Phytochemical and Pharmacological Investigations of *Uraria lagopodies* DC. and *Urena lobata* L.

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ABSTRACT: Easy availability and reduced side effects of plants and plant-derived herbal preparations are the gifts of nature and are useful since ancient time for the treatment of various diseases. From this view point two Bangladeshi plants, *Uraria lagopodies* DC. and *Urena lobata* L. have been evaluated for their possible pharmacological activities. Preliminary phytochemical screenings with the crude ethanol extracts demonstrated the presence of alkaloids, glycosides, tannins, saponins, steroids and reducing sugars. The crude extract of *U. lagopodies* significantly ($p<0.001$) increased the charcoal evacuation by 375 min at 500 mg/kg body weight of mice when compared to the standard, Loperamide by 391.8 min at 5 mg/kg b.w. In the acetic acid induced analgesic activity test, the oral administration of ethanol extract of *U. lobata* produced significant ($p<0.05$) pain relieving activity (number of writhing 32) at 500 mg/kg body weight as compared to standard diclofenac-Na. Both the crude extracts showed mild anti-inflammatory and membrane stabilizing activities during the heat induced anti-inflammatory test and membrane stabilization screening. A dose dependent biological activity was observed during the study period.

Key words: *Uraria lagopodies*, *Urena lobata*, antimotility, analgesic, antiinflammatory

INTRODUCTION

Medicinal plants contain bioactive compounds which over the years have been exploited in traditional medical practice for treatment of various ailments.[1] Bangladesh being a developing country largely depends on folk medicines which are provided by the medicinal plants. *Uraria lagopodies* DC. (Bengali name: Lata chakuley, Gurkha chakulia; Family: Fabaceae) is a terrestrial, perennial, erect herb, and minor weed in rice fields that can attain upto 150 cm in height.[2] On the other hand, *Urena lobata* L. (Bengali name: Bun ochra, Aramina gacch; Family: Malvaceae) is a wild flowering shrub as well as weed in paddy fields.[2] In Bangladesh they are mostly found in Chittagong, Naogaon, Jessore and Tangail districts and are native to the Indian continent, South-east China, Taiwan, South-east Asia and Northern Australia. The plant possesses antidiabetic, antidiarrhoeal, antioxidant, antibacterial, hepatoprotective and immunomodulatory activities.[3,4] Traditionally theses two plants are used as abortifacient, oxytocic, anti-implantation, antidiarrhoeal and antimicrobial agents in the southeast areas of Bangladesh. As part of our continuing investigations on medicinal plants of Bangladesh,[5,6] we studied the antimotility, analgesic, anti-inflammatory and membrane stabilizing activities of *U. lagopodies* and *U. lobata* in addition to the results of preliminary phytochemical screenings of the above plants growing in Bangladesh.

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MATERIALS AND METHODS

Collection and identification. The aerial parts of *U. lagopodies* and *U. lobata* were collected from the hilly areas of Foy's Lake, Chittagong, Bangladesh in October, 2010 and were identified by the experts in the Forest Research Institute, Chittagong, Bangladesh where voucher specimens (BFRIH-5073 for *U. lagopodies* and BFRIH-7011 for *U. lobata*) have been maintained.

Extraction. The aerial parts of each plant were first air-dried followed by drying in on oven at 35°C and ground to coarse powder with a grinder. The powder (125 g) of each plant was separately extracted with 95% ethanol (500 ml) at room temperature for 7 days with occasional shaking and stirring. Both the extracts were filtered off through filter paper (Whatman filter paper no. 1) and the filtrates were separately concentrated with a rotary evaporator at reduced temperature and pressure to yield 8.11g (6 .49%) and 7.06 g (5.65%) extractive for *U. lagopodies* and *U. lobata*, respectively.

