In-vivo Antidiarrheal and *In-vitro* Antimicrobial Activities of the Leaf Extracts of *Bauhinia acuminata*

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Abstract:

This study evaluated the antidiarrheal and the antimicrobial study of the plant, *Bauhinia Acuminata* (Fabaceae). The study suggested that methanolic extracts was safe up to 2000mg/kg. The crude methanolic extract of *B. Acuminata* showed a significance antidiarrheal activity at dose of 200 mg/kg and 400mg/kg- body weight as compared to the standard antidiarrheal agent Loperamide (dose:1mg/kg-body weight). At a dose of 200 mg/kg and 400mg/kg- body weight showed significant ($P< 0.01$) reduction in animal model in magnesium sulphate induced enteropooling by 50.66% to 66.66% respectively. In castor oil induced diarrhea, it showed reduction in dose dependent manner. Anti-diarrheal activity was present in the methanolic extract (200mg/kg and 400mg/kg) which indicate that the crude drug acted by causing decreased intestinal motility by 41.89% to 58.33% respectively. Any kind of antimicrobial activity was not shown by the methanolic extract of leaf of this plant *B. acuminata.* This result suggests that *B. acuminate* leaves extract could be used for the treatment of diarrhoea.

Keywords: Antidiarrheal, Antimicrobial, *Bauhinia Acuminata*, Leaf extract

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1. Introduction

Diarrhea, an important health problem whole over the world especially in developing countries, Every year, around more than 5-8 million deaths in infant and children under 5 years [1]. Several folk medicines those are collected from plant are used to treat infectious diseases such as diarrhea, urinary tract infection, cutaneous abscesses, bronchitis and parasitic diseases [2]. According to world health organization (WHO) about 80% people used plant as traditional medicine [3]. The death caused by diarrhea is highly visible in tropical and subtropical countries [4]. Local people usually relay on various herb sources to diarrhea and other infectious diseases. The World Health Organization also encouraged the studies for the treatment and prevention diarrheal disease treatment depending on traditional medicinal practices [5]. FDA has also approved the botanical drug, Fulyzac, as the first antidiarrheal drug for the HIV/ AIDs patients [6]. This exaltation of the health authorities towards the plant medicines is elevating for the research and development of this particular field. The plants are also a rich source of anti-microbial agent. People from indigenous group are using this plant as a vital source of medicine of treating diseases caused by microbes. The search of better alternative medicines by exploration of our vegetation is thus a worthwhile attempt [7].

Bauhinia acuminata (Fabaceae), an evergreen large shrub, grows in disturbed areas of Southeast Asia such as Indonesia, Malaysia or the Philippines [8]. In Bangladesh, it grows in hilly forests of Chittagong and Sylhet. It grows two to three meters tall. Leaves with petioles 1.5-4 cm long; blades ovate, broadly ovate or sub orbicular, divided about 1/3 their length, membranous, glabrous adaxially, densely puberulent abaxially, base cordate to rounded, apex of lobes acute. They are 6 to 15 centimeters long and broad, with the apical cleft up to 5 cm deep. The flowers

are fragrant, 8 to 12 centimeters in diameter, with five white petals, ten yellow-tipped stamens and a green stigma [9]. The species occurs in deciduous forests and scrub. Several chemical compounds including phthalic acid, palmitic acid, three phthalic acid esters, gallic acid, ursolic acid were identified from the leaves of *B. acuminata* [10]. In this study, we report the anti-diarrheal activity and the antimicrobial activity of the methanol leaf extract of this plant.

2. Materials and Methods

2.1. Collection of plant sample

The plant, *B.acuminata* was collected in August 2013 from the Batali hill, Lalkhan bazaar, Chittagong. A voucher specimen is deposited in the Bangladesh National Herbarium, Mirpur, Dhaka and is tagged with the accession number-38305.

2.2. Preparation of plant extract

The amount collected was 1.5 kg from which the leaves were separated. The leaves were initially dried in the air and finally in mechanical drier at 60-70° C. The dried samples were ground to coarse powder with a mechanical grinder and powdered samples were kept in clean closed glass containers pending extraction. During grinding of the sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other foreign material deposited on the grinder. For the purpose of extraction, 100gm of the powder was soaked in 500ml methanol and the process of shaking was performed for 7 days on a shaker machine and manually as well. The plant extract was filtered with filter paper with the help of Buchner funnel. Finally, the filtrate was concentrated by evaporating the solvent using a water bath at a temperature of 40° C. A paste-like deep green colored concentrate was obtained.

2.3. Drugs and chemicals

The drugs such as loperamide and kanamycin were collected from this company Opsonin Pharmaceuticals Ltd, Dhaka, Bangladesh. The instant nutrient agar medium (Difco) were

brought from Himedia laboratories, private limited, Mumbai, India. Chemicals were obtained from Merck limited, Worli and Mumbai, India.

2.4. Experimental animal

Adult Swiss albino mice (30 - 35 days age) were collected from the icddrb, Mohakhali, Dhaka. The mice weighing about 20-40 grams, was housed in colony cages (4 mice per cage) at an ambient temperature of 25° to 27° C with 12 hours light and dark cycles having proper ventilation in the room. The mice were kept male and female in separate cages. They were fed normal diets purchased commercially from the vendors and water *ad libitum*. The animal was allowed to acclimatize to the laboratory environment for the one week and then divided into groups for experiments.

2.5. Antidiarrheal activity screening

2.5.1. Castor oil-induced diarrhea in mice

Overnight fasted four mice were kept in four groups cases. No I: (Control) mice of this cage received 1 ml 2% v/v aqueous Tween 80 orally. No II: (Standard drug treated). Mice of this cage were treated with the reference drug, loperamide at the dose of 3 mg/kg body weight, orally. No III, IV: (MELI treated) Mice of this cage were treated with methanolic extract of *B. acuminata* leaves at the doses of 200 and 400 mg/kg body weight by oral route respectively suspended in 2% v/v aqueous Tween 80. After one hour of dosing, all the mice were treated with 0.5 ml of castor oil orally by gavage and observed for consistency of faecal material. The numbers of wet faecal droppings were measured for four hours after castor oil administration. Characteristic diarrheal droppings were noted in transparent plastic dishes placed beneath the individual perforated mice cages [11].

2.5.2. Magnesium sulphate induced diarrhea

Diarrhea was induced by oral administration of magnesium sulphate at the dose of 2mg /kg to the animals 30 min after pre-treatment with vehicle (1% Tween 80 in water, 10 ml/kg) to the group, loperamide (3 mg/kg) of the standard group, and the methanol extract of leaves of *B. acuminata*

at the doses of 200 and 400 mg/kg to the test groups. All the administrations were carried out through the oral route. Percent inhibition (PI) was calculated as above [12].

2.6. Antimicrobial screening

Antimicrobial screening was performed using disc diffusion method. Sterile filter paper disc (5mm in diameter) were made and handled under laminar air flow. Sample solutions of desired concentrations (100µg/disc, 300µg/disc and 500µg/disc) were applied to the disc with the help of the micropipette in an aseptic condition [13].

2.7. Statistical analysis

Results were expressed as mean ± S.E. Mean values were evaluated by One Way ANOVA followed by Dunnett's multiple comparisons. Statistical significance was accepted at $P < 0.001$, $P < 0.01$ and $P < 0.05$.

3. Results

3.1. Antidiarrheal study

3.1.1. Castor oil induced diarrhea

In the castor oil-induced diarrheal experiment in mice, the methanol extract of leaves of *Bauhinia acuminata* at the doses of 200 and 400 mg/kg, reduced the total number of faeces as well as the total number of diarrheal faeces in a dose dependent manner (Table 1).

Table 1: Castor oil induced diarrheal model

Group	Total number of feces	% inhibition of defecation	Total number of diarrheal feces	% inhibition
Control	24.33±0.57	----	20.33±0.57	----
Loperamide	9.66±0.57	62.5±0.5	6.33±0.57	75.66±0.57
MEBA200mg/kg	18.66±0.57	25.66±0.57	14.33±0.57	41.89±0.19
MEBA400mg/kg	14.33±0.57	41.67±0.19	10.66±0.57	58.33±0.38

*MEBA= methanolic extract *Bauhinia acuminate* and Values are mean±SD.

3.1.2. Magnesium sulphate induced enteropooling

In the Magnesium sulphate induced diarrheal model in mice, the methanolic extract of leaves of *Bauhinia acuminata* above dose levels significantly ($P < 0.01$) reduced the extent of diarrhea in test animals (Table 2). Both the doses were shown to reduce the total number of faeces and wet faeces when compared to the control.

