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A comparative study of thrombolytic effects of methanolic extract of *Bridelia stipularis* and *Aglaonema hookerianum* leaf

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Abstract
Objective: To evaluate thrombolytic activities of crude methanol extract of *B. stipularis* and *A. hookerianum* Leaf.
Methods: The thrombolytic activity was evaluated using the in vitro clot lysis model. In a brief, venous blood drawn from five healthy volunteers was allowed to form clots which were weighed and treated with the test plant materials to disrupt the clots. Weight of clot after and before treatment provided a percentage of clot lysis and compared the result with streptokinase as positive control and water as negative control.
Results: In thrombolytic study, it is found that *B. stipularis* and *A. hookerianum* showed 33.42 ± 3.37% and 24.72 ± 2.75% of clot lysis respectively. Among the herbs studied *B. stipularis,* showed very significant ($p < 0.001$) percentage (%) of clot lysis than the *A. hookerianum* compared to reference drug streptokinase (63.54 ± 2.61%).
Conclusion: The results of the study demonstrated that the leaf of the plants contains promising thrombolytic activity *in vitro* when tested on human blood. However, further study is needed to see its potentiality as a thrombolytic agent.

Keywords: *Bridelia stipularis*, *Aglaonema hookerianum*, Thrombolytic, Clot lysis, Streptokinase.

1. Introduction
Bridelia stipularis [1] also known as *Clutia stipularis L.* is a woody climbers or scandent shrubs. It is distributed in Bangladesh, China, Bhutan, Brunei, Cambodia, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Philippines, Singapore, Sri Lanka, Thailand, Timor, Vietnam [2]. In Bangladesh, it is found in forests of Chittagong, Chittagong Hill Tracts, Sylhet, Gazipur, Cox's Bazar, Sayadpur. Tribal name of this plants are familiar as Bangaribhanga gaas (Chakma), Pat khowi, So mui, Si Ooyaza (Marma) [3]. Different parts of this plant are applicable for various treatments such as the roots are used as medicine for reducing inflammation and as an astringent antidiarrheal. The fruits are used to induce vomiting and as an antitoxic [2]. Leaf extract is taken for the treatment of allergies [4] in children (Chakma). Plant is used in pleurisy and exudation. Bark decoction is given to children for cough, fever and asthma and as gargle for sores in mouth. Fresh tender leaves are used for the treatment of jaundice and anaemia due to pregnancy. Leaf powder and warm leaf poultice are applied to white spots in the skin (Marma) [5]. Another essential medicinal plant is *Aglaonema hookerianum* which is a herb. It is distributed in Bangladesh, North eastern India and Myanmar. In Bangladesh, it is found in the forests of Sylhet, Chittagong and Chittagong Hill Tracts. Vernacular name of this plant is known as *Chekhov*, *Khaichcha Parabol*, *Meggey* (Marma); *Hatharikhiethok* (Tripura), *Lykho* (Khumi) and *Gach Petic*, *Shakkosala*, *Sikkachalal* (Chakma). The species is also used in the treatment of cirrhosis, flatulence, hyper acidity (gastritis) and tetanus [6] and conjunctivitis and constipation [7]. Extracted juice of spathe of this plant is used for stomachache by various tribes such as Khumi, Marma and Tripura [8].

2. Materials and Methods
2.1 Plant collection and identification
Leaf of *B. stipularis* and *A. hookerianum* were collected from different parts of the Chittagong region, Bangladesh. The plants were identified by Dr. Shaikh Bokhtear Uddin, Taxonomist and Associate Professor, Department of Botany, University of Chittagong.

2.2 Chemicals and drugs
To the commercially available lyophilized Streptokinase vial (Durakinase, Dongkook Pharma. Co. Ltd, South Korea) of 15 00000 I.U., 5 ml sterile distilled water was added and mixed

properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for in vitro thrombolysis. Absolute methanol (99.50%) was purchased from Sigma-Aldrich, Munich, Germany.

2.3 Extract preparation
Both of the plant materials were dried and ground (Moulinex Blender AK-241, Moulinex, France) into powder (40-80 mesh, 500 g) and soaked for 7 days with 2–3 days interval in 2.0 L of ethanol at room temperature (23 ± 0.5°C). Filtrate obtained through cheesecloth and Whatman filter paper No. 1 was concentrated under reduced pressure at the temperature below 50°C using a rotary evaporator (RE 200, Sterling, UK). The extracts (yield 4.4–5.6% W/W) were all placed in glass petri dishes (90 X 15mm, Pyrex, Germany). A 100mg each of the extracts was suspended in 10 ml distilled water and the suspension was shaken vigorously on a vortex mixer. The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.22-µm syringe filter. A 100 µl of this aqueous preparation was added to the microcentrifuge tubes containing the clots to check thrombolytic activity.

2.4 Thrombolytic activity
2.4.1 Blood specimen
Whole blood (2 ml) was drawn from healthy human volunteers (n = 5) without a history of oral contraceptive or anticoagulant therapy. A 500 µl of blood was transferred to each of the three previously weighed microcentrifuge tubes to form clots.

