OPEN ACCESS Pharmacologia

ISSN 2044-4648
DOI: 10.5567/pharmacologia.2017.78.82

Short Communication
Thrombolysis Potential of Methanol Extracts from the Five Medicinal Plants Leaf, Available in Bangladesh

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Abstract

Background and Objective: Blood clots block the blood vessels due to the several reasons that might be leading to the several cardiovascular and cerebrovascular disorders. Considering the adverse effects of modern medicine, the discovery and development of drugs by utilizing the medicinal plant sources are an alternative option. The present study was carried out to compare the thrombolysis activity among the five different methanol extracts from the five Bangladeshi medicinal plants. **Materials and Methods:** An *in vitro* thrombolytic model was used to investigate the clot lysis activity of medicinal herbs using streptokinase as a positive control and water as a negative control. In brief, venous blood drawn from ten healthy volunteers was permitted to form clots which were weighed and treated with the test plant extracts to disrupt the clots. Weight of clot after and before treatment presented a percentage of clot lysis. Statistical comparisons were performed using one-way ANOVA followed by Tukey/Tukey-Kramer (equal/unequal observations). **Results:** Among the all tested herb extracts, the maximum percentage of clot lysis activity was reached by *Jacquemontia paniculata* (*J. paniculata*) (60.753%) and *P. adenophylla* (54.057%), respectively. In this study, all of the herb extracts showed very significant ($p<0.001$) clot lysis activity compared with streptokinase as a positive control. **Conclusion:** On the basis of the experiment, all methanol extracts of the medicinal plants possessed blood clots lysis activity.

Key words: Thrombolysis, medicinal plants, clot lysis, *J. paniculata*, *P. adenophylla*

Citation: Md. Jakaria, Mukimul Islam, Md. Shariful Islam, Mohammad Belal Talukder, Chayan Dhar Clinton and Mohammed Ibrahim, 2017. Thrombolysis potential of methanol extracts from the five medicinal plants leaf, available in Bangladesh. Pharmacologia, 8: 78-82.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Thrombosis is the formation of an atypical mass inside the vascular system of a living animal[1]. It is a serious happening in the arterial diseases linked with myocardial infarction and stroke and venous thromboembolic disorder report for substantial morbidity and mortality. In addition, venous thrombosis is the second foremost cause of death in patients with cancer[2].

Clinical thrombolytic agents such as urokinase (UK), streptokinase (SK) or tissue plasminogen activators (t-PA) are broadly indicated in the coronary artery thrombosis for the management of severe or massive deep venous thrombosis, pulmonary embolism, myocardial infarction and occluded intravenous or dialysis cannulas[3]. Due to lesser cost, UK and SK are commonly used in India, Bangladesh and other developing countries[4] as compared to other thrombolytic drugs, but the use was combined with elevated risk of bleeding intracranial hemorrhage, severe anaphylactic reaction and lacks specificity[5,6]. The therapy for thrombolysis with recombinant t-PA was effective in acute myocardial infarction, however, the treatment was inadequate by a fairly sluggish reperfusion rate and frequent early reocclusions. Moreover, the platelet-rich thrombi are extremely resistant to lysis by t-PA[7]. Further, fucoidan as a thrombolytic agent, a branched sulfated fucan extracted from brown seaweeds with anticoagulant and antithrombotic effects mediated via straight thrombin inhibition has been reported newly[3]. On the other hand, herbal drugs are safe because it has been coming from natural sources. The acceptability of traditional herbal drugs increased day by day due to their barely credible pharmacological activities, cost-effective viability and less side effects in different health care management system[8,9].

