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THROMBOLYTIC POTENTIALS OF SOME MEDICINAL PLANTS USED BY THE LOCAL PEOPLE FOR CARDIOVASCULAR DISEASES IN BANGLADESH

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Abstract

Cardiovascular diseases (CVDs) are one of the major causes of death in the world. Medicinal plants with thrombolytic properties may be used as an alternative to modern medicines for CVDs. The present study was aimed to evaluate the thrombolytic potential of six medicinal plants available in Bangladesh using an *in vitro* clot lysis method where streptokinase and ethanol were used as a positive and negative control, respectively. Ethanolic extract at a dose of 10 mg/ml of Arjun tree (*Terminalia arjuna*), Garlic (*Allium sativum*), Elephant apple (*Dillenia indica*), Amla (*Phyllanthus emblica*), Yellow mombin (*Spondias pinnata*) and Burmese grape (*Baccaurea ramiflora*) showed 14.18 ± 1.23%, 10.72 ± 0.78%, 8.25 ± 0.42%, 7.08 ± 0.64%, 5.42 ± 0.47% and 2.47 ± 0.19% clot lysis, respectively, whereas the standard drug streptokinase lysed 41.11±0.31% clot at a dose of 30,000 IU. From the data, it is evident that ethanolic extracts of six selected medicinal plants possess a moderate to insignificant thrombolytic activities. Among these plants, Arjun tree and Garlic exhibited the highest thrombolytic activity and the Burmese grape showed the lowest thrombolytic activity. Through our study, it could be concluded that Arjun tree, Garlic, and Elephant apple might be used as traditional healing purposes of CVDs. However, further animal studies will prove the scientific justification of their uses. Conservation efforts should be given for Arjun tree, Elephant apple, Yellow mombin, Burmese grape, and Amla to save these plants from extinction in nature.

Introduction

The thrombolytic disorder is one of the major causes of morbidity in Bangladesh (Islam and Mojumder, 2013). Thrombus development inside the blood vessels inhibits bloodstream through the circulatory system leading to high blood pressure, stroke to the heart, anoxia, atherosclerosis, angina, ischemic heart disease, thromboembolism, myocardial and cerebral infarction (Khatun *et al*., 2016). Management of cerebral venous sinus thrombosis patients is highly expensive and widely used thrombolytic drugs have limitations to some extend (Ali *et al.* 2014).

In the absence of thrombolytic drugs, local people have long been using medicinal plants for the management of cardiovascular diseases (Uddin *et al.* 2019). In most cases, scientific validation of ethnobotanical uses of medicinal plants for cardiac management is less common. Moreover, herbal medicines are prescribed by indigenous physicians and play an important role in maintaining primary healthcare in many developing and underdeveloped countries (Ghosh, 2003). Medicinal plant products are sometimes recognized as safe because they are "natural" (Demrow *et al.*, 1995). The convincing proof is that dietary consumption phytoconstituents having anticoagulant properties can lessen the risks of thromboembolic diseases (Lee *et al.*, 2012; Manicam *et al.*, 2010).

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Globally, the researches on antithrombolytic activity of different plant species have been initiated (Ijiri *et al*., 2016; Ijiri *et al*. 2016 and Yamamoto *et al.*, 2013). Bangladesh as sub-tropical country possesses a number of useful medicinal plant having cardio protective properties. As a result, indigenous physicians recommend their use for treatment of chest diseases, high cholesterol, blood pressure and other CVDs (Uddin *et al.*, 2019). *Allium sativum* was used for gastric, cold, fever, chest pain, reduced pressure and ringworm (Uddin *et al.*, 2015a,b; Haque *et al*., 2017). *Terminalia arjuna* was reported for the treatment of heartache from different area from Bangladesh (Uddin *et al.*, 2012, Uddin and Hassan, 2014). *Baccaurea ramiflora* was reported for antioxidant properties (Ullah *et al.*, 2012). *Phyllanthus emblica* is used for the treatment of heart disease (Khatun and Rahman, 2018). Verification of scientific validity of local uses of medicinal plants in Bangladesh is in preliminary stage. So an ethnobotanical approach for the scientific validations of local uses of medicinal plants for CVDs management is essential. In the present study an attempt was taken to evaluate thrombolytic activity of six selected medicinal plant species available in Bangladesh using *in vitro* clot lysis model.

