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Phytochemial investigation and evaluation of antiemetic & anthelmintic activities of *Polygonum lapathifolium* roots extract

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Abstract

The present study was designed to explore the phytochemical constituents, anti-emetic & anthelmintic effect of methanolic extract of *Polygonum lapathifolium* roots. The phytochemical screening shows the presence of alkaloids, phytosterols, triterpins, flavonoids. & Saponin, those are responsible for antiviral, antibacterial, antiallergic, antihypertensive, antiarrythmic, hepatoprotective, anti-inflammatory effects in mammals. In vitro anthelmintic activity test (using *Pheretima posthuma* model), the parameters like: time of paralysis and time of death were determined by using the extracts at the concentrations of 20, 40, 60, 80 and 100 mg/ml. The roots extracts exhibited significant anthelmintic activity at a concentration of 60mg/ml. Observations were comparable with piperazine citrate (10mg/ml) as standard reference. In anti-emetic test, emesis was induced by the oral administration of copper sulphate (50mg/kg) using chick emesis model. Roots extract (150mg/kg orally) showed statistically significant antiemetic effect (89.37% Inhibition) compared with reference drug metoclopramide (50mg/kg intraperitoneally) which showed 82.48% antiemetic activity.

Key-Words: *Polygonum lapathifolium*, *Pheretima posthuma*, Piperizine citrate, Chick, Metoclopramide, Copper sulphate

Introduction

The family Polygonaceae consists of several important medicinal plants with wide range of biological activities and interesting phyto-chemical constituents. Various plants of Polygonaceae used in the management of GI complication and helminthiasis treatment in traditional medicine[1-2]. *P. lapathifolium* (s.l.) generally known as Knotweed belongs to the family Polygonaceae, is 2'-5' tall annual herb [3-4]. *P. lapathifolium* is a widespread weed in Britain and it is probably present in all vice-counties. It is regarded as native in Europe and Asia and as a naturalized alien in America and Australasia[5]. The genus also grows in northern temperate regions including Bangladesh, India, Britain, S. Africa [3-4].The plant grows in swampy areas, roadsides, floodplains, waste places [6]. The round stems are hairless and somewhat swollen near the base of the leaves. The stems are sometimes glabrolls but are more usually very slightly pubescent.

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The leaves of *P. lapathifolium* are lanceolate to linear-lanceolate and have smooth margins. The alternate leaves are up to 2'-10' long and broader than those of the preceding. At the base of each petiole of the leaves, there is a membraneous sheath (ocrea) that wraps around the stem [5,7]. The peduncles of *P. lapathifolium* are glandular with stalked glands. The upper stems terminate in spike-like racemes of flowers. The small flowers are densely crowded together along the length of the raceme. They are usually pink ,white or greenish white, and less often light pink. Each flower is about 1/8" long, consisting of 5 sepals and no petals. Because the flowers usually don't open fully, the inner sepals are often difficult to observe [8]. The seeds are in fact nuts which fall from the parent plant with the dead perianth still attached. Each seed is dark brown or black, rather flat and oval in shape, and up to 2 mm. across having a smooth shiny surface. Roots are blackish in color usually 3-5 cm in length and10-20mm in width. They are cluster in nature and contain more than 100 roots are in a cluster [5,8].The whole plant has antiseptic and astringent property. An infusion of root has been used in the treatment of stomach complaints

and fevers. The plant is also applied externally on burns. Young leaves & seeds are used as raw or cooked form. The plant produces a soft white mass which is used for bathing and washing clothes [4]. The selection of plant *P. lapathifolium* was based on its availability, therapeutic value and the degree of research work, which is not done mostly in earlier. Keeping in mind about the adverse effects of synthetic drugs available in the market, *P. lapathifolium* roots extract were used for the screening of different pharmacological activities and active constituents present in the extract.

Material and Methods

Collection & Identification of **Plant material**

Plant sample of P. lapathifolium were collected from Noakhali Science and Technology University campus, Sonapur, Noakhali, in September 2012. The plant was identified by the expert of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh (Identification number-37924).

Preparation of the plant materials

The collected plant parts (Roots) were separated from undesirable materials or plants or plant parts & washed thoroughly with water several times. During collection any type of adulteration was strictly prohibited. They were sun-dried for one week and then dried in an oven at reduced temperature (not more than 50^0C) to make it suitable for grinding. The coarse powder was then stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Extraction of the plant material

The dried and ground plant powder of P. lapathifolium (roots -250 gm) were soaked in 1.3 liter methanol. Plant powders were kept in separate desiccators at room temperature with occasional stirring and shaking for 20 days. The extract was then filtered through filter-cloth. The filtrate was kept to dry in fresh and clean air to afford a greenish mass of biological investigation.

