

Evaluation of Antibacterial and Antidiarrhoeal Activities of *Feronia limonia* Leaf Extract

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ABSTRACT

The present study was carried out to investigate possible antibacterial and antidiarrhoeal activities of ethanol extract of *Feronia limonia* leaves. Phytochemical analysis of the crude extract was performed to detect presence of different kinds of phytoconstituents. The antibacterial activity was investigated against four Gram positive and four Gram negative bacteria by using disc diffusion method. The plant extract showed moderate antibacterial activity against Gram positive bacteria namely *Staphylococcus saprophyticus* and *Staphylococcus pyogenes* and all tested Gram negative bacteria namely *Escherichia coli*, *Shigella boydii*, *Shigella dysentery* and *Shigella flexneri* in dose dependant manner. The results of castor oil-induced diarrhoeal study showed that *Feronia limonia* extract significantly reduced the severity & frequency of diarrhoea in mice at a higher dose of 500 mg/kg compared with the standard drug loperamide (25 mg/kg). The present study clearly supports the medicinal value of this plant. The overall results indicate the possibility of presence of some active principles in the plant extract possessