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Assessment of Thrombolytic Activity of Five Bangladeshi Medicinal Plants: Potential Source for Thrombolytic Compounds

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SMMH and INK designed the study, wrote the protocol, and wrote the first draft of the manuscript. All managed the literature searches, analyses of the study performed the analysis. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: The aim of our project work was to assess the thrombolytic activity of five common Bangladeshi plant extract in different solvent. Five plants are *Geodorum densiflorum* (Shankhamul), *Pistia stratiotes* (Topa Pana), *Smilax zeylanica* (Kumarilata), *Pandanus foetidus* (Keya) & *Tabernaemontana coronaria* (Tagar). Plants were collected and air dried separately for three weeks. They were ground into a coarse powder. Cold extractions were performed for all plants by using different solvents.
Place and Duration of Study: Department of Pharmacy, University of Chittagong and University of Science and Technology Chittagong, November, 2013.
Methodology: Fresh blood was collected from healthy individuals ten volunteers (*n*=10). Blood was allowed to form clots in a pre-weighed sterile micro-centrifuge eppendorf tubes. After clot serum was removed and blood clot was weighed then blood clot was allowed to lysis by streptokinase. After lysis fluid was removed and the remaining of blood clot was again weighed along with the tube. Percentage of blood clot lysis was calculated on the basis of the weight difference. Weight difference of tubes obtained by weighing before and after clot lyses of blood. % clot lysis=(Weight after clot lysis/ Weight of clot before lysis)×100. This method was repeated for all extracts.

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Result: Among the herbs studied *Pandanus foetidus* (C), *Pandanus foetidus* (PE), *Smilax zeylanica* (E) and *Pistia stratiotes*-Root (M) showed significant % of clot lysis 47.54% 41.49%, 43.35% and 35.85% respectively with reference to standard, streptokinase (70.24%).
Conclusion: These extracts lyse the blood clots *In-vitro*, however, we need to know *In-vivo* clot dissolving property. Further systemic research on these plants and may be a potential source of thrombolytic agent in future.

Keywords: Thrombolytic agent; *Geodorum densiflorum*; *Pistia stratiotes*; *Smilax zeylanica*; *Pandanus foetidus; Tabernaemontana coronaria.*

1. INTRODUCTION

Different epidemiologic studies and research proved that plants have different medicinal values and plants are having been in using as traditional medicine. Experimentally proved that foods has anti-thrombotic effect could reduce risk of thrombosis. Herbs showing thrombolytic activity have been studied and some significant observations have been reported earlier [1]. Based on traditional use we had selected following five common medicinal plants to evaluate the thrombolytic study.

Geodorum densiflorum (Lam.) Schltr. (Family: Orchidaceae) is one of the traditionally and medicinally important ground orchids of Bangladesh. Rhizomes of *Geodorum densiflorum* are used as traditional medicine for the treatment of various diseases like used to regularize menstrual cycle in women [2]. Phytochemical analyses of the crude extract contain carbohydrate, alkaloid, glycoside and steroids [3].

Pistia stratiotes L. (Family: Araceae) is having medicinal value and used to treat skin inflammations, dysuria, boils, dysentery, asthma and ringworms [4]. The plant is laxative, cooling and demulcent in nature. Juice of the plant is useful in eye and ear diseases. Leaves are considered as diuretic, antiseptic, antitubercular, antidysenteric and anthelmintic; used for the treatment eczema, leprosy, piles, ulcers, syphilis, cough and asthma. It is also used as a poultice in hemorrhoids. Leaves extract by decoction is prescribed in urinary tract diseases. Stratioside I and C13 norterpene glucoside is the major constituent of the plant. Leaves contain proteins, essential amino acids, a steroid, stigmatane, three new sitosterol acyl glycosides, minerals, Vicenin, lucenin, cyanidin-3-glucoside and two unidentified compounds also isolated from the plant [5-6].

Medicinal *Pandanus foetidus* (keya) belongs to family *Pandanaceae* is a very important medicinal plant. Leaves are alexiteric, tonic and aphrodisiacin in nature. Leaves used in treatment of leprosy, small-pox, syphilis, scabies, leucoderma and diabetes. The oil and otto obtained from the bracts are used as stimulant, antispasmodic and also used in headache and rheumatism. The root is considered as diuretic, depurative and tonic [5]. Bracts of flowers yield an essential oil (0.1-0.3%) contains terpinenol [5], methyl ether of β-phenylethyl alcohol as active phyto-constituent (70%), benzyl benzoate, benzyl salicylate, benzyl acetate, benzyl alcohol, geraniol, linalool, linalyl acetate, bromostyrene, guaicol, phenylethyl alcohol and aldehydes as active phytochemicals [7].

