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Phytochemical Screenings, Thrombolytic Activity, Membrane Stabilizing Activity and Cytotoxic Properties of *Polygonum hydropiper*

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ABSTRACT

Polygonum hydropiper of the family Polygonaceae has been considered as an important plant in the traditional Ayurvedic and in the indigenous medical system in Bangladesh. As membrane stabilizing activity, thrombolytic activity and cytotoxic activity of *Polygonum hydropiper* were not studied earlier, our aim is to find out those activities of *Polygonum hydropiper*. In the present study the phytochemical screening, thrombolytic activity, membrane stabilizing activity and cytotoxic activity of *Polygonum hydropiper* was evaluated. For phytochemical screening, some common standard tests those are available for phytochemical screening was done. Cytotoxicity test was done by Brine shrimp lethality bioassay. The thrombolytic and membrane stabilizing activities were assessed by using human erythrocyte and the results were compared with standard streptokinase (SK) and standard anti-inflammatory drug, Acetyl Salicylic Acid (ASA) or aspirin, respectively. Carbohydrates, steroids, flavonoids and saponins were present in different solvent extracts. The extracts demonstrated significant toxicity to *A. salina* with LC_{50} values ranging from 1.59 to 3.39 μg mL^{-1} and LC_{90} values ranging from 32.36 to 85.11 μg mL^{-1} as compared to standard Vincristine sulphate (VS, LC_{50} value 0.927 μg mL^{-1} and LC_{90} value 6.310 μg mL^{-1}). The ethanol soluble fraction of ethanol extract of *Polygonum hydropiper* revealed highest thrombolytic activity 43.08%. For hypotonic solution induced haemolysis, at a concentration of 1.0 mg mL^{-1}, the Methanolic Extract (ME) inhibited 85.06% haemolysis of RBCs. Our study demonstrates that *Polygonum hydropiper* possesses significant thrombolytic and membrane stabilizing activity and shows moderate cytotoxic activity.

Key words: *Polygonum hydropiper*, phytochemical screenings, thrombolytic activity, membrane stabilizing activity, cytotoxic properties

INTRODUCTION

For decades, the utilization of herbal plants has drawn avalanche of interest as they could accommodate therapeutic response and are promising candidate to be developed as pharmaceutical products. Presently, complication has arises in severity and extent in combating bacterial and fungal infections in behalf of the development of bacteria and fungi resistant to many current antibiotics (Aderogba *et al*., 2005; Rabaud *et al*., 1997). Free radicals are responsible for initiating many serious diseases (Malorni *et al*., 1998; Robert and Meunier, 1998; Pauli *et al*., 2005;

Shah, 2005; Rios and Recio, 2005). These free radicals drive oxidative stress and transform the pathophysiological condition of the patient by acting on immune system. It has been known that phenolic and flavonoid compounds of the plant extracts are responsible for antioxidant and antibacterial effects (Da-Silva *et al*., 2006; Majhenic *et al*., 2007; Pereira *et al*., 2007).

Polygonum hydropiper (Fam. Polygonaceae) locally known as Bishkatali, is an erect or ascending herb with stem decumbent at base, nodes below swollen, linear-lanceolate leaves and small pink flowers in very slender erect or decurved racemes, commonly found in wet places, particularly near the banks of canals and ditches all over the country.

Aerial parts of *P. hydropiper* contain several flavonols and flavones glycosides, including quercetin, quercitrin, kaempferol, rutin, persicarin and its methyl ester, hyperoside, rhamnacin and its ester, a dialdehyde sesquiterpene, tadeonal (polygodial) and its isomers, iso-tadeonal and confertifolin, iso-rhamnazin (Yoshiyasu *et al*., 1983; Yoshiyasu, 1985; Haraguchi *et al*., 1996; Murai *et al*., 1983). Acrid essential oil and dimethyl anthraquinones are present in the leaves (Chopra *et al*., 1969). Tendines, a glucoside, polygopiperin, an inactive alkaloid, tannins and a number of organic acids are also found in this herb. Tannins, ellagic acid methyl ether, gallic acid, anthraquinone and oxymethyl anthraquinone, quercetin glycosides and iso-coumarin polygonolide are restrained in roots (Barnes and Loder, 1962; Furuta *et al*., 1986).

