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Evaluation of Phytochemical and Pharmacological Properties of *Dillenia indica* Linn. Leaves

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Abstract: The crude methanol extract of the roots of *Dillenia indica* Linn. (Family: Dilleniaceae) was investigated for its possible analgesic, antidiarrhoeal and GI motility tests in animal models. The extract produced significant writhing inhibition in acetic acid-induced writhing in mice at the oral dose of 250 and 500 mg kg⁻¹ b.wt. (p<0.01) comparable to the standard drug diclofenac sodium at the dose of 25 mg kg⁻¹ of body weight. The crude extract produced significant antidiarrhoeal effect at the dose of 500 mg kg⁻¹ of body weight comparable to that produced by loperamide, used as standard drug. The extract also reduced significantly the charcoal induced Gastro Intestinal (GI) motility in mice; decreased the movement of GI tract in comparison to control animals. This study has found a base stone to step ahead for further researches to make them pharmaceutically useful.

Key words: *Dillenia indica* Linn, phytochemical study, pharmacological study, ethanolic extraction and herb

INTRODUCTION

Dillenia indica Linn. (commonly called elephant apple) for its pharmacognostic and pharmacological activities although, it is widely used as food and for medicinal purposes (Abdille *et al.*, 2005; Kumar *et al.*, 2009; Shome *et al.*, 1979, 1980). Leaves of *Dillenia indica* Linn. are taken for investigation in the study. The fruits of this plant reported as potential anti-leukemic activities (Kumar *et al.*, 2009). Banerji *et al.* (1975) isolated pentacyclic triterpenoids from *Dillenia indica*. Two another new compounds dihydro-isorhamnetin and dillenetin have been isolated by Haque *et al.* (2008). The leaves are extracted with ethanol and the phytochemical properties of *Dillenia indica* Linn. leaves were explored. A number of chemical investigations have been performed on this plant, as for example, Parvin *et al.* (2009) reported four new compounds from *Dillenia indica*; i.e., lupeol, betulinalhyde, betulinic acid and stigmasterol. Anti-inflammatory activity was found by the carrageenan-induced edema and acetic acid induced capillary permeability method by Yeshwante *et al.* (2009). Antinociceptive activity of the extracts was discovered by the acetic acid induced writhing method (Koster *et al.*, 1959). An important application of *Dillenia indica* Linn. in traditional medicine is its antidiarrhoeal activity. Liquid extract of the leaves are still used as herbal medication for diarrhea. This increased our interest to derive the antidiarrhoeal properties together with its influence in Gastro Intestinal (GI) motility. However, no significant

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biological activity has yet been reported for this plant. The objective of the present study was to investigate the antinociceptive, antidiarrhoeal and GI activity tests of the crude extracts of leaves of *Dillenia indica* Linn.

MATERIALS AND METHODS

Plant Material Collection and Extraction

Dillenia indica Linn. plants were collected from the domestic areas around Bagerhat district, Bangladesh in September 2006 and were taxonomically identified by the experts at the Bangladesh National Herbarium (accession No: 30414). About 400 g of dried powdered plant material was taken in a clean, flat-bottomed glass container and soaked in 1,500 mL of 80% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by clean cotton followed by a filtration through Whatmann filter paper. The filtrate then obtained and concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd., UK) to get the crude extract.

Animals Used

Young Swiss-albino mice of either sex, weighing 20-25 g, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for the test. The animals were kept at animal house (Pharmacy Discipline, Khulna University) for adaptation after their purchase under standard laboratory conditions (relative humidity 55-65%, room temperature $25.0 \pm 2.0^\circ\text{C}$ and 12 h light-dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water.

Phytochemical Tests

Desirable amount of *Dillenia indica* Linn. extract was solubilized in water for phytochemical tests. The extract solution was tested for alkaloids, glycosides, steroids, gums, flavonoids, saponins, sugars and tannins according to the protocol described by Trease and Evans (1983).

Determination of Antinociceptive Activity

Analgesic activity of *Dillenia indica* Linn. extract was compared to the inhibition of writhing of a standard analgesic agent (diclofenac sodium). Experimental control mice were administered with 10 mL kg^{-1} 1% Tween80 (Alpha labchem) with water; positive control mice were administered with 25 mg kg^{-1} b.wt. diclofenac-sodium (Diclofenac) solution made to 2.5 mL with water and two test concentrations (250 and 500 mg kg^{-1} b.wt.) of the crude extract of *Dillenia indica* Linn. was triturated by addition of small amount of Tween80 and water was slowly added to make the final volume of the test solutions to 2.5 mL. Four groups (Group 1, 2, 3 and 4) of experimental animals were randomly selected with 5 animals in each group for each treatment. Mice were carefully administered with Tween80, diclofenac sodium and the test solutions by feeding needle. Thirty minutes interval was given to ensure proper absorption. During the time test mice were noted for any unwanted reactions. Acetic acid (0.7%) at a dose of 0.1 mg/10 g was administered intraperitoneally to induce pain sensation. After an interval of 10 minutes which was given for the absorption of acetic acid, numbers of writhing were calculated for 10 min.

Determination of Antidiarrhoeal Activity

Antidiarrhoeal screening of the *Dillenia indica* Linn. leaf extract was carried by the method described by Awouters *et al.* (1978) and Gricilda and Molly (2001). Animals were screened for diarrheal activity by administering 0.3 mL castor oil (Hospital pharmacy, Khulna medical college) and those showing diarrhoeal activity were selected for the experiment. The animals were divided as five mice per group for experimental control (1% Tween 80, 10 mL kg⁻¹), positive control (3 mg kg⁻¹ b.wt. of loperamide) and for test group (500 mg kg⁻¹ b.wt. of leaf extract). Forty minutes after treatment, mice were administered with 0.3 mL castor oil to induce diarrhoea. Immediately the mice were placed in individual cages, floor lined with blotting paper. The floor lining was changed every hour for the next 4 h of the observation period. Non-infective diarrhoea are characterised by looseness of bowel (Peters, 1910). Hence, we count the total number of diarrheic faecal output and latent time for each of sample set. A numerical score based on stool consistency was assigned as follows: normal stool = 1 and watery stool = 2.

Determination of GI Motility Influence

Two groups (experimental control and sample) of five mice per group were selected on random and were starved 24 h prior to experiment but, allowed free access to water. Control group were administered with vehicle 1% Tween80, 10 mg kg⁻¹ and the test group with 500 mg kg⁻¹ b.wt. After 30 min both groups received charcoal meal (3% suspension of deactivated charcoal in 0.5% aqueous methyl cellulose). Thirty minutes after the administration of charcoal meal, the animals were sacrificed, abdomen was opened and distance moved by the charcoal from pylorus to caecum was determined and expressed as percentage of the total length of the small intestine (Akah *et al.*, 1999).

RESULTS

Phytochemical Test

The results manifest the presence of glycoside, steroids, flavonoids, saponins and reducing sugars from crude extract of the leaves (Table 1).

Antinociceptive Activity Test

Table 2 showed the effect of the methanolic extract of *Dillenia indica* acetic acid induced writhing response method in mice. Healthy mice were divided into groups of 5 for each treatment and were administered with respective doses of control and test. After an absorption period of 30 min, the mice were administered with peritoneal injection of 0.7% acetic acid and writhing effect was counted for 10 min after 10 min. Inhibition of antinociceptive activity of the plant extract was compared against inhibition of writhing effect of standard analgesic agent diclofenac sodium. Oneway ANOVA was used from online tool

Table 1: Phytochemical properties of *Dillenia indica* Linn. crude extract

Compounds	Observation
Alkaloids	- ve
Glycosides	+ve
Steroids	+ve
Gums	- ve
Flavonoids	+ve
Saponins	+ve
Reducing sugars	+ve
Tannins	- ve

+ve: Presence, -ve: Absence

Table 2: Effects of *Dillenia indica* Linn. crude extract on writhing effect on acetic acid induced mice

Treatments	Dose (mg kg ⁻¹)	Mean writhing	Inhibition (%)	SD	p-value (One way ANOVA)*
Experimental control (1% Tween80)	10	34.0±3.32	-	3.32	<0.01
Positive control (Diclofenac sodium)	25	13.6±3.05	60.00	3.05	<0.01
DILCE	250	17.4±3.97	48.82	3.98	<0.01
DILCE	500	15.0±4.40	55.88	4.36	<0.01