Smilax zeylanica L. belongs to family Smilacaceae. In the traditional system of medicine, the plant was used in veneral diseases treatment, the healing of wounds, swellings, abscesses, in rheumatism, pain in lower extremities, skin diseases, leucorrhoea, colic, dysentery,

dysuria and fever [4,5]. Phytochemical research has reported that it contains 1-3% steroidal saponins, phytosterols, starch, resin, sarsapic acid, and minerals [8]. Leaves and roots contain diosgenin [9]. Roots of *Smilax zeylanica* L. contain large amounts of phyto-chemicals like tannin, saponin, 31-norcycloartenol, beta-sitosterol, parillin, phenolic acid, and potassium nitrate. The saponin, on hydrolysis, yields the sapogenin, sarsasapogenin and asparagenin [10].

Tabernaemontana divaricata (Linn) Family: Apocynaceae (Synonym-*Tabernaemontana coronaria, Ervatamia coronaria*), commonly known as Togor, dudhphul in Bangladesh. This plant is traditionally used to treat various diseases like abdominal tumors, arthralgia, asthma, diarrhea, epilepsy, eye infections, fever, fractures, headache, inflammation, leprosy, mania, oedema, paralysis, piles, rabies, rheumatic pain, skin diseases, ulceration and vomiting. It is also used as antihelmintic, antihypertensive, aphrodisiac, diuretic, emmenagogue, hair growth promoter, as purgative, remedy against poisons and tonic for brain, liver & spleen [11-15]. The phytochemistry and a number of chemical constituents such as alkaloids, terpenoids, steroids, flavonoids, phenyl propanoids, phenolic acids and enzymes from the leaves, stems, and roots have been reported previously [16-20].

Because of the historical interest in the many uses for *Geodorum densiflorum*, *Pistia stratiotes*, *Smilax zeylanica*, *Pandanus foetidus* & *Tabernaemontana coronaria* their uses in traditional medicine, we are interested to check the thrombolytic activity of these plant, by using an *In-vitro* procedure.

2. MATERIALS AND METHODS

2.1 Collection and Identification

Different parts of *Geodorum densiflorum* (whole plant), *Pistia stratiotes* (leaves & roots), *Smilax zeylanica* (leaves), *Pandanus foetidus* (leaves) & *Tabernaemontana coronaria* (leaves) was collected at their fully mature form in the month of October, 2013. The parts of plant were identified by using standard taxonomical methods and identified by Syedul Alam, Scientific officer, Forest Botany Division, BFRI, Chittagong, Bangladesh. After identification plants voucher specimens have been kept for future reference in department of pharmacy, University of Science and Technology Chittagong. After cleaning, the plant parts of selected plant were taken and air dried for 10 days.

2.2 Extraction of Different Parts of Plant

Cold extraction was done by using ethanol, pet ether, ethyl acetate, chloroform and methanol as solvent. The leaves were dried under shade and ground. The ground crude drug was soaked in sufficient amount of solvent for one week then filtered through a cotton plug followed by Whitman filter paper number 1. The solvent was evaporated under vacuum at room temperature to yield semisolid. The extract was then preserved in a refrigerator till further use [21].

2.3 Herbal Preparation

100mg of each dry extract was mixed with 10mL distilled water and shaken vigorously on a vortex mixer to dissolve in and to form uniform solution. The concentration of herbal extract solution stands 10mg/mL. Then the solution was kept aside overnight and then the insoluble

supernatant was removed by filtration through a filter paper (Whatmann No. 1). The clear solution was used for *In vitro* evaluation of clot lysis activity on blood [22,23].

2.4 Streptokinase (SK) Solution Preparations

Lyophilized Streptokinase vials of 1500000 I.U commercially available in market (manufacturer Polamin Werk Gmb H, Herdecke, Germany). One vial was collected and 5mL sterile distilled water was added, mixed properly. This suspension was used as a standard stock from which 100µL (30,000 I.U) was used for *In vitro* thrombolysis activity evaluation [22,23].

2.5 Specimen

5mL blood was drawn from healthy human volunteers (n=10) without a history of oral contraceptive or anticoagulant therapy (following a protocol, approved by the Institutional Ethics Committee of Central India Institute of Medical Sciences, Nagpur). 500µL of blood was transferred to each of the ten previously weighed eppendorf tubes to form clots [22,23].