Jacquemontia paniculata (Burm.f.) Hallier f. belongs to family Convolvulaceae. *Jacquemontia* is one of the superior genera with approximately 120 species[10]. Psychotria adenophylla Wall. (Family: Rubiaceae). Traditionally, roots used in mouth sore and rheumatism[11]. According to the plant list and Wikipedia, *Cheilocostus speciosus* (J.Konig) C.Specht or (syn. *Costus speciosus*) was probably the top identified species in the genus *Cheilocostus* (family: Costaceae). The hexane extract of *C. speciosus* rhizome have anti-hyperglycemic, hypolipidemic activity, hepatic antioxidant enzyme activity, affects neurotransmitters and monoamine oxidase activity, anti-inflammatory, antipyretic properties and hepatoprotective activity[12]. *Primula sikkimensis* Hook. (Family: Primulaceae) is an endemic species in the Himalaya-Hengduan Mountains (HMM) region and is the only species in Primula sect[13]. *Dioscorea pentaphylla* Linn (Family: Dioscoreaceae), potentially used as vegetables, tubers are boiled and eaten, leaf paste mixed with mustard oil was rubbed on the effective part to treat rheumatism[14-17].

Nowadays, natural medicines from plant sources have been shown versatile pharmacological activities in various disease model. The pharmacological activities due to the different phytochemicals that are available in different parts of plants. Considering the thrombosis model, the aim of the present study was to investigate *in vitro* thrombolysis activity of five Bangladeshi medicinal herbs such as *J. paniculata*, *P. adenophylla*, *C. speciosus*, *P. sikkimensis* and *D. pentaphylla*.

MATERIALS AND METHODS

Plant collection and identification: The leaves of the plant of *J. paniculata*, *P. adenophylla*, *C. speciosus*, *P. sikkimensis* and *D. pentaphylla* were collected from different parts of Chittagong region, Bangladesh. All plants were identified by Prof. Dr. Shaikh Bokhtear Uddin, Department of Botany, University of Chittagong. All experiments and collection of plant specimens during October, 2015 to April, 2016.

Preparation of crude extract: The fresh leaves of all herbs were separated, washed and air dried at room temperature (24±2°C) for about 10 days. Then the leaves were grounded into coarse powder (250 g) with a mechanical grinder (Moulinex three-in-one grinder, China). The coarse powder emerged using methanol and shaking by rotary shaker apparatus for 7 days. The tincture was collected using Buchner funnel. Methanol was evaporated at a temperature below 45°C then 25 g of the concentrated crude extract was weighed and stored at 4°C temperatures.

Streptokinase (SK): Thrombolysis activities of all plant extracts were tested by a method using streptokinase (SK: 15,00,000 I.U.) as a reference standard. Five milliliters sterile distilled water was added to streptokinase vial and mixed properly. From this suspension, 100 µL (30,000 I.U) was used for *in vitro* thrombolysis[18].

Sample preparation: A hundred milligrams each of the extracts was suspended in 10 mL distilled water and the suspension was shaken vigorously by using a vortex mixer. Then, the suspension was reserved during the night and decanted to remove the soluble supernatant, which was filtered.

Blood sample: For this test, blood samples (Five mL) were drawn from healthy human volunteers (n = 10) with maintaining aseptic circumstance devoid of a history of oral contraceptive or anticoagulant therapy. The study protocol was accepted by the Planning and Development Committee (Grant No. Pharmacy P&D 68/09-15), Department of Pharmacy, International Islamic University Chittagong, Bangladesh.

Thrombolysis activity: An *in vitro* thrombolytic model used to investigate the thrombolysis activity[18]. Venous blood was drawn from every volunteer which were taken in ten different pre-weighed sterile microcentrifuge tubes and permitted to incubate at 37°C for 45 min. Later than clot formation, fluid was wholly removed from all microcentrifuge tubes and determined clot weight by subtracting the weight of clot containing tube from the weight of tube alone. In addition, 100 µL of streptokinase (SK) used as a positive control but 100 µL of distilled water as a negative control. Moreover, 100 µL of every sample also independently added to the microcentrifuge tubes. All the tubes were followed by incubated at 37°C for 90 min and observed for clot lysis. Later than incubation, the released fluid was discarded and tubes were again weighed to observe the difference in weight after clot disruption. The percentage of clot lysis was calculated as following[6]:

$$\text{Clot lysis (\%)} = \frac{\text{wt. of released clot}}{\text{Clot wt.}} \times 100$$