Materials and Methods

Selection of plant material

Based on ethnomedicinal information locally used in Thankurgaon and Dinajpur district, the six most commonly used medicinal plants for the management of CVDs were selected. These were Arjun tree (*Terminalia arjuna*), Burmese grape (*Baccaurea ramiflora*), Elephant apple (*Dillenia indica*), Garlic (*Allium sativum*), Amla (*Phyllanthus emblica*), Yellow mombin (*Spondias pinnata*) (Plate 1). Then bark of Arjun tree, bulb of Garlic, fruits of Burmese grape**,** Elephant apple, Amla and Yellow mombin were collected from study area and were brought in Plant taxonomy laboratory during 2018. Identities of these plants were confirmed using traditional herbarium techniques (Alexiades, 1996; Hyland, 1972). Voucher specimens of these species were preserved in Dhaka University Salar Khan Herbarium.

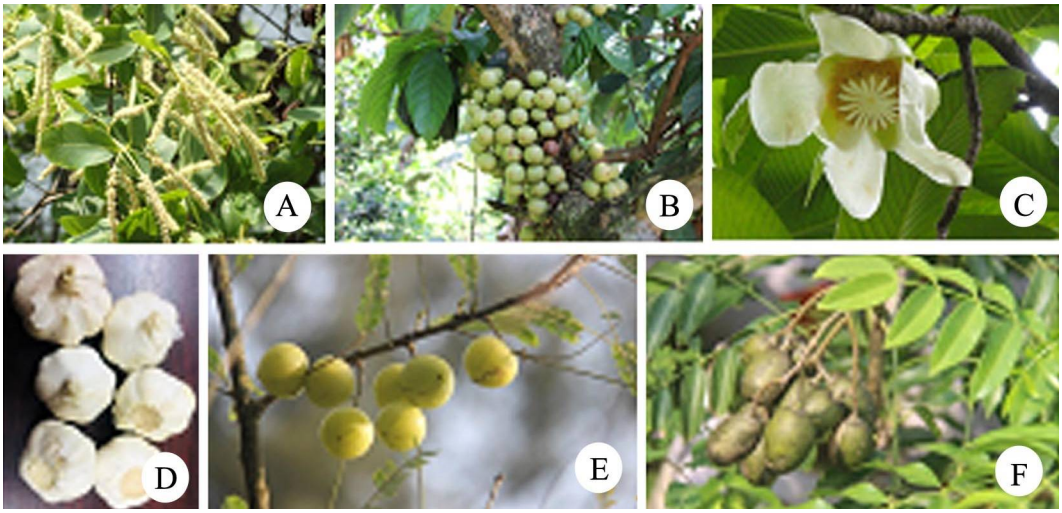

Plate 1. A. Arjun tree (*Terminalia arjuna*) B. Burmese grape (*Baccaurea ramiflora*) C. Elephant apple (*Dillenia indica*) D. Garlic (*Allium sativum*) E. Amla (*Phyllanthus emblica*) F. Yellow mombin (*Spondias pinnata*)

Preparation of plant materials

Immediately after collection, specified parts (barks, bulbs and fruits) of the plants were washed with clean water to remove filth and dirt materials. After proper washing these parts were cut into small pieces, then shade dried for several days. Then these materials were ground into coarse powder using high capacity grinding machine and preserved in a locked container at room temperature for further experimental analysis.

Streptokinase (SK)

The commercially available lyophilized streptokinase (SK) (S-kinase, Popular Pharmaceuticals Ltd., Bangladesh) of 15, 00, 000I.U per vial used as a positive control. Then, 5 ml of sterile water for injection was added to streptokinase vial and mixed thoroughly. From this suspension 100µl (30,000 I.U) was used as positive control in *in vitro* thrombolysis assay (Prasad *et al.* 2007).

Crude extracts preparation

At first 100 gm powdered materials from each plant were taken in three clean, round bottomed flasks and soaked in 300 ml of 70% ethanol. The containers with its content were sealed by foil and kept for a period of 5 days with occasional shaking and stirring. The mixture was then filtered with Whatman's filter paper. Then the filtered extracts were collected and dried at low temperature employing vacuum to make crude extracts.

Plant extracts preparation

The evaluations of thrombolytic activities of all plant extractives were done using streptokinase (SK) as a reference standard drug (Ali *et al.* 2014). At first 100 mg crude extracts of each plants was suspended in 10 ml of 70% ethanol and the suspension was shaken vigorously with a vortex mixture. Then the suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered with Whatman's filter paper. Later this preparation from each was added to the microcentrifuge tubes containing the clots for checking thrombolytic activities.

Collection of blood sample

Venous blood was drawn from healthy human volunteers irrespective of gender by maintaining aseptic condition. Then blood samples were immediately transferred to pre-weighted and pre-labelled sterile microcentrifuge tubes (0.5 ml to each centrifuge tube) to form clots.