2.6 *In vitro* Thrombolytic Study

Experimental design for *In-vitro* thrombolytic study was carried out earlier and reported [22,23]. Venous blood was drawn from healthy volunteers and 500µL was transferred in each pre-weighed sterile eppendorf tube. Then all tubes are incubated in incubator at 37ºC for 45 minutes. After clot formation, serum was completely aspirated out without disturbing the clot formed. Each tube with clot was again weighed (Clot weight=weight of clot containing tube–weight of tube alone). Each eppendorf tube containing clot was properly labeled and 100µL (10µg/µL) of plant extract was added to all the tubes and then incubated at 37ºC for 90 minutes. After incubation, fluid obtained was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. Streptokinase and water were used as a positive and negative (non thrombolytic) control respectively. The experiment was repeated for five times with the blood samples of different volunteers. Percent clot lysis was calculated as below.

$$\text{Percent clot lysis} = (\text{Weight after clot lysis} / \text{Weight of clot before lysis}) \times 100$$

2.7 Statistical Analysis

The significance between % clot lysis by different plant extract by means of weight difference was tested. Data calculation performed by the paired t-test. Data are expressed as mean± standard deviation.

3. RESULTS

Thrombolytic activity was evaluated by following *In-vitro* thrombolytic model for all plant extract in different solvent. The significance between % clot lysis by herbal extract by means of weight difference was tested by the paired t-test analysis. Data are expressed as mean±standard deviation. P- Values are calculated in comparison with negative control (water). All results are summarized in (Table 1 and Fig. 1).

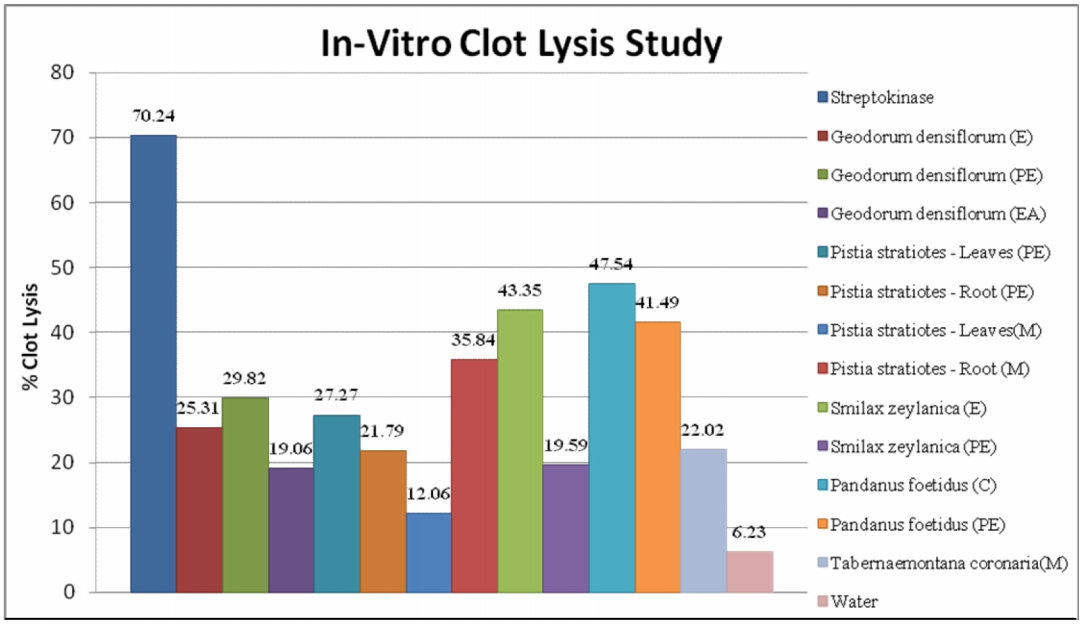

Fig. 1. *In-vitro* Percent Clot lysis by streptokinase, water and various herbal preparations

E: Extract of ethanol, PE: Extract of pet ether, EA: Extract of ethyl acetate, C: Extract of chloroform, M: Extract of methanol

