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In vitro Cytotoxic, Membrane Stabilizing and Thrombolytic Activities of *Polygonum glabrum* Willd

Mohammad Firoz Khan[1], Sikder Nahidul Islam Rabbi[1], Fahima Aktar[2] and Md. Hassan Kawsar[1]

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

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Abstract

The crude methanol extract of leaves of *Polygonum glabrum* Willd and its Kupchan fractions were screened for cytotoxic, membrane stabilizing and thrombolytic activities. Among all fractions, the crude methanol extract showed significant cytotoxic activity having LC_{50} value 0.74 ± 0.045 µg/ml. Moreover, in hypotonic solution- and heat- induced conditions, the crude methanol extract inhibited hemolysis of human erythrocyte by 79.21 ± 0.44% and 84.87±0.23%, respectively as compared to 71.9 ± 0.73% and 42.12 ± 0.37% demonstrated by the standard acetyl salicylic acid. On the other hand, in thrombolytic activity assay the methanol extract demonstrated highest clot lysis value of 35.17 ± 0.42%.

Key words: *Polygonum glabrum*, cytotoxic, membrane stabilizing and thrombolytic.

Introduction

The plant *Polygonum glabrum willd* (Family- Polygonaceae, Common Name- Denseflower knotweed) is an erect, glabrous herb, 70-100cm in height. Annual herb, dilated at nodes, rarely branched (Jamal et al., 2011). *P. glabrum* have been used as folk medicine and as ingredient in various Ayurvedic preparations (Jamal *et al.,* 2011). A decoction of the plant has been used as a foot and leg soak in the treatment of rheumatism (Shiddamallayya *et al.*, 2010; Khare *et al.,* 2007). The leaves of *P. glabrum* are used as anthelminthes and antimalarial agent in Sudan (Hashim and Kamali, 2009). The leaves and roots are used as colic and febrifuge in piles and jaundice (Shiddamallayya *et al.*, 2010). In South India the leaf extract of *P. glabrum* are used to treat dysentery (Soudahmini *et al.*, 2005).

Previous phytochemical studies of *P. glabrum* revealed that the chloroform soluble fraction contains alkaloids, carbohydrates and flavonoids (Sivakumar *et al.*, 2011).

Since this plant has important medicinal properties and based on its availability, therapeutic value and the degree of research work, which is not done mostly in earlier the present study has been undertaken.

Materials and Methods

Plant materials: The leaves of *P. glabrum* were collected from Khulna and a voucher specimen of the plant sample has been deposited in the Department of Botany, University of Dhaka for future reference.

Extraction and fractionation: The collected plant parts were sun dried for several days and then oven dried for 24 hours at 40°C to facilitate grinding. The powdered whole plant (500 gm) of *P. glabrum* was extracted with about 1.5 L methanol for 7 days 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 concentrated methanol extract (ME) was partitionated by modified Kupchan method (Van Wagenen *et al*., 1993) and the resultant partitionates i.e., methanol extract (ME), petroleum ether (PE), carbon tetrachloride (CT), chloroform (CL) and aqueous (AQ) soluble materials were used for different biological screenings.

Cytotoxic activity: This technique was applied for the determination of general toxic property of the plant extractives using the method of Meyer *et al.* (1982) and McLaughlin *et al*. (1998) against *Artemia salina* in a 1-day in vivo assay. Vincristine sulphate was used as positive control.

Correspondence to: Md. Hassan Kawsar;.Tel.: 880-2-9854301; E-mail: hassankawsar@yahoo.com

stabilizing activity of the extractives was assessed by evaluating their ability to inhibit hypotonic solution and heat induced hemolysis of human erythrocytes following the method developed by Omale *et al*. (2008).

Thrombolytic activity: Whole blood was drawn from healthy volunteers without a history of oral contraceptive or anticoagulant therapy and 1.0 ml of blood was transferred to the previously weighed microcentrifuge tubes and was allowed to clot.

