

Assessment of antidiarrhoeal, analgesic and antibacterial activity of ethanolic extract of *Ecbolium linneanum* (Acanthaceae) leaves

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ABSTRACT: The ethanolic extract of whole plant of *Ecbolium linneanum* (Acanthaceae) was assessed for its possible antidiarrhoeal, analgesic and antibacterial activity. Preliminary phytochemical screening of the ethanolic extract revealed the presence of alkaloids, glycosides, flavonoids and gums. The extract showed a significant ($P < 0.01$ and $P < 0.001$) antidiarrhoeal activity against castor oil induce diarrhea in mice in which it decreased the frequency of defecation and increased the mean latent period at the dose of 250 mg/kg and 500 mg/kg body weight. In acetic acid induced writhing in mice, the ethanolic extract (250 and 500 mg/kg) exhibited significant ($p < 0.01$ & $p < 0.001$) inhibition of writhing reflex 36.20% and 54.48% respectively compared to standard Diclofenac-Na (75.51%) at the dose of 25 mg/kg. The ethanolic extract showed standard antibacterial activity against both gram-positive and gram-negative bacteria. All the results tend to justify the traditional uses of the plant.

Keywords: *Ecbolium linneanum*, Acanthaceae, Antidiarrhoeal, Analgesic, Antibacterial

I. INTRODUCTION

Plants are used as food, flavor, cosmetic, ornamental, fumigant, insect deterrent, and medicine (1). Over centuries and decades our ancestors relied on the herbal products as therapeutic which can be traced back to at least 5,000 years (2). According to World Health Organization about 80% of the world population depends on the natural product for their health due to minimal side effect and cost effective (3). *Ecbolium linneanum* belongs to the family Acanthaceae commonly referred to as Blue Fox Tail or Blue Justicia in English and Neel Kantha in Bengali. Locally it is known as Bakos, Udajati etc. *Ecbolium linneanum* is a shrubby plant, with 4-sided flower-spikes at the end of branches. Leaves are often 3 by 1/3 in tip triangular, obtuse, base narrowed, glabrous or obscurely puberulous; petiole 0–1/6 inch long; leaves varying from narrowly oblong to broadly ovate, also much in size. Bracts are oval, entire, mucronate whole length 3/4 inch; stalk linear-cylindric; head compressed, 1/3 in diameter, containing 2 thin discoid margined slightly rough seeds. Flowers are large, greenish blue. Upper lip of the flower is linear, reflexed (4). *Ecbolium linneanum* has been reported to possess many ritual uses such as in jaundice, menorrhoea, rheumatism (5) and anti-inflammatory activity (6). Root juice is used as anti-helminthic and also to treat premenstrual colic (7). This Plant is also used in gout and dysuria, decoction of leaves for stricture (8). The roots and leaves are used against tumors and 50% ethanolic extract of the plants are used to treat cardiovascular disease (9). Glycoflavones have also been reported previously from this plant (10). The phytochemicals that give the plant its unique biological activity are luteolin, orientin, vitexin and isoorientin which have been isolated from the ethanolic extract of the roots, flowers and leaves of the plant (10). A lignin named as ecbolin A, has been isolated from the chloroform extract of roots (11). Phytochemical analysis of the ethanolic extract of *Ecbolium linneanum* indicated the presence of Alkaloid, glycoside, gums, and flavonoids. Traditionally leaf poultice used for leprosy. Decoction of leaves and flowers are taken internally as diuretic and gonorrhoea. Bark root is used for pulmonary problems. The decoction made from its aerial parts is used in infectious diarrhoea and dysentery. Antioxidant bioactive compounds from plant sources are used to reduce the incidence of diseases (12). Although plants may possess pharmacological properties, they may also be toxic or mutagenic. The toxic effects of most widely used medicinal plants are not well documented. Moreover, one might expect plants used in traditional medicine to be safe over a long period (13). Moreover, an evaluation of the toxicity, cytotoxicity and mutagenicity are important in all scientific studies (14). Earlier no literature is currently available to authenticate analgesic, antidiarrhoeal and antibacterial activities from ethanolic extract of *Ecbolium linneanum*, therefore the present study is a part about pharmacological screening

of this medicinal plant and designed to provide scientific evidence for its use as a traditional folk remedy by scrutinizing the antidiarrhoeal, analgesic and antibacterial activities.

