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Evaluation of Thrombolytic and Cytotoxic activities of an Ornamental medicinal plant: *Byttneria pilosa*

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Abstract **Purpose:** The rapidly growing incidence of ischemic stroke caused by thrombosis of the arterial vessels is one of the major factors of death in the present world. The aim of this study was to investigate whether the chosen herbal preparations possess thrombolytic activity or not and aimed to find out its toxicity. **Methods:** An in vitro thrombolytic model was used to check the clot lysis effect of the crude extract of *B. pilosa*, streptokinase was used as a positive control and water as a negative control. In another part, we used Brine shrimp lethality bioassay method to measure the cytotoxic potency of the plant extract. **Results:** In the in vitro thrombolytic model, methanolic extract of *B. pilosa* showed significant ($p < 0.002$) clot lysis activity with 46.20 ± 2.274% when compared with positive control Streptokinase (82.60 ± 2.45%) and negative control distilled water (11.29 ± 0.677%). Other part of our study showed moderate or little bit low activity with LC_{50} of 216.7µg/ml. **Conclusions:** Our study suggests that thrombolytic activity of *B. pilosa* could be considered as very promising and beneficial for the Bangladeshi traditional medicine. Lower effects in cytotoxic activity finding may be due to insufficient quantities of toxic metabolite or antitumor component in the extract. In vivo clot dissolving property and active components of the extract for clot lysis could lead the plants for their therapeutic uses. However, further work will establish whether, the phytochemicals from this plant could be incorporated as a thrombolytic agent for the improvement of the patients suffering from diseases like atherosclerosis or embolism.

Keywords: *antitumor, atherosclerosis, B. pilosa, Brine shrimp, thrombolysis*

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1. Introduction

In the recent few years, there has been a growing interest among the investigator, researchers working with different extracts from traditional medicinal plants, to find out potential sources of new thrombolytic agents [1,2], as well as they also have equal interest on cytotoxic plant extract [3]. Working with different medicinal plants extract showed that they can lyses thrombus as streptokinase do [4,5]. Some of the plant extract also increase lethality of the cell due to their known cytotoxic effect. Brine shrimp lethality bioassay is performed for evaluating the level of toxicity according to the method of Persoone, 1980 and Goldstein et al., 1974.

If a blood clot (thrombus) developed in the circulatory system, it will cause vascular blockage and may leads to serious consequences of atherothrombotic diseases such as acute myocardial or cerebral infarction, and ultimantely leads to death. Commonly used thrombolytic agents are alteplase, streptokinase, urokinase and tissue plasminogen activator (TPA) to dissolve clots [6]. All available thrombolytic agents still have some shortcomings, including the limited fibrin specificity and bleeding tendency. Because of these shortcomings, attempts are in progress to develop better-quality recombinant alternatives of these drugs [7]. Since the ancient era, herbal preparations have been used for the management of several diseases. Herbal products are often alleged as safe [8]. Epidemiologic studies have delivered data that diets with experimentally proved anti-thrombotic effect could ease hazard of thrombosis. With the proper identification and thorough analysis of herbs and their components possessing anti-thrombotic activity, renewed herbal medicine could be established so far [9,10].

Keeping the fact to find out new molecule under consideration, we attempted to establish physiochemical standards of the plant *Byttneria pilosa* Roxbs. (Locally known as Harjora, Tribal Name, Salam Vra (Marma) and is belongs to the family of Sterculiaceae [11]. *Byttneria pilosa*, a large woody climber with grooved, strigose, branchlets. Leaves are suborbicular, palmately 3-lobed, pilose on both surfaces. The plant is very popular in tribal

community of Bangladesh like Chakma, Marma, Khumi etc. for its medicinal benefit. The root of this plant is chewed or juice is tropically applied as antidote in case of poisoning [12], stem paste is applied to boils and leaves infusion is used in bath in the treatment of scabies [13,14] (Chakma).

2. Materials and Method

2.1. Preparation of Extract [15]

B. pilosa leaves were collected from hilly region of Chittagong district of Bangladesh in May 2013 and were identified by the taxonomist Associate Prof. Md. Sheikh Bakhtiar Uddin, University of Chittagong, and Bangladesh National Herbarium, Chittagong branch. One voucher specimen was deposited in Herbarium and the accession number is 36186. After isolating the leaves parts of *B. pilosa* form collected sample, it was dried in open air, under a shed for approximately 15 days, and then the sample grounded to coarse powder with the help of suitable grinder. About 850 gm of that powder material was then soaked with 4 liter of methanol in a sealed container for another 7 days. Then the mixture was filtered through Whatman filter paper. After 7 to 8 days evaporation of methanol occurred and concentrated methanolic extract was acquired. It rendered a greenish black color. The greenish black color extract was designated as crude extract of methanol. From 900 gm of powdered *B. pilosa* finally we yields 17 gm of *B. pilosa*. Therefore, percent yield is {(17/900) X 100%} =1.89%.

