Evaluation of Thrombolytic Activity of Four Bangladeshi Medicinal Plants, as a Possible Renewable Source for Thrombolytic Compounds

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Abstract: Four Bangladeshi medicinal plants *Sansevieria trifasciata*, *Justica gendarussa*, *Hydnocarpus kurzii* and *Mesua nagassarium* have been investigated for their *in vitro* thrombolytic activity. The clot lysis activity was assessed by addition of the test material to the pre-clotted blood and incubation for 90 min. at 37°C and was expressed as % lysis of clot. Each of the plant was extracted with methanol at room temperature and the concentrated methanolic extract was fractionated by the modified Kupchan partitioning method to provide pet-ether, carbon tetrachloride, chloroform and aqueous soluble fractions. Among the four plants the aqueous soluble fraction of *M. nagassarium,* carbon tetrachloride soluble fraction of *H. Kurzii* , aqueous soluble fraction of methanolic extract of *S. trifasciata* exhibited highest thrombolytic activity with clot lysis value of 50.86%, 47.50%, and 47.10% respectively. However, the pet ether and carbon tetrachloride soluble fraction of methanolic extract of *J. gendarussa* demonstrated significant thrombolytic activity as evident from 45.93% and 45.47% lysis of clot, respectively. Standard streptokinase was used as positive control which exhibited 61.50% lysis of clot while the negative control water revealed 2.56% lysis of clot.

Keywords: Sansevieria trifasciata, Justica gendarussa, *Hydnocarpus kurzii*, Mesua nagassarium, thrombolytic, streptokinase.

1. INTRODUCTION

Mesua nagassarium (Bengali - nagesar, nageswar**,** Family- Clusiaceae) is a medium-sized or fairly large evergreen tree up to 36 m tall. A mixture of pounded kernels and seed oil isolated from this plant is used for poultice as wounds. The seed-oil is used for treating itch and other skin eruptions, dandruff and rheumatism [1]. The flowers are known to be useful for the treatment of severe colds, bleeding haemorrhoids, dysentery with mucus, excessive thirst, excessive perspiration, cough and digestion, rheumatism and iron induced lipid peroxidation [2, 3]. Phenolic extract of seed oil of *M. nagassarium* revealed potent anti asthmatic effect [4]. The methanolic extract and its pet-ether and carbon tetrachloride fractions showed the significant antimicrobial activity [5].

Justica gendarussa (Bengali – bishjaron, Family- Acanthaceae) is a shade loving, quick growing, evergreen scented shrub found through out India and also in all Asian countries like Malaysia, Indonesia, srilanka [6, 7]. The plant is used in traditional medicinal practice for chronic rheumatism, inflammations, bronchitis, vaginal discharges, dyspepsia, eye diseases and fever [6, 8]. Justicia found to contain lignans, naturally occurring phenolic dimmers [8] and triterpenoids [9]. Lignans have been used as lead compounds for the development of anti rheumatic agents [8].

Sansevieria trifasciata (Bangle-ghorchhokor, English-bowstring hemp, mother-in-law's tongue, Family- Agavaceae), is a perennial seamless herb with erect leaves arising from an underground rhizome. Leaves are thick, flat, fibrous, and smooth in texture, up to 1 m long, with thin pointed apices, the blade of light green colour with small white lines running perpendicular to the growth of the leaf. Flowers are 6-parted, with green and white perianth parts, fragrant, bone on terminal racemes. *S. trifasciata* is a common ornamental garden plant which is widely cultivated throughout the warmer regions of the world. Phytochemical screening of this plant has shown the presence of N-butyl-4-ol-N-propylphthalate, pregnane glycosides, and steroidal sapogenins [10]. The leaf sap is applied directly to infected sores, cuts and grazes. It is also used to treat fungal and scabies infection [11].

Hydnocarpus kurzii belonging to the Family- Achariaceae, also placed in Flacourtiaceae [12] is a tree attaining the height of 40-50 feet. The fixed oil

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expressed from the ripe seeds is known as hydnocarpus oil, Kalaw tree Oil, leprosy oil [13]. The oil and the crushed seed have long been used in Southeast Asia to treat various skin diseases like scabies, eczema, psoriasis, scrofula, ringworm, and intestinal worms and it has been shown that the active principles of the oil (hydnocarpic and chaulmoogric acids) are strongly antibacterial. For this reason Caulmoogra is employed in Hindu medicine to treat leprosy. The bark contains principles capable of reducing fevers. Seeds are usually applied externally as a dressing for skin diseases combined with walnut oil and pork lard for ringworm; with calomel and sesame oil for leprosy; and with sulfur and camphor for scabies. In India the seeds are considered to be an alternative tonic [14]. There are several thrombolytic drugs obtained from various sources. Some are modified further with the use of recombinant technology [15] in order to make these thrombolytic drugs more site specific and effective.

Previously less attention has been focused on clot lysing activity of *M. nagassarium, S. trifasciata*, *J. gendarussa*, and *H. kurzii*. As they contain a variety of water soluble phytoconstituents, they may have affect as thrombolysis. Thus, the aim of this study was to examine the *in vitro* thrombolytic potentiality of these four medicinal plants of Bangladesh.

2. MATERIALS AND METHODS

2.1. Plant Materials

Leaves of *S. trifasciata*, *H. kurzii* and *M. nagassarium were* collected from Dhaka while *J. gendarussa* was collected from Gazipur in the month of January 2011 and voucher specimens for each of the collections (DACB 35490, , 35491, 35158 and 35489 respectively) have been deposited in Bangladesh National Herbarium (BNH) for future references. The Leaves were first separated from the plants, cleaned, cut into small pieces and air- dried for several days. Then they were dried in oven at 40 ^{o}C to facilitate size reduction through grinding.

2.2. Extraction

The air-dried and powdered leaves of the plants (400 mg each) were separately soaked in methanol (1.5 L each) for 15 days at room temperature with occasional shaking and stirring. It was then filtered through a fresh cotton plug and finally with a Whatman No.1 filter paper. The volume of the filtrate then was reduced using a Buchii Rotary evaporator at low temperature and pressure and Subsequent evaporation of solvents afforded extracts of *S. trifasciata* (8.5 g), *J. gendarussa* (11.5 g), *H. kurzii* (10.5 g) and *M. nagassarium (*12.5 g*)*. An aliquot of 400 gm of each of the concentrated methanolic extract (MES) was separately partitioned by modified Kupchan method [16] and subsequent evaporation of solvents yielded different fractions as mentioned in Table **1**.

2.3. Streptokinase (SK)

Commercially available lyophilized Altepase (Streptokinase) vial (Beacon pharmaceutical Ltd) of 15, 00,000 I.U., was collected and 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100µl (30,000 I.U) was used for *in vitro* thrombolysis.

2.4. Blood Sample

Whole blood (n=10) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and 1ml of blood was transferred to the previously weighed microcentrifuge tubes and was allowed to form clots.

2.5. Thrombolytic Activity

The thrombolytic activity of all extractives was evaluated by the method developed by Daginawala

Table 1: Different Partitionates of *J. gendarussa, S. trifasciata, H. kurzii, K. pinnaata* Obtained by Kupchan Partitioning of 5 gm of Crude Extract

Partitionates	*J. gendarussa*	*S. trifasciata*	*H. kurzii*	*K. pinnaata*
PESF	730 mg	750 mg	720 mg	720 mg
CTSF	500 mg	520 mg	550 mg	600 mg
CHSF	480 mg	470 mg	410 mg	580 mg
AQS	1200 mg	1100 mg	1350 mg	1420 mg

PESF= Pet-ether soluble fraction of methanolic extract, CTSF= Carbon tetrachloride soluble fraction of methanolic extract, CHSF= Chloroform soluble fraction of methanolic extract , AQSF= Aqueous soluble fraction of methanolic extract