Worm Collection and Authentication

Earthworms, *Pheretima posthuma* (Annelida), were collected from moist soil at Noakhali Science & Technology University, Noakhali Dhaka and washed with normal saline to remove soil and fecal matter. Earthworms were identified by Fisheries & Marin Science Dept, Noakhali Science & Technology University. The earthworms of 4-6 cm in length and 0.2-0.3 cm in width were used for the experimental protocol.

Animals

Young male chicks, 2- 4 days of age, weighing from 32-52 gm were obtained from a poultry local store. After 24 hrs fasting, the antiemetic activity was evaluated. All chicks were kept under laboratory conditions at room temperature with 12h light and dark cycles. All animal experiments were carried out in accordance with the acts of the Animal Ethical Committee of NSTU Research Cell, Noakhali Science and Technology University.

Chemicals

Piperazine Citrate was purchased from GlaxoSmithKline (BD) Limited. Unless stated otherwise, all other reagents were from Sigma Chemicals limited. Copper sulfate was purchased from Scharlau Chem-ie S.A. Barcelona, Spain. Metoclopramide hydrochloride was purchased from. Dimethyl sulfoxide (DMSO), Polyoxy-ethylene sorbitan monooleate (Tween 80) and methanol were purchased from Merck, Darm-stadt, Germany. Acetic anhydride, Sulphuric acid, lead acetate, Nitric acid, Copper acetate were also purchased from Merck, Darm-stadt, Germany.

Phytochemical Screening

Preliminary phytochemical study was screened for presence of alkaloid, phenols, phytosterols, Saponins, proteins and aminoacids, flavonoids ,diterpenes & triterpenes. These were identified by characteristic colour changes using standard procedures [9-10]

Detection of alkaloids

Hager's Test: Extracts were dissolved individually in dilute Hydrochloric acid and the solutions were filtered. Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids was confirmed by the formation of yellow colored precipitate.

Detection of phytosterols

Libermann Burchard.s test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of diterpenes

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicated the presence of diterpenes.

Detection of triterpenes

Libermann-Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled and then conc. sulphuric acid was added. Formation of brown ring at the junction confirmed the presence of phytosterols.

Detection of flavonoids

Lead acetate Test: Extracts were treated with 4-5 drops of lead acetate solution. Formation of yellow color

precipitate indicates the presence of flavonoids.

Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicated the presence of phenols.

Detection of proteins and aminoacids

Xanthoproteic Test: The extracts were treated with 4-5 drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

Detection of saponins

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. Foam was produced which remained for 10 minutes and confirmed the presence of saponins.

Anthelminic activity

The anthelmintic assay was carried as per the method of Ajaiyeoba et al. [11] with minor modifications. In this experiment, *P. lapathifolium* were used because of its anatomical and physiological similarity with intestinal roundworm parasites of human beings and they are belonging to same group of Annelida. All the test solutions and standard drug solutions were prepared freshly before starting the experiment. Piperazine citrate (10mg/ml) was used as reference standard while saline water served as a control. The earthworms were divided into different groups with equal size & each group containing six worms. 60 ml formulations containing five different concentrations of methanolic extracts of *P. lapathifolium* (20, 40, 60, 80 and 100 mg/ml in distilled water) roots were prepared. All the test solution and standard solution were prepared freshly before starting experiments. The time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. The times of death of the worms were recorded after ascertaining that worms neither moved when shaken vigorously or when dipped in warm water (50^0C).

Anti-emetic activity

The antiemetic effect was determined by calculating the mean decrease in number of retching following the protocols of Akita et al [12], 1998. The 4 days old yang chicks were divided into four groups of five chicks each and each chick was kept in a large beaker at 25^0C for 10 minutes. The extracts of *P. lapathifolium* roots were dissolved in 0.9% saline containing 5% DMSO and 1% Tween 80 and administered at a dose of 150 mg / kg orally and volume of 10 ml / kg to the test animal on the basis of their body weights. Control group received only saline 0.9%. After 10 minutes copper sulphate was administered orally at 50 mg / kg, then the number of retching was observed during next ten minutes. Metoclopramide was used as a standard drug (50 mg/kg .b.w intraperitoneally).

The antiemetic effect was assessed as the decrease in number of retches in the treated group in contrast to the control. The inhibition (%) was calculated as follows:

$$\text{Inhibition (\%)} = \frac{A - B}{A} \times 100$$

Where A is the control frequency of retching and B is the frequency of retching of the treated group.