Preliminary phytochemical screenings. The extractives were subjected to various tests (Table 1) for determining the chemical nature of the phyto constituents.[7,8]

Antimotility activity. In the *charcoal-meal* defecation method[9] the experimental animals (Swiss albino mice) were divided into six groups each containing five mice. After fasting for 180 min, the mice were administered with loperamide (*IMOTIL*, 2 mg/Cap. Square Pharmaceuticals Ltd., Bangladesh) (5 mg/kg b.w. orally) as positive control, 1% Tween-80 as negative control group and 250 mg/kg b.w. and 500 mg/kg b.w. extracts dispersed in 1% Tween-80 as the test drugs. After 90 min, 0.3 ml of an aqueous suspension of 5% charcoal was administered to each animal orally. Sixty min later the mice had free access to food and the animals were observed at 5 min intervals until faeces with charcoal were eliminated and observed for 450 min. Charcoal was observed on the faeces using normal light when it was easily visible, or using a microscope to help the detection of black spots. The results were based on the time for the charcoal to be eliminated.

Analgesic activity. In analgesic activity test by acetic acid-induced writhing method in mice,[10] the extracts dispersed in Tween-80; Tween-80 and diclofenac-Na (*CLOFENAC*, 50 mg/Tab., Square Pharmaceuticals Ltd., Bangladesh) were given orally by means of a feeding tube. After 30 min acute pain was induced by intra-peritoneal (i.p.) administration of acetic acid solution (0.7%, 15 ml/kg b.w.) to each mouse. The number of squirms (writhing) due to pain in the abdominal cavity was calculated for 20 min after administration of acetic acid.

Anti-inflammatory activity. The anti-inflammatory activity of crude extracts was determined by using inhibition of albumin denaturation technique[11,12] with minor modifications. 1% egg albumin (1 ml) was transferred to six test tubes. For positive control (tube 1), 0.1 mg/ml aspirin (*Merck*, India) was added. To the negative control tube (tube 2), 1 ml ethanol and for the tests (tubes 3 and 4 for *U. lagopodies* and tubes 5 and 6 for *U. lobata*), two doses (250 and 500 mg/kg) were added. The pH of the reaction mixtures were adjusted (7.4±0.2) by using small amount of phosphate buffer. The samples were incubated at 37°C for 20 min and the turbidity of the samples was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated as follows:

$$\% \text{ inhibition} = (A_{control} - A_{sample}) \times 100 / A_{control}$$

Membrane stabilization activity. The test was conducted by certain modifications of the method designed by Shinde.[13] The reaction mixtures (2 ml of each) comprising of 1 ml 0.1 mg aspirin (*Merck*, India) and 1 ml of 10% RBC suspension (for positive control), for the test samples 1 ml of 10% RBC suspension was added to 1 ml of sample (at 250 and 500 mg/kg doses) and for the negative control group 1 ml ethanol was added to 1 ml of 10% RBCs suspension. Then all the tubes were treated with 1.0 ml of 0.5% NaCl solution in water. The pH (7.4±0.2) of the reaction mixtures was adjusted by phosphate buffer. The reaction tubes were incubated in a water bath at 56°C for 30 min. After cooling the reaction mixtures were centrifuged at 2500 rpm for 5 min and

the absorbance of the supernatants was taken at 556 nm after filtration. The test was repeated for three times. The percentage inhibition of haemolysis was calculated as follows:

% inhibition = $(A_{control} - A_{sample}) \times 100 / A_{control}$

Statistical analysis. The primary data obtained from the experiments were manipulated as the source of responses. For each of the extracts, three samples were prepared for each of the bioassays. Data were expressed as mean ± SEM (standard error of mean) followed by Least Significant Difference (LSD) testing. Differences were considered statistically significant when $p < 0.5$.

RESULTS AND DISCUSSION

The preliminary phytochemical screenings of the crude extractives with various chemical reagents demonstrated the presence of alkaloids, glycosides, saponins, tannins, steroids and reducing sugars (Table 1).

Table 1. Chemical constituents present in extracts of *U. lagopodies* and *U. lobata.*

Test for	Test name/reagent	Crude ethanol extract of	
		U. lagopodies DC.	*U. lobata* L.
Alkaloids	Mayer's test	+	+
	Dragendorff's reagent	+	+
	Wagner's reagent	+	-
	Hager's reagent	+	+
	Tannic acid test	+	+
Glycosides	Salkowski test	+	+
	Liebermann–Burchard test	+	+
Steroids	Salkowski test	-	+
	Liebermann–Burchard test	-	+
Tannins	Ferric chloride	+	+
	Potassium dichromate test	+	+
Saponins	Shaking test for foaminess	-	+
Reducing sugar	Fehling's solution	+	+
	Benedict's solution	+	+

(+) = present; (-) = absent

In antimotility activity test by charcoal meal defecation, the extracts produced significant delay for the total elimination of charcoal from the intestinal cavity of the mice. The time taken for charcoal meal defecation by the *U. lagopodies* and *U. lobata* extractives at a dose 500 mg/kg b.w. was 375 min and 356 min, respectively. The half dose (250 mg/kg b.w.) of the crude extracts also delayed the defecation period in comparison to the standard drug, loperamide (Table 2).

