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Uraria lagopodies DC. shows Antimicrobial, Antioxidant, Antidiarrhoeal, Antidepressant and Cytotoxic Activities

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An ethanol extract of the aerial parts of *Uraria lagopodies* demonstrated moderate to strong antimicrobial activity against some pathogenic bacteria and fungi with zone of inhibition of 10.0-25.0 mm as compared to 21.0-31.0 exhibited by the standard drugs, Ciprofloxacin and Fluconazole. In MIC determination by serial dilution method, the extract revealed MIC value of 31.25 µg/ml against *Salmonella paratyphi* and *Trichophyton* spp. *Ex-vivo* brine shrimp lethality bioassay showed mild to moderate cytotoxicity of the crude extract when compared to Vincristine sulphate. In the DPPH assay, the extract produced strong free radical scavenging activity with IC_{50} 58.0 µg/ml. During screening for antidiarrhoeal and antidepressant properties, significant and dose dependent activities were observed in mice.

Uraria lagopodies DC. (Bengali name: Lata chakuley, Gurkha chakulia; Family: Fabaceae) a terrestrial, perennial, erect herb, up to 150 cm tall and minor weed in rice fields (Ghani, 2003). Although there is no written evidence about its traditional uses, it is used as abortifacient, oxytocic, antiimplantation and antidiarrhoeal agent in the southeast area of Bangladesh. As a part of our continuous studies on medicinal plants of Bangladesh (Kaisar *et al*., 2011; Rokeya *et al*., 2010), the present study was conducted to evaluate the antimicrobial sensitivity, general toxic properties to lower animal, antidiarrhoeal and antidepressant activities of ethanolic extract of the targeted plant and to search logical evidence for its folk uses. We, herein, report the antioxidant, antidiarrhoeal, antidepressant as well as the antimicrobial activities of an ethanolic extract of *U. lagopodies* against some microorganisms.

The aerial parts of *U. lagopodies* was collected from the Foy's Lake area, Chittagong, Bangladesh in October, 2010. A voucher specimen No. BFRIH 317 has been deposited in the Forest Research Institute Chittagong, Bangladesh.

About 125 gm of the powdered material was soaked in 500 ml of ethanol and was kept for 7 days with occasional shaking and stirring. The extract was filtered off through Whatman filter paper no. 1. Besides that the powdered material was also subjected hot extraction with a Soxhlet apparatus. Then the extract was combined with the hot extract. This was then concentrated with a rotary evaporator at 40ºC under reduced pressure to yield 12.5 gm (10%).

The antibacterial and antifungal activities of the crude extract were evaluated by the disc diffusion method (Bauer *et al.,* 1966) against some pathogenic bacteria and fungi (Table 1) using Ciprofloxacin and Fluconazole as standards. The organisms were obtained as pure culture from the Faculty of Biology, University of Chittagong, Bangladesh. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm. The experiment was carried out in triplicate and the results have been shown as mean ± SEM.

The minimum inhibitory concentration (MIC) of the combined ethanol extract of *U. lagopodies* was determined by the serial tube dilution technique (Andrews,

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2001) in broth medium, containing graded concentration of the plant extract inoculated with the test organisms.

Brine shrimp lethality bioassay technique (Meyer *et al.,* 1982) was applied for determination of general toxic properties of the plant extract. DMSO solutions of the sample were applied against *Artemia salina* in a one-day ex-vivo assay. For the experiment, 4 mg of ethanol crude extract was dissolved in DMSO and solution of varying concentrations (10.5, 9.0, 7.5, 6.0, 4.4, 3.0, 1.5, 0.75, 0.375 and 0.1875 µg/ml) were obtained by serial dilution. Here Vincristine sulphate was used as positive control.

The free radical scavenging activity of the crude extract on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was determined by comparing the DPPH inhibitory capacity of the extract (Brand-Williams *et al.,* 1995). A series of concentration (1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 µg/ml) were made with the extractives by using the respective solvents and 0.004 % (w/v) DPPH solution in ethanol. Then the reaction mixtures were allowed to stand for 20 min and the absorbance was determined at 517 nm with UV-VIS spectrophotometer and from these values the corresponding percentages of inhibitions were calculated by using the following equation:

$$\% \text{ inhibition} = [1- (A_{sample} \div A_{ontrol}) \times 100]$$

where A_{sample} and $A_{control}$ are the absorbance of the sample and blank, respectively.

Then % inhibitions were plotted against respective concentration and from the graph the IC_{50} was calculated by using Ascorbic acid as standard antioxidant.

In antidiarrhoeal activity test by castor oil-induced diarrhoea in *Swiss-albino* mice (Shoba and Thomas, 2001) of either sex (bw 18-25 g) the animals were divided into negative control, positive control and two test groups containing five mice in each. The mice of negative control group received 1% Tween-80 (10 ml/kg, p.o.) while those of positive control group received Loperamide (3 mg/kg, p.o.). The mice in test groups received the ethanol extract at 250 mg/kg or 500 mg/kg orally. Acute diarrhoea was produced by oral administration of 0.4 ml of castor oil to each mouse and the latency period and total diarrheic secretion were counted for 4 h.

In the Phenobarbital-induced sleeping time test (Williamson *et al.,* 1996) randomly selected young *Swiss-albino* mice of either sex (bw. 18-25 g) the animals were grouped into negative control, positive control and test animal. The mice of negative control group received 1% Tween-80 at a dose of 10 ml/kg body weight orally and those of the positive control group received Diazepam (1 mg/kg i.p.) The mice in the test groups received the extract at 250 or 500 mg/kg body weight orally. Thirty minutes later, Phenobarbital (40 mg/kg, i.p.) was administered to all groups to induce sleep. The animals were observed for the latent period (time between Phenobarbital administration to loss of righting reflex) and duration of sleep (time between the loss and recovery of righting reflex).

