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In-Vitro Cytotoxic and Thrombolytic Potential of *Pothos scandens* L

A.T.M Yusuf[1], Mohammad Mamun Ur Rashid[2]*, Mohammed Mainul Hassan[2], Mohammed Aktar Sayeed[2], Mohammed Abdullah Jainul[2]

1. Department of Pharmacy, North South University, Dhaka, Bangladesh.
2. Department of Pharmacy, International Islamic University Chittagong, Chittagnong-4203, Bangladesh. [E-mail: rmamun26@yahoo.com Tel: +8801671322745]

The methanolic extract of leaf of *Pothos scandens was* subjected to screening for cytotoxic activity by *in-vitro* brine shrimp lethality bioassay and thrombolytic activity. In brine shrimp bioassay, the crude methanolic extract of leaf showed strong cytotoxic activity with LC_{50} value of 14.195µg/ml compared to that of 0.305µg/ml exhibited by standard vincristine sulphate. During assay for thrombolytic activity, the methanolic extract of *P. scandens* revealed 19.451±1.711% lysis of clot while standard streptokinase (SK) and water, used as positive and negative controls, demonstrated 69.480± 2.651% and 3.0695± 0.497% lysis of clot, respectively.

Keyword: *Pothos scandens,* Brine Shrimp Lethality, Thrombolytic, % lysis of clot, Vincristine, Streptokinase.

1. Introduction

Herbal medicines are assumed to be of great importance in the primary healthcare of individuals and communities in many developing countries [1]. For thousands of years, these natural plant products have been utilized for human healthcare in the form of drugs, antioxidants, flavours, fragrances, dyes, insecticides and pheromones. However, during the last century the use of synthetic drugs led to a decline in the use of plant-derived compounds, so that the synthetic drugs would perhaps completely replace the use of traditional plant-derived medicines[2].

Pothos scandens (*P. scandens*) is a medicinal aroid, which belongs to the family Araceae. The bruised root of the plant is applied to promote healing of abscesses, after being fried in oil. The people of Indian subcontinent use an infusion of the leaves of this plant as a bath for curing convulsions and epilepsy. Apart from that, the stem is also widely used to treat asthma, after being cut up with camphor and smoked like tobacco. It has been also reported that the whole plant is used against various health problems and disorders such as diarrhea[3], cancer[4], small pox[5], muscle catches, sprains[6] and bone fracture[7]. Recently, Sainuddin reported that ethanolic extract of *P. scandens* is affective in wound healing[8] and Thankarajan Sajeesh informed about antioxidant and antipyretic activity of *P. scandens*[9]. Even though the whole plant possesses so many medicinal properties, extensive research on phytochemical and pharmacological investigations of this plant have never been carried out. Therefore, the present study has been done to analyze the *in-vitro* cytotoxic and thrombolytic activity.

2. Materials and Methods

2.1 Plant Materials

The leaves of *P. scandens* 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-4331, Bangladesh.

2.2 Reagents and Chemicals

All chemicals i.e. methanol, *DMSO* and other reagents used in these experiments were of the highest analytical grade.

Vincristine sulfate (2mg/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.

2.3 Extraction of Plant Materials

Extraction of plant leaves was done by using organic solvent [10].The fresh leaves of *P. scandens* were cut, washed and air dried at room temperature ($24^{o}\pm2^{o}C$) for about 10 days. Dried leaves were macerated into coarse powder. Dried powder (500 gm) 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 $45^{o}C$ and concentrated extract was weighed 29 gm, stored at $4^{o}C$.

2.4 *In-vitro* Cytotoxic Test

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds[11,12]. Here 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[11]. 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 12.5, 25, 50, 100, 200 and 400 µg/ml. A vial containing 50 µL DMSO diluted to 5 mL was used as a control. Standard vincristine sulphate 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:

$$\% \text{ Mortality} = N_t * N_0/100$$

Where, N_t = Number of killed nauplii after 24 hrs of incubation,

N_0 =Number of total nauplii transferred i.e 10.

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

2.5 *In-vitro* Thrombolytic Test [13]

The thombolytic activity of this extractive was evaluated by the method of Prasad and collaborators (2006)[13] 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 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 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 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. The differences in weights taken before and after clot lysis were expressed as percentage of clot lysis as shown below:

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

2.6 Statistical Analysis:

Statistical analysis was performed using SPSS 15. All the results of thrombolytic test were expressed as mean ± standard error of mean (S.E.M.).

3. Results and Discussions:

The methanolic extract of *P. scandens possesses* cytotoxic activity. The LC_{50} values obtained from brine shrimp lethality bioassay was 14.195µg/ml (Table1 and Figure 1) whereas Vincristine sulfate showed 0.305µg/ml (Table 1 and Figure 2).

Table 1: Cytotoxic activity of Methanolic Extract of *P. scandens* and Vincristine Sulfate.