2.2. Collection of Blood Sample

Whole blood sample (n=20) of 4 ml were collected from the healthy volunteers without a history of oral contraceptive or anticoagulant therapy. For each treatment, ten tubes were taken and experiment was repeated thrice. The blood was withdrawn from median cubital vein. The ethical committee of our institution (International Islamic University Chittagong) approved the whole process; the consent number was Pharm-P&D-46/07'13-04.

2.3. Volunteer's Agreement Form

An agreement form was supplied to each volunteer that contains the full explanation of our purpose and the procedure of sampling. Each of them was suggested to read it carefully and if they found disagree with any point they can quit immediately.

2.4. Test for Thrombolytic Activity

A 100 mg of the crude extracts was suspended in 10 ml of distilled water and for proper suspension; it was shaken on a vortex mixer thoroughly. The suspension kept at least for 10-12 hour and draw off the soluble supernatant through a 0.22-µm syringe filter. A 100 µl of the earlier aqueous preparation was added to the microcentrifuge tubes containing the clots to check thrombolytic activity [16].

Experiments for clot lysis were carried as reported previously [17]. Briefly, 4 ml of venous blood strained from the healthy volunteers was dispersed in nine different previously weighed sterile microcentrifuge tubes (0.5 mL/tube), three for each different groups, and incubated at 37°C for 45 minutes. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight. To each microcentrifuge tube containing pre-weighed clot, 100 µl of crude extracts was added. Same procedure was followed for positive control, 100 µl of Streptokinase and a negative control, 100 µl of distilled water. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, released fluid was removed and tubes were again weighed to observe the difference in weight after clot lysis. Difference obtained in weight taken before and after clot, lysis was expressed as percentage of clot lysis.

2.5. Test for Cytotoxic Property

Methanol extract was subjected to cytotoxic study. A 1mg of crude sample was taken and a stock solution of 1000µg/ml was prepared with dimethyl sulfoxide (DMSO). A series of solutions of different concentrations were prepared from the stock solution by serial dilution method and the concentrations were as - 1000µg/ml, 500µg/ml, 200µg/ml, 100µg/ml, 75µg/ml and 50µg/ml. Then the samples were subjected to brine shrimp lethality bioassay [18,19] for cytotoxic studies. In each test tube, containing different concentrations of test sample, 10 brine shrimp nauplii (*Artemia salina*) were added. One control group was used in this study, to validate the method as well as the result due to the activity of the test agent. DMSO was added to each of three premarked glass vials containing 5ml of simulated seawater and 10 shrimp nauplii to use as negative control group. After 24 hours, the test tubes were observed, the numbers of survived nauplii in each test tube were counted, and the results were noted. From this, the percentage of lethality of brine shrimp nauplii was calculated at each concentration for the extract.

2.6. Statistical Analysis

The significance between % clot lysis by Streptokinase and plant extracts was tested by the paired t-test analysis using the software GraphPad prism 6. Data are expressed as mean ± standard deviation. The considered significant limit was $p < 0.05$. The calculation of EC_{50} was also made using the same software.

3. Result and Discussion

3.1. Thrombolytic Assay Result

Streptokinase as positive control (100µl) after 90 minutes of incubation at 37°C it showed 82.60% clot lysis with $p<0.0002$. The water as negative control showed only 11.29% clot lysis. The methanolic extract of *B. pilosa* showed 46.20% (significant $p< 0.002$) of clot lysis. Percent clot lysis obtained after treating the clots crude extract and standard control is shown in Table 1 and graphically represented in Figure 1.

Morphological and angiographic studies have established that the formation of coagulation at sites of atherosclerotic lesions is the major cause of the development of complications of atherosclerosis, which

are at present supposed to be one of the leading causes of morbidity and mortality all over the world [20]. Thrombogenicity of the atheroma is determined mainly by the stability of a fibrous cap and contents of tissue factor that activates the clotting cataract when exposed to regular flow of blood [21]. These components work together with each other and with the blood vessel wall and under physiological conditions the blood flow to tissues is unimpaired by clotting [22]. Under pathophysiological conditions when platelets, vessel wall and plasma proteins (primary haemostasis) activated, coagulation occurs. In that situation, there is evidence of cardiological study that thrombus treated with antiplatelet agents speeds up and seems to improve survival rate [23]. Moreover, epidemiologic studies in recent years with natural product have provided evidence that experimentally proven thrombolytic/fibrinolytic agents from natural sources have capability to reduce the risk of thrombosis more than others [24,25,26,27].