Statistical analysis: Data were expressed as the mean±standard error of mean (SEM). Statistical comparisons were performed using one-way ANOVA followed by Tukey/Tukey-Kramer (equal/unequal observations). The values obtained were compared with the control group and considered statistically significant when $p<0.001$, $p<0.01$ and $p<0.05$. All statistical analysis was performed using MaxStat Lite 3.60 version software (MaxStat Software, Oliver Wurl Grüner Weg 1726441 Jever-OT Cleverns, Germany).

RESULTS AND DISCUSSION

The thrombolysis activity of five different medicinal plants was investigated as a part of searching of drugs for the disorders of the cardiovascular system. Addition of streptokinase as a positive control with the clots along with 90 min of incubation at 37°C, showed 75% clot lysis but distilled water as a negative control treated-clot showed only negligible clot lysis (4.097%).

Table 1: Thrombolysis activity of five medicinal herbs

Treatments/control	Clot lysis (%)
Distill water	4.097±0.055
Streptokinase	75.000±0.289
MEJP	60.753±0.946
MEPA	54.057±0.627
MECS	18.200±0.351
MEPS	17.907±0.459
MEDP	10.237±0.368

Clot lysis by water, streptokinase and various herb extracts. Data were expressed as mean±standard error of mean (SEM) and n = 3. The values obtained were compared with the control group and considered statistically significant where $p<0.001$

Among the all tested herb extracts, the maximum percentage of clot lysis activity was reached by *J. paniculata* (60.753%) and *P. adenophylla* (54.057%), respectively. On the contrary, *C. speciosus*, *P. sikkimensis* and *D. pentaphylla* showed relatively lower percentage of clot lysis activities as follows: 18.200, 17.907 and 10.237% respectively. In this study, all of the extracts showed very significantly ($p<0.001$) clot lysis activity compared with streptokinase as a positive control that shown in Table 1.

At the present time, phytopharmacological analysis has created a new field to the discovery of drugs from plant sources, which were effective in remedial of several ailments and transformed the consideration in herbal medicines. It was projected that an about 30% of the pharmaceuticals are prepared from plants sources[19]. There are a number of research works have been conducted to discover the plants and natural food sources and their supplements having antithrombotic (anticoagulant and antiplatelet) result and there was an indication that consuming such food leads to prevention of coronary diseases and stroke[6,20-23]. Even though there were numerous thrombolytic drugs with those obtained by recombinant DNA technology, but side effects related to some of these drugs that lead to further complications have been reported[24-27]. In this study, greatest thrombolysis activity was revealed by crude methanol extracts of *J. paniculate* and *P. adenophylla*. It has been accounted that flavonoids might have significant potentials of displaying thrombolytic activity[28,29]. Therefore, many flavonoids in both plants, as well as other efficacious plants, might be a reason for their thrombolysis potentiality.

CONCLUSION

With the respect to the experiment results, all extract produced pharmacological activities in induced thrombosis model. This is a preliminary study, so, deeper research studies concerning *in vitro* and *in vivo* models are recommended.

Then, isolation and characterization of responsible compounds for the thrombolytic activity should be carried out. It is expected that study data might be helpful to conduct further studies.

SIGNIFICANCE STATEMENTS

This study was designed to compare the thrombolysis potential among the different methanol extracts from five different plants, geographically available in Bangladesh. It has been found that investigated plant extracts have produced thrombolysis effect. Among the five plants, *J. paniculata* and *P. adenophylla* were shown greatest activities. This preliminary study might be helpful to set and conduct the deeper study and might be contributed to drug discovery for diseases due to the thrombus formation.

ACKNOWLEDGMENT

The authors are grateful to Department of Pharmacy, International Islamic University Chittagong (IIUC) for providing necessary logistic support to carry out the study.

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