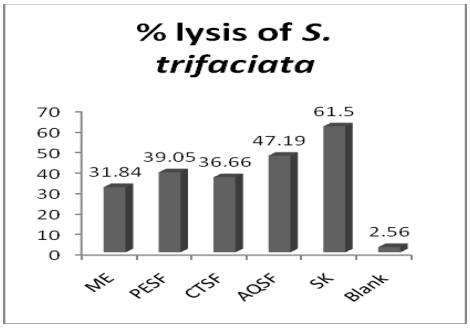

Figure 1: Thrombolytic activity of *S. trifasciata.*

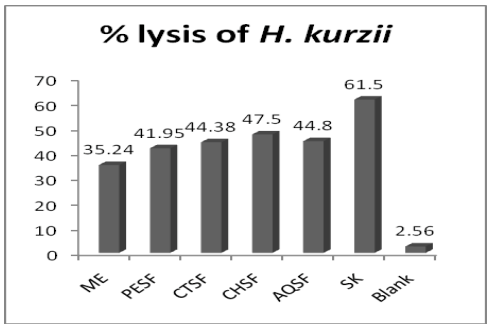

Figure 2: Thrombolytic activity of *H. kurzii.*

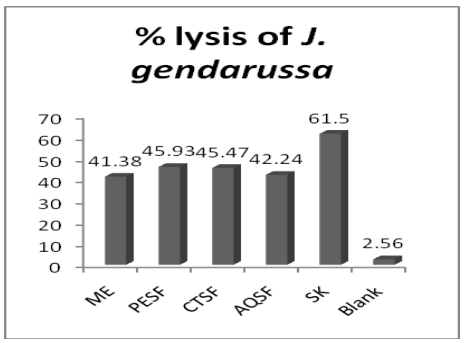

Figure 3: Thrombolytic activity of *J. gendarussa.*

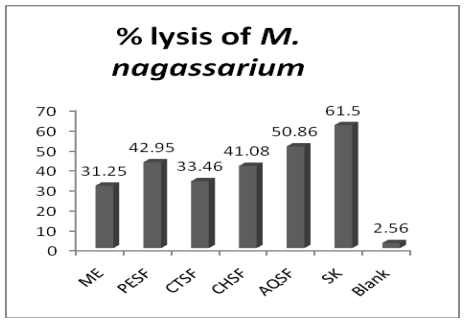

Figure 4: Thrombolytic activity of *M. nagassarium*

[17] using streptokinase (SK) as the standard substance. In the dry extract (100 mg) from each plant was suspended in 10 ml of distilled water and it was kept overnight. Then the soluble supernatant was decanted and filtered through a 0.22-micron syringe filter. For clot lysis venous blood drawn from healthy volunteers was distributed in different pre weighed sterile microcentrifuge tube (1 ml/tube) and incubated at 37 °C for 45 minutes. After clot formation, the 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).

To each microcentrifuge tube containing pre-weighed clot, 100µl aqueous solution of different partitionates and crude extract was added separately. Then, 100µl of streptokinase (SK) and 100 mg of distilled water were separately added to the control tube as positive and negative controls respectively. All the tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, the released of fluid 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 as shown below:

% of clot lysis = (wt of released clot /clot wt) × 100

2.6. Statistical Analysis

The significance between % lysis of clot by Streptokinase and each of the extracts of four plants by means of weight difference was carried out five times in blood samples of fifty different healthy volunteers and results are expressed as mean ± standard deviation.

3. RESULTS AND DISCUSSION

As a part of discovery of cardio protective drugs from natural resources the extractives of *S. trifasciata*, *J. gendarussa*, *H. kurzii* and *M. nagassarium* were assessed for thrombolytic activity and the results are presented in Table **2**. Addition of 100µl SK, a positive control (30,000 I.U.), to the clots and subsequent incubation for 90 minutes at 37°C, showed 61.50% lysis of clot. On the other hand, distilled water was treated as negative control which exhibited a negligible percentages of lysis of clot (2.56%). The mean difference in clot lysis percentage between positive and negative control was found statistically very significant.