Table 1. Result of thrombolytic assay

Treatment groups	Percent of clot lysis
Water	11.29 ± 0.677
Streptokinase	82.60 ± 2.45*
B. pilosa	46.20 ± 2.274**

Values presented as mean ± SD, *p<0.0002 and **p<0.002, the result obtained from two-tailed t-test and each group was compared with negative control, the extract results statistically significant when compared with water.

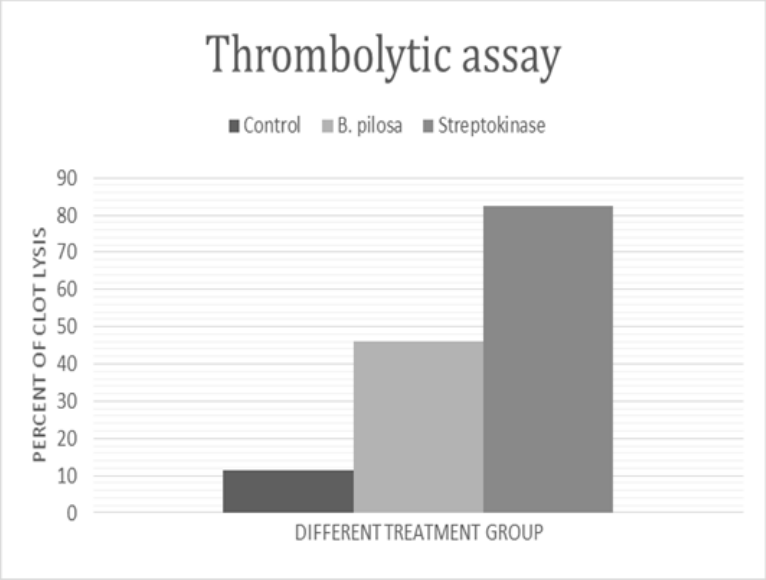

Figure 1. Graphical representation of thrombolytic assay of methanolic extract of *B. pilosa* leaves, the percent of thrombus lysed by our plant extract was 46.2% that was comparatively close to Streptokinase (82.6%)

The pharmaceuticals are available at present are approved by the Food and Drug Administration (FDA) are certainly from plant sources. Based on the reported immunomodulatory effects, the most important role that plants are playing are efficacy and safety [28,29]. In our present study, the extracts of *B. pilosa* showed the thrombolytic activity and had the significant activity. There is evidence that bacterial contaminants of plants have plasminogen receptors and they can bind plasminogen. The cell surface binds this plasminogen and easily activate to plasmin that could lead to fibrinolysis [30] though there is other plant species that could exert their thrombolytic or fibrinolytic effects via their content of certain fibrinolytic proteases enzymes. Individual chemical component-activity relationship, which can explore the other new clue for the observed thrombolytic effects of this plant part, will be the next step of the research follow-up of our continuous study.

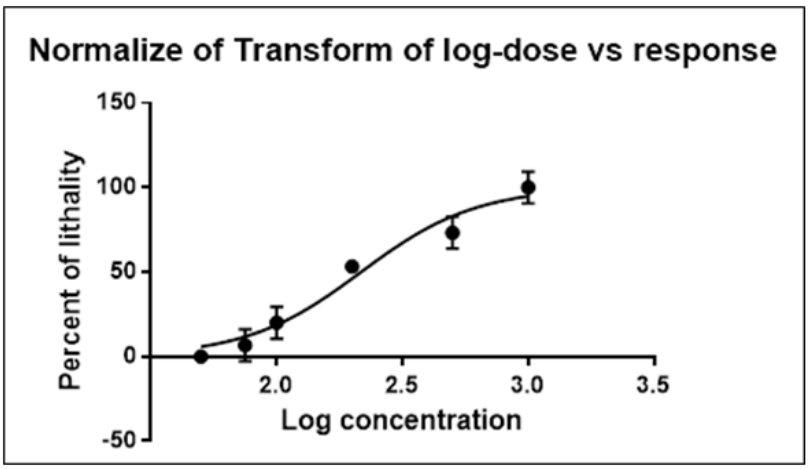

Figure 2. Schematic representation of percent of mortality done by *B. pilosa* in Brine shrimp lethality bioassay, the observed toxic concentration was 216.7µg/ml

3.2. Cytotoxic assay

In the bioassay, the methanol extracts showed lethality indicating the biological activity of the compound present in the extract. Test samples showed different mortality rate at different concentrations. For the extract, the number of nauplii died and percent of mortality were counted. The LC_{50} value for the extract was calculated from Figure 2. The observed value was not so lethal and it was 216.7µg/ml.