2.4.2 Clot lysis
Experiments for clot lysis were carried as reported earlier [9]. Briefly, 2 ml venous blood drawn from the healthy volunteers was distributed in three different pre weighed sterile microcentrifuge tube (0.5 ml/tube) and incubated at 37°C for 45 min. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). To each microcentrifuge tube containing pre-weighed clot, 100 µl of methanol extracts of both plants
(*B. stipularis* and *A. hookerianum*) were added separately. As a positive control, 100 µl of streptokinase and as a negative control, 100 µl of distilled water were separately added to the control tubes numbered. All the tubes were then incubated at 37°C for 90 min and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference in weight before and after clot lysis was expressed as percentage of clot lysis as shown below:
Percent (%) of clot lysis = (Weight of released clot /clot weight) ×100
The experiment was repeated with the blood samples of the 5 volunteers.

2.4.3 Statistical analysis
The significance between percent (%) of clot lysis by streptokinase and plant extracts was tested by paired t-test analysis using the software SPSS, version 20.0 (SPSS for Windows, Version 20.0, IBM Corporation, New York, USA). Data are expressed as mean ± standard deviation. The mean difference between positive and negative control was considered significant at $P < 0.05$.

3. Results
3.1 Thrombolytic activity
Addition of 100 µl streptokinase (positive control) to the clots along with 90 minutes of incubation at 37°C, showed 63.54 ± 2.61% clot lysis. However, distilled water (negative control) treated-clots showed only negligible clot lysis (4.21 ± 0.73%). The mean difference in clot lysis percentage between positive and negative control was very significant (p value < 0.05). Treatment of clots with *B. stipularis* and *A. hookerianum* extracts provided the clot lysis 33.42 ± 3.37% and 24.72 ± 2.75%, respectively. The mean percentage of clot lysis by *B. stipularis* and *A. hookerianum* was statistically significant (p value < 0.001). *B. stipularis* showed relatively higher percentage of clot lysis than *A. hookerianum*, although the values were significant (p value < 0.001) compared to those of both positive control (streptokinase) and negative control (water). Percent clot lysis obtained after treating the clots with both methanolic extracts and appropriate controls is shown in Figure 1.

Table 1: Effect of both extracts (10 mg/ml) on in-vitro clot lysis

Treatment	% of clot lysis (Mean ± S. D.)
Streptokinase(Positive Control)	63.54 ± 2.61**
Distilled water (Negative Control)	4.21 ± 0.73**
BS	33.42 ± 3.37*
AH	24.72 ± 2.75*

Statistical representation of the effective clot lysis percentage by herbal preparations, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) done by paired t-test analysis; clot lysis % is represented as mean ± S.D. *P<0.001, **P<0.05 compared to control.

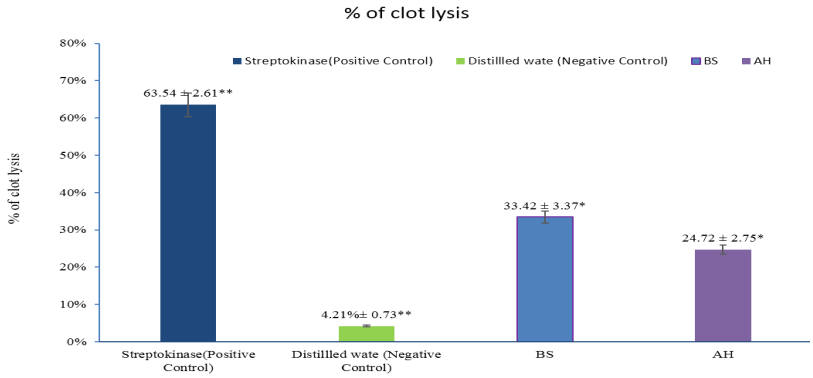

Fig 1: Clot lysis by streptokinase, water and both of methanolic extracts. Effects of drugs on dissolution of clots prepared from blood of normal individuals. Maximum clot lysis (63.54 ± 2.61%) was observed in clot treated with streptokinase (streptokinase). Both of the plants extract shows 33.42 ± 3.37% and 24.72 ± 2.75% clot lysis, respectively. Water (as a negative control) showed 4.21 ± 0.73% clot lysis. *BS=*Bridelia stipularis*, *AH= *Aglaonema hookerianum.*

4. Discussion

According to the thrombolytic study, both the methanolic plant extract showed promising activity. Compared to the clot lysis percentage obtained through streptokinase and also water, an extremely significant ($p< 0.05$) thrombolytic study was observed after treating the clots to *B. stipularis* and *A. hookerianum* extract. It is established that there are some bacterial pollutants of plants that have plasminogen receptors which certain for plasminogen. Cell surface certain of plasminogen is instantly activated to plasmin that could lead to fibrinolysis [10]. Bacterial plasminogen activator: staphylokinase, streptokinase, act as cofactor molecules that cause exosite formation and improve the substrate performance towards the enzyme. Staphylokinase activates plasminogen to be in a position to break down clots, also damages the extracellular matrix and fibrin particles that keep cell together [11-12]. From the above study, methanolic extract *B. stipularis* and *A. hookerianum* exhibited 33.42 ± 3.37% and 24.72 ± 2.75% clot lysis, respectively. In this study, *B. stipularis* showed more significant activity than *A. hookerianum*. Further work will found whether or not, phytochemicals derivative from this plant could be incorporated as a thrombolytic agent for the progress of the patients suffering from coronary atherothrombotic diseases.

5. Conflict of interest statement

We declare that we have no conflict of interest.

6. Acknowledgements

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