

RESEARCH ARTICLE

EVALUATION OF CYTOTOXIC AND THROMBOLYTIC ACTIVITIES OF METHANOLIC EXTRACT OF THE FLOWERS OF *SIDA ACUTA*

Mohammad Mamun Ur Rashid¹, Mohammad Robiul Hossain², Md. Naimul Islam², A.T.M. Mostafa Kamal² and A.T.M. Yusuf^{1*}

¹Department of Pharmaceutical Sciences, School of Health and Life Sciences, North South University, Dhaka, Bangladesh

²Department of Pharmacy, International Islamic University Chittagong, Chittagong-4203, Bangladesh

*E-mail: yusuf_pharma@yahoo.com

Tel.: +8801819917163.

Received: May 21, 2014 / Revised: Oct 12, 2014 / Accepted: Oct 13, 2014

The methanolic extract of flowers of *Sida acuta* (MESAF) was subjected to cytotoxic activity evaluation by *in vitro* brine shrimp lethality bioassay and thrombolytic activity. In brine shrimp bioassay, the crude methanolic extract of flowers showed significant cytotoxic activity with LC₅₀ value of 74.20 µg/ml compared to 0.96 µg/ml, exhibited by standard vincristine sulphate. During thrombolytic activity evaluation, the methanolic extract of *S. acuta* flower showed 24.733±8.017% lysis of clot while standard streptokinase (SK) and water, used as positive and negative controls, demonstrated 79.062±6.197% and 1.54±0.876% lysis of clot, respectively.

Key words: *Sida acuta*, Brine Shrimp bioassay, Thrombolytic activity, Cytotoxic activity, Streptokinase.

INTRODUCTION

All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into 'primary metabolites' such as sugars and fats, which are found in all plants; and 'secondary metabolites', compounds which are found in a smaller range of plants, serving a more specific function. These secondary metabolites can have therapeutic actions in humans which can be refined to produce drugs (Meskin Mark, 2002) and presence of these active constituents contribute for medicinal value of plants (Dey *et al* 2012; Deb *et al* 2013; Arjariya and Nema, 2014).

Sida acuta is a small, erect, perennial shrub, branching profusely from the base. It belongs to the family 'Malvaceae' and has wide application in Nigeria folk medicine. Some herbalist have claimed the traditional use of this plant to cure infections such as malaria, ulcer, fever,

gonorrhoea, abortion, breast cancer following inflammation, wound infections (Kayode, 2006; Edeoga *et al* 2005). The leaf part is the most frequently used against various infections (Figure 1).



Fig. 1. *Sida acuta* flower (Wireweed)

Keeping in view the medicinal properties of *Sida acuta*, present investigation was directed toward the cytotoxic and thrombolytic evaluation of methanolic extracts of *S. acuta* flowers.

MATERIALS AND METHODS

Plant material

The flowers of *S. acuta* were collected from Chittagong hill tracts area and it is authenticated by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong, Bangladesh.

Reagents and chemicals

All chemicals *i.e.* methanol, DMSO and other reagents used in these experiments were of the highest analytical grade. Vincristine sulfate (2 mg/vial; Techno Drugs Limited, Bangladesh) and Streptokinase (1.5 million unit/vial, Sanofi-Aventis Bangladesh Limited) were used as positive control for *in-vitro* cytotoxic test and thrombolytic test respectively. In case of brine shrimp lethality bioassay (cytotoxic test), DMSO was used as negative control, while water was used for thrombolytic test.

Extraction of plant materials

Extraction of flowers of *S. acuta* was done by using organic solvent (Ghani, 2005). The fresh flowers of *S. acuta* were cut, washed and air dried at room temperature ($24\pm 2^\circ\text{C}$) for about 10 days. Dried leaves were macerated into coarse powder. Dried powder (500 g) was then, extracted using methanol. Then, methanolic extract was shaken by rotary shaking apparatus for 7 days. The extract was collected using Buckner funnel. The methanol was evaporated at a temperature below 45°C and concentrated extract was weighed 35 g and stored at 4°C .

In vitro cytotoxic test

Brine shrimp lethality bioassay was used in the bioassay for the bioactive compounds (Meyer *et al* 1982; Zhao *et al* 1992). Simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The dried cyst of the brine shrimp were collected from an aquarium shop (Chittagong, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) with strong aeration for 48 hours day/dark cycles to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using Meyer method (Meyer *et al* 1982). The test sample (extract) were prepared by dissolving them in DMSO (not more than 50 μl

in 5 ml solution) plus sea water (3.8% NaCl in water) to attain concentrations of 2.5, 5, 10, 20, 40, 60 and 80 $\mu\text{g/ml}$. A vial containing 50 μl DMSO diluted to 5 ml was used as a control. Standard vincristine sulphate (Concentrations, 1.25, 2.5, 5, 10, 20, 40 and 60 $\mu\text{g/ml}$) was used as positive control.

