Bangladesh Pharmaceutical Journal ***17****(1): 43-45, 2014*

Membrane Stabilizing and Thrombolytic Activities of *Sida rhombifolia* L.

Mohammd Firoz Khan[1], Sikder Nahidul Islam Rabbi[1], Md. Aslam Hossain[2] 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

Abstract

The crude methanol extract of whole plant of *Sida rhombifolia* and different fractions generated from it were tested for membrane stabilizing and thrombolytic activities. In hypotonic solution- and heat-induced conditions, the chloroform soluble partitionates inhibited haemolysis of human erythrocyte by 22.11±0.14% and 64.71±0.08%, respectively as compared to 71.97±0.51% and 40.12±0.29% demonstrated by the standard acetyl salicylic acid (ASA). Moreover, the pet ether soluble materials revealed highest thrombolytic activity with clot lysis value of 19.51±0.03% as compared to 66.98±0.11% exhibited by the standard streptokinase.

Key words: *Sida rhombifolia*, membrane stabilization, thrombolysis.

Introduction

Sida rhombifolia L. (Bengali Name- Lal Berela, Family- Malvaceae) is a small erect undershrub which grows all over Bangladesh (Rahman *et al.*, 2003). It is used in the Indian system of medicine, Ayurveda for the treatment of fever and as diuretic (Kirtikar and Basu, 1990), rheumatism and neurological complaints, including epilepsy (Warrier, 1994). The roots of *S. rhombifolia* were used to treat diarrhoea and indigestion by Australian aborigines (Cribb and Cribb, 1982). In Europe, the root of this species has also been used for treating tuberculosis (Mills, 1994). The whole plant, in combination with other drugs, is prescribed as an anitdote to snake and scorpion venom (Kirtikar and Basu, 1980).

Previous phytochemical investigations of *S. rhombifolia* resulted in the isolation of stigmasterol, β-sitosterol, *n*-hexacos-11-enoic acid (Woldeyes *et al.*, 2012) and daucosterol (Akhtaruzaman *et al.*, 2009).

Since this plant has important medicinal properties, the present study has been undertaken as part of our regular research program (Ferdous *et. al.*, 2012, Sikder *et al.*, 2013) and we, herein, report the results of preliminary studies of membrane stabilizing and thrombolytic activities of whole plant of *S. rhombifolia* for the first time.

Materials and Methods

Plant materials: The whole plant materials (roots, stems and leaves) of *S. rhombifolia* were collected from Tangail and a voucher specimen (DUSH- 6780) for this collection has been deposited in the Salar Khan Herberium of the Department of Botany, University of Dhaka for future reference.

Extraction and fractionation: The collected palnt parts were sun dried for several days and then oven dried for 24 hours at 40 °C to facilitate grinding. The powdered whole plant (1.75kg) of *S. rhombifolia* was extracted with 4.0 L methanol for 7 days and then filtered through a cotton plug followed by Whatman number 1 filter paper. The extract was then concentrated by using a rotary evaporator at reduced temperature (40-45 °C) and pressure. The concetrated aqueous methanol extract (ME) was partitionated by the modified Kupchan method (Van Wagenen *et al.*, 1993) and the resultant patitionates i.e., pet ether (PE), carbon tetrachloride (CT), chloroform (CL) and aqueous (AQ) soluble materials were used for our current inveatigation.

Biological Investigations

i. *Membrane stabilizing activity:* The membrane stabilizing activity of the extractives was determined with human erythrocytes by following the method developed by Omale *et al*. (2008).

Correspondence to: Mohammad A. Rashid; Tel.: 880-2-9661900, Extn. 8137; Fax: 880-2-8615583; E-mail: rashidma@du.ac.bd

ii. *Thrombolytic activity:* The blood was drawn from healthy volunteers without a history of oral contraceptive 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. The extractive (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 containing the clot was again weighed to determine the clot weight (clot weight = weight of clot contaning tube – weight of tube alone). The ethical clearance for the experiment was obtained from the institutional ethical review committee and was performed by following the safe animal handling protocol.

To each microcentrifuge tube with the pre-weighed clot, 100 µl aqueous solution 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,

$$= (\text{wt of clot after release of fluid/clot wt}) \times 100$$

Streptokinase (SK): 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* thrombolysis studies.

Statistical Analysis: Three replicates of each sample were used for each assay to facilitate statistical analysis and the values are reported as mean ± SD.

Results and Discussion

At 1.0 mg/ml, different partitionates of *S. rhombifolia* protected the haemolysis of RBC induced by hypotonic solution and heat as compared to the standard acetyl salicylic acid. The chloroform soluble fraction inhibited 22.11±0.14% and 64.71±0.08% of haemolysis of RBC induced by hypotonic solution and heat as compared to 71.97±0.51% and 40.12±0.29% demonstrated by acetyl salicylic acid, respectively (Table 1).