II. MATERIALS AND METHODS

Plant material collection and extraction - *Ecbolium linneanum* was collected from Jessore area in Bangladesh. The time of collection was January 11, 2010 at the daytime (15). The fresh plants were collected from the road side area. During collection, any type of adulteration was strictly prohibited. The plant was identified by Bangladesh National Herbarium, Mirpur, Dhaka (Access No. 35053). A voucher specimen has been deposited in Pharmacy Discipline, Khulna University, and Khulna, Bangladesh. The collected plant parts were separated from undesirable materials or plants or plant parts. They were sun-dried for one week after cutting into small pieces. The plant parts were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis started. About 120 gm of powered material was taken in a clean, flat bottomed glass container and soaked in 600 ml of 95% ethanol. The container with its contents was sealed and kept for a period of 14 days accompanying occasional shaking and stirring. The whole mixture then experienced a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper (Bibby RE200, Sterilin Ltd., UK).

Animals - Young Swiss-albino mice of either sex, weighing 20-25 gram, purchased from the Animal Research Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B) were used for laxative activity 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 $21.0 \pm 2.0^{\circ}\text{C}$ and 12 hours light/dark cycle) and fed with standard diets and had free access to tap water.

Chemicals - The standard drugs Loperamide, Diclofenac-Na and Mecillinam were collected from the local Pharmacy, Khulna. Ethanol was used for extraction as a solvent.

III. PHARMACOLOGICAL STUDIES

Antidiarrhoeal activity - Antidiarrhoeal activity of ethanolic extract of leaves of *Ecbolium linneanum* was tested by using the model of castor oil induced diarrhoea in mice (16). The animals were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhoea were selected for the final experiment. The animals were divided into control, positive control and test groups containing five mice in each group. Control group received vehicle (1% Tween 80 in water) at a dose of 10 ml/kg body weight orally. The positive control group received Loperamide at the dose of 50 mg/kg orally; test groups received the ethanolic extract of leaves of *Ecbolium linneanum* at the doses of 250 mg/kg and 500 mg/kg body weight orally. Each animal was placed in an individual cage, the floor of which was lined with blotting paper. The floor lining was changed every hour. Diarrhoea was induced by oral administration of 0.5 ml castor oil to each mouse, 40 minutes after the above treatments. During an observation period of 4 hour, the total number of fecal output and the number of diarrheic faeces excreted by the animals were recorded. A numerical score based on stool consistency was assigned as follows: normal stool=1 and watery stool=2.

Analgesic activity - Analgesic activity of ethanolic extract of leaves of *Ecbolium linneanum* was tested by using the writhing method of acetic acid induced algia in mice (17). The test consists of injecting the 0.7% acetic acid solution intraperitoneally and then observing the animal for specific contraction of body referred as 'writhing'. A comparison of writhing was made between positive control (Diclofenac-Na), control and test sample given orally 30 minutes prior to acetic acid injection. Experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III, group-IV consisting of 5 mice in each group. Each group received a particular treatment i.e. control, positive control and the two doses of the extract. Each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. Then each group was treated with intraperitoneally administered 0.2 ml of a 3% acetic acid solution. To prepare suspension of the test samples at the doses of 500 mg/kg and 250 mg/kg per body weight, 250 mg and 125mg of samples were measured respectively. The extract was triturated in unidirectional manner by the addition of small amount of tween-80. After proper mixing of extract and tween-80, the distilled water was slowly added. The final volume of the suspension was made 5ml. To stabilize the suspension, it was shaken well by vortex mixture. For the preparation of Diclofenac sodium at the dose of 25 mg/kg-body weight, 6.25 mg of Diclofenac sodium was taken and a suspension of 2.5 ml. was made.

Antibacterial activity - Antibacterial activity of *Ecbolium linneanum* was tested by using the disc diffusion method (18). In this method-measured amount of the test samples are dissolved in definite volumes of solvent to prepare solutions of desired concentration ($\mu\text{g/ml}$). The sterile Matricel (BBL, Cocksville, USA) filter paper discs are impregnated with known amount of test substances using micropipette and dried. Disk of sample, positive control and negative control are then placed in petridishes (120 mm in diameter) containing a suitable agar medium seeded with the test organisms using sterile transfer loop for anti-microbial screening . The plates are then kept at 40°C for facilitating maximum diffusion. The plates are then kept in an incubator for 12-18 hour to allow the growth of the microorganisms. If the test material has any anti-microbial activity, it will inhibit the growth of microorganism giving a clear, distinct zone called “zone of inhibition”. The antibacterial activity of the test agent is determined by measuring the diameter of the zone of inhibition in term of millimeter and compared with the standard antibiotic. The experiments are carried out duplicate manner.

