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In vitro thrombolytic activity of ethanolic extract of leaves of *Amaranthus spinosus* (Katamarish)

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Abstract
Thrombus formed in blood vessel results in thrombo-occlusive disease such as myocardial infarction or cerebral infarction. The aim of the study is to determine the thrombolytic potentiality of ethanolic extracts of *Amaranthus spinosus* (Katamarish). In that study streptokinase used as a positive control and water used as a negative control. By using an *in vitro* thrombolytic model *Amaranthus spinosus* (Katamarish) showed 41.87% thrombolytic activity. Besides Streptokinase showed 86.20% thrombolytic and distilled water showed 5.20% clotlysis activity. From our study it was found that *Amaranthus spinosus* possesses thrombolytic properties that could lyses blood clots when subjected to *in vitro* study. But *In vivo* clotlysis activities and active components for clotlysis properties need to be discovered further. Our present *in vitro* studies proposed that further studies need to be carried out for using Katamarish as thrombolytic agent to the treatment of the patient suffering from thrombo-occlusive diseases.

Keywords: Thrombolytic activity, *Amaranthus spinosus*, Streptokinase (SK), Thrombo-occlusive disease

1. Introduction
Plants are the important source of diverse range of bioactive principles .Plant kingdom that having ingredients which contain therapeutic value. This therapeutic values that are used for the treatment of various diseases. That's why plants are continuously screened to obtain the therapeutic value. The natural product which have therapeutic activity used as treatment purpose for several diseases from the ancient age of human civilization. Besides medicinal plants serve as an important therapeutic agents as well as important active ingredients for the manufacture of modern medicines [1-2].

Amaranthus spinosus commonly known as Katamarish (Family: Amaranthaceae – Amaranth family) has a long history of medicinal use in Ayurvedic system. *Amaranthus spinosus* possesses many medicinal properties like astringent, diaphoretic, diuretic, emollient, febrifuge, galactogogue etc. *Amaranthus spinosus also* used in the treatment of internal bleeding, diarrhea, excessive menstruation, snake bites, boils, stomach disorders, ulcerated mouths, vaginal discharges, nosebleeds and wounds [3].

Thrombolytic agents act by converting plasminogen, an inactive protein in the blood circulation, to plasmin, an active protein that breaks down the key proteins that form blood clots (fibrinogen and fibrin). These agents can act as before the clot fully forms and hardens, which establishes a therapeutic window of about four hours from the onset of clot formation. Early diagnosis is very much essential in case of thrombo-occlusive diseases. Thrombolytic agents like streptokinase are used to treat already formed clots in blood vessels. But we know that synthetic drug possesses lots of serious side effects, so everyone would like to use different herbal preparation to treat diseases and want to get ride from such severe side effects of drug. From the long ancient time different herbal formulation or medicinal plants are used to treat the diseases. As different medicinal plants and their parts are used to treat from old age, we have gathered the knowledge of thrombolytic activity of different medicinal plants and as a result we like to study the herbs or medicinal plants having any thrombolytic activity or not. The purpose of the study is to determine the thrombolytic activity of Katamarish thus can be used as thrombolytic agent for the treatment of thrombo occlusive diseases [4-5].

2. Materials and Methods
Collection Drying & Preparation of the plant sample
The plants namely *Amaranthus spinosus* was collected from Chittagong. Ethanol extract was purchased from Merck India. All other reagents used were of analytical grade.

The leaves of *Amaranthus Spinosus* (Katamarish) were taken from the plant and air dried for 10 days, then kept in an oven at 45 °C for 72hours.

The dried was ground into coarse powder with the grinder and was stored in suitable extraction jar container for extraction purpose [6].

Preparation of Extract

Powder (300 g) was taken on a glass jar on which ethanol (900 ml) was poured up to 1-inch height above the sample surface as it can sufficiently cover the sample surface. To prevent the contact with air, the jar was closed properly with plastic cover and aluminium foil and kept for three (3) days. To get better extraction the Jar was shaked in several times during the process. After the extraction process the extract was filtered with cotton filter and collected in a beaker. The *Amaranthus spinosus* leaf extract was concentrated by evaporating the solvent using water bath at a temperature of 60 °C [6-7].

Thrombolytic Study

In vitro thrombolytic activity of *Amaranthus spinosus* was carried out by using ethanolic extract, Streptokinase (SK) a standard clot lysis agent is used as a positive control and distilled water is used as negative control.

To the commercially available lyophilized SK vial (Polamin Werk GmbH, Herdecke, Germany) of 15, 00,000 I.U., 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for *in vitro* thrombolysis.