The brine shrimp lethality bioassay is very useful to assess the bioactivity of the plant extracts which in most cases correlates reasonably well with cytotoxic and anti-tumor properties [31]. LC_{50} values of *B. pilosa* revealed its considerable cytotoxic potency. Sufficient amount of phenolics and flavonoids may be present and it might be responsible for its promising cytotoxic activity [32,33] and the possible mechanism of cytotoxicity against brine shrimp nauplii due to poisonous effect on cell mitosis.

4. Conclusion

In the conclusion, it can be termed that the extracts of the *B. pilosa* can be used to design thrombolytic agent due to its good activity against coagulation. This extract also has minor cytotoxic effectiveness, so it is suggested to investigate for its antimicrobial property. Further work is needed to isolate the metabolites that lysed the thrombi. This *in vitro* study demonstrated that folk medicine could be as effective as modern medicine to reduce risk of cardiogenic problem as well as other risk factor like deep vein thrombosis. The study we made is primary investigation but it creates an opportunity to develop a new thrombolytic molecule.

Conflict of Interest

The authors have declared that there is no conflict of interest.

Author's Contribution

MYI designed the current project, performed the experiments and wrote the manuscript; PA carried out the experimental process; and also responsible for data interpretation, statistical analysis; NAJ helped in experiments and preparing the manuscript; SMS participated in experiments and data collection; MS edited the manuscript. All authors read and approved the final version of the manuscript.

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References

[1] Elumalai A, Eswariah MC, Chowdary VCH, Kumar R, Anusha M and Naresh K; Screening of thrombolytic activity of *Bougainvillea glabra* leaves extract by *In-Vitro*; Asian J Res Pharm Sci; 2012; 2(4):134-136.

[2] Emran TB, Rahman MA, Uddin MMN, Rahman MM, Dash R, Layzu C and Uddin MZ; Effects of organic extracts and their different fractions of five Bangladeshi plants on in vitro thrombolysis; BMC Compl Alt Med; 2015; 15:128-36.

[3] Hossain MK, Hassan MM, Parvin MN, Hasan MM, Islam MS and Haque MA; Antimicrobial, cytotoxic and thrombolytic activity of *Cassia senna* leaves (family: Fabaceae); J App Pharm Sci; 2012; 2(6): 186-190.

[4] Gennaro AR. Remington; The Science and Practice of Pharmacy; Thrombolytic agents; 20 th ed. Lippincott Williams & Wilkins; New York; 2000; 1256-1257.

[5] Sweta P, Rajpal SK, Jayant YD, Hemant JP, Girdhar MD and Hatim FD; Development of an in vitro model to study clot lysis activity of thrombolytic drugs; Throm J; 2006; 4(14):1-4.

[6] Collen D; Coronary thrombolysis: streptokinase or recombinant tissue-type plasminogen activator; Ann Intern Med; 1990; 112: 529-538.

[7] Marder VJ; Recombinant streptokinase – opportunity for an improved agent; Blood Coagul Fibrin; 1993; 4: 1039-1040.

[8] 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; 1995; 91:1182-1188.

[9] Basta G, Lupi C, Lazzerini G, Chiarelli P, L'Abbate A, Rovai D; Therapeutic effect of diagnostic ultrasound on enzymatic thrombolysis: An in vitro study on blood of normal subjects and patients with coronary artery disease; Thromb Haemost; 2004; 91: 1078-1083.

[10] Yamamoto J, Yamada K, Naemura A, Yamashita T and Arai R; Testing various herbs for antithrombotic effect; Nutrition; 2005; 21:580-587.

[11] http://www.ebbd.info/byttneria-pilosa.html; cited 6 April 2015.

[12] Islam A, Siddik AB, Hanee U, Guha A, Zaman F, Mokarroma U, Zahan H, Jabber S, Naurin S, Kabir H, Jahan S and Rahmatullah M; Ethnomedicinal practices of Chakma tribal healer practicing among a Marma community in Rangamati district, Bangladesh; World J Pharm Pharma Sci; 2015; 4(3): 180-188.

[13] Rahman MA; Indigenous knowledge of herbal medicine in Bangladesh, 3 treatment of skin diseases by tribal communities of the hill tracts districts; Bangladesh J Bot; 2010; 39(2): 169-177.

