- Short communication

Bioactivities of *Digera muricata* (L.) Mart. Available in Bangladesh

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Digera muricata (L.) Mart. (Bengali name: Latamouri or Gungutiya; Family: Amaranthaceace) is an annual herb which is widely distributed in Jessore and other western districts of Bangladesh.[1] It is an important medicinal plant[2] used in urinary disorders[3] and bowel complaints. It is also used as mild astringent and expectorant.[4,5] The leaves are used for treatment of diabetes[6] and constipation.[7] The flower and seeds are used to treat urinary discharges.[8] The plant is considered as a famine food because of rich source of nutrients.[9] The present study was designed to evaluate analgesic, anti-diarrheal, anti-depressant, cytotoxic, membrane stabilizing and thrombolytic activities of methanol extract of aerial parts of *D. muricata* and its Kupchan partitionates, and we, herein, report the results of our preliminary studies.

The aerial parts of *D. muricata* were collected from Bandarban district, Chittagong, Bangladesh and identified in Bangladesh National Herbarium, where a voucher specimen (Accession No. DACB-10781) has been deposited for future reference. The plant samples were dried and then ground to a coarse powder. The powdered material (650 g) was soaked in 1.8 liter of methanol for 15 days and filtered through Whatman filter paper number 1. Then the concentrated filtrate (5 g) was subjected to Kupchan partitioning protocol[10] to yield petroleum ether, carbon tetrachloride, dichloromethane and aqueous soluble fractions.

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For bioassays, Swiss Albino mice (25 ± 5 g) of either sex, aged 4-5 weeks, were purchased from the Animal House of the International Centre for Diarrheal Diseases and Research, Bangladesh (icddr,b). The animals (for each test) were divided into four groups of six animals each: Group I (negative control), Group II (positive control), and Group III and Group IV (experimental groups).

The analgesic activity of the crude methanol extract of *D. muricata* was evaluated in mice by acetic acid-induced writhing test[11], tail immersion test[12] and hot plate test[13], while the antidiarrheal activity was determined in mice by castor oil-induced diarrhea,[14] gastrointestinal motility test[15] and castor oil-induced enteropooling[16]method, respectively. The methanol extract was also subjected to evaluate anti-depressant activity following the method of Williamson *et al.*[17] using normal saline water containing 1% Tween-80 (10 ml/kg, b.w.) and diazepam (1 mg/kg, b.w.) as negative and positive control, respectively.

The general toxic properties of dimethylsul-foxide solution of the crude extract and different Kupchan partitionates were determined against *Artemia salina* in a single day assay by using vincristine sulphate as positive control.[18] The thrombolytic activity of the plant extractives was evaluated following the method developed by Prasad *et al.*[19] by using streptokinase as standard. On the other hand, membrane stabilizing activity was assessed by using hypotonic solution- and heat-induced hemolysis of human erythrocytes.[20]

Results have been presented as mean ± standard error of mean (SEM) and were determined by one way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison tests for inter group comparisons. The statistically significant difference was considered at the level of $p< 0.05$.

In acetic acid-induced writhing test, the crude extract conferred 63.48 and 66.09% protection at the dose of 200- and 400-mg/kg b.w., respectively as compared to 74.80% exhibited by standard diclofenac-Na (Table 1). In tail immersion test, the crude extract at the dose of 200- and 400-mg/kg b.w. produced significant increase in pain reaction time of 3.94, 5.11; 6.90, 7.30 and 7.33, 9.63 at 30 min, 60 min, and 90 min, respectively and this result was comparable to diclofenac-Na (Table1). On the other hand, in hot plate test, the crude extract increased pain reaction time and this effect was dose-dependent (Table 1).

The plant extract at doses of 200- and 400-mg/kg bw inhibited castor oil induced-diarrhea in mice by 61.44% and 65.72%, respectively (Table 2). In castor oil-induced enteropooling method, the crude extract produced dose-dependent reduction in intestinal weight and volume. The sample extract at the doses of 200- and 400-mg/kg, bw reduced intestinal weight by 65.15%, 82.73%, respectively. Similarly, the sample extract at the same condition reduced intestinal volume by 65.86% and 82.33%, respectively (Table 2). In gastrointestinal motility test, the methanolic extract of *D. muricata* inhibited mice intestinal motility by 61.68% and 72.40% at the doses of 200- and 400-mg/kg b.w., respectively (Table 2). The effect of *D. muricata* in mice was comparable to the antidiarrheal drug.