In the antimicrobial screening, the crude extract of *U. lagopodies*, at 250 µg/disc, displayed strong antibacterial activity against *Salmonella paratyphi* (25.0 mm), *S. typhi* (22.0 mm), *Shigella dysenteriae* (22.0 mm) and *Vibrio cholerae* (22.0 mm) while the growth of fungus *Microsporum* spp. (15.0 mm) was moderately inhibited (Table 1).

Table 1. Antimicrobial activity of *U. lagopodies*, Ciprofloxacin and Fluconazole

Test organisms	Diameter of zone of inhibition (mm)	
	U. lagopodies (250 µgm/disc)	Standard (30 µg/disc)
Gram positive bacteria		Ciprofloxacin
Bacillus subtilis	19.0 ± 0.93[a]	27.0 ± 0.89
Gram negative bacteria		
Salmonella paratyphi	25.0 ± 0.73[c]	29.0 ± 1.13
S. typhi	22.0 ± 1.33[b]	30.0 ± 1.82
Shigella dysenteriae	22.0 ± 0.89[b]	30.0 ± 1.82
S. sonnei	20.0 ± 0.89[a]	29.0 ± 1.13
Vibrio cholerae	22.0 ± 1.22[a]	29.0 ± 0.20
Fungi		**Fluconazole**
Aspergillus niger	10.0 ± 0.70[a]	27.0 ± 1.08
Candida albicans	12.0 ± 0.93[a]	26.0 ± 0.70
Cryptococcus neoformans	10.0 ± 0.89[a]	21.0 ± 1.27
Microsporum spp.	15.0 ± 0.93[c]	23.0 ± 1.24
Trichophyton spp.	13.0 ± 0.53[a]	29.0 ± 1.47

[a]$p<0.01$, [b]$p<0.02$, [c]$p<0.05$; The diameters of zone of inhibition are expressed as mean ± SEM (n=3); SEM: standard error of mean.

During the MIC determination, the crude extract displayed low MIC values (31.25-62.50 µg/ml) against *Salmonella paratyphi, S. typhi* and *Trichophyton* spp. which suggested the presence of strong antimicrobial compounds in the extractive.

In brine shrimp lethality bioassay, the LC_{50} and LC_{90} of the crude extract of *U. lagopodies* were found to be 5.76 µg/ml and 10.50 µg/ml as compared to 0.45 µg/ml and 0.89 µg/ml, respectively exhibited by Vincristine sulphate.

In the *in-vitro* antioxidant screening, the crude extract produced significant inhibition of DPPH action with IC_{50} for the extract to be 58.0 µg/ml in comparison to the standard, Ascorbic acid (22.5 µg/ml) (Table 2).

Table 2. DPPH inhibitory activity of Ascorbic acid and *U. lagopodies* extract

Conc. (µg/ml)		10	20	30	40	50	60	70	80	90	100	IC_{50}
%	Ascorbic acid	31.6	37.7	57.8	71.5	75.2	89.6	91.1	94.2	96.9	97.1	22.5
I_{DPPH}	*U. lagopodies*	10.4	12.4	20.8	26.1	30.2	46.6	69.0	80.6	90.0	91.9	58.0

% I_{DPPH} : % Inhibition of DPPH = $[(A_{control} - A_{sample}) \times 100] \div A_{control}$; A: Absorbance

In castor oil induced diarrhea in mice, the ethanol extract of *U. lagopodies* revealed a dose dependent but moderate antidiarrhoeal response. The onset of action and the total diarrheac faces were 1.18 h and 9.0 at a dose of 500 mg/kg body weight. The half dose of the crude extract also prolonged the latency period and reduced the defecation when compared to the standard Loperamide (Table 3).

Table 3. Antidiarrhoeal activity of *U. lagopodies* extract and Loperamide

Sample	TLP (h)	TNF
Loperamide (3 mg/kg, b.w.)	1.35 ± 6.02^a	6.4 ± 1.15^b
U. lagopodies (250 mg/kg, b.w.)	1.03 ± 1.94^b	13.0 ± 0.79^b
U. lagopodies (500 mg/kg, b.w.)	1.18 ± 1.94^b	9.0 ± 1.37^b

$^a p<0.02$, $^b p<0.05$; TLP:Total latent period (Mean latent period ± SEM); TNF: Total number of faeces (Mean defecation ± SEM)

In Phenobarbital induced hypnosis test, the plant extract produced moderate onset of action and also prolonged duration of sleep. The 250 mg/kg dose was found to be half fold active as compared to 500 mg/kg body weight and standard Phenobarbital (Table 4).

Table 4. CNS depressant activity of *U. lagopodies* extract and Phenobarbital

Sample	TLP (min)	TST (min)
Penobarbital (40 mg/kg, b.w.)	5.4 ± 0.57^a	47.0 ± 3.04^a
EUL (250 mg/kg, b.w.)	21.4 ± 2.73^b	19.4 ± 1.82^a
EUL (500 mg/kg, b.w.)	16.0 ± 2.42^c	33.4 ± 2.79^c

$^a p<0.01$, $^b p<0.02$, $^c p<0.10$; TLP: Total latent period (Mean latent period ± SEM); TST: Total sleeping time (Mean sleep time ± SEM); EUL: Ethanol extract of *U. lagopodies*

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