Then, matured shrimps were applied to each of all experimental and control vials. After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial were counted. From this data, the percent (%) of mortality of the brine shrimp nauplii was calculated for each concentration using the following formula:

$$\% \text{ Mortality} = N_t \times N_o / 100$$

where N_t = Number of killed nauplii after 24 h of incubation, N_o = Number of total nauplii transferred *i.e.* 10

The LC_{50} (Median lethal concentration) was then, determined using Microsoft Excel 2007.

In vitro thrombolytic test

The thrombolytic activity of plant extract was evaluated by the method of Prasad and collaborators (Prasad *et al* 2006) using streptokinase as standard. The dry crude extract (10 mg) was suspended in 10 ml of distilled water and it was kept overnight. Then the soluble supernatant was decanted and filtered. Aliquots (5 ml) of venous blood were drawn from healthy volunteers which were distributed in five different pre weighed sterile micro centrifuge tube (1 ml/tube) and incubated at 37°C for 45 min. 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 preweighed clot, 100 μl aqueous solutions of different partitionates along with the crude extract was added separately.

As a positive control, 100 μl of streptokinase (SK) and as a negative non thrombolytic control, 100 μl of distilled water were separately added to the control tubes. All the tubes were then incubated at 37°C for 90 min 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. The differences in weights taken

before and after clot lysis were expressed as percentage of clot lysis as shown below:

$$\% \text{ Clot lysis} = (\text{Wt. of released clot} / \text{Clot wt.}) \times 100$$

Statistical analysis

Statistical analysis was performed using SPSS 15. The significance between % clot lysis by herbal extract by means of weight difference was tested

by the paired t-test analysis. Data are expressed as mean \pm standard deviation.

RESULTS AND DISCUSSION

The methanolic extract of *S. acuta* flowers possessed cytotoxic activity. The LC₅₀ values obtained from brine shrimp lethality bioassay was 74.2 $\mu\text{g/ml}$ (Table 1, Figure 1) whereas for vincristine sulfate, LC₅₀ value was 0.96 $\mu\text{g/ml}$.

Table 1. Data of *in-vitro* brine shrimp lethality bioassay of MESAF and vincristine sulphate

MESAF						
Concentration ($\mu\text{g/ml}$)	Log C	No. of nauplii taken	No. of nauplii alive	No. of nauplii death	% Mortality	LC ₅₀ ($\mu\text{g/ml}$)
2.5	0.39794	10	10	0	0	74.2
5	0.69897	10	9	1	10	
10	1	10	8	2	20	
20	1.30103	10	8	2	20	
40	1.60206	10	7	3	30	
60	1.77815	10	6	4	40	
80	1.90309	10	3	7	70	
Vincristine sulphate						
Concentration ($\mu\text{g/ml}$)	Log C	No. of nauplii taken	No. of nauplii alive	No. of nauplii death	% Mortality	LC ₅₀ ($\mu\text{g/ml}$)
1.25	0.09691	10	6	4	40	0.96
2.5	0.39794	10	3	7	70	
5	0.69897	10	2	8	80	
10	1	10	1	9	90	
20	1.30103	10	1	9	90	
40	1.60206	10	0	10	100	
60	1.778151	10	0	10	100	

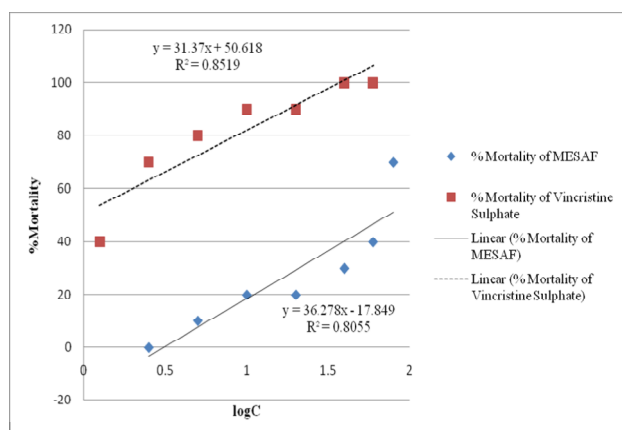


Fig 1. Determination of LC₅₀ value for MESAF and vincristine sulfate from linear correlation between logarithmic concentrations vs % of mortality

Addition of 100 μl SK, a positive control (30,000 I.U.) to the clots along with 90 min of incubation at 37°C, showed 79.062 % clot lysis. Clots when treated with 100 μl sterile distilled water

(negative control), showed only negligible clot lysis (1.54 %).

