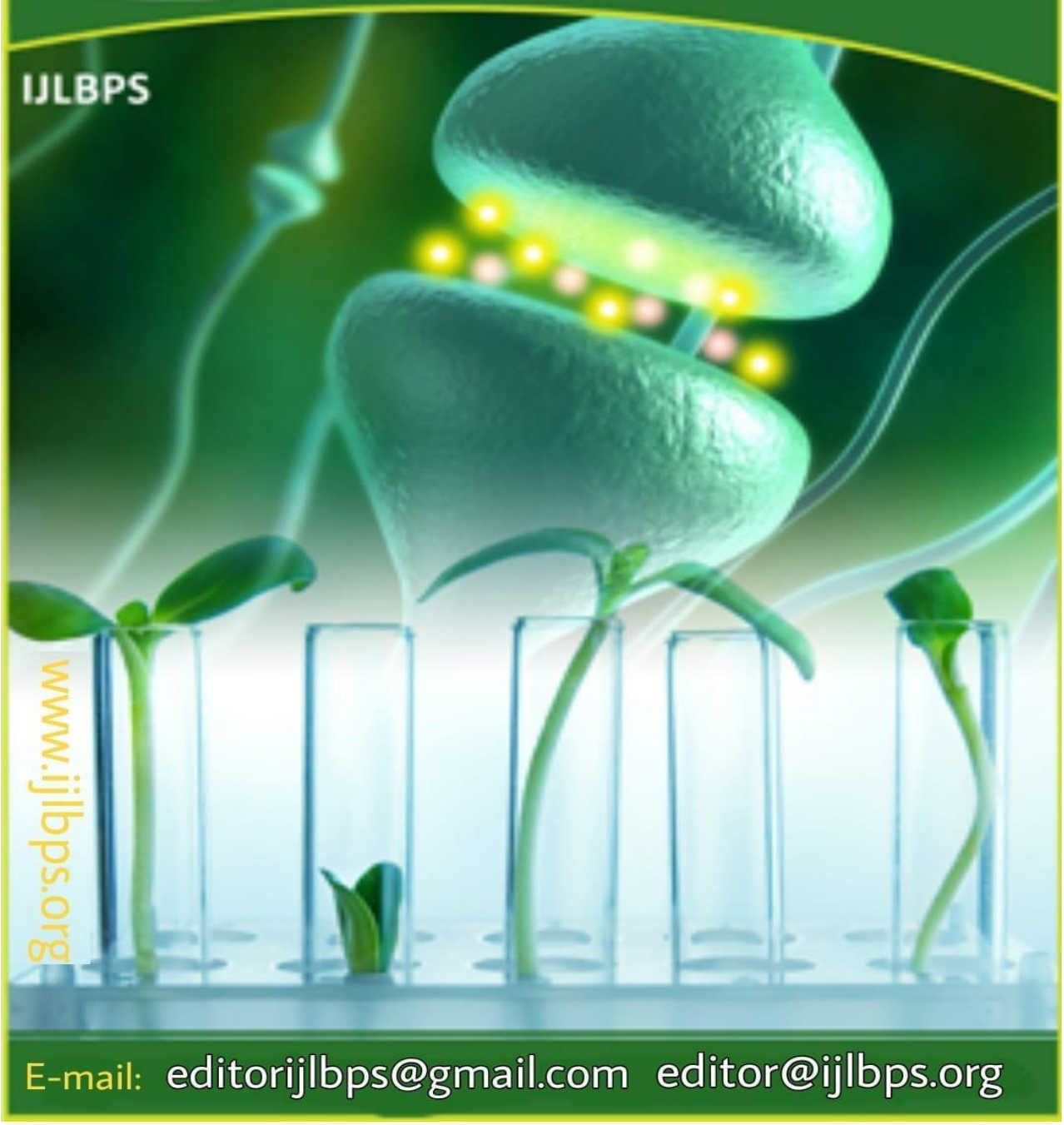
ISSN 2395-650X

International Journal of Life Sciences Biotechnology Pharma Sciences



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International Journal of Life Sciences Biotechnology Pharma Sciences