Statistical analysis

In case of Anthelmintic activity test, the experimental data were calculated as mean ± SEM, evaluated by unpaired one way ANOVA, test values of $P<0.01$ were considered statistical significant. All numerical data are expressed as the mean ± standard error of mean (SEM). In case of anti-emetic test, statistical analysis was carried out using student's t-test and differences between means were considered to be significant when $p < 0.05$.

Results and Discussion

Phytochemical screening

By preliminary phytochemical screening it was found that roots extract contain alkaloids, phytosterols, triterpins, flavonoids. & Saponin. (Table .1)

Antiemetic activity

Result of the antiemetic activity of methanolic extract of *P. lapathifolium* roots were given in Table 2. After administration of a dose of 50 mg/kg BW Metoclopramide and the extracts of roots (150/ kg BW), the numbers of retches were reduced. The group of chicks treated with Metoclopramide was found to have 12 retches as compared to the 64 retches of control group, thus Metoclopramide reduced the retches by 81.25%. The chicks treated with root extracts inhibited the retches up to 89.37%.Therefore, methanolic extracts of root inhibited emesis to an extant greater than Metoclopramide at 50 mg/kg (Table 2 & Fig. 1).

Anthelmintic activity

The methanolic extracts of roots showed a significant anthelmintic activity in dose dependent manner (Table 3, Fig. 2).In case of roots extract, the paralysis time at different concentrations, including 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml ,100 mg/ml and was 46.83,22.50, 15.16, 11.16, 5.83 minutes respectively, whereas death time was 59.66, 37.33, 32.50, 24.50, and 14.00 minutes respectively .The paralysis and dead time for standard piperazine citrate at a concentration of 10 mg/ml were 20.33 and 34.16 minutes, respectively.

Antiemetic activity

On the basis of these results it may be concluded that extract of roots have anti-emetic potential and are comparable with that of Metoclopramide (the reference

drug). Although the results are significant but the mode of action is not known.

However, as the oral copper sulphate induces emesis by peripheral action [13], and the extracts were able to effectively prevent its effect, it could be implied that these extracts have a peripheral anti-emetic action. This study also justifies the traditional use of *P. lapathifolium* in G.I.T complaints. From chemical point of view, roots of *P. lapathifolium* contain alkaloids and showed highest activity as compared to standard. Therefore, it may be said that alkaloidal contents may play some role in anti-emetic effect [14].

Retching may occur after administration of cancer chemotherapeutic agents .Chemotherapy-induced nausea and vomiting (CINV) is a common side-effect of many cancer treatments. Nausea and vomiting are two of the most feared cancer treatment-related side effects for cancer patients and their families. It has also been established that the peripheral 5- HT4 receptors play an important role in copper sulfate induced emesis [15]. Chemotherapeutic agents or their metabolites can directly activate the medullary chemoreceptor trigger zone or vomiting center or act peripherally by causing cell damage in the gastro-intestinal tract and releasing serotonin from enterochromaffin cells of the small intestinal mucosa. The released serotonin activates 5-HT receptors on vagal and splanchnic afferent fibers, which then carry sensory signals to the medulla, leading to the emetic response [13,16]. Metoclopramide, which has already been known to elicit antiemetic activity through acceleration of gastrointestinal tract movement [15], was found to be less effective than roots extract. *P. lapathifolium* reduces copper sulfate induced retching in young chicks, possibly by peripheral action as the oral copper sulfate induces emesis by peripheral action through excitation of visceral afferent nerve fibers of the gastrointestinal tract [17]. The observed antiemetic activity of *P. lapathifolium* flowers and roots extracts may be attributed to its alkaloid and terpenes constituents. Until now, no other research papers are found to the antiemetic activity of *P. lapathifolium* flowers and roots extracts and thus provides scientific basis for its use in folk medicine for the management of GI complication. Further studies are required to determine the exact mode of action and the active compounds responsible for this effect.

Anthelmintic activity

The anthelmintic activity of methanolic extracts was comparable with that of standard drug (piperazine citrate).The methanolic extracts of *P. lapathifolium* demonstrated paralysis as well as death of worms in a less time as compared to piperazin citrate especially at higher concentration of 100 mg/ml. From the Table No.3 & Fig. 2 , where the concentration of 40,60, 80,100 mg/ml & 60, 80,100 mg/ml of flowers and roots extracts showed a significant anthelmintic activity respectively comparable with the standard drug at concentration of 10mg/ml. of piperizine citrate. Methanolic extract of roots produces dose-dependent paralysis ranging from loss of motility to loss of response to external stimuli, which gradually progressed to death . The results illustrated that the significant anthelmintic property of *P. lapathifolium* roots might be due to the presence of alkaloids, flavanoids and triterpenoids. Various studies stated that these phytochemicals have anthelmintic properties [18-20]. Further attention has to be carried out for isolation and characterization of the active components to establish an effective drug resource scientifically.