*(VassarStats, 2009); DILCE: *Dillenia indica* Linn. Crude extract. 30 min after treatment, 0.7% acetic acid was injected i.p., 10 min after injection writhing responses was recorded for 10 min. N = 5

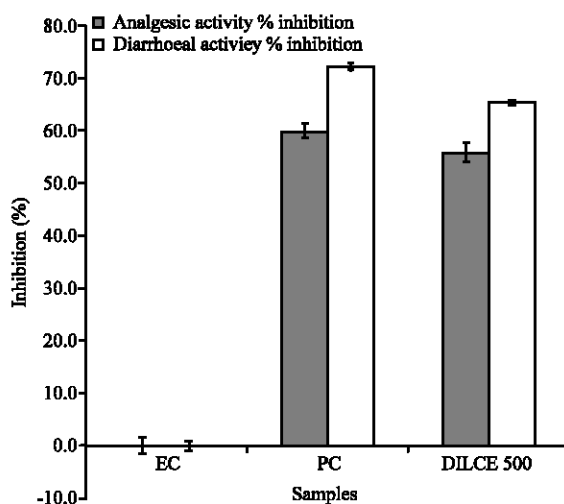


Fig. 1: Antinociceptive and Antidiarrhoeal activity of *Dillenia indica* Linn. extract. The samples were tested as quintuplicates and the percentage inhibition was tested in comparison with the mice with EC and PC. EC: Experimental control, PC: Position control, DILCE 250 and DILCE 500. *Dillenic indica* Linn. Crude extract dose: 500 mg kg⁻¹ b.wt.

(VassarStats, 2009). The ethanolic extract showed significant inhibition of writhing when compared to the control (Fig. 1). At dose of 250 and 500 mg kg⁻¹ of b.wt., the extracts produced 48.82 and 55.88% inhibition in test animals, respectively. The results were found to be statistically significant (p<0.01) and were comparable to the standard drug diclofenac sodium, which showed about 60% writhing inhibition at dose of 25 mg kg⁻¹ (p<0.01).

Antidiarrhoeal and GI Motility Determination

Antidiarrhoeal activity of the methanol extract of *Dillenia indica* Linn extract was tested by castor oil-induced diarrhea in mice. Diarrheal initiation time and the number of stools excreted by the animals in 4 h were collected. The extract caused an increase in latent period (1.1 h) i.e., delayed the onset of diarrhoeal episode of 500 mg kg⁻¹ body of weight significantly (p<0.01) which was comparable to the standard drug loperamide at the dose of 50 mg kg⁻¹ b.wt. in which the resulted value was 1.5 h (p<0.01) (Table 3). The selected concentration of the extract also showed a good diarrheal inhibition with 65.28%. Loperamide, standard antidiarrheal agent showed an inhibition of 72.22%. The latent period for the initiation of stool excretion was noted. This was 1.104 h, which is 0.418 h earlier than loperamide treated mice but, 0.42 h latter than experimental control mice.

Table 3: Effects of *Dillenia indica* Linn. crude extract on inhibition of castor oil diarrhoea

Treatments	Dose (mg kg ⁻¹)	Latent period (h)	Mean No. of stools* ¹	Inhibition (%)	SD	p-value (Oneway ANOVA)*
Experimental control (1% Tween80)	10	0.684±0.19	3.60	-	0.91	
Positive control (Loperamide)	25	1.522±0.57	1.00	72.22	0.67	<0.01
DILCE	500	1.104±0.41	1.25	65.28	0.41	<0.01

*(VassarStats, 2009); DILCE: *Dillenia indica* Linn. Crude extract. Forty min after treatment, 0.3 mL castor oil was administered orally. Latent period of castor oil induced diarrhea was noted. Number of stools excreted for the next 4 h were noted. *1: Mean number of stools was an average number of stools for 4 h for each treatment. % inhibition, SD and p-value was also calculated with respect to the number of stools. N = 5