Pothos scandens					
Concentration, C, µg/ml	**Log C**	**No. of nauplii taken**	**No.of nauplii Death**	**% mortality**	**LC_{50}, µg/ml**
12.5	1.09691	20	10	50	14.195
25	1.39794	20	12	60	
50	1.69897	20	13	65	
100	2	20	15	75	
200	2.30103	20	19	95	
400	2.60206	20	20	100	
Vincristine sulfate					
Concentration, C, µg/ml	**LogC**	**No. of nauplii taken**	**No. of naplii Dead**	**% mortality**	**LC_{50} µg/ml**
1.25	0.09691	10	6	60	0.305
2.5	0.39794	10	8	80	
5	0.69897	10	8	80	
10	1	10	9	90	
20	1.30103	10	10	100	
40	1.60206	10	10	100	

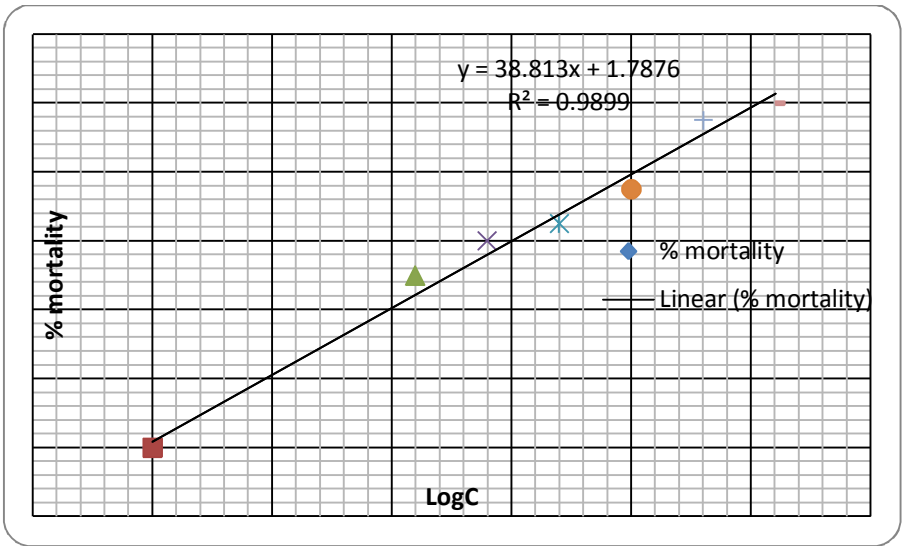

Fig 1: Determination of LC50 Value for Extract of *P. scandens* leaves from Linear Correlation between Logarithmic Concentrations Versus % of mortality.

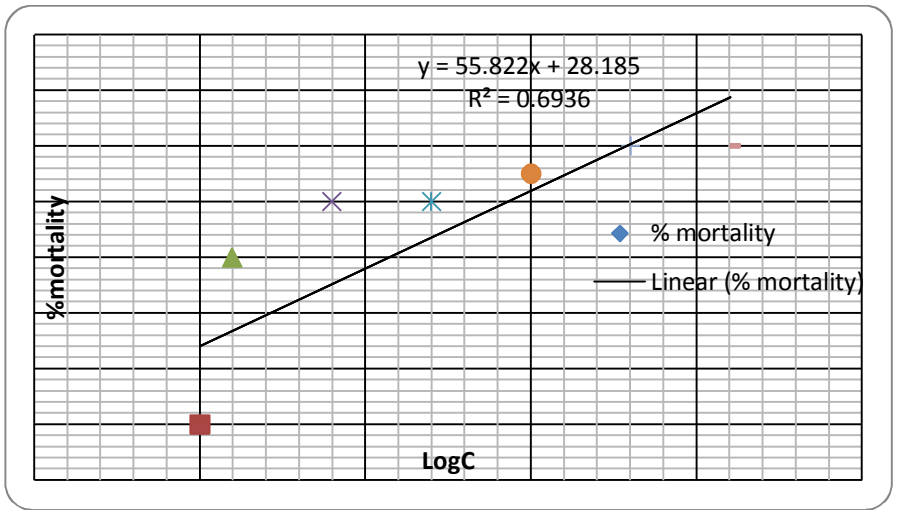

Fig 2: Determination of LC50 value for Vincristine Sulfate from Linear Correlation between Logarithmic Concentrations Versus % Of Mortality.

The methanolic extract of *P. scandens* (MEPS) showed significant thrombolytic activity with 19.451±1.711% (Table 2, Figure 3) lysis of clot. The positive control (Streptokinase) showed 69.480±2.651% and negative non thrombolytic control (distilled water) showed 3.0695± 0.497%lysis of clot.