Table 2: Magnesium sulphate induced diarrheal model

Group	Total number of feces	% inhibition of defecation	Total number of diarrheal feces	% inhibition
Control	14.33±0.57	----	12.66±0.57	----
Loperamide	3.66±0.57	78.86±0.24	1.66±0.57	91.67±0.20
MEBA200mg/kg	9.66±0.57	35.71±0.16	6.33±0.57	50.66±0.57
MEBA400mg/kg	7.33±0.57	50.33±0.57	4.66±0.57	66.66±0.19

*MEBA= methanolic extract *Bauhinia acuminate* and Values are mean±SD.

3.2. Antimicrobial study

The antibacterial screening of *Bauhinia acuminata* (Methanolic extract) showed no antimicrobial activity against the various bacterial strains. The result is as follows given in the Table 3.

Table 3: The zones of inhibition against selective bacteria of *Bauhinia acuminata*

Name of the Bacteria	Zone of inhibition			
	Kanamycin disc(30µg/disc)	*Bauhinia acuminata* (Methanol extract)		
		30µg/disc (mm)	50µg/disc (mm)	100µg/disc (mm)
Staphylococccus agalactiae	30 mm	0	0	0
Bacillus cereus	28 mm	0	0	0
Shigella boydii	30 mm	0	0	0
Shigella sonnci	30 mm	0	0	0
Pseudomonas aeruginosa	27 mm	0	0	0
Eschericia coli	30 mm	0	0	0
Proteus mirabilis	24 mm	0	0	0

4. Discussion

Diarrhea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hurry resulting in an excess loss of fluid in the faeces. In some diarrhea the secretory component predominates, while other diarrhea is characterized by hyper motility. Castor oil causes diarrhea due to its active metabolite, ricinoleic acid. This stimulates

peristaltic activity in the small intestine while magnesium sulphate induces saline catheresis, leading to changes in the electrolyte permeability of the intestinal mucosa. Castor oil and magnesium sulphate reported to induce diarrhea by increasing the volume of intestinal contents by preventing the reabsorption of water. The result shows in fig-1 and fig-2. The liberation of ricinoleic acid results in irritation and inflammation of intestinal mucosa leading to release of prostaglandin.

