



Thrombolytic Activity of *Syzygium cumini* Seed Extract: An *In-Vitro* Evaluation

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Abstract

The aim of the present study was to evaluate the thrombolytic potential of the ethanolic seed extract of *Syzygium cumini*. Sterile microcentrifuge tubes were weighed and labeled properly. 2.5 ml of fresh blood was drawn from each human volunteer and 500µl blood freshly collected blood was then transferred to separate previously weighed microcentrifuge tubes. They were then kept to form clot. Each properly labeled blood filled microcentrifuge tube was then incubated at 37°C for 45 minutes. After clot formation, serum was withdrawn completely, without disturbing the clot. After that each tube was weighed again to get the weight of clot. Clot weight = weight of clot filled tube – weight of empty tube. To each microcentrifuge tube containing clot, 100µg/ml, 250µg/ml of ethanolic seed extract of *Syzygium cumini* (100µl) were added. In the control, 100µl of distilled water was added and in the standard 100µl of Streptokinase was added. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, supernatant fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference between previous weight and at now was noted to obtain the percent of clot lysis. The ethanolic seed extract (250µg/ml) of *Syzygium cumini* exerted 34% clot lysis from clotted blood in thrombolytic activity test while for standard (streptokinase) and control are 79% and 3% respectively. The findings of the present study revealed that the ethanolic seed extract of *Syzygium cumini* possess moderate thrombolytic activity.

Keywords

Syzygium cumini, Ethanolic, thrombolytic activity, seed & clot lysis.

INTRODUCTION

Herbal drugs are the plants or plant parts or an extract or mixture of these. Herbalism is the art or practice of using herbs and herbal preparations to maintain health and to prevent, alleviate, or cure disease. Herbal drugs are one type of dietary supplement. Medicinal plants frequently used as raw

materials for extraction of active ingredients which used in the synthesis of different drugs.

Thrombosis is the formation of a blood clot (thrombus) inside a blood vessel, obstructing the flow of blood. Any injury to the vessel's wall includes trauma, surgery, infection or turbulent flow at bifurcations can induce thrombosis. The main

mechanism is exposure of tissue factor to the blood coagulation system. Arterial thrombosis can embolize and is a major cause of arterial embolism, potentially causing infarction of almost any organ in the body like stroke, myocardial infarction.

Syzygium cumini is one of the best known species and it is very often cultivated. It is commonly known as jambolan, black plum, jamun, java plum, Indian blackberry, Portuguese plum etc. The bark is acrid, sweet, digestive, astringent to the bowels, anthelmintic and used for the treatment of sore throat, bronchitis, asthma, thirst, biliousness, dysentery and ulcers. It is also a good blood purifier. After conducting literature survey it has been found that the seeds of the plant *Syzygium cumini* is associated with a huge number of pharmacological activities such as antibacterial [1], anti-cancer [2, 3], anti-diabetic [4, 5], antifungal [6, 7], anti-hyperlipidemic [8], anti-inflammatory [9] antioxidant [10], antiviral [11], cardioprotective [12], hepatoprotective [13], inhibits lipid peroxidation [14] etc.

The literature review on *Syzygium cumini* has revealed that this plant is associated with good number of pharmacological activities and it is also

used as traditional medicine. The seeds of this plant have not been evaluated scientifically for *in-vitro* thrombolytic activity. Thus it was thought that it would be worthwhile to evaluate the thrombolytic potential of the ethanolic seed extract of *Syzygium cumini*.

MATERIALS AND METHODS

Collection of plant materials

The whole seeds of *Syzygium cumini* were collected from market of Hailakandi (Assam) and were identified by experts in India National Herbarium. The collected seeds were ground to coarse powder with a mechanical grinder.

Extraction procedure

The seed powder was subjected to soxhlet extraction using ethanol as solvent. Extraction was performed at room temperature.

Evaporation of the Solvent

The filtrate (ethanolic extract) was obtained by evaporating under ceiling. Water bath was used to concentrate the resultant filtrate in the semisolid form (figure 1). This gummy concentrate was designated as crude extract or ethanol extract and stored in a desiccator.



Figure 1: Ethanolic extract of *Syzygium cumini*

Method for evaluation of *in-vitro* thrombolytic activity

Preparation of extract solutions

100mg ethanolic extract of was suspended in 100ml distilled water and the mixture was shaken vigorously to make it soluble and a concentration of 1mg/mL was obtained. This solution was further diluted to obtain a concentration of 100 μ g/ml and 250 μ g/ml for performing the thrombolytic study.

Preparation of Standard solution

To the commercially available lyophilized streptokinase (SK) vial of 15,00,000 I.U., 5 ml sterilize distilled water was added and was properly mixed. This suspension was used as a stock. From this stock

solution 100 μ l (30,000 I.U) was used for performing the *in-vitro* thrombolysis [15].