100 mg ethanolic extract of *Amaranthus spinosus*, was suspended in 10 ml distilled water and the suspension was shaken vigorously on a vortex mixer. The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a paper filter. 100 µl of this aqueous preparation of herbs was added to the appendorf tubes containing the clots to check thrombolytic activity.

Whole blood (4 ml) was drawn from healthy human volunteer without a history of oral contraceptive or anticoagulant therapy and then transferred in eight different pre weighed appendorf tube (0.5 ml/tube) and incubated at 37 °C for 45 minutes. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone) [8].

To each appendorf tube containing pre-weighed clot, 100 µl of aqueous extract of herb *Amaranthus spinosus* was added. As a negative control, 100 µl of distilled water was added to the control tube numbered. All the tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, fluid released 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 clotlysis [9-11].

$$\text{Percentage of clotlysis} = \frac{\text{Weight of clot after lysis}}{\text{Weight of clot before lysis}} \text{X } 100$$

3. Result

Table 1: Determination of percentage (%) of clotlysis after adding ethanolic extract of *Amaranthus spinosus*

Number of Tube	Concentration of negative control, positive control and plant extracts	Weight of tube with clot (gm.) (X1)	Weight of clot before Lysis (gm.) (A)	Weight of tube with clot after lysis (gm.) (X2)	Weight of lysis clot (gm.) B=(X1-X2)	Percentage of lysis (%) = (B*100) /A
01	100	1.059	0.121	1.053	0.006	5.20 (Water)
02	100	1.366	0.446	0.986	0.380	85.20 (SK)
03	100	1.361	0.411	1.234	0.129	31.38 (Sample)
04	150	1.314	0.345	1.156	0.158	45.79 (Sample)
05	200	1.186	0.250	1.071	0.115	46.00 (Sample)
06	250	1.341	0.416	1.157	0.204	49.03 (Sample)
07	300	1.208	0.273	1.093	0.115	42.12 (Sample)
08	350	1.482	0.507	1.268	0.214	42.20 (Sample)
09	400	1.353	0.425	1.194	0.159	37.41 (Sample)
10	450	1.404	0.434	1.226	0.178	41.01 (Sample)

Table 2: *In vitro* Clotlysis effect of extract of *Amaranthus spinosus*, Streptokinase, Distilled water.

Streptokinase (Positive control)	*Amaranthus spinosus* (Sample))	Water (Negative control)
85.20%	41.87%	5.20%