IV. RESULTS

Antidiarrhoeal activity - Antidiarrhoeal activity of the ethanolic extract of *Ecbolium linnaenum* was tested by castor oil induced diarrhoea in mice (16). The extract caused a significant increase in latent period [1.18 ($p<0.005$) and 2.04 ($p<0.001$) hour] i.e. slow the onset of diarrhoeal episode at the doses of 250 and 500 mg/kg of body weight respectively as compared to the standard antidiarrhoeal agent Loperamide where the mean latent period was 2.49 hours (Table 1). The extract also significantly decreased the frequency of defecation at the doses of 250 mg/kg and 500 mg/kg of body weight respectively where the average mean numbers of stool at the 1st, 2nd, 3rd, 4th hour of study were 6.8 hours and 5.8 hours respectively which was comparable to the standard drug Loperamide where the average mean numbers of stool at the 1st, 2nd, 3rd, 4th hour of study were 9.8 hours respectively.

Analgesic activity - Analgesic activity of *Ecbolium linneanum* was tested by acetic acid-induced writhing model in mice. The extract produced 63.80% writhing at a dose of 250 mg/kg body weight and 45.51% writhing at dose of 500 mg/kg body weight. At the same time the plant extract produced 36.20% ($P<0.01$) and 54.48% ($P<0.001$) writhing inhibition (Protection) at the doses of 250 mg/kg and 500 mg/kg respectively, which is comparable to the standard drug Diclofenac-Na 75.51% ($P<0.001$) at the dose of 25 mg/kg (Table 2)

Antibacterial activity - Antibacterial activity of *Ecbolium linneanum* was tested by using the disc diffusion method (18). The antibacterial activity was assessed against a panel of 10 pathogenic bacterial strains (both gram positive and gram negative) at the dose of 250 and 500 $\mu\text{g/disc}$, and the results were compared with the activity of the positive control, Mecillinam (25 $\mu\text{g/disc}$) (Table 3). At 250 $\mu\text{g/disc}$ the extract showed activity against *S. typh*e (8 mm), *E. coli* (7 mm), *S. sonnei* (9 mm), *S. boydii* (7 mm), *E. faecalis* (8 mm), *S. agal*ectiae (10 mm) and *S. saprophyticus* (6 mm). At 500 $\mu\text{g/}$ disc it showed activity against *S. typh*e (10 mm), *E. coli* (10 mm), *S. flexneri* (8 mm), *S. sonnei* (14 mm), *S. boydii* (9 mm), *E. faecalis* (10 mm), *S. agal*ectiae (13 mm), *S. pyogens* (7 mm) and *S. saprophyticus* (7 mm).

TABLES

Table 1: Result of the antidiarrhoeal effect of the ethanolic extract of leaves of *Ecbolium linneanum*

Treatment	Total number of stools in 4 hour	Mean on of Stools in 4 hour	Mean of latent period (hour)
Control (1% Tween 80 in water; 10 mg/kg)	43	9.8	0.46
Positive control (Loperamide 50 mg/kg)	12	4	2.49
Test group-1 250 mg/kg body weight	34	6.8	1.18
Test group-2 500 mg/kg body weight	29	5.8	2.04

Table 2: Result of the analgesic effect of the ethanolic extract of leaves of *Ecbolium linneanum*

Animal group/ Treatment	Number of writhes (% writhing)	Inhibition (%)
Control 1% tween-80 in water, p.o.	145±4.39 (100)	-
Positive control Diclofenac sodium 25 mg/kg, p.o.	35.5±2.16* (24.48)	75.51
Test group - 1 Ethanolic extract 250 mg/kg, p.o.	92.5±2.63** (63.80)	36.20
Test group - 2 Ethanolic extract 500 mg/kg, p.o	66±0.98* (45.51)	54.48

Values are expressed as Mean S.E.M (n=5), * $P < 0.001$, ** $P < 0.01$, % = Percentage, p.o. = per oral.