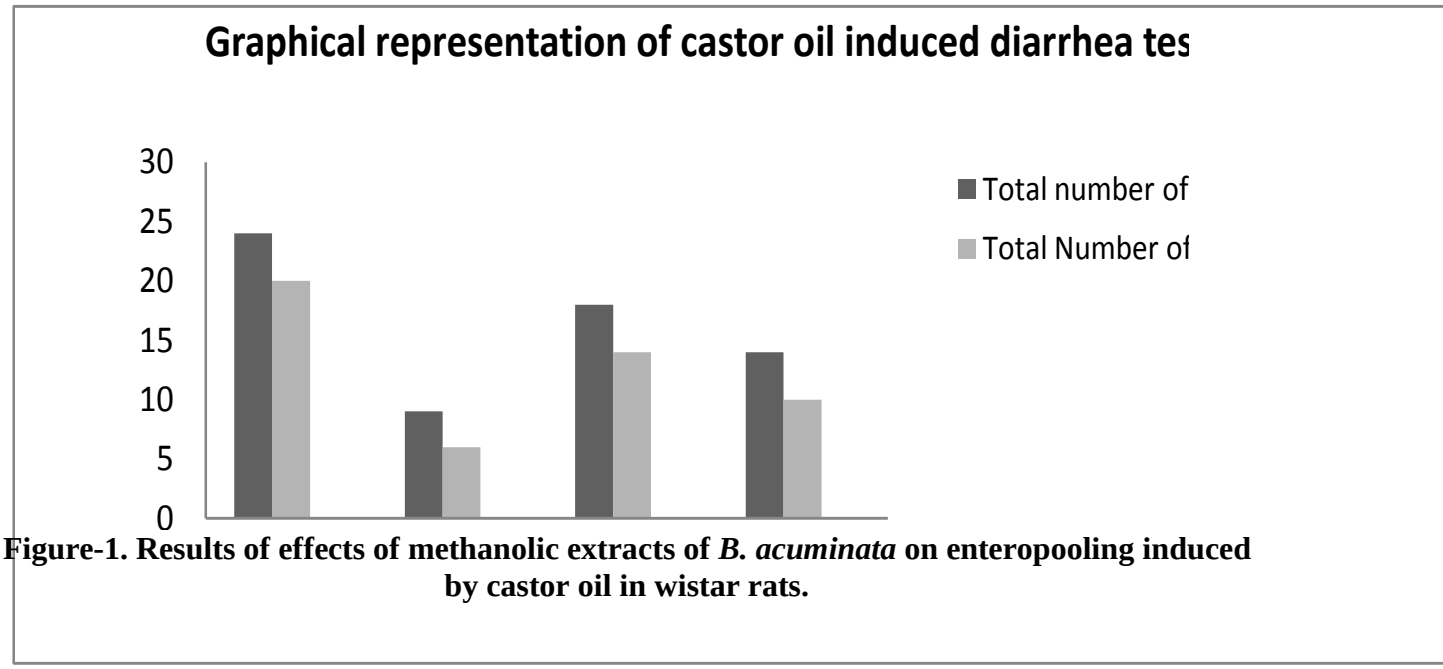

Figure-1. Results of effects of methanolic extracts of *B. acuminata* on enteropooling induced by castor oil in wistar rats.

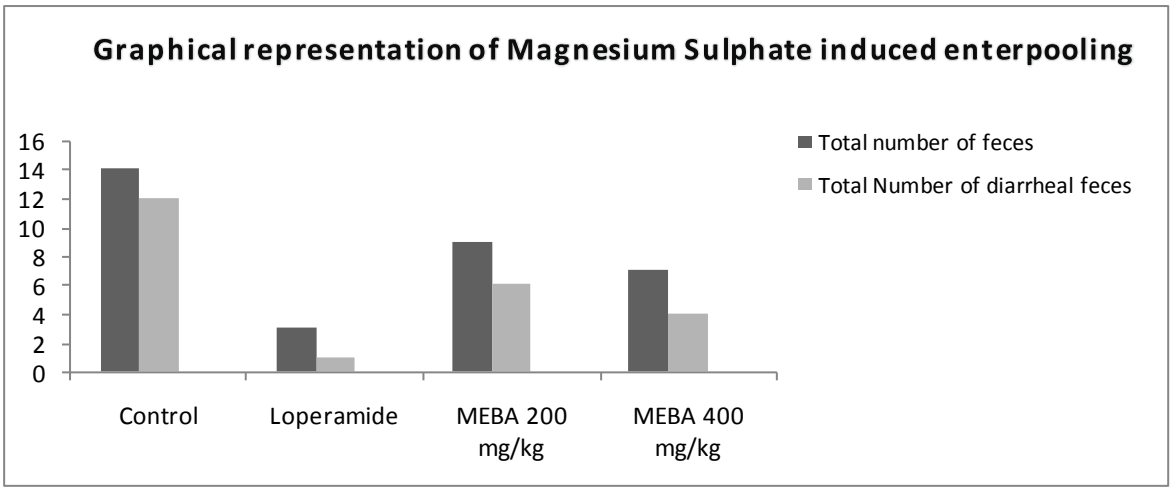

Figure-2. Results of effects of methanolic extracts of *B. acuminata* on enteropooling induced by magnesium sulphate.

In this study, the methanolic extract of *B. acuminata* leaves exhibited a significant dose-dependent antidiarrheal activity. The results were comparable to that of the standard drug loperamide (1mg/kg) with regard to the severity of diarrhea. Above observations suggest that the extract in graded doses reduces diarrhea by inhibiting peristalsis and gastrointestinal motility and castor oil induced enteropooling. It is equally effective in the prevention and curing of diarrhea. Any one of the above constitute may be useful for the treatment of diarrhea and also may enhance intestinal absorption of $Na^+$and water.

The methanolic extract of leaves of the plant *B. acuminata* showed no antimicrobial activity.

5. Conclusion

In animals pretreated with methanolic extracts of *B. acuminata* showed significant inhibitory activity against castor oil induced diarrhea and inhibited significantly magnesium sulphate induced enteropooling in mice. Potential anti-diarrheal phytochemicals may isolate from this plant.

Conflict of interest statement

We declare that we have no conflict of interest.

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