In addition, the plant extract considerably shortened the time for onset of sleep and prolonged the duration of phenobarbitone-induced sleep in mice. This anti-depressant activity was comparable to the reference drug diazepam (Table 3).

Table 1. Analgesic activity of *D. muricata* extract in mice in acetic acid-induced writhing-, tail immersion- and hot plate-tests.

Treatment	Acetic acid-induced writhing method	Tail immersion test		Hot plate test	
	% Inhibition of writhing	% Elongation of pain reaction time		% Elongation of pain reaction time	
		60 min	90 min	60 min	90 min
Normal saline (10 ml/kg, b.w.)	--	--	--	--	--
Diclofenac-Na (50 mg/kg, b.w.)	74.80%	221.69%	460.91%	138.90%	143.31%
ME (200 mg/kg, b.w.)	63.48%	177.11%	33.18%	89.64%	88.91%
ME (400 mg/kg, b.w.)	66.09%	193.17%	337.73%	110.99%	117.15%

Each value is presented as mean ± SEM (n=6); ME = Methanolic extract of *D. muricata*

Table 2. Antidiarrheal effect of *D. muricata* extract as observed in mice by castor oil-induced diarrhea, castor oil-induced enteropooling and gastrointestinal motility test.

Treatment	Castor oil-induced method	Castor oil-induced enteropooling		Gastrointestinal motility test
	% Inhibition of diarrhea	% Inhibition of intestinal weight	% Inhibition of intestinal volume	% Inhibition of intestinal motility
Normal saline (10 ml/kg, b.w.)	--	--	--	—
Loperamide (5 mg/kg, b.w.)	81.41%	75.61%	78.71%	75.67%
ME (200 mg/kg, b.w.)	61.44%	65.15%	65.86%	61.68%
ME (400 mg/kg, b.w.)	65.72%	82.73%	82.33%	72.40%

Each value is presented as mean ± SEM (n=6)

Table 3. Anti-depressant activity of *D. muricata* extract in mice.

Treatment	Time of onset of sleep (min)	Total sleeping time (min)
Control	80.00 ± 4.63	49.33 ± 6.12
Diazepam (1 mg/kg, b.w.)	23.00 ± 0.75	187.33 ± 1.11
ME (200 mg/kg, b.w.)	29.00 ± 0.82	132.33 ± 9.13
ME (400 mg/kg, b.w.)	22.67 ± 1.54	151.33 ± 19.05

Each value is presented as mean ± SEM (n=6)

Table 4. Cytotoxic (LC_{50} µg/ml), thrombolytic (% clot lysis) and membrane stabilizing activities of different Kupchan fractions of *D. muricata*.

Samples	LC_{50} (µg/ml)	% Clot lysis	% Inhibition of hemolysis	
			Hypotonic solution induced	Heat-induced
ME	1.54 ± 0.05	17.59 ± 0.85	43.70 ± 0.20	51.63 ± 0.74
PESF	0.51 ± 0.03	24.27 ± 0.92	42.02 ± 1.84	65.42 ± 0.73
CTSF	71.35 ± 1.90	20.62 ± 1.13	28.17 ± 0.24	58.37 ± 1.23
DCSF	1.23 ± 0.12	9.60 ± 0.34	31.52 ± 1.48	28.48 ± 1.77
AQSF	6.45 ± 0.76	46.11 ± 1.09	88.20 ± 0.82	72.62 ± 1.10
VS	0.451 ± 0.07	--	--	--
SK	--	66.53 ± 0.25	--	--
ASA	--	--	72.38 ± 1.31	41.95 ± 0.55

Each value is presented as mean ± SEM (n=3). Here, PESF= Petroleum ether soluble fraction, CTSF= Carbon tetrachloride soluble fraction, DCSF= Dichloromethane soluble fraction, AQSF= Aqueous soluble fraction of *D. muricata*, VS = Vincristine sulfate, SK = Streptokinase; ASA = Acetyl salicyclic acid.

Finally, it can be concluded that the methanol extract of *D. muricata* and its Kupchan partitionates possess noticeable analgesic, antidiarrheal, anti-depressant and cytotoxic activities, and there by supports the traditional uses of the plant in various diseases. Here, we focused a preliminary screening which will require further detailed investigation of bioactive compounds responsible for these actions.

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