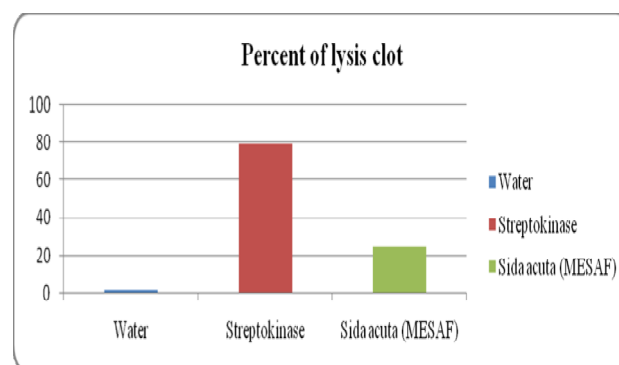


Fig. 2. Thrombolysis of MESAF, streptokinase and water

The mean difference in clot lysis percentage between positive and negative control was very significant (p value < 0.001). The *in vitro* thrombolytic activity study revealed that the methanolic extracts of *S. acuta* flowers (MESAF)

showed 24.733% clot lysis and when compared with the negative control (water) the mean clot lysis % difference was significant (p value <0.001). % Clot lysis obtained after treating clots with these two extracts and appropriate controls

is shown in **Figure 2**. Statistical representation of the effective clot lysis percentage by MESAF, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) is shown in **Table 2**.

Table 2. Percent (%) of lysis clot of MESAF, SK and water

Group	Percent of lysis clot
Water	1.54±0.876
Streptokinase	79.062±6.197*
<i>Sida acuta</i> (MESAF)	24.733±8.017*

Statistical representation of the effective clot lysis percentage by herbal preparations, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) done by paired t-test analysis; % clot lysis is represented as mean ± S.D. and * $p < 0.001$, significant compared to control.

CONCLUSIONS

From results of brine shrimp lethality bioassay, it was revealed that the methanolic extract of *S. acuta* flowers has good cytotoxic activity. *In-vitro* clot lysis study results indicated that *S. acuta* has moderate clot lysis activity. So, it can be concluded that plant extract can be considered as a potential source of natural cytotoxic as well as thrombolytic agents. In context, it would be interesting to investigate the causative components/mechanism for clot lysis by the plant extracts and for brine shrimp lethality bioassay. Moreover, the plant extract should

thoroughly investigated phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentialities.

ACKNOWLEDGEMENT

Authors would like to acknowledge the support by the Department of Pharmacy, International Islamic University Chittagong (IIUC), Chittagong, Bangladesh for this work. Also, authors are thankful to Mr. Kazi Ashfak Ahmed Chowdhury, Lecturer, International Islamic University, Chittagong for his cooperation.

REFERENCES

- Arjariya S, Nema N. Estimation of antipyretic, palliative and anti-inflammatory potential of methanolic extract of *Terminalia catappa*. *Bull. Pharm. Res.* 2014;4(1):37-42.
- Deb L, Bhattacharjee C, Shetty SR, Dutta A. Evaluation of anti-diabetic potential of the *Syzygium cuminii* (linn) skeels by reverse pharmacological approaches. *Bull. Pharm. Res.* 2013;3(3):135-45.
- Dey TK, Emran TB, Saha D, Rahman MA, Zahid Hosen SM, Chowdhury N. Antioxidant activity of ethanolic extract of *Cassia hirsuta* (L.) leaves. *Bull. Pharm. Res.* 2012;2(2): 78-82.
- Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotech.* 2005;4(7):685-8.
- Ghani A. Textbook of Pharmacognosy (Part 1), 1st edition, Institution of Medical Technology, Dhaka, Bangladesh: 2005; 263-4.
- Kayode J. Conversion of indigenous medicinal botanicals in Ekiti state, Nigeria. *J. Zhejiang Univ. Sci. B* 2006;7(9): 713-8. [DOI: 10.1631/jzus.2006.B0713]
- Meskin Mark S. Phytochemicals in Nutrition and Health. CRC Press, Florida, USA: 2002; 123.
- Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plants constituents. *Planta Med.* 1982;45(5):31-4. [DOI: 10.1055/s-2007-971236]
- Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM and Dagainawala HF. Development of an *in vitro* model to study clot lysis activity of thrombolytic drugs. *Thromb. J.* 2006;4:14. [DOI: 10.1186/1477-9560-4-14]
- Zhao G, Hui Y, Rupprecht JK, McLaughlin JL, Wood KV. Additional bioactive compounds and trilobacin, a novel highly cytotoxic acetogenin, from the bark of *Asimina triloba*. *J. Nat. Prod.* 1992;55(3):347-56. [DOI: 10.1021/np50081a011]
- www.eattheweeds.com/sida-wireweed