In this study, the aqueous soluble fraction of methanolic extract of *M. nagassarium* exhibited highest thrombolytic activity (50.86%). However, significant thrombolytic activity was demonstrated by the chloroform soluble fraction of methanolic extract of *H. Kurzii* (47.5%), aqueous soluble fraction of methanolic extract of *S. trifasciata* (47.1%), pet ether and carbon

Table 2: Thrombolytic Activity (in Terms of % of Clot Lysis) of *M. nagassarium, H. Kurzii, S. trifacicata, J.gendarussa*

M. nagassarium				
Fractions	W1	W2	W3	% of lysis
ME	0.898±0.02	1.5804±0.01	1.3672±0.01	31.24±0.72
PESF	0.896±0.02	1.4114±0.03	1.190±0.01	42.95±0.71
CTSF	0.8882±0.02	1.4674±0.04	1.2736±0.03	33.46±0.59
CHSF	0.9014±0.03	1.7126±0.01	1.3794±0.01	41.08±1.00
AQSF	0.8934±0.02	1.68±0.01	1.28±0.01	50.86±0.87
H. Kurzii				
Fractions	W1	W2	W3	% of lysis
ME	0.9022±0.02	1.583±0.04	1.3332±0.02	35.24±0.75
PESF	0.8866±0.01	1.399±0.01	1.1854±0.01	41.95±1.30
CTSF	0.8964±0.02	1.545±0.01	1.2614±0.01	44.38±1.44
CHSF	0.892±0.02	1.726±0.01	1.334±0.01	47.5±0.73
AQSF	0.8998±0.01	1.683±0.01	1.3298±0.01	44.80±1.18
S. trifacicata				
Fractions	W1	W2	W3	% of lysis
ME	0.8772±0.02	1.128±0.2	1.193±0.1	31.84±0.86
PESF	0.8894±0.02	1.185±0.1	1.1988±0.09	39.05±1.16
CTSF	0.8736±0.02	1.721±0.10	1.3674±0.06	36.66±0.07
AQSF	0.9128±0.01	1.244±0.11	1.1806±0.06	47.19±1.59
J. gendarussa				
Fractions	W1	W2	W3	% of lysis
ME	0.9082±0.01	1.357±0.13	1.2798±0.08	41.38±1.30
PESF	0.8928±0.02	1.39±0.08	1.2204±0.05	45.93±0.47
CTSF	0.8992±0.01	1.733±0.02	1.3514±0.01	45.47±1.14
AQSF	0.8884±0.02	1.438±0.06	1.2738±0.03	42.24±0.48
Blank (water) and Streptokinase				
	W1	W2	W3	% of lysis
Blank	0.888±.001	1.473±.02	1.113±.01	2.56±0.79
SK	0.885±.009	1.467±.02	1.453±.02	61.5±0.17

ME=Methanolic extract, PESF= Pet-ether soluble fraction of methanolic extract, CTSF= Carbon tetrachloride soluble fraction of methanolic extract, CHSF= Chloroform soluble fraction of methanolic extract, AQSF= Aqueous soluble fraction of methanolic extract

W_1 = Weight of micro centrifuge tube (500 (μl/tube) alone; W_2 = Weight of clot containing tube; W_3 = Weight of clot containing tube after clot disruption; SK = Streptokinase

tetrachloride soluble fraction of methanolic extract of *J. gendarussa* (45.93% and 45.47%, respectively), aqueous and carbon tetrachloride *soluble* fraction of methanolic extract of *H. Kurzii* (44.80% and 44.38%, respectively).

Pet ether and carbon tetrachloride soluble fractions of methanolic extract of *S. trifasciata* on the other hand, demonstrated 39.05% and 36.66% lysis of clot, respectively.

CONCLUSION

This is an important finding which may have important implications in cardiovascular health. In addition, this finding may indicate the possibility of developing novel thrombolytic compounds from *S. trifacicata, J. gendarussa, H. Kurzii* and *M. nagassarium* extractives. Further studies are underway to isolate and characterize the compounds responsible for thrombolytic activity.

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