Table 1. Percentage (%) inhibition of heat- and hypotonic solution- induced haemolysis of erythrocyte membrane by standard and different partitionates of *S. rhombifolia*.

Samples	% Inhibition of haemolysis	
	Heat induced	Hypotonic solution induced
Hypotonic medium	-	-
ME	56.89 ± 0.23	8.56 ± 0.42
CL	64.71 ± 0.08	22.11 ± 0.14
CT	40.64 ± 0.15	6.18 ± 0.52
PE	38.51 ± 0.66	21.46 ± 0.21
ASA	40.12 ± 0.29	71.97 ± 0.51

ME= Methanol extract, CL= Chloroform soluble fraction, CT= Carbon tetrachloride soluble fraction, PE= Pet ether soluble fraction, ASA= Acetyl salicylic acid

As a part of discovery of cardio protective drugs from natural resources, methanol extract of *S. rhombifolia* was subjected to assay for thrombolytic activity and the results are presented in Table 2. 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±0.11% lysis of clot. On the other hand, distilled water was treated as negative control which exhibited a negligible percentage of lysis of clot 3.14±0.31%. The mean difference of in percentage of clot lysis between positive and negative control was found to be statistically significant. In this study, pet ether soluble fraction of *S. rhombifolia* displayed highest thrombolytic activity (19.51±0.03%).

It is clearly evident from the above findings that *S. rhombifolia* has significant membrane stabilizing and thrombolytic activities. Therefore, the plant is a good candidate for further studies to isolate the bioactive principles.

Table 2. Thrombolytic activity (in terms of % clot lysis) of *S. rhombifolia*.

Sample	% of clot lysis
Blank	3.14 ± 0.31
SK	66.98 ± 0.11
ME	12.15 ± 0.04
CL	6.85 ± 0.08
CT	8.42 ± 0.10
PE	19.51 ± 0.03

SK = Streptokinase (positive control), ME= Methanol extract, CL= Chloroform soluble fraction, CT= Carbon tetrachloride soluble fraction, PE= Pet ether soluble fraction, Blank= Water as negative control.

References

Chowdhury, A., Alam, M.A., Rahman, M.S. and Rashid, M.A. 2009. Phytochemical and biological investigations of *Sida rhomboidea* Linn. *The Internet Journal of Alternative Medicine* **7** (2).

Cribb, A.B. and Cribb, J.W. 1982. Useful Wild Plants in Australia. Fontana Books, Australia, p. 109.

Daginawala, H.F., Prasad, S., Kashyap, R.S., Deopujari, J.W., Purohit, H.J. and Taori, G.M. 2006. Development of an *in vitro* model to study clot lysis activity of thrombolytic drugs. *Thrombosis J*. **4**, p. 14.

Ferdous M.R., Rashid R.B., Sikder M.A., Aktar F. and Rashid M.A. 2012. Preliminary *in vitro* biological and phytochemical screenings of Parmentiera cereifera Seem. *Bang Pharm. J*. **15**(2), 103-106.

Kirtikar, K.R. and Basu, B.D. 1980. Indian medicinal plants, B. Singh and M.P. Sing publishers, India, Vol. 1, 2nd ed. p. 310.

Kirtikar, K.R., Basu, B.D. 1990 Indian Medicinal Plant, Vol. 1. International Book Distributor, Dehradun, India, p. 309-310.

Mills, S. 1994. The Complete Guide to Modern Herbalism. Thorsons, London, p. 215.

Omale, J. and Okafor, P.N. 2008. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata*. *Afr. J. Biotechnol*. **7**, 3129-3133.

Rahman, M.M., Khan, Salar, M. and Hossain, S. 2003. Flora of Bangladesh, Malvaceae - Ministry of Environment and Forest Govt. of the People's Republic of Bangladesh, p. 47.

Sikder M.A., Sharmin T., Rahman A. M., Haque M.R., Rahman M. S. and Rashid M.A. 2013. Screenings of four medicinal plants of Bangladesh for bioactivities. *Dhaka Univ. J. Pharm. Sci.* **12**(1), 59-62.

Woldeyes, S., Adane, L., Tariku, Y., Muleta, D. and Begashaw, T. 2012. Evaluation of antibacterial activities of compounds isolated from *Sida rhombifolia* Linn. (Malvaceae). *Nat. Prod. Chem. Res.* **1**,101-108.

Warrier, P.K. 1994. Compendium of 500 Species, Vol. 5. Orient Longman, Hyderabad, pp. 135-137.