswariah CM, Chowdary V, Kumar R, Anusha M, Naresh K, Screening of Thrombolytic Activity of *Bougainvillea glabra* Leaves Extract by In-Vitro, *Asian Journal of Research in Pharmaceutical Sciences*, 2(4), 2012, 134–136.
- Sheeja E, Edwin E, Amal RA, Gupta VB, Rana AC, Pharmacognostical and preliminary phytochemical studies on *Bougainvillea glabra* Choisy, *Planta Indica*, 1, 2005, 33–36.
- Andrea B, Letizia P, Fabiola O, Paola V, Luigi B, Battelli MG, New ribosome inactivating proteins with polynucleotide: adenosine glycosidase and antiviral activities from *Basella rubra* L. and *Bougainvillea spectabilis* Willd, *Planta*, 203, 1997, 422–429.
- Umamaheswari A, Shreevidya R, Nuni A, In vitro Antibacterial Activity of *Bougainvillea spectabilis* Leaves Extracts, *Advan Biol Res*, 2, 2008, 01–05.
- Saikia H, Das S, Antidiabetic action of *Bougainvillea spectabilis* (leaves) in normal and alloxan induced diabetic albino rats, *Indian Drugs*, 46, 2009, 391–397.
- Narayanan CR, Joshi DD, Mujumdar AM, Dhokne VV, Pinitol- A new Antidiabetic compound from the leaves of *Bougainvillea spectabilis*, *Current Science* 56, 1987, 139–141.
- Mishra N, Joshi S, Tandon VL, Munjal A, Evaluation of Antifertility Potential of aqueous extract of *Bougainvillea spectabilis* leaves in swiss albino mice, *International Journal of Pharmaceutical Science and Drug Research*, 1, 2009, 19–23.



18. Ali MS, Ibrahim SA, Ahmed F, Pervez MK, Colour versus bioactivity in the flowers of *Bougainvillea spectabilis* (Nyctaginaceae), Natural Product Research, 19, 2005, 1-5.
19. Joshi DD, Mujumdar AM, Narayanan CR. Anti-inflammatory activity of *Bougainvillea spectabilis* leaves. Indian Journal of Pharmaceutical Sciences, 46, 1984, 187-188.
20. Saikia H, Lama A, Effect of *Bougainvillea spectabilis* Leaves on Serum Lipids in Albino Rats Fed with High Fat Diet. International Journal of Pharmaceutical Sciences and Drug Research 3(2), 2011, 141-145.
21. Malomo SO, Adebayo JO, Arise RO, Olorunniji FJ, Egwim EC, Effects of Ethanolic Extract of *Bougainvillea spectabilis* Leaves on some Liver and Kidney Function Indices in Rats, Phytochemistry & Pharmacology-III, 17, 2006, 261-272.
22. Sherwani SK, Khan MM, Munir S, Ziaullah, Kazmi SU, Anthelmintic potential of crude extract of *Camellia sinensis* (Green tea). International Research Journal of Pharmacy (2013) (Accepted).
23. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Dagainawala HF, Effect of *Fagonia Arabica* (Dhamasa) on in vitro thrombolysis, BMC Complementary and Alternative Medicine, 7(36), 2007, 1-6.
24. Dagainawala HF, Prasad S, Kashyap RS, Deopujari JY, Purohit HJ and Taori GM. Development of an in vitro model to study clot lysis activity of thrombolytic drugs. Thrombosis Journal, 4, 2006, 14.
25. Chowdhury SN, Badrul AM, Haque ASMT, Zahan R, Mazumder ME and Haque ME, In vitro Free Radical Scavenging and Thrombolytic Activities of Bangladeshi Aquatic Plant *Aponogeton undulatus* Roxb, Global Journal of Pharmacology, 5(1), 2011, 27-32.
26. Kawsar MH, Sikder MA, Rana MS, Nimmi I and Rashid MA, Studies of Thrombolytic and cytotoxic properties of two asteraceous plants of Bangladesh, Bang. Pharm. J 14, 2011, 103-106.
27. Sarker R, Sharmin T, Chowdhury SR and Islam F, Thrombolytic Activity and Preliminary Cytotoxicity of Five Different Fractions of Methanol Extract of *Allamanda cathartica* Leaf, Journal of Applied Pharmaceutical Science, 02(07), 2012, 129-132.
28. Elumalai A, Eswariah CM, Chowdary V, Kumar R, Anusha M, Naresh K, Screening of Thrombolytic Activity of *Bougainvillea glabra* Leaves Extract by *In-Vitro*, Asian Journal of Research in Pharmaceutical Sciences, 2(4), 2012, 134-136.
29. Apu AS, Chowdhury FA, Khatun F, Jamaluddin ATM, Pathan A and Pal A, Phytochemical Screening and *In vitro* Evaluation of Pharmacological Activities of *Aphanamixis polystachya* (Wall) Parker Fruit Extracts, Tropical Journal of Pharmaceutical Research, 12(1), 2013, 111-116.
30. Wardlaw JM, Berge E, Zoppo DG, Yamaguchi T, Thrombolysis for acute ischemic stroke, Stroke 35, 2004, 2914-2915.
31. Capstick T, Henry MT, Efficacy of thrombolytic agents in the treatment of PE, European Respiratory Journal, 26, 2005, 864-874.
32. Giuseppina B, Cristiana L, Guido L, Piero C, Antonio LA, Daniele R, Therapeutic effect of diagnostic ultrasound on enzymatic thrombolysis. An *in vitro* study on blood of normal subjects and patients with coronary artery disease. Journal of Thrombosis and Haemostasis, 91, 2004, 1078-1083.
33. Ansari AV, Siddiqui HH, Singh SP, Antithrombotic and Thrombolytic activity of *Terminalia bellerica* fruit extracts, Research Journal of Pharmaceutical, Biological and Chemical Sciences 3(2), 2012, 471.
34. Mannan A, Kawser MJ, Ahmed AMA, Islam NN, Alam SMM, Emon MAEK, Gupta SD, Assessment of antibacterial, thrombolytic and cytotoxic potential of *Cassia alata* seed oil. Journal of Applied Pharmaceutical Sciences, 1(9), 2011, 56-59.
35. Dwivedi S. *Terminalia arjuna* Wight & Arn. A useful drug for cardiovascular disorders. J Ethnopharmacol (2012).
36. Sumi H, Hamada H, Nakanishi K, Hiratani H, Enhancement of the fibrinolytic activity in plasma by oral administration of NK, Acta Haematology, 84, 1990, 139-143.
37. Tsuchida SN, Ensslen S, Schafer C, Woltje M, Denecke B, Moser M, Graber S, Wakabayashi S, Koide T, Jahn DW: Enhanced blood coagulation and fibrinolysis in mice lacking histidine-rich glycoprotein (HRG). Journal of Thrombosis and Haemostasis, 2005, 3(5), 865-872.
38. Strief TW: *In vitro* simulation of therapeutic thrombolysis with microtiter plate clot-lysis assay. Clinical and Applied Thrombosis/ Hemostasis 2006.

Source of Support: Nil, Conflict of Interest: None.