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EVALUATION OF THROMBOLYTIC ACTIVITY OF THREE ETHNOMEDICINAL PLANTS IN BANGLADESH

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Abstract

The current study was designed to explore the thrombolytic activity of *Allium cepa*, *Cinnamomum tamala* and *Ipomoea aquatica*. All of them showed thrombolytic activity which was determined as part of a new probe of cardioprotective drugs from medicinal plants. It showed that the addition of 100 µl of streptokinase solution as a positive thrombolytic control (30000 I.U.) to blood clots with 90 min incubation at 37ºC showed 68.79 % of clot lysis. In contrast, distilled water considered as negative non-thrombolytic control revealed insignificant level of clot lysis activity. All the tested extracts showed mild to moderate thrombolytic activity. Among these, *C. tamala* leaf extract showed highest (58.99 %) thrombolytic activity for clot lysis. Based on the outcomes, it is perceived that all the three plants revealed thrombolytic activity which is needed for further analysis to validate the active components of these extracts.

In the circulatory system, development of blood clot or thrombus due to the failure of hemostasis causes vascular blockage and severe consequences in atherothrombotic illnesses which consist of acute myocardial or cerebral infarction, at times leading to death. Cerebral venous sinus thrombosis is a typical problem with severe suffering and death (Capecchi *et al.* 2018). Commercially available thrombolytic agents are streptokinase, urokinase, alteplase and anistreplase, that can be used clinically to dissolve blood clots (Ali *et al.* 2014). Several assay procedures were developed, but the *in vitro* model involving streptokinase as standard showed superior consistency in the outcome of the experiment (Prasad *et al*. 2006). Herbal supplements obtained from various plant parts used as traditional medicines for the treatment of various ailments. Herbal products are realized as safe because they are natural and available with minimal side effects (Nasim *et al.* 2022).

Allium cepa L. is an important vegetable crop with significant medicinal, nutritional and functional properties. It is also known as onion used as spice all over the world. Phytochemically onion extract is rich in bioactive compounds such as flavonoids and organosulfur compounds. These polyphenolics are expected to exhibit a large number of beneficial effects such as antioxidant, antiplatelet, antidiabetic, anti-inflammatory properties (Nile *et al*. 2017, González-de-Peredo *et al*. 2021).

Cinnamomum tamala belongs to the family of Lauraceae. It is used as spice that possesses diuretic, carminative and stimulant action. The plant has medicinal properties that can offer health benefits in the treatment of digestive issues, infections, stress or anxiety. Phytochemical study revealed the presence of alkaloids, tannins, amino acids, reducing sugar, and steroids in the crude extract of *C. tamala* leaf (Ahmed *et al*. 2013). *Ipomoea aquatica* Forsk or water spinach belongs to convolvulaceae is a green leafy vegetable which is extensively investigated as natural antioxidant due to its capacity to reduce the risk of certain diseases such as cancer, heart diseases

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and stroke (Dewanjee *et al.* 2015). It is one of the richest sources of carotenoids and chlorophylls. The leaf extract showed potent antioxidant, cytotoxic and membrane stabilizing potential (James *et al.* 2009). Although several ethnomedicinal plants are used traditionally in cardiovascular conditions, few have been systematically evaluated for thrombolytic activity. Therefore, the objective of this study was to investigate the thrombolytic activity of these plants.

The leaves of *A. cepa, C. tamala* and *I. aquatica* were collected from Dhaka local market. The leaves of these plants were washed with clean water, leaves were cut into small pieces and then dried in the sun for several days. These dried materials were then ground into powder using a high-performance grinder and stored in a closed container at room temperature for further analysis for the experiment.

The plant materials (100 g) were placed in three clean conical flasks and soaked in 300 ml of 70% methanol. The containers and their contents were sealed with aluminum foil and stored for 5 days with occasional stirring and shaking. The entire mixture was then filtered with Whatman filter paper. Then the filtered extracts were collected and dried at low temperature using a rotary evaporator to prepare the crude extracts.

The thrombolytic activity of all the plant extracts was evaluated with a method using streptokinase (SK) as a reference standard (Prasad *et al.* 2006, Ali *et al.* 2014). First, 100 mg of crude extracts from each sample was suspended in 10 ml of 70% ethanol and the suspension was vigorously stirred with a vortex mixer. The suspension was then stored overnight in a conical flask and decanted to remove soluble supernatant, which was filtered through Whatman filter paper and stored in a beaker. Subsequently, this preparation of each plant sample was added to the microcentrifuge tubes containing the blood clots to monitor the thrombolytic activity.

