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BIOLOGICAL ACTIVITIES OF *POLYGONUM FLACCIDUM*

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ABSTRACT: *Polygonum flaccidum* (Polygonaceae) is an annual herb widely distributed in Bangladesh, India, Bhutan, Sri Lanka and Afghanistan. Objectives of the present study were to determine antioxidant, anthelmintic, cytotoxic, insecticidal and antibacterial activities of methanol extract of *P. flaccidum*. Antioxidant activity in terms of free radical scavenging ability was determined by DPPH free radical scavenging assay. Cytotoxic activity was performed by brine shrimp (*Artemia salina*) lethality bioassay. Anthelmintic efficacy was determined using adult Bangladeshi earthworm. Insecticidal activity was tested against rice weevil *Sitophilus oryzae*. Antibacterial activity was tested by disc diffusion method. The extract exhibited significant antioxidant activity by scavenging DPPH free radical. The IC₅₀ value of the extract displayed 4.91 µg/ml while standard ascorbic acid showed 2.69 µg/ml. The extract exhibited potent anthelmintic activity by causing paralysis (30 min) and death (45 min) of worms and the effect was found to be dose dependent. In insecticidal study, the lethal effect of methanol extract on *Sitophilus oryzae* was found to be dose dependent manner. In cytotoxicity study, LC₅₀ value of the methanol extract was 1.5 µg/ml while the LC₅₀ value of vincristine sulphate was 0.52 µg/ml. On the other hand, the methanol extract also showed remarkable antibacterial activity against both the gram positive and negative bacteria but inactive against fungi. Further studies on isolation of constituents and their bio-efficacies *in vitro* and *in vivo* are under investigation.

INTRODUCTION: *Polygonum flaccidum* (Commonly known Lalbishkatali) a Bangladeshi annual herb, is well known for its analgesic, anti-inflammatory, diuretic, purgative and insecticidal properties^{1, 2}. This plant has also been used traditionally to treat snake-bites. Previous phytochemical studies on this species revealed the presence of epoxides, flavonoids, steroids and terpenoids^{1, 3}. Since this plant has important medicinal properties, the present study has been undertaken and we, herein, report the antioxidant, anthelmintic and cytotoxic, properties of *P. flaccidum* for the first time and we also report the insecticidal and antibacterial activities. Aerial parts of *Polygonum flaccidum* was collected from the local area of Savar, Dhaka during January 2014.

The collected plant was then identified by the taxonomist of Jahangirnagar University Herbarium, Savar, Dhaka and a voucher specimen (DACB: 39,317) has been deposited for future reference.

The powdered aerial parts (1 kg) of *P. flaccidum* were extracted by cold extraction method with 6 L of methanol for 7 days at room temperature with occasional stirring. The extract was then filtered and evaporated on rotary evaporator under reduced pressure to obtain 48.59 gm extract which was used for biological screening.

The antioxidant activity (free radical scavenging activity) of the extract on the stable radical 1, 1-diphenyl- 2-picrylhydrazyl (DPPH) was determined by following standard protocol developed by Brand-Williams^{4,5}. Then % inhibitions were plotted against respective concentrations used and from the graph IC₅₀ was calculated by using Ascorbic acid (AA), a potential antioxidant as positive control. The experiments were carried out

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in triplicate and the results have been shown as mean \pm SEM (standard error of mean).

Adult Bangladeshi earthworms (*Pheretima posthuma*) were used to assess anthelmintic effect of extract. The worms were washed using normal saline (0.85%) to remove extraneous matter. Six worms of equal size (6 cm long) were transferred into normal saline (0.9% NaCl) containing standard drug (Pyrantel pamoate 10 mg/kg) and different concentrations of extract (25, 50 and 100 mg/ml). The time taken for paralysis of worms was noted when no movement was observed (except when the worms were shaken vigorously). The death time was taken when worms failed to exhibit movement on shaking vigorously or on dipping in slight hot water (50°C). Normal saline served as control ⁶.

The insecticidal effect of different concentrations of extract (5, 10, 20, 40, 50, 60, 70 and 80 mg/ml) was tested against *Sitophilus oryzae* also called rice weevil because of its habitat, we collected rice weevil from old rice godown, Dhaka. Briefly, ten insects were placed in beakers containing extract. A control was kept without adding extract. The insecticidal effect of extract was determined by counting the number of dead insect after 12 and 24 hrs. Dead insects were identified on the basis of no movement after probing with a needle in siphon or cervical region. The experiment was repeated triplicate and average mortality (%) was noted ^{7, 8}.

For cytotoxicity screening, crude methanol extract was dissolved in DMSO ^{9,10}. The test samples were then applied against *Artemia salina* in a 1- day *in vitro* assay. The experiments were carried out in triplicate using vincristine sulphate as standard and the results have been shown as mean \pm SEM (standard error of mean).