Traditionally juice of leaves is reported to be useful in pain, headache, toothache, liver enlargement, gastric ulcer, dysentery, loss of appetite and dismenorrhoea. Juice of paste is applied to wounds, skin diseases and painful carbuncles. Root is used as stimulant. Preparation of the plant is used to cause premature abortion. Leaf paste is also capable of stopping external haemorrhage (Ghani, 2003).

The aim of present study is to evaluate bioactivities including phytochemical screening, thrombolytic activity, membrane stabilizing activity and cytotoxic activity by using brine shrimp lethality assay of the plant extractives.

MATERIALS AND METHODS

Plant materials collection and identification: Whole plant sample of *Polygonum hydropiper* was collected from Tongi, Gazipur, Dhaka, Bangladesh in June 2012. Then the plant sample was submitted to The National Herbarium of Bangladesh, Mirpur, Dhaka for its identification. One week later its voucher specimen was collected after its identification (Accession No. 37756) which was identified and authenticated by taxonomist of the National Herbarium of Bangladesh.

Plant materials preparation: Whole plant of *P. hydropiper* was separated in different parts and sun dried for 7 days. Then leaves were taken and oven dried for 3 h at 40°C temperatures and then 800 g dried leaves were grinded in coarse powder using high capacity grinding machine. About 715 g of grinded powders were sieved to get fine powder. Finally 630 g of fine powder obtained which was then stored in air tight container with necessary marking for identification and kept in cool, dark and dry place for further investigation.

Powdered leaves (15 g) were successively extracted in Soxhlet extractor at elevated temperature (40-60°C) by using 300 mL of methanol (solvent). Then by same process, ethanol, chloroform, petroleum ether and n-Hexane were extracted too. All extracts were filtered individually by Watman filter paper. Then all extracts were poured on petri dishes individually to evaporate liquid and proper dry. After drying, crude extracts were stored in those petri dishes and kept in refrigerator (0-4°C) for future investigation.

Preliminary phytochemical screening: Methanol extract (0.5 g) of *P. hydropiper* was dissolved in 50 mL of methanol and was subjected to preliminary phytochemical screenings for determining nature of phytoconstituents (Horbone, 1998; Kokate, 2001).

Thrombolytic activity: The thrombolytic activity of all extracts was evaluated by the method developed by Prasad *et al.* (2006) and using streptokinase (SK) as the standard.

Blood samples of volunteers: Blood (n = 6) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy.

Streptokinase (SK): Commercially available lyophilized streptokinase (15,00,000 I.U.) vial (Trade name: S-Kinase from Popular Pharmaceuticals Ltd., Tongi, Bangladesh) was used as standard in this test. On this vial 5 mL 0.9% NaCl was added with powdered streptokinase and mixed properly. The concentration the streptokinase became 30,000 I.U. which was used reference standard for *in vitro* thrombolysis.

Membrane stabilizing activity: The erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane (Omale and Okafor, 2008). The membrane stabilizing activity of the extractives was assessed by using hypotonic solution-induced and heat-induced mice erythrocyte haemolysis (Shinde *et al.*, 1999).

To prepare the erythrocyte suspension, 10 mL blood was obtained from healthy human volunteers and was taken in syringes containing anticoagulant EDTA (3.1% Na-citrate). The blood was centrifuged for 10 min at 3000 g and blood cells were washed three times with solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4) through centrifugation for 10 min at 3000 g.