Table 4: GI track motility of experimental control and DILCE

Treatments	Dose (mg kg ⁻¹)	Total GI track length mean(mm)	Charcoal meal traverse (mm)	SE	p-value (One way ANOVA)*
Experimental control (1% Tween80)	10	43.36±1.55	14.75±1.52	0.91	
DILCE	500	42.70±1.68	11.1±2.76	0.41	<0.01

*(VassarStats, 2009); DILCE: *Dillenia indica* Linn. Crude extract. Thirty min after ingestion of charcoal, animals were treated and they were sacrificed after 30 min of treatment. Traverse of charcoal meal from pylorus to caecum. N = 5. Treatment, 0.7% acetic acid was injected i.p., 10 min after injection writhing responses was recorded for 10 min

Antidiarrhoeal activity would be directly related to the motility of GI tract. To explore the influence of extract on the GI motility, animals treated with the test dose were sacrificed and the movement of charcoal meal from pylorus to caecum were measured which, is then compared to the experimental control animals. The movement was about 14.75 and 11.1 mm for the experimental control and for the test sample respectively. For the test group the movement was significantly reduced in compare to control group, i.e., for test group the value was (p<0.01) (Table 4).

DISCUSSION

Plants are employed as important source of medication in many traditional medications (Grover *et al.*, 2002; Keung and Vallee, 1998; Neves *et al.*, 2009). *Dillenia indica* Linn. commonly found tree plant was taken to explore the phytochemical and pharmacological properties as there was very limited work done on the plant. The leaves, which are mostly used as a source of medication in traditional medicines was considered to examine the properties of the plant.

Dried leaves of *Dillenia indica* Linn. were powdered and extracted with ethanol. The extract was dried and the powdered form, solubilized with solvents was used for the experiments. The foremost curiosity while working with a plant drug is to know the chemical substituent present in it. Phytochemical tests revealed the presence of reducing sugars, steroids, glycosides, flavonoids and saponins.

Antinociceptive activity was explored with two different concentrations of 250 and 500 mg kg⁻¹ b.wt. Antinociceptive activity of *Dillenia indica* was tested by acetic acid induced writhing model in mice. Acetic acid-induced writhing model causing pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes analgesia by liberation of endogeneous substances, which in turn excite the pain nerve endings (Taesotikul *et al.*, 2003). Increased levels of PGE₂ and PGF_{2α} in the peritoneal fluid have been reported to be responsible for pain production caused by intraperitoneal administration of acetic acid (Derardt *et al.*, 1980). The methanol extract of *Dillenia indica*

showed significant writhing inhibition in compare to the standard drug diclofenac sodium (Table 2). According to the basis of this result it can be concluded that the extract possesses antinociceptive activity.

Antidiarrhoeal activity of the extract of *Dillenia indica* was tested by using the model of castor oil-induced diarrhoea in mice (Chatterjee, 1993). Castor oil mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinolic acid remains in the intestine and produces its absorptive or secretory effect. The ricinolic acid thus liberated readily forms of ricinoleate salts with sodium and potassium in the lumen of the intestine. The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. Generally ricinoleate salts stimulates the intestinal epithelial cells adenyl cyclase (Racusen and Binder, 1979) or released prostaglandin (Beubler and Juan, 1979). The extract caused and increased in latent period and decreased the frequency of defecation as well as the number of total stool count. Obtained the results of castor oil-induced diarrhoea, it can be concluded that the extract contains antidiarrhoeal activity.

Antidiarrhoeal results increased the interest to further check the motility of GI track. The results explain the antidiarrhoeal action of the extract. In normal diarrhoeal condition GI motility will be less. Charcoal meal, which was used to determine GI motility, moved 14.75 and 11.1 mm, respectively with the control mice and sample treated models. This influence on the GI motility is highly credential towards the antidiarrhoeal activity of the leaf extract

In conclusion, it could be suggested that the methanol extract of *Dillenia indica* possesses antinociceptive nad antidiarrhoeal activities. These facts indicate the scientific basis of *Dillenia indica* Linn. being used as a traditional medicine. However, further experiments may help to determine the pharmaceutical potentialities of the plant as a medicine.

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