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Research Article

Comparative Evaluation of *In Vitro* Thrombolytic Activity of Four Medicinal Plants

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ABSTRACT

Thrombolytic drugs are mainly used for the treatment of myocardial infarction, which occurs due to thrombus formation and causes tissue necrosis. Currently plasminogen activator (t-PA), streptokinase, urokinase are used, but the major drawback was adverse effects like hemorrhage, allergic reactions and lack of specificity.The present study was aimed to investigate *in vitro* thrombolytic activity of methanolic and aquoes extract of Phyllanthus niruri, Nyctanthes arbor- tristis, Syzygium cumini and Clome gynandra, where streptokinase and water were used as positive and negative control. Among the medicinal plants Nyctanthes arbor- tristis(aqueous) extract exhibited highest thrombolytic activity by clot lysis of 14.2%, followed by Cleome gynandra(aqueous) extract with 7.7% of clot lysis and Phyllanthus niruri(methanol) extract with 6.7% clot lysis when compared with Standard streptokinase showed clot lysis of 22.3% while the negative control water 2.2% lysis of clot.From our findings it was observed that among all plants Nyctanthes arbor- tristis(aqueous) extract showed highest thrombolytic activity. Therefore, further studies required to isolate active component(s).

Keywords: Thrombolytics; Clot lysis; Steptokinase

INTRODUCTION

Thrombus or embolus hampers the blood flow in the blood vessels therefore tissue lack normal blood flow and oxygen. Lack of oxygen causes necrosis of particular area which may result in complication like Myocardial infarction. Thrombolytic drugs used for dissolution of clot, which include tissue Plasminogen Activator (t-PA), urokinase, streptokinase etc. act by activating plasminogen which can cause lysis fibrin. All available thrombolytic agents have significant limitations like fewer outcomes, lack of specificity and efficiency at low doses and on other hand if given in large doses their safety is questionable and bleeding tendency at times leading to death [1]. Drugs based on herbs have become a common form of therapy due to less adverse effects. Today, they are one of the promising agents in the field of herbal therapeutics [2] *Phyllanthus niruri* is a widespread tropical plant commonly found in coastal areas, known by the common names gale of the wind, stonebreaker. It belongs to the *Phyllanthus* genus of Family Phyllanthace,it is an important plant of Indian Ayurvedic system of medicine in which it is used for problems of the stomach, genitourinary system, liver, kidney and spleen [3,4] . *Syzigium cumini* is also known as java pulp, jamun, jambal, Indian black pulp. It belongs to family Myrtaceae; traditionally the jambul fruits, leaves, seeds, and bark are all used in ayurvedic medicine. The bark contains tannins and carbohydrates, accounting for its long-term use as an astringent to combat ailments like dysentery and seeds used for diabetes, inflammation [5].

Nyctanthes arbor-tristis is known as Night- flowering Jasmine. The leaves have been used in Ayurvedic medicine and Homoeopathy for sciatica, arthritis, fevers, and as a laxative. Its leaves are also blend into a paste and used in skin related troubles, especially ringworm. The fresh leaves are boiled in mustard oil and used externally for treating ringworm. The leaf juice is mixed with common salt to treat intestinal worms. Coral jasmine is used in

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the treatment of fungal skin infection, dry cough. [6-8]. *Cleome gynandra* is used as a medicinal plant and can be found in all over world. It belongs to the family of *Capparaceae*, it also known as wild spider flower, African spider flower, cat whiskers, and Bastard mustard. Various parts of the plant are used as a folk therapy for various activities like free radical scavangic activity, Anti-cancer, Immuno modulatory effect, Anti-microbial, Anti-diabetic, Anti-diarrhoeal, Anthelmintic, Anti-urolithiatic effect [9].

MATERIALS AND METHODS

Collection of plant materials

Leaves of *Phyllanthusniruri*, *Nyctant hesarbor-tristis*, *Syzygium cumini* and *Clome gynandra* were collected from Eluru and Tadepalligudem, during the month of February 2016. The above plants were authentificated by botany department of Dr. Rajeshekar Reddy horticulture university Leaves were collected from the plants by hand pricking method and were washed with clean water to discard dirt materials. They were dried under shade for 4-5 days. After the leaves are dried they were ground into coarse powder and preserved in a closed container at 25°C for further use.

Preparation of extracts of plant material

The extraction was performed using cold maceration technique. 25 gms powder of each plant was weighed and soaked in 250 ml of methanol and 250 ml of water separately for about 3 days at room temperature with occasional stirring. After 3 days the solution was filtered through filter cloth followed by Whatman's filter paper and the filtrate thus obtained was collected into a beaker. This filtrate was concentrated by heating at a temperature of 60°C-70°C. The filtrate was completely dried; flakes of the extract were obtained. These were collected into amber colored container and preserved.

Preparation of standard (streptokinase) solution

To the commercially available lyophilized SK vial (Thromboflux) 15,00,000 I.U. used as a standard which was collected from Bharat serum and vaccines Ltd, Ambernath (Figure 1). 5 ml 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* thrombolytic activity [10].

Preparation of extract for thrombolytic test

The thrombolytic activities of all methanolic and aqueous leaf extracts were evaluated by a method using Streptokinase (SK) as a reference standard. 100 mg of methanolic extract and 100 mg of aqueous extract of all plants were dissolved separately in 10 ml of methanol and distilled water respectively (Figure 2). They were kept overnight. Then the solution were filtered and further used for study [11].

Specimen

4 ml venous blood was collected from healthy human volunteers (n=4) by maintaining aseptic condition without a history of oral contraceptive or anticoagulant therapy. 0.5 ml of blood was blood was transferred to each tube according to protocol accepted by institutional committee of Sri Vasavi institute of pharmaceutical sciences.

