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In vitro Thrombolytic Activity of *Albizia lebbeck* Benth.

Saiful Islam Sohaily[1], Md. Mohidur Rahman[1], Mohammad Firoz Khan[1] and Mohammad A. Rashid[2]

[1]Department of Pharmacy, State University of Bangladesh, Dhaka-1205, Bangladesh
[2]Department of Pharmaceutical Chemistry, University of Dhaka, Dhaka-1000, Bangladesh

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The crude methanol extract of bark of *Albizia lebbeck* Benth. and its Kupchan fractions were screened for thrombolytic activity. Among all partitionates methanol soluble fraction (MESF) of *A. lebbeck* showed 54.13 ± 0.30% clot lysis as compared to 66.98 ± 0.15% demonstrated by the standard Streptokinase (SK).

A. lebbeck Benth. (Bengali Name- Shirish, Kalo koroi, Family- Fabaceae) is native to Indomalaya, New Guinea and Northern Australia and widely cultivated and naturalised in other tropical and subtropical regions. *A. lebbeck* is an unarmed deciduous tree of 12-21 m height that grows all over Bangladesh. The bark of *A. lebbeck* is used medicinally to treat inflammation (Lowry *et al.* 1994). It is an astringent and also used by some cultures to treat boils, cough, eye problems, flu, gingivitis, lung problems and abdominal tumors (Duke, 2008). *A. lebbeck* is also psychoactive (Ratsch, 2004). Previous phytochemical investigations with *A. lebbeck* revealed the occurrences of glycosides (Varshney, 1976), alkaloids (Dixit and Misra, 1997), terpenoids, steroids, saponins (Pal *et al*., 1995), anthraquinone and other phenolics (Deshpande and Shastri, 1997) and lupeol, stigmasterol, 4-hydroxy-3-methoxycinnamic, *trans-p*-coumaric acid (Hussain *et al*., 2008). Since this plant has important medicinal properties, the present study has been undertaken as part of our regular research program (Amran *et al*., 2013; Sikder *et al*., 2013) and we, herein, report thrombolytic activity of the bark of *A. lebbeck* for the first time.

The bark of *A. lebbeck* was collected from Chittagong. The collected barks were cut into small pieces, sun dried for several days and then oven dried for 24 hours at 40 °C to facilitate grinding. The powdered material (600g) was extracted with 1.5 L methanol for 7 days at room temperature and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was then concentrated by using a rotary evaporator at reduced temperature (40-45°C) and pressure. The concetrated methanol extract (ME) was partitionated by the modified Kupchan method (Van Wagenen *et al*., 1993) and the crude extract along with the resultant patitionates petroleum ether (PE), carbon tetrachloride (CT), and aqueous (AQ) soluble materials were used for screening of thrombolytic activity.

For determining thrombolytic activity blood was drawn from healthy human volunteers without a history of oral contraceptives or anticoagulant therapy and 1.0 ml of venous blood was transferred to the previously weighed microcentrifuge tubes and was allowed to clot.

The thrombolytic activity of all extractives was evaluated by the method developed by Daginawala (2006) using streptokinase (SK) as the standard substance. Commercially available lyophilized Alteplase (Streptokinase) vial (Beacon Pharmaceuticals Ltd.) of 15, 00, 000 IU, was collected and 5 ml sterile distilled water was added to it and mixed properly. This suspension was used as a stock from which 100 µl (30,000 IU) was used for *in vitro* thrombolytic studies. The crude extract and each partitionates (100 mg) were 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 containing the clot was again weighed to determine the weight of clot (clot weight = weight of clot contaning tube - weight of tube alone).

Correspondence to: Mohammad A. Rashid; Tel.: +880-2-9661900, Extn. - 8137; Fax: +880-2-9667222; E-mail: rashidma@du.ac.bd

Then to each microcentrifuge tube with the pre-weighed clot, 100 µl aqueous solutions of different partitionates and crude extract was added separately. Then 100 µl of streptokinase and 100 µl of distilled water were separately added to the positive and negative control tubes, respectively. All tubes were then incubated at 37° C for 90 minutes and observed for lysis of clot, if any. After incubation, the released 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 clot after release of fluid/clot wt) × 100

For statistical analysis three replicates of each sample were used for assay and the values are reported as mean ± SD.

The methanol extract of *A. lebbeck* as well as Kupchan partitionates derived from it were subjected to assay for thrombolytic activity and the results are presented in Table 1. Addition of 100 µl SK, a positive control (30,000 IU), to the clots and subsequent incubation for 90 minutes at 37 °C showed 66.98 ± 0.15% lysis of clot. On the other hand, distilled water was treated as negative control produced negligible percentage of lysis of clot. The mean difference in percentage of clot lysis between positive and negative control was found to be statistically significant. In this study, the crude methanol extract (ME) showed highest percentage of clot lysis (54.13 ± 0.30%).

It is clearly evident from the findings that *A. lebbeck* has thrombolytic activity. Therefore, the plant is a good candidate for further studies to isolate bioactive principles of antithrombotic function for developing new cardioprotective drugs.

Table 1. Thrombolytic activity of *A. lebbeck*.

Sample	% clot lysis
ME	54.13 ± 0.30
PE	22.61 ± 0.54
CT	9.50 ± 0.65
AQ	8.29 ± 0.20
Water (Blank)	5.30 ± 0.25
SK	66.98 ± 0.15

ME = Methanol extract; PE= Petroleum ether soluble fraction; CT= Carbon tetrachloride soluble fraction; AQ= Aqueous soluble fraction; Water= negative control (blank); SK=Streptokinase (positive control).

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