Graphical representation: 01

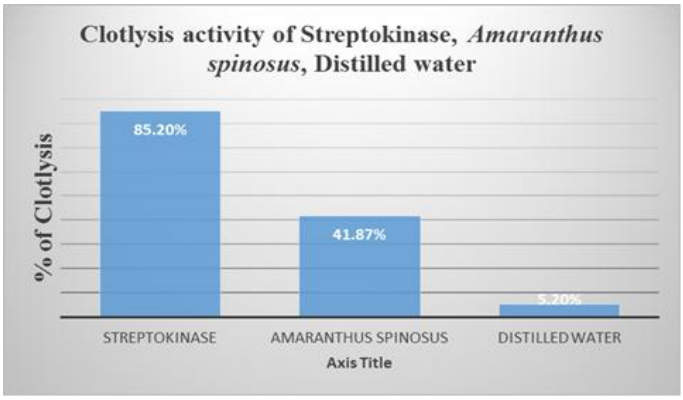

Fig 1: Clotlysis by Streptokinase, water and *Amaranthus spinosus* preparations

Graphical representation: 02

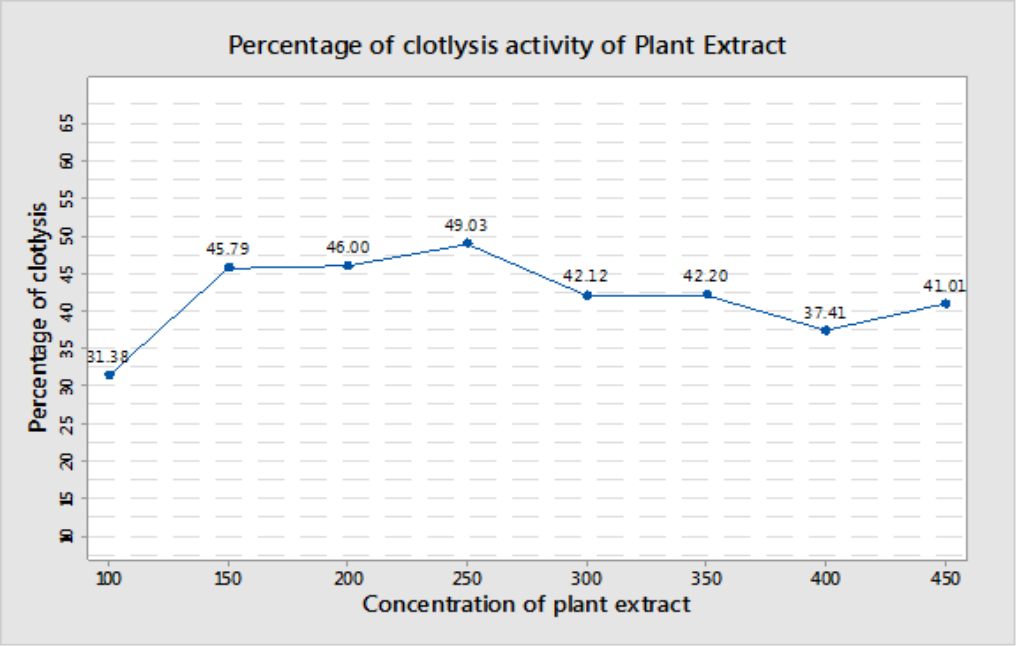

Fig 2: Percentage of Clotlysis activity of extract of *Amaranthus spinosus* preparations

Descriptive Statistics: Percentage of Clotlysis

Variable	N	Mean± SEM	StDev	Minimum	Median	Maximum
Percentage of Clotlysis	8	41.8 ± 1.96	5.54	31.38	42.16	49.03

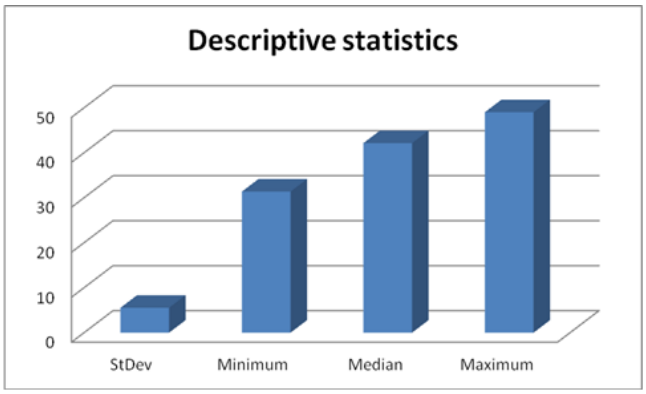

Fig 3: Graphical Representation of descriptive Statistics of extract of *Amaranthus spinosus*

4. Discussion

Herbal preparations with adequate dose can lead to a better option for curing various ailments. By advancing Phytochemistry and identification of plant compounds that are effective for the treatment of certain diseases have recommenced the interest in herbal medicine. As a part of discovery of drugs for Thrombo-occlusive disease from natural resource of the extract of the *Amaranthus spinosus* for thrombolytic activity and result are presented in table 1& table 2. After addition of 100 µl of streptokinase, a positive control (30000I.U.) to the clots along with 90 minutes of incubation at 37 ºC, showed 86.2% clotlysis**.** Clots when treated with 100 µl sterile distilled water (negative control) showed insignificant clotlysis (5.20%) activity. After treatment of clot with *Amaranthus spinosus* a significant thrombolytic activity was observed (41.87%). The aim of the present study was to find out the herbal preparation of *Amaranthus spinosus* clot lysis potentiality. The evaluation of the positive control (streptokinase) with negative control (Distilled water) clearly demonstrated that clot dissolution does not occur when water was added to the clot. Encouraged by the result of the positive control, we compared eight different concentrations of the test sample in the same way with the negative control and observed significant thrombolytic activity. However it could be predicted that these phytochemicals may be responsible for clot lysis activity. That's why further study may need to carry on this leaves extract to find out a therapeutic activity used to treat thrombo-occlusive disease and able to play an important potentiality in the Thrombolytic field.

5. Conclusion

From the above discussion it can be cleared that plant leaves of *Amaranthus Spinosus* may have significant thrombolytic potential that shows 41.87% clotlysis activity. Although *Amaranthus spinosus* lyses blood clots found on *in vitro* study, however, *in vivo* clot dissolving properties yet to be discovered. Besides this study indicate that *Amaranthus spinosus* may be incorporated as a thrombolytic agent for the improvement of the patients suffering from thrombo-occlusive diseases. More studies need to perform on the extract to develop the medicinal and pharmaceutical potentiality.

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