[14] Rahman MA, Uddin SB and Wilcock CC; Medicinal plants used by Chakma tribe in the hill tracts districts of Bangladesh; Indian J Trad Know; 2007; 6(3): 508-517.

[15] A. Ghani; 1st ed.; Textbook of Pharmacognosy; Institution of Medical Technology, Dhaka, Bangladesh; 2005.

[16] Rahman MA, Sultana R, Emran TB, Islam MS, Chakma JS, Rashid HU, et al; Effects of organic extracts of six Bangladeshi plants on in vitro thrombolysis and cytotoxicity; BMC Compl Alt Med; 2013; 13(25):1472-6882.

[17] Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM and Daginawala HF; Development of an in vitro model to study clot lysis activity of thrombolytic drugs; Thromb J; 2006; 4:14.

[18] Goldstein AL and Kalkan SM; Principles of Drug Action; 2nd ed; Willey Biochemical Health Publications; 1974; 376-381.

[19] Meyer BB, Ferringi NR, Futman FJ, Jacobsen LB, Nichols DE and Mclaughlin JL; Brine shrimp a convenient general bioassay for active plant constituents; Planta Medica; 1982; 5: 31-34.

[20] Libby P, Ridker PM and Hansson GK; Progress and challenges in translating the biology of atherosclerosis; Nature; 2011; 473: 317-325.

[21] Fuentes E, Guzmán L, Alarcón M, Moore R and Palomo I; Thrombolytic/fibrinolytic mechanism of natural products; Fibrinolysis and Thrombolysis; chapter 5; 2014; 107-121.

[22] Ananyeva NM; Kouiavskaia DV; Shima M and Saenko EL; Intrinsic pathway of blood coagulation contributes to thrombogenicity of atherosclerotic plaque; Blood; 2002; 99: 4475-4485.

[23] Zinkstok SM, Vermeulen M, Stam J, de Haan RJ and Roos YB; Antiplatelet therapy in combination with rt-PA thrombolysis in ischemic stroke (ARTIS): rationale and design of a randomized controlled trial; Cerebrovasc Dis; 2010; 29: 79-81.

[24] Rahman MA, Sultana R, Bin Emran T, Islam MS; Chakma JS, Rashid HU and Hasan CM; Effects of organic extracts of six Bangladeshi plants on in vitro thrombolysis and cytotoxicity; BMC Compl Alt Med; 2013; 13: 25.

[25] Yamada K, Naemura A, Sawashita N, Noguchi Y and Yamamoto J; An onion variety has natural antithrombotic effect as assessed by thrombosis/thrombolysis models in rodents; Thromb Res; 2004; 114: 213-220.

[26] Suzuki Y, Kondo K, Matsumoto Y, Zhao BQ, Otsuguro K, Maeda T, Tsukamoto Y, Urano T and Umemura K; Dietary supplementation of fermented soybean, natto, suppresses intimal thickening and modulates the lysis of mural thrombi after endothelial injury in rat femoral artery; Life Sci; 2003; 73: 1289-1298.

[27] Rajput MS, Mathur V, Agrawal P, Chandrawanshi HK and Pilaniya U; Fibrinolytic activity of kaempferol isolated from the fruits of *Lagenaria siceraria* (Molina) Standley; Nat Prod Res; 2011; 25: 1870-1875.

[28] Licciardi PV and Underwood JR; Plant-derived medicines: a novel class of immunological adjuvants; Int Immunopharmacol; 2011; 11(3): 390-8.

[29] Potterat O and Hamburger M; Drug discovery and development with plant-derived compounds; Prog Drug Res; 2008; 65(45): 47-118.

[30] Verstraete M; Third-generation thrombolytic drugs; Am J Med; 2000; 109(1):52-8.

[31] McLauglin JL, Chang CJ and Smith DL; Simple bench-top bioassays (brine shrimp and potato discs) for the discovery of plant antitumour compounds. In: Human Medicinal Agents from Plants; Kinghorn AD and Balandrin MF; (Eds.), ACS Symposium 534, American Chemical Society, Washington, D. C.; 1993: 112-137.

[32] Moreira MD, Picanço MC, Barbosa LC, Guedes RN, Barros EC and Campos MR; Compounds from *Ageratum conyzoides*: isolation, structural elucidation and insecticidal activity; Pest Manag Sci; 2007; 63: 615-621.

[33] Okwori AEJ, Dina CO, Junaid S, Okeke IO, Adetunji JA and Olabode AO; Antibacterial activities of *Ageratum conyzoides* extracts on selected bacterial pathogens; Int J Micro; 2007; 4: 1937-1949.