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Table 1 : Results of different group tests of *P. lapathifolium* roots extract

Extract	Alkaloid	Phytosterols	Diterpins &Triterpens	Amino acid & protein	Flavonoids	Phenolic compounds	Saponin
Roots Extracts	+	+	- +	-	+	-	+

+= Presence, - = Absence

Table 2: Antiemetic activities of methanolic extracts of *P. lapathifolium* roots extract

Drug / dose	No. of retches (Mean±S.E.M)	% Inhibition
Control (10ml/kg)	64.00±1.58	--
Metoclopramide (50 mg/kg)	12±1.89	81.25%
P. lapathifolium roots extracts (150mg/kg)	6.80±0.66	89.37%
*S.E.M=Standard Error Mean		

Table 3: Anthelmintic activity of methanolic extracts of *P. lapathifolium* roots extract

Serial No	Concentration (mg/ml)	Time taken for paralysis in min. (Mean±S.E.M)	Time taken for Death in min. (Mean±S.E.M)
Control	-	-	-
Piperazine Citrae	10	20.33±1.45	34.16±1.88
P. lapathifolium roots extracts	20	41.00±2.22	63.00± 1.59
	40	19.66±0.42	39.33±1.22
	60	13.33 ±1.05	26.66±1.54
	80	9.33±0.66	20.50±0.76
	100	5.66±0.66	15.85±1.42

*S.E.M=Standard Error Mean

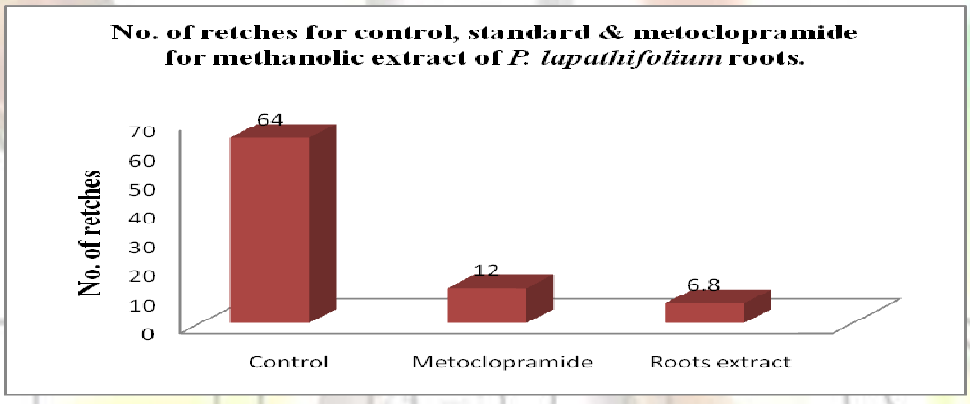

Fig. 1: No. of retches for control, standard and methanolic extracts of *P. lapathifolium* roots

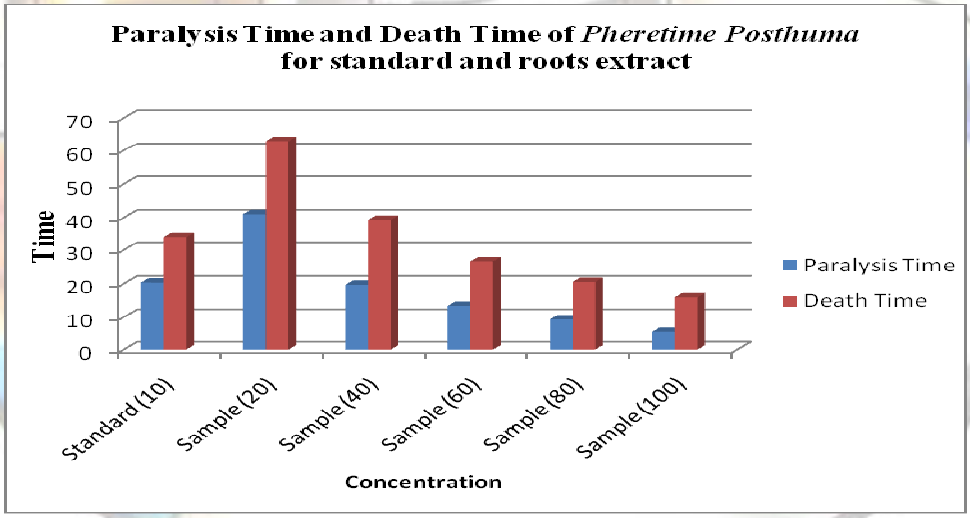

Fig. 2: Paralysis Time and Death Time of *P. posthuma* for standard and roots extracts