Hypotonic solution-induced haemolysis: The test sample consisted of stock erythrocyte (RBC) suspension (500 μL) mixed with 5 mL of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing all kinds of extracts (1.0 mg mL^{-1}) and standard acetyl salicylic acid (0.1 mg mL^{-1}). The control sample consisted of 500 μL of RBCs mixed with hypotonic-buffered saline alone. The mixture was incubated for 10 min at room temperature, centrifuged for 10 min at 3000 g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of either haemolysis or membrane stabilization was calculated using the following equation:

$$\text{Inhibition of haemolysis\%} = 100\times(OD_1\text{-}OD_2/OD_1)$$

where, OD_1 is optical density of hypotonic-buffered saline solution alone (control) and OD_2 is optical density of test sample in hypotonic solution.

Heat-induced haemolysis: Fresh human blood (10 mL) was collected and transferred to the centrifuged tubes containing anticoagulant EDTA (1 mL of 3.1% Na-citrate). The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline (0.9% NaCl). The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline(0.9% NaCl).

The reaction mixture (2 mL) consisted of 1 mL of all kinds of extracts (1.0 mg mL^{-1}) or test samples and 1 mL of 10% RBCs suspension, instead of drug only saline was added to the control test tube. Aspirin or acetyl salicylic acid (0.1 mg mL^{-1}) was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56°C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates. The percentage inhibition or acceleration of hemolysis in tests and was calculated according to the equation:

$$\text{Inhibition of hemolysis\%} = 100\times [1- (OD_2\text{-}OD_1/ OD_3\text{-}OD_1)]$$

where, OD_1 is optical density of unheated test sample, OD_2 is optical density of heated test sample and OD_3 is optical density of heated control sample.

Brine shrimp lethality bioassay: Brine shrimp lethality bioassay (Meyer *et al*., 1982; McLaughlin *et al*., 1998) technique was applied for the determination of general toxic properties of the plant extractives. DMSO solutions of the samples were applied against Artemia salina Leach in a 1-day *in vivo* assay For the experiment.

Statistical analysis: Data were statistically analyzed with the help of Microsoft office excel-2010.

RESULTS AND DISCUSSION

Preliminary phytochemical screening: Previously, the presence of triterpenes and/or steroids, coumarins, flavonoids, polyphenols, tannins and saponins were evaluated in different extracts of *P. hydropiperoides* in different research studies (Jacome *et al*., 2004). Here, Phytochemical screening of the crude extracts of the leaves of *Polygonum hydropiper* (L.) revealed the presence of different kinds of chemical groups summarized in the Table 1.

Thrombolytic activity: As a part of discovery of cardio-protective drugs from natural sources of leaf extractives of *P. hydropiper* were assessed for thrombolytic activity and the results are presented in Table 2. Addition of 100 µL SK, a positive control (30,000 I.U.), to the clots and subsequent incubation for 90 min at 37°C, showed 59.20% lysis of clot. At the same time, distilled

Table 1: Result of chemical group test of leaf extracts of *Polygonum hydropiper*

	Name of extracts				
Name of tests	Methanol	Ethanol	Chloroform	Pet-ether	n-Hexane
Alkaloids	-	-	-	-	-
Carbohydrates	++	++	++	++	++
Flavonoids	+	+	+	+	+
Glycosides	+++	++	-	-	-
Phenols	+	+	-	-	-
Saponins	+	+	+	+	+
Steroids	++	++	+++	+	+
Tannins	++	++	-	-	-

+++: Presence in high concentration, ++: Presence in moderate concentration, +: Presence in low concentration, -: Absence

Table 2: Clot lysis % by different extracts of *Polygonum hydropiper*

Name of samples	Clot lyses (%)
Control	3.215±0.52
Standard (SK)	59.20±0.800
Methanol extract	24.43±1.660
Ethanol extract	43.08±1.290
Chloroform extract	36.48±1.590
Petroleum ether extract	40.60±1.500
n-Hexane extract	20.15±1.270

SK: Streptokinase

Table 3: Effect of extractives of *Polygonum hydropiper* on hypotonic solution (A) and heat induced (B) of erythrocyte membrane