Table 1. Effect of herbal extracts on *In vitro* clot lysis

Herb/Drug	Mean±S.D. (Clot lysis %)	"t" value	"P" value when compared to negative control (water)
Streptokinase	70.24±1.98	101.76	<0.0009
Geodorum densiflorum (E)	25.31±2.15	28.38	<0.0001
Geodorum densiflorum (PE)	29.82±2.63	28.42	<0.0001
Geodorum densiflorum (EA)	19.06±1.69	24.24	<0.0001
Pistia stratiotes leaves (PE)	27.27±2.83	24.21	<0.0001
Pistia stratiotes root (PE)	21.79±1.19	37.08	<0.0001
Pistia stratiotes leaves (M)	12.06±3.56	5.77	0.0003
Pistia stratiotes root (M)	35.84±2.28	41.11	<0.0001
Smilax zeylanica leaves (E)	43.35±4.26	27.71	<0.0001
Smilax zeylanica leaves (PE)	19.59±2.67	15.89	<0.0001
Pandanus foetidus leaves (C)	47.54±4.25	30.82	<0.0001
Pandanus foetidus leaves (PE)	41.49±3.27	34.23	<0.0001
Tabernaemontana coronaria leaves (M)	22.02±2.13	23.55	<0.0001

E: Extract of ethanol, PE: Extract of pet ether, EA: Extract of ethyl acetate, C: Extract of chloroform, M: Extract of methanol

Statistical calculations of the effective clot lysis in percentage by all plant extract preparations, positive control (Streptokinase) and negative control (sterile distilled water)

done by paired t-test analysis. Percent clot lysis is represented as mean±S.D. and p values of all herbal preparations were considered as significant (<0.05).

4. DISCUSSION

Since ancient times, herbal preparations have been used for the treatment of several diseases. The leaves, twigs, stem, bark and underground parts of plants are most often used for traditional medicines. Considerable efforts have been directed towards the discovery and development of natural products from various plant and animal sources which have anti-platelet [24], anticoagulant [25,26], anti-thrombotic [27] and thrombolytic activity.

Addition of 100µL SK, a positive control (30,000 I.U.) to the clots along with 90 minutes of incubation at 37ºC, showed 70.24% clot lysis and when treated with 100µL sterile distilled water (negative control) showed only negligible clot lysis (6.23%). The mean difference in clot lysis percentage between positive and negative control was very significant (p value<0.0001).

After treatment of clots with 100µL of extract *Geodorum* densiflorum whole plant with different solvents ethanol, pet ether and ethyl acetate shows moderate to negligible amount of clot lysis, i.e. 25.31%, 29.82%, 19.06%. Again *Pistia stratiotes* (PE)-Leaves, *Pistia stratiotes* (PE)-Root, *Pistia stratiotes* (M)-Leaves and *Pistia stratiotes* (M) root shows 27.27%, 21.79%, 12.06% and 35.84% clot lysis respectively. Here *Pistia stratiotes* leaves pet ether extract lyses clot more than ethanol extract and root methanol extract lyses clot more than leaves methanol extract. *Smilax zeylanica* leaves ethanolic extract shows more significant clot lysis than pet ether leaves extract, i.e. 43.35% and 19.59% respectively. But after treatment of clots with 100µl of *Pandanus foetidus* leaves chloroform and pet ether extract shows more significant 47.54%, 41.49% clot lysis respectively. *Tabernaemontana coronaria* leaves methanolic extract shows moderate to negligible amount clot lysis, i.e. 22.02%. All experimental results are compared with the negative control (water) the mean clot lysis % difference was significant p value <0.0001 (Table 1 and Fig. 1).

The comparison of positive control with negative control clearly demonstrated that clot dissolution did not occur when water was added to the clot. When compared with the clot lysis percentage obtained through water (negative control), a significant thrombolytic activity was observed after treating the clots with *Pandanus foetidus* (C), *Pandanus foetidus* (PE), *Smilax zeylanica* (E) and *Pistia stratiotes*-Root (M) extracts and % clot lysis was 47.54% 41.49%, 43.35% and 35.85% respectively. We get p value<.0001 and that can be considered as significant. The literature review of *Pandanus foetidus* , *Pandanus foetidus, Smilax zeylanica* and *Pistia stratiotes* crude drugs are phytochemically rich and different types of functional chemical groups are present and may responsible for significant clot lysis.

5. CONCLUSION

From this experiment, it can be concluded that *Pandanus foetidus* (C), *Pandanus foetidus* (PE), *Smilax zeylanica* (E) and *Pistia stratiotes*-Root (M) have got the very good potential as a candidate for future thrombolytic agent. Others show less % of clot lysis. These extracts lyses blood clots *in-vitro*, however, further research on *In-vivo* clots lysis has to perform.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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