Volume 11 Issue2,April 2023

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

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Article Info

Received: 09- 01-2023 Revised: 10 -02-2023 Accepted: 2-03-2023

Abstract

The goals of this investigation were to identify the phytochemical components of Polygonum lapathifolium roots and to test the efficacy of a methanolic extract of these roots as both an anti-emetic and an anthelmintic. Alkaloids, phytosterols, triterpenes, flavonoids, and saponin were detected in the phytochemical analysis, and these compounds are responsible for the plant's antiviral, antibacterial, antiallergic, antihypertensive, antiarrhythmic, hepatoprotective, and antiinflammatory activities in animals. The extracts were tested at doses of 20, 40, 60, 80, and 100 mg/ml for their anthelmintic efficacy in an in vitro worm model (Pheretima posthuma). At 60mg/ml, the anthelmintic activity of the root extracts was statistically significant. The results were consistent with those obtained using piperazine citrate (10mg/ml)as a control. The chick emesis model was used to administer copper sulfate (50mg/kg) orally to induce vomiting for the anti-emetic test. When compared to the reference medicine metoclopramide (50mg/kg intraperitoneally), roots extract (150mg/kg orally) demonstrated statistically significant antiemetic efficacy (89.37% Inhibition).

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

Introduction

There are a number of useful medicinal plants in the family Polygonaceae, each with its own unique set of biological properties and phytochemical components. Traditional medicine uses several different Polygonaceae herbs for treating helminthiasis and gastrointestinal (GI) complications.1-2. Knotweed, or Polygonum lapathifolium (s.l.), is a 2–5-foot-tall annual plant in the family Polygonaceae. 3-4. P. lapathifolium is a common weed in the United Kingdom; it is most likely found in every single county. It is considered a native species in Europe and Asia but an alien species in the Americas and Australia5. Northern temperate locations, such as Bangladesh, India, Britain, and South Africa, are also suitable for this genus's growth. 3-4.Wetlands, ditches, and abandoned lots are ideal conditions for this plant's growth. Six, the leaves are rounded, the stems are hairless, and the leaf bases are somewhat enlarged. The stems may be glabrolls, although they're most often only barely entering puberty. 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.

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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

flavonoids are present if there is a precipitate.

Analyzing for Phenols

Three to four drops of ferric chloride solution were used to treat extracts in the ferric chloride test. The presence of phenols was revealed by the development of a bluish-black tint.Protein and amino acid detectionFor the xanthoproteic test, we added 4-5 drops of concentrated nitric acid to the extracts. Proteins are present when a yellow hue forms.The Search for SaponinsShaking 0.5 g of extract with 2 cc of water produced foam for analysis. There was foam, and it lasted for at least 10 minutes, proving the existence of saponins.Action against worms

The anthelmintic test was performed using a modified version of the procedure described by Ajaiyeoba et al. 11. Because they are both roundworms and belong to the Annelida phylum, P. lapathifolium were utilized in this study because of their morphological and physiological similarities to human intestinal roundworm parasites. Standard medication solutions and test solutions were both freshly made before the experiment began. The control group consisted of salt water and the reference standard was piperazine citrate (10mg/ml). There were six worms in each group of earthworms, and the groupings were all of similar size. P. lapathifolium root methanol extracts (20, 40, 60, 80, and 100 mg/ml in distilled water) were formulated into five distinct 60 ml dosage forms. Before beginning any experiments, new batches of both the test solution and the standard solution were made. Paralysis was diagnosed when the worms showed no signs of life at all, not even when they were violently disturbed. After determining that the worms did not respond to being shook forcefully or being submerged in heated water (500C), the timings of death were recorded.

Mean reduction in vomiting episodes was used to quantify the antiemetic efficacy, as described in the procedures of Akita et al., 1998, 12, 1998. The yang chicks, aged four days, were split into four groups of five, with each group housed in a separate big beaker heated to 250C for 10 minutes. Root extracts of P. lapathifolium were diluted to a volume of 10 ml / kg and a dosage of 150 mg / kg by orally administering the saline solution containing 5% DMSO and 1% Tween 80. The placebo group was given 0.9% salt water. After 10 minutes, 50 mg/kg of copper sulfate was given orally, and the number of vomiting episodes was recorded during the next 10 minutes. The usual dose of metoclopramide (50 mg/kg.b.w. intraperitoneally) was administered. 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 50mg/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 forits 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 an increased concentration of citrate, such as 100 mg/ml. Table No.3 and Fig. 2 reveal that flower and root extracts at 40, 60, 80, and 100 mg/ml and 60, 80, and 100 mg/ml, respectively, exhibited considerable anthelmintic activity that was on par with that of the standard medication piperizine citrate at a concentration of 10 mg/ml. Root extract in methanol causes paralysis that is dose-dependent, beginning with inability to move and progressing via an inability to respond to stimuli to eventual death. The findings suggested that alkaloids, flavanoids, and triterpenoids could be responsible for the powerful anthelmintic function of P. lapathifolium roots. These phytochemicals have been shown to have anthelmintic effects in studies ranging from 18–20. More research is needed to isolate and characterize the active components in order to provide a reliable pharmacological resource.

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