Group	Concentration (mg mL^{-1})	(A) Haemolysis inhibition (%)	(B) Haemolysis inhibition (%)
Control	Only		
Hypotonic solution	-	-	
Standard (Acetyl salicylic acid)	0.1	92.13±0.012	73.79±0.03
Methanol extract	1	85.06±0.010	57.90±0.09
Ethanol extract	1	86.1±0.0090	55.61±0.03
Chloroform extract	1	87.3±0.0170	76.45±0.05

water was treated as negative control which exhibited negligible lysis of clot (3.215%). In this study, after treatment of clots with 100 µL methanolic, ethanolic, chloroform, pet ether, n-Hexane extract of, clot lysis 24.43, 43.08, 36.48, 40.60 and 20.15% was obtained respectively. In this study, the ethanol soluble fraction of ethanol extract of *Polygonum hydropiper* revealed highest thrombolytic activity 43.08%, whereas methanolic, chloroform, pet ether, n-Hexane extract of *Polygonum hydropiper* (24.43, 36.48, 40.60 and 20.15%) displayed moderate thrombolytic activities.

On the basis of the result obtained in this present study we can say that the *Polygonum hydropiper* leaves extract have mild thrombolytic activity compared to standard. So, in comparison with standard, *Polygonum hydropiper* can be further use as a mild thrombolytic agent. In previous, the thrombolytic activity of this plant was not carried out.

Membrane stabilizing activity: The whole plant extractives of *P. hydropiper* at concentration of 1.0 mg mL^{-1}, were tested against lysis of human erythrocyte membrane induced by hypotonic solution as well as heat and compared with the standard Acetyl Salicylic Acid (ASA) (0.10 mg mL^{-1}) (Table 3). For hypotonic solution induced haemolysis, at a concentration of 1.0 mg mL^{-1}, the methanolic extract (ME) inhibited 85.06% haemolysis of RBCs as compared to 92.13% produced by acetyl salicylic acid (0.10 mg mL^{-1}). The ethanol and chloroform soluble extractives also revealed significant inhibition of haemolysis of RBCs. To confirm the membrane stabilizing activity of *Polygonum hydropiper* observed in the above mentioned model (Omale and Okafor, 2008), experiments were performed on the erythrocyte membrane. A possible explanation for the stabilizing activity of the extractives due to an increase in the surface area/volume ratio of the cells which could be brought about by an expansion of membrane or shrinkage of the cell and an interaction with membrane proteins. The present investigation suggests that the membrane stabilizing activity of *Polygonum hydropiper* may be playing a significant role in its anti-inflammatory activity. Membrane stabilizing activity of *Polygonum hydropiper* was not tested previously.

Table 4: LC_{50} and LC_{90} values of the five extracts of *Polygonum Hydropiper* and standard

Test samples	LC_{50}	LC_{90}
Vincristine (Standard)	0.927	6.310
Methanol extract (ME)	3.090	85.113
Ethanol extract (EE)	2.089	60.256
Chloroform extract (CE)	3.311	32.359
Pet-ether extract (PEE)	3.389	77.625
n-hexane extract (n-HE)	1.585	48.977

Brine shrimp lethality bioassay: In the brine shrimp lethality bioassay, the LC_{50} values of ME, EE, CE, PEE and n-HE of *P. hydropiper* were found to be 3.09, 2.089, 3.311, 3.389 and 1.585 μg mL^{-1}, respectively and LC_{90} values of ME, EE, CE, PEE and n-HE of *P. hydropiper* were found to be 85.113, 60.256, 32.359, 77.625 and 48.977 (Table 4). LC_{50} and LC_{90} were calculated by plotting graph in Microsoft office excel-2010 grade sheet.

CONCLUSION

The phytochemical screening revealed the presence of carbohydrates, flavonoids, glycosides, phenols, saponins, steroids and tannins. In percent mortality of Brine Shrimp nauplii produced by the extracts of *Polygonum hydropiper* indicates the presence of cytotoxic principles in these extracts. After thrombolytic and membrane stabilizing activity significant results are shown in the present study.

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