Table 2. Antimotility activity of *U. lagopodies* and *U. lobata* extractives.

Sample	Time until charcoal defecation (min)
Loperamide (5mg/kg b.w.)	391.8±9.59[b]
U. lagopodies (250 mg/kg b.w.)	303.6±7.13[a]
U. lagopodies (500 mg/kg b.w.)	375.0±11.92[a]
U. lobata (250 mg/kg b.w.)	295.8±22.64[b]
U. lobata (500 mg/kg b.w.)	355.8±21.99[b]

[a]$p<0.001$, [b]$p<0.05$; Total charcoal defecation: (MDP ± SEM) (where, n= 5)

During acetic acid-induced writhing test for the analgesic activity in mice, *U. lagopodies* and *U.*

lobata at 500 mg/kg b.w. demonstrated significant ($p<0.05$) analgesic activity as compared to the standard drug, Diclofenac-Na. Total writhing by the *U. lagopodies* and *U. lobata* extractives at 500 mg/kg b.w. were 33.8 and 32.0 respectively, while for the standard drug it was 29.2 (Table 3).

In the anti-inflammatory activity and membrane stabilization tests, *U. lagopodies* at 500 mg/kg b.w. produced mild anti-inflammatory and membrane stabilization activities. The percentage of inhibition of protein denaturation and inhibition of haemolysis were 19.46% and 5.59%, respectively and in the case of *U. lobata* the values were 48.55% and 19.78%, respectively. On the other hand, the lower dose (250 mg/kg b.w.) produced a dose dependent activity as compare to the standard, acetyl salicylic acid (Table 4).

Table 3. Analgesic activity of *U. lagopodies* and *U. lobata* extractives.

Sample	Number of writhing
Diclofenac-Na (25 mg/kg b.w.)	29.2 ± 4.307^{b}
U. lagopodies (250 mg/kg b.w.)	39.6 ± 4.425^{b}
U. lagopodies (500 mg/kg b.w.)	33.8 ± 6.219^{b}
U. lobata (250 mg/kg b.w.)	37.6 ± 4.575^{b}
U. lobata (500 mg/kg b.w.)	32.0 ± 5.853^{b}

$^{a}p<0.001$, $^{b}p<0.05$; Number of writhing: (Mean writhing ± SEM) (where, n= 5)

Table 4. Anti-inflammatory and membrane stabilization activities of *U. lagopodies* and *U. lobata* extractives.

Heat induced anti-inflammatory		Heat induced membrane stabilization
Sample	TIPD	TIH
Acetyl salicylic acid (0.1 mg b.w.)	52.35 ± 0.00041d	35.82 ± 0.00795a
U. lagopodies (250 mg/kg b.w.)	6.04 ± 0.00122a	2.61 ± 0.00248a
U. lagopodies (500 mg/kg b.w.)	19.46 ± 0.00187a	5.59 ± 0.00286a
U. lobata (250 mg/kg b.w.)	12.75 ± 0.00141a	16.79 ± 0.00147b
U. lobata (500 mg/kg b.w.)	48.55 ± 0.00216d	19.78 ± 0.00736c

$^{a}p<0.01$, $^{b}p<0.02$, $^{c}p<0.05$, $^{d}p<0.5$; TIPD: Total inhibition of protein denaturation (% MIPD ± SEM); TIH: Total inhibition of haemolysis (% IMHLs ± SEM) (where, n=3)

CONCLUSION

The present study showed that the ethanol extract of *U. lagopodies* and *U. lobata* exhibited significant antidiarroheal and analgesic as well as mild anti-inflammatory and membrane stabilizing activities when compared to the corresponding standard drugs. So these plants could be a source of medicines and further studies are required to isolate the bioactive principles.

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