Thrombolytic activity procedure

Micro centrifuge tubes were taken and empty weight of each tube was noted. Note it as W1. (W1=empty weight of micro centrifuge tube). Then 0.5 ml of blood was transferred to each tube and it was incubated at 37°C for 45 minutes for clot formation. After clot formation, fluids were completely released from each micro centrifuge tubes and weigh the tubes along with the clot formed. Note it as W2. (W2= weight of micro centrifuge tube with clot – empty weight). As a positive control, 100 µl of streptokinase (SK) was used and as a negative control 100 µl of distilled water was used. 100 µl of each samples (methanolic and aqueous) were separately added to the micro centrifuge tubes containing clot (Figure 3). All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, the released fluid was discarded and tubes were again weighed to observe the difference in weight after clot disruption, Note it as W3. Finally percentage of clot lysis was determined as followings: Wt of released clot = (W2-W3), Clot weight = (W2-W1). Therefore (W3-W2) / (W2-W2) × 100 gives us % clot lysis. % of clot lysis = (released clot /clot wt) × 100 [11].

Statistical analysis

All values are expressed as average % clot lysis for three replicates.

Figure 1: Microcentrifuge tubes.

Figure 2: Micro centrifuge tube with blood clot.

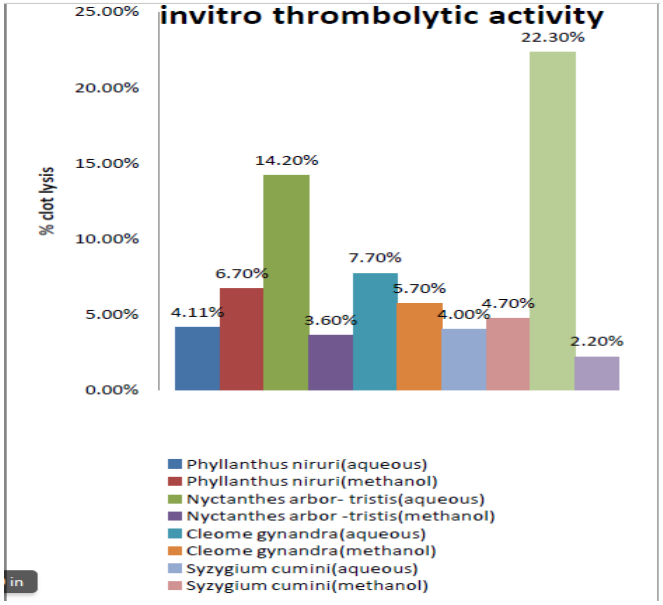

Figure 3: Comparative thrombolytic activity.

RESULTS AND DISCUSSION

A number of studies conducted earlier by different researchers reveals that plants also possess thrombolytic active principles. A study was done using *Boganvellia spectata* leaves contain high content of phytochemical constituents like flavanoids , alkaloids , terpiniods which are responsible for thrombolytic potential. The present study was carried out to investigate thrombolytic activity of *Ncyranthus arbortritis*, *clome gynandra* , *phyllanthus niruri*, *syzyuim cumini* , where Streptokinase (SK), a known thrombolytic drug is used as a positive control [9]. Water, on the other hand, was used as a negative control. Among the four medicinal plants *Nyctanthes arbor- tristis(aqueous)* extract exhibited highest thrombolytic activity with 14.2% of clot lysis , followed by *Cleome gynandra(aqueous)* extract with 7.7% of clot lysis and *Phyllanthus niruri(methanol)* extract with 6.7% clot lysis when compared with Standard streptokinase showed clot lysis of 22.3% while the negative control water 2.2% lysis of clot. By comparising the results of positive control with negative control clearly confirmed that clot dissolution does not occur when water was added to the clot. All the above plants contain flavonids, alkaloids and terpinoids as phytochemical constituents hence these plants also possess antioxidant property (Table 1). This might be a reason for their thrombolytic potential.phytochemical constituents like flavonids, phenols are rich in *Nyctanthes arbor- tristis*, when compared with other plants, and thus it exhibited the highest thrombolytic potential.

Table 1: Effect of different plant extracts on *in vitro* thrombolytic activity.

Plant name	empty weight(w1) in grams	clot+empty weight(w2) in grams	clot weight(w3) in grams	Avg.% clot lysis
Phyllanthus niruri (aqueous)	1	1.73	1.70	4.11%
Phyllanthus niruri (methanol)	0.96	1.746	1.693	6.7%
Nyctanthes arbor-tristis (aqueous)	1.04	1.46	1.40	14.2%
Nyctanthes arbor-tristis (methanol)	0.973	1.53	1.51	3.6%
Cleome gynandra (aqueous)	0.97	1.49	1.45	7.7%
Cleome gynandra (methanol)	0.96	1.65	1.61	5.7%
Syzygium cumini (aqueous)	1.00	1.50	1.48	4.0%
Syzygium cumini (methanol)	0.99	1.62	1.59	4.7%
Steptokinase (Std)	0.99	1.66	1.51	22.3%
Control (distill water)	0.90	1.34	1.33	2.2%

CONCLUSION

In conclusion from our results, it can be demonstrated that our findings may help scientific community in developing novel thrombolytic compounds with less side effects from *Ncyranthus arbortritis*, *clome gynandra*, *phyllanthus niruri*, *syzyuim cumini* extracts. Further studies are necessary to isolate and characterize the compounds and to explore the possible mechanism of action for thrombolytic activity. The present study was aimed to investigate *in vitro* thrombolytic activity of methanolic and aquoes extract of *Phyllanthus niruri, Nyctanthes arbor- tristis, Syzygium cumini* and *Clome gynandra,* where streptokinase and water were used as positive and negative control.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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