Sampling of blood

Five human volunteers were selected for this study. Sterile microcentrifuge tubes were weighed and labeled properly. 2.5 ml of fresh blood was drawn from each human volunteer and 500 μ l blood freshly collected blood was then transferred to separate previously weighed microcentrifuge tubes. They were then kept to form clot.

Assay [15-17]

Each properly labeled blood filled microcentrifuge tube was then incubated at 37 $^{\circ}$ C for 45 minutes. After clot formation, serum was withdrawn completely, without disturbing the clot. After that

each tube was weighed again to get the weight of clot.

Clot weight = Weight of clot filled tube – Weight of empty tube

To each microcentrifuge tube containing clot, 100µl of 100µg/ml, 250µg/ml of ethanolic seed extract of *Syzygium cumini* were added.

In the control, 100µl of distilled water was added and in the standard 100µl of streptokinase was added.

All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, supernatant fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. The difference between previous weight and at now was noted to obtain the percent of clot lysis by using the formula mentioned below.

% of clot lysis= (Weight of lysed clot/weight of clot before lysis) ×100



Figure 2: Clot lysis in thrombolytic activity

RESULTS AND DISCUSSIONS

Results of thrombolytic activity

The results of thrombolytic activity for the different dilutions of ethanolic seed extract of *Syzygium*

cumini, standard (streptokinase) and control are mentioned in table 1 and figure 3.

Table 1: Percentage clot lysis by ethanolic seed extracts and standard drug (streptokinase) and control

Sl. No	Dose	% Clot Lysis
1	Extract 100µg/ml	28±.102%
2	Extract 250µg/ml	34±.212%
3	Streptokinase 100µl (Standard)	79±.199%
4	Distilled water (control)	3±.168%

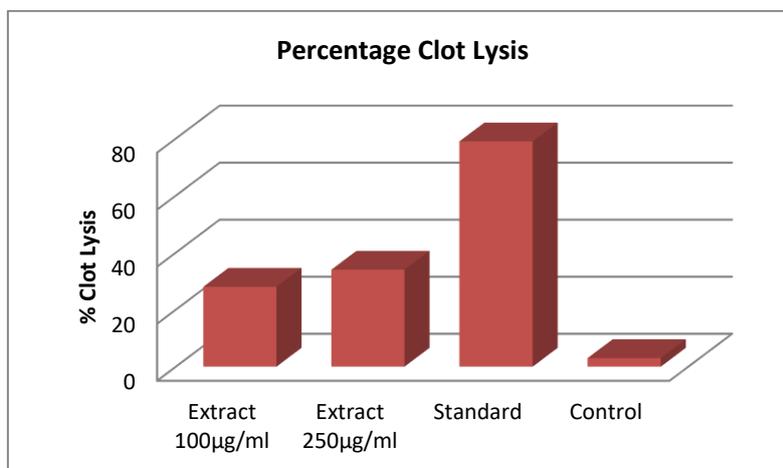


Figure 3: Graph showing percentage clot lysis by ethanolic seed extracts and standard drug (streptokinase) and control

Discussions on Thrombolytic activity

Thrombolytic agents work by activating the enzyme plasminogen, which digests the clot. This makes the clot soluble and subject to further proteolysis by other enzymes and restores blood flow over occluded blood vessels. Thus thrombolytic agents are useful for the treatment of myocardial infarction, thromboembolic strokes etc. Unfortunately, these drugs are shown to have several adverse effects related to such as bleeding and embolism, which lead to further complications. In order to discover new sources of herbs and natural foods and their supplements having antithrombotic effect (anticoagulant and antiplatelet) a number of studies have been conducted by various researchers and the study confirmed that consuming such food leads to prevention of coronary events and stroke [18].

The aim of the present study was to conclude that whether this plant extract has clot lytic activity. Streptokinase (SK), a known thrombolytic drug is used as a standard drug [15]. Water, on the other hand, was designated as a control. In our thrombolytic assay, when we compared the percentage clot lysis by our extract with the percentage clot lysis by streptokinase (standard) and water (control). Extract 100µg/ml showed 28% clot lysis, extract 250µg/ml showed 34% clot lysis and standard drug streptokinase 100µl showed 79% clot lysis (Table 1 and figure 3). The comparison of standard with control clearly demonstrated that clot dissolution does not occur when water was added to the clot. On the basis of the results obtained in this present study we can say that the extract has moderate thrombolytic activity compared to control (water).

The plant *Syzygium cumini* contains several phytochemical constituents belonging to categories such as alkaloids, carbohydrates, glycosides, tannins, triterpenes and flavonoids etc. The moderate thrombolytic activity of the extract of *Syzygium cumini* may be due the presence of such phytoconstituents which has potential to dissolve blood clot.

CONCLUSION

From this study, it can be concluded that the ethanolic seed extract of *Syzygium cumini* possess moderate thrombolytic activity. This is only a preliminary study and to make final comment the extract should be thoroughly investigated phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentialities.

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