The thrombolytic activity of all extracts was evaluated by the method developed by Daginawala (2006) using streptokinase (SK) as the standard substance. The 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 containing the clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube - weight of tube alone).

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 were separately added to the control tube as positive and negative controls respectively. All tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. 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 released clot/clot wt) × 100

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

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

Result and Discussion

The methanol extract of *P. glabrum* as well as different Kupchan partitionates derived from it were subjected to assay for cytotoxic, membrane stabilizing and thrombolytic activities.

The median lethal concentration (LC_{50}) of the test samples after 24 hours was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration and the best-fit line was obtained from the graph by means of regression analysis. Among all the partitionates of crude methanol extract of *P. glabrum*, the crude methanol extract exhibited highest lethality having LC_{50} value 0.74 ± 0.045 µg/ml (Table 1).

Table 1. LC_{50} values of standard and different partitionates of *P. glabrum* in brine shrimp lethality bioassay.

Test samples	Regression line	R^2	LC_{50} (µg/ml)
VS	y = 30.79x + 60.65	0.973	0.471± 0.032
PE	y = 36.03x + 22.04	0.851	5.97 ± 0.222
CT	y = 36.64x + 10.29	0.926	2.04 ± 0.235
CL	y = 42.07x + 2.511	0.928	13.45 ± 0.155
ME	y = 21.74x + 52.87	0.883	0.74 ± 0.045
AQ	y = 35.43x + 7.798	0.961	15.53 ± 0.065

VS= Vincristine sulfate, PE= Pet ether soluble fraction, CT= Carbon tetrachloride soluble fraction, CL= Chloroform soluble fraction, ME= Methanol extract, AQ= Aqueous soluble fraction.

At concentration 1.0 mg/ml, the different partitionate fractions of *P. glabrum* protected the hemolysis of RBC induced by hypotonic solution and heat as compared to the standard acetyl salicylic acid. The crude methanol extract inhibited 79.21±0.44% and 84.87±0.23% of hemolysis of RBC induced by hypotonic solution and heat as compared to 71.9±0.73% and 42.12±0.37% by acetyl salicylic acid, respectively (Table 2).

As a part of discovery of cardio protective drugs from natural resources, methanol extracts of *P. glabrum* was assessed for thrombolytic activity and the results are presented in Table 3. Addition of 100 µl SK, a positive control (30,000 IU), to the clots and subsequent incubation for 90 minutes at 37°C, showed 65.16 ± 0.48% lysis of clot. On the other hand, distilled water was treated as negative control which exhibited a negligible percentage of lysis of clot 2.41 ± 0.27%. The mean difference of in clot lysis percentage between positive and negative control was found statistically very significant. In this study, methanol extract of *P. glabrum* exhibited highest thrombolytic activity (35.17 ± 0.42%).

Table 2. Percentage (%) inhibition of heat and hypotonic solution induced hemolysis of erythrocyte membrane by standard and different partitionates of *P. glabrum.*

Samples	% Inhibition of hemolysis	
	Heat induced	Hypotonic solution induced
CL	11.78 ± 0.12	24.63 ± 0.61
ME	84.87 ± 0.23	79.21 ± 0.44
CT	18.10 ± 0.15	24.63 ± 0.32
PE	76.60 ± 0.88	1.48 ± 0.04
AQ	5.43 ± 0.76	42.47 ± 0.36
ASA	42.12 ± 0.37	71.9 ± 0.73

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

Table 3. Thrombolytic activity of *P. glabrum.*

Sample	% of lysis
Blank	2.41 ± 0.27
SK	65.16 ± 0.48
CL	14.85 ± 0.08
ME	35.17 ± 0.42
CT	15.63 ± 0.33
PE	20.68 ± 0.51
AQ	17.70 ± 0.12

SK = streptokinase, CL= Chloroform soluble fraction, ME= Methanol extract, CT= Carbon tetrachloride soluble fraction, PE= Pet ether soluble fraction, AQ= Aqueous soluble fraction.

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