Table 3: Result of the antibacterial activity of the ethanolic extract of leaves of *Ecbolium linneanum*

Serial no	Bacterial strain	Type of Bacterial strain	Diameter of Zone of Inhibition in millimeter (mm)			
			Blank	Mecillinam (25 µg/disc)	Ethanolic extract of leaf of <i>Ecbolium linneanum</i>	
					(250µg/disc)	(500µg/disc)
01	<i>Salmonella typh</i> e	Gram (-)	-	8	8	7
02	<i>Enterococcus coli</i>	Gram (-)	-	11	7	10
03	<i>Shigella flexneri</i>	Gram (-)	-	14	-	8
04	<i>Shigella sonnei</i>	Gram (-)	-	27	9	14
05	<i>Shigella boydii</i>	Gram (-)	-	14	7	9
06	<i>Shigella dysenterae</i>	Gram (-)	-	6	-	-
07	<i>Enterococcus faecalis</i>	Gram (+)	-	31	8	10
08	<i>Streptococcus agalactiae</i>	Gram (+)	-	32	10	9
09	<i>Streptococcus pyogens</i>	Gram (+)	-	13	-	7
10	<i>Streptococcus saprophyticus</i>	Gram (+)	-	12	6	7

Gram (-):-Gram Negative Bacteria; Gram (+):-Gram Positive Bacteria; (-):- No inhibition

V. CONCLUSION

Antidiarrhoeal activity of the ethanolic extract of *Ecbolium linneanum* was tested using the model by castor oil induced diarrhoea in mice (16). Castor oil, which is used to induce diarrhoea in mice, mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinoleic acid remains in the intestine and produces its anti absorptive or secretory effect. The ricinoleic acid thus liberated readily forms ricinoleate salts with sodium and potassium in the lumen of the intestine (19). The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface (20). Most agreed view is that ricinoleate salts stimulates the intestinal epithelial cell's adenyl cyclase (21) or release prostaglandin, which results in an increase in the net secretion of water and electrolytes in the small intestine (22). The ethanolic extract of leaves of *Ecbolium linneanum* significantly and dose dependently decreased the onset of diarrhea in mice at he tested doses. The maximum effect was found at 500 mg/kg of body weight. On the basis of the result of castor oil induced diarrhoea, it can be concluded that the ethanolic extract of *Ecbolium linneanum* might possess a significant antidiarrhoeal activity.

Moreover pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain acts as a warning signal against disturbances of the body and has a proactive function. Analgesic activity of *Ecbolium linneanum* was tested by acetic acid-induced writhing model in mice. The acetic acid-induced mouse writhing test has been used extensively to qualify analgesic agents that have peripheral analgesic action (23). The peripheral analgesic effect of the plant's extract may be mediated via inhibition of cyclooxygenases and/or lipooxygenase and other inflammatory mediators. According to this hypothesis, acetic acid-induced writhing and hot-plate test methods are useful techniques for the evaluation of peripherally- and centrally-acting analgesic drugs, respectively. Diclofenac-Na, a non-steroidal anti-inflammatory drug (NSAIDs), is commonly employed in the treatment and/or management of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis, and for its anti-inflammatory and analgesic effects. Diclofenac reduces inflammation, swelling and arthritic pain by inhibiting prostaglandins synthesis and/or

production. The drug also affects polymorphonuclear leukocytes function in vitro, thereby reducing chemotaxis, superoxide toxic radical formation, oxygen-derived free radical generation, and neutral protease production. Diclofenac has also been reported to suppress inflammation induced by various phlogistic agents in experimental animal models. Acetic acid, which is used to induce, writhing in mice, causes analgesia by liberation of endogenous substances, which then excite the pain nerve endings. The extract produced significant writhing inhibition at the doses of 250 mg/kg and 500 mg/kg respectively, which is comparable to the standard drug Diclofenac sodium at the dose of 25 mg/kg. Based on this, it could be concluded that the Ethanolic extract of *Ecbolium linneanum* possess analgesic activity.

Plant containing Quercetagenin-7-arabinosyl-galactoside, a flavonoid has been used extensively to treat infectious disease (24). The flavone baicalein is reported to be largely responsible for antimicrobial effects (25). Flavonoid rich plant extracts from species of *Hypericum* (26), *Capsella* (27) and *Chromolaena* (28) have been reported to possess antibacterial activity. Many other phytochemical preparations with high flavonoid content have also been reported to exhibit antibacterial activity (29-37). The antibacterial activity of *Ecbolium linneanum* is probably due to the presence of flavonoid that revealed in phytochemical studies. The zone of inhibition varies within the ranges of 6-10 mm and 7-14 mm at the dose of 250 and 500µg/disc respectively. The highest zone of inhibition was found against *Shigella sonnei* (14 mm) at 500µg/disc. As it showed a moderate activity against *E. coli*, *E. faecalis* and *S. agalactiae*, the results support the traditional use of this plant as a remedy of infectious skin infections and gonorrhoea.

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