Table 2: Percent (%) of lysis clot of MEPS, SK and Water

Volunteers	MEPS				SK				Water			
	% lysis of Clot	Average	S.D	SEM	% lysis of Clot	Average	S.D	SEM	% lysis of Clot	Average	S.D	SEM
1	21.569	19.451	5.411	1.711	70.89	69.480	8.384	2.651	5.12	3.0695	1.571	0.497
2	22.695				59.5				2.25			
3	26.982				85.2				1.75			
4	11.689				78.9				0			
5	16.327				69.755				2.75			
6	11.947				72.95				4.45			
7	19.292				71.25				5.05			
8	19.232				60.65				3.45			
9	17.485				60.2				3.125			
10	27.294				65.5				2.75			

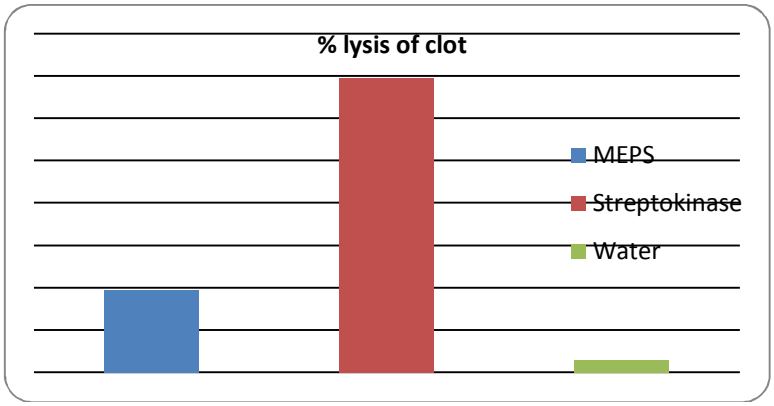

Fig 3: Thrombolysis of MEPS, Streptokinase and Water

4. Conclusion

As apparent from our results of brine shrimp lethality bioassay it can be revealed that the methanolic extracts of *Pothos scandens* has very good cytotoxic activity. From *in-vitro* clot lysis study, we demonstrated that *P. scandens* has moderate clot lysis activity. So that, we may assume that these extracts can be considered as a potential source of natural Cytotoxic as well as thrombolytic agent. In context of the above discussion it would be interesting to investigate the causative components/mechanism for clot lysis by these plant extracts and for brine shrimp lethality. This is only a preliminary study and to make final comment the extract should thoroughly investigated phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentialities.

5. References

1. Ghosh A. Herbal folk remedies of Bankura and Medinipur districts, West Bengal (India). Indian J Tradit Knowl 2003; 2:393-396.
2. Gomez-Galera S, Pelacho AM, Gene A, Capell T, Christou P. The genetic manipulation of medicinal and aromatic plants. Plant Cell Rep 2007; 26: 1689-1715.
3. Sangwoo L, Chunjie X, Shengji P. Ethnobotanical survey of medicinal plants at periodic markets of Honghe Prefecture in Yunnan Province, SW China. J Ethnopharmacol 2008; 117: 362-377.

4. Angkhana I, Shengji P, Balslev H, Wong PW, Trisonthi C. A comparative study on medicinal plants used in Akha's traditional medicine in China and Thailand, cultural coherence or ecological divergence? J Ethnopharmacol 2008; 116: 508-517.
5. Baharuddin S, Mansor M. Medicinal aroids conservation: A case study of floral garden, School of Biological Scie Ces, Universiti Sains Malaysia. Proceedings of the 4th IMT-GT UNINET Conference. 2002; 216-219.
6. Bhandary MJ, Chandrashekar KR, Kaveriappa KM. Medical ethnobotany of the Siddis of Uttara Kannada district, Karnataka, India. J Ethnopharmacol 1995; 47: 149-158.
7. Das AK, Dutta BK, Sharma GD. Medicinal plants used by different tribes of Cachar district, Assam. Indian J Tradit Knowl 2008; 7(3): 446-454.
8. Sainuddin T, Mohammed HKP. Formulation & pharmacological evaluation of herbal gel of Pothos scandens Linn. Webmed Central Wound Healing 2010; 1(12): WMC001344.
9. Sajeesh T, Arunachalam K, Parimelazhagan T. Antioxidant and antipyretic studies on Pothos scandens L. Asian Pacific J Tropical Med 2011; 889-899
10. Ghani, A; Textbook Of Pharmacognosy (Part-1);Edition-1; Institution Of Medical Technology; Mirpur-12. Dhaka-1000; Bangladesh; P(263-264).
11. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen JE, Nichols DE and JL McLaughlin. Brine shrimp: A convenient general bioassay for active plants constituents. J. Med. Plant Res 1982; 45: 31-34.
12. Zhao GY, Hui, JK Rupprecht, JL McLaughlin and KV Wood. Additional bioactive compounds and trilobacin, a novel highly cytotoxic acetogenin, from the bark of Asimina triloba. J. Nat. Prod 1992; 55: 347-356.
13. Prasad S., Kashyap R.S., Deopujari J.Y., Purohit H.J., Tori G.M. and Daginawala H.F., (2006). Development of an in-vitro method to study clot lysis activity of thrombolytic drugs, Thrombosis Journal, 4(2): 1-4.