The antibacterial activity of the extract was determined by the disc diffusion method ^{11,12} against 2 gram positive bacteria, 2 gram negative bacteria and 2 fungal strains. The organisms were collected as pure cultures from the Institute of Nutrition and Food Sciences (INFS), University of Dhaka. The experiments were carried out in triplicate using ciprofloxacin as standard and the results have been shown as mean \pm SEM (standard error of mean).

Statistical differences between extract activities were determined using ANOVA followed by Least Significant Difference (LSD) testing. Data were expressed as mean \pm SEM (standard error of mean). Differences were considered statistically significant when $p < 0.5$.

The methanol extract of *P.flaccidum* exhibited significant antioxidant activity with the IC₅₀ value of 4.91 μ g/ml against DPPH free radical while standard ascorbic acid showed 2.69 μ g/ml (**Table 1**).

TABLE 1: IC₅₀ VALUES OF STANDARD AND METHANOL EXTRACT OF *P. FLACCIDUM* IN DPPH ASSAY

Sample	Regression equation	R ²	LC ₅₀ (μ g/ml)
PF	$y = 12.991x - 13.77$	0.9203	4.91
AA	$y = 10.649x + 21.375$	0.6968	2.69

PF= *Polygonum flaccidum*, AA= Ascorbic Acid

The potent anthelmintic activity was observed at the concentration of 100 mg/ml (**Table 2**). At this concentration, the time required for paralysis and death of earth worms was only 30 and 45 minutes

respectively whereas time taken for paralysis and death by the standard drug Pyrantel pamoate (10 mg/ml) was 26 and 35 minutes respectively.

TABLE 2: ANTHELMINTIC ACTIVITY OF *P.FLACCIDUM*

Group	Dose (mg/ml)	No. of worms	Time taken for paralysis (min)	Time taken for death (min)
Control	20 ml	6	-----	-----
Standard	10	6	26 \pm 2	35 \pm 3
	25	6	44 \pm 3	73 \pm 2
	50	6	39 \pm 2	48 \pm 3
	100	6	30 \pm 3	45 \pm 2
PF	100	6	30 \pm 3	45 \pm 2

The methanol extract of *P. flaccidum* exhibited potent insecticidal activity with the IC₅₀ value of 66.31 µg/ml and 6.43 µg/ml against *Sitophilus*

oryzae after 12hrs and 24 hrs respectively. (Table 3).

TABLE 3: LC₅₀ VALUES OF METHANOL EXTRACT OF *P. FLACCIDUM* IN INSECTICIDAL ACTIVITY AFTER 12HRS AND 24 HRS

Time	Regression equation	R ²	LC ₅₀ (µg/ml)
12hr	y = 0.3855x + 24.434	0.8969	66.31
24hr	y = 8764x + 42.692	0.8992	6.43

Table 4 shows the results of the brine shrimp lethality assay after 24 hr exposure to the samples and the positive control vincristine sulfate. The positive control, compared with the negative control (DMSO) was lethal, depicting significant mortality to the shrimp. The median lethal concentration (LC₅₀) of the test samples after 24 hr

was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the graph by means of regression analysis. The LC₅₀ value of methanol extract of *P. flaccidum* was 0.15 µg/ml.

TABLE 4: RESULTS OF CYTOTOXICITY SCREENING OF *P. FLACCIDUM*

Sample	Regression equation	R ²	LC ₅₀ (µg/ml)
PF	y = 19.624x + 48.999	0.9897	0.15
VS	y = 32.614x + 59.22	0.942	0.52

VS= Vincristine sulfate, PF= *Polygonum flaccidum*

The methanol extract of *P. flaccidum* was tested for antibacterial and antifungal activities against a number of gram positive and gram negative bacteria as well as some fungi. The extract showed

the remarkable antibacterial activity against both the gram positive and negative bacteria but inactive against fungi.

TABLE 5: ANTIMICROBIAL ACTIVITY OF *P. FLACCIDUM*

Microorganism		Determination of zone of inhibition in mm	
		PF(300 µg/disc)	Cipro (5 µg/disc)
Gram (+ve)	<i>Staphylococcus aureus</i>	14	22
	<i>Bacillus. megaterium</i>	9	15
Gram (-ve)	<i>Escherichia coli</i>	10	18
	<i>Pseudomonas aureus</i>	11	21
Fungi	<i>Aspergillus niger</i>	0	0
	<i>Aspergillus flavus</i>	0	0

PF= *Polygonum flaccidum*, Cipro = Ciprofloxacin, 0 =No Zone of inhibition

On the basis of the above biological screenings and folk medicinal uses it can be concluded that this plant has significant medicinal properties. It is also clearly evident from the above findings that the whole plant of *Polygonum flaccidum* has high antioxidant, anthelmintic, cytotoxic, insecticidal potential and remarkable antibacterial activities. Therefore, the plant is a good candidate for further studies to isolate the bioactive principles.

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