During thrombolytic assay, the microcentrifuge tubes along with the blood samples were centrifuged at 2000 rpm for 5 min so that the serum could be separated by easy removal from the centrifuge tube. The centrifuge tubes were then placed at simulated body temperature, *i.e.* at 37°C for 45 min in a heat-controlled incubator. Aliquots (5 ml) of venous blood were taken from healthy volunteers (n=5) regardless of sex while maintaining an aseptic condition, which were distributed into five different pre-weighed sterile microcentrifuge tubes (l ml/tube) and incubated for 45 min at 37°C. After formation of the clot, the serum was completely removed without disturbing the clot and each microcentrifuge tube having clot was again weighed to determine the clot weight. Then, weight of clotted blood was taken by subtracting the pre-weighted (W_1) tube from the weight of clot containing tube (W_2). In each microcentrifuge tube containing a pre-weighed clot, 100 µl of aqueous solutions of different distributions were added separately with the crude extract. As a positive control, 100 µl of streptokinase (SK) stock solution and as a non-thrombolytic negative control, 100 µl of distilled water was added separately to the control tubes. All tubes were then incubated for 90 min at 37°C and observed for clot lysis. After incubation, the released liquid was removed and the tubes reweighed to observe the difference in weight after the clot was ruptured (Zaman *et al.* 2015). The difference in weight measured before and after clot lysis was expressed as a percentage of clot lysis with the formula:

$$\% \text{ clot lysis} = \frac{W_2 - W_3}{W_2 - W_1} \times 100\%.$$

(W_1 = Weight of vial alone; W_2 = Weight of clot containing vial; W_3 = Weight of clot containing vial after clot disruption).

Thrombolytic activity was determined as part of a new probe of cardioprotective drugs from medicinal plants *A. cepa, C. tamala* and *I. aquatica*. It showed that the addition of 100 µl of streptokinase stock solution as a positive thrombolytic control (30000 I. U) to the blood clots with 90 min of incubation at 37°C showed 68.79% clot lysis. On the other hand, distilled water was

treated as negative non-thrombolytic control which exhibited negligible percentages of lysis of clot (2.04%). The mean difference in clot lysis percentages between positive and negative control was found statistically significant.

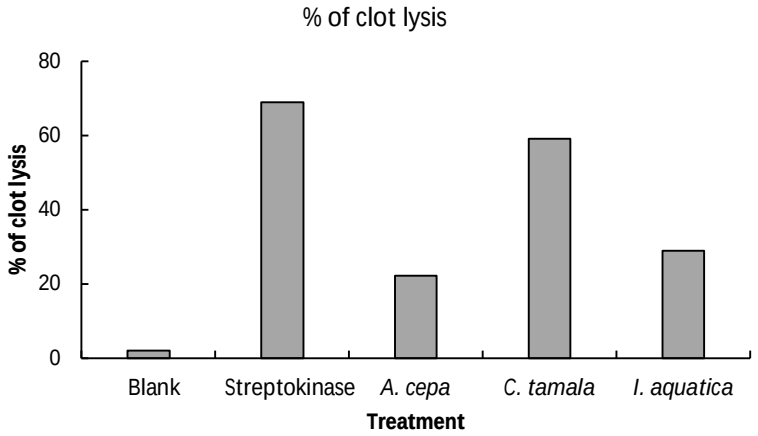

Fig. 1. Comparative thrombolytic activity of *Allium cepa*, *Cinnamomum tamala* and *Ipomoea aquatica* leaf extract and standard streptokinase.

As shown in Table 1, treatment of blood clots with *A. cepa*, *C. tamala* and *I. aquatica* leaf extracts provided the clot lysis 22.24, 58.99 and 28.96%, respectively compared to the positive control and negative control. Fig.1 represents comparative thrombolytic activity with the extracts of *A. cepa*, *C. tamala* and *I. aquatica*. Among the extracts, *C. tamala* showed the highest thrombolytic activity (58.99%) rather than *I. aquatica* and *A. cepa*. Similar thrombolytic effect was reported by *C. tamala* leaf extract (Al-Mamun *et al*. 2012).

Table 1. Thrombolytic activity of methanol extract of *A. cepa, C. tamala,* and *I. aqutica* leaf.

Test sample	W_1	W_2	W_3	$W_4=W_2-W_1$	$W_5=W_2-W_3$	% of clot lysis
Blank	5293.4	5907.6	5895.1	614.2	12.5	2.04
Streptokinase	5038.7	5573.7	5205.7	535	368	68.79
A. cepa	5173.4	5812.3	5670.2	638.9	142.1	22.24
C. tamala	5012.2	5470.2	5200	458	270.2	58.99
I. aquatica	4804	5063	4988	259	75	28.96

W_1: Weight of vial alone, W_2: Weight of clot containing vial, W_3: Weight of clot containing vial after clot disruption.

It can be concluded that the plant extracts of *A.cepa*, *C. tamala* and *I. aquatica* can be used to check the efficacy of thrombolytic agents due to their moderate thrombolytic activity. Further study is needed to identify the bioactive compounds responsible for thrombolytic activity.

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