In vitro thrombolytic activity

The thrombolytic activity in terms of *in vitro* clot lysis was carried as reported earlier (Prasad *et al.* 2007, Ali *et al.* 2014).

Preparation of clots

At first the micro centrifuge tubes along with blood samples were centrifuged at 2000 rpm for 5 min to let the serum separate above the easy removal from the centrifuge tube. Then the centrifuge tubes were incubated in simulated body temperature i.e. at 37°C for 45 minutes in temperature-controlled incubator.

Clot lysis

After incubation, blood clot was formed at the bottom of each centrifuge tube. Then the serum was completely removed from each centrifuge tube without disturbing the formed clot. After removing the serum, the clot containing tubes were weighted again to determine the clot weight. Then weight of clotted blood (ΔW) was taken by subtracting the pre-weighted (W_1) from the

weight of clot containing tube (W_2) as, $\Delta W = W_2 - W_1$ (Zaman *et al.* 2015). The equation for calculating clot weight is as following: clot weight = weight of clot containing tube – weight of empty tube.

Then 100µl of each extractive was added in each micro centrifuge tubes, where streptokinase was applied as a positive thrombolytic control and ethanol was applied as a negative thrombolytic control respectively. All the centrifuge tubes were again incubated at 37°C for 90min to observe clot lysis. After incubation, the centrifuge tubes were taken out from the incubator and the obtained fluid was removed. The tubes were again weighted 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 (Zaman *et al.* 2015). So, percentage of clot lysis was determined as following equation:

$$\% \text{ of clot lysis} = \frac{\text{Weight of lysis}}{\text{Weight of clot before lysis}} \times 100.$$

Statistical analysis

The statistical analysis was carried out by JMP version 4. The values were analyzed as mean ± SEM and expressed as percentages. All values were expressed as mean ± SEM for nine replicates. Data were analyzed by one-way ANOVA. A p value ≤ 0.0001 was considered to be statistically significant.

Results and Discussion

The results of clot lysis using plant extracts, streptokinase and ethanol were presented in the Table 1. The inclusion of 100µl of streptokinase, positive control (30000 IU) to the clot along with 90 minutes of incubation at 37°C temperature obtained 41.11 ± 0.31% clot lysis. When 100 µl of ethanol as negative control was added to clot, it showed negligible amount lysis (1.36 ± 0.02) of clot. The main differences in clot lysis percentage between positive and negative control are statistically very significant. After treatment of blood clots with 10mg/ml of ethanolic extract of Arjun tree (*Terminalia arjuna* (Roxb. *ex* DC.) Wight & Arn.), Garlic (*Allium sativum* L.) and Elephant apple (*Dillenia indica* L.), it was found that 14.18 ± 1.23% clot has been lysed by Arjun extract 10.72 ± 0.78% by garlic extract and 8.25 ± 0.42% by elephant apple extract. On the other hand, Amla (*Phyllanthus emblica* L.) extract, Yellow mombin (*Spondias pinnata* (L. f.) Kurz) Burmese grape (*Baccaurea ramiflora* Lour.) extract lysed 7.08 ± 0.64%, 5.42 ± 0.47%, 2.47 ± 0.19% of clots, respectively (Table 1). So compare with the standard it is evident that ethanolic extracts of the former three extract possess a moderate thrombolytic potentials. But the later three extractives exhibited insignificant lysis of clots. Among the tested plant extracts, extract of Arjun tree and Garlic exhibited highest thrombolytic activity and extract of Burmese grape showed lowest thrombolytic activity in comparison to native control (Table 1).

This study seems to be the preliminary attempt to justify the potentials of plants for clots lysis based on ethnobotanical information of medicinal plants. The plants including Arjun tree, Garlic, Elephant apple, Amla, Yellow mombin and Burmese grape used in the research have long been used by the local people in CVDs management based on their forefather long experience (Uddin *et al*., 2001; Uddin *et al*., 2004; Uddin *et al*., 2006; Roy *et al*., 2008; Yusuf *et al*., 2009; Uddin, 2013; Uddin and Hassan, 2014; Uddin *et al*., 2017; Ghani, 2003; Fahad *et al*., 2014). The local people did not have any scientific evidence of the use of these plants. They followed the knowledge of their ancestors about plant uses as true. Among the six plants used in the present study, Arjun tree and Garlic were most commonly used by locals in the management of CVDs which has been proven some extend by the current scientific evaluation. The daily intake of garlic

is effective for prevention of arterial thrombotic disorders (Ijiri *et al.*, 2016), whereas the bark of Arjun tree also showed beneficial effect in coronary artery diseases (Dwivedi, 2007). Apart from these, the use of sour fruits among the local people for the management of CVDs could also be noticed (Uddin *et al.*, 2019). In the present study we have found some evidences of the ability of clot lysis from Elephant apple, Amla, Yellow mombin and Burmese grape which was proved traditional knowledge of local people.

Table 1. Clot lysis values of six medicinal plants (in terms of % of clot lysis).

Scientific name and voucher number	English/Bengali name	Family	% of clot lysis after using plant extract
Terminalia arjuna (Roxb. ex DC.) Wight & Arn., ABR-01	Arjun tree/Arjun	Combretaceae	14.18 ± 1.23***
Allium sativum L., FYM-36	Garlic/Roshun	Liliaceae	10.72 ± 0.78***
Dillenia indica L., ABR-12	Elephant apple/ Chalta	Dilleniaceae	8.25 ± 0.42***
Phyllanthus emblica L., FYM-07	Amla/Amloki	Euphorbiaceae	7.08 ± 0.64***
Spondias pinnata (L. f.) Kurz, FYM-86	Yellow mombin/Amra	Anacardiaceae	5.42 ± 0.47***
Baccaurea ramiflora Lour., ABR-87	Burmese grape/ Lotkon	Euphorbiaceae	2.47 ± 0.19***
Streptokinase			41.62 ± 0.40*
Ethanol			1.36 ± 0.02**

Results represented in means ± SEM (n = 9); Level of Significance, *** $p<0.0001$,** $p<0.001$, * $p<0.05$ comparing with standard Streptokinase (41.62%).

Considering the preliminary results of the present research, the potential of the plants can be proved with certainty by carrying out long term research which could be a milestone in the discovery of new medicines for the management of CVDs from the medicinal plants. Once upon a time, there were plenty of medicinal plants including Arjun tree, Elephant apple, Amla, Yellow mombin and Burmese grape availalbe in Bangladesh. Due to anthropogenic pressure, lack of awareness, and development activities, currently, such medicinal plants were not encountered in nature easily. Since their folk uses have been proven scientifically to some extend true, it is matter of time before elimination, these plants are to be protected in nature. Arjun tree, Garlic, Elephant apple, Amla, Yellow mombin and Burmese grape can be used in future as an alternative source of medicine to modern drugs if clot lysis power will be proven further using modern scientific tools. From the present evaluation, it can be established that our findings may have substantial implications in CVDs management. The results are also supported the medicinal uses of these six medicinal plants in Bangladesh. The findings may direct the opportunity of developing new thrombolytic compounds from Arjun tree, Garlic and Elephant apple extracts.

This study investigated some medicinal plants and spices to explore their thrombolytic potential. Traditionally, medicinal plants, spices, herbs etc. are used from the history of mankind. Scientific evaluation of those has yielded many plant derived drugs which are available in the market. It is noteworthy that about one of three drugs is discovered from natural sources (Leta *et al.*, 2002; Gillman *et al.*, 1995). Many studies have been directed by various researchers to find out the herbs, species, plants and natural foods possessing antithrombotic properties. There is

evidence that consuming such materials lead to prevention of CVDs including coronary thromboembolism and stroke (Ratnasooriya *et al*., 2008; Joshipura *et al.,* 1999. Liu *et al.,* 2000, Bazzano *et al.,* 2002). Though there are several commercially available thrombolytic drugs including those obtained by recombinant DNA technology, but adverse effects related to some of these drugs have been reported (Baruah *et al*., 2006; Gallus, 1998; Wardlaw *et al*., 2004; Capstick and Henry, 2005). Therefore, as an alternative if herbal preparations are taken appropriately may provide better effect in curing many ailments including CVDs. But there is also a concern about the toxicities of many plants. Such concerns may be overcome by toxicities studies to set up a safe mode of uses of those plants for treatment and preventive purposes (Krishnaraju *et al.,* 2006; Collen, 1996). Applications of drugs in the management of CVDs are well established as the complete mechanisms of the commercially available drugs have been confirmed scientifically. On the contrary, though natural products are considered to be safe, in most cases their applications are doubted because there is lacking in the scientific proofs and mode of actions of many traditional medicinal plants.

In our finding, ethanolic extract of Arjun tree, Garlic and Elephant apple showed moderate thrombolytic effects while the other three plants extracts exhibited insignificant effects. In conclusion, it can be inferred that consumption of those extracts showed moderate thrombolytic effects may reduce the risk of CVDs. However, further studies are needed to set up their quantity of consumption, forms to be taken, and frequency to be taken. Moreover, the mode of actions and toxicities are to be set up by animal studies. These demand further studies in *in vivo* experimental models. As the six selected medicinal plant species were showed thrombolytic activity, conservation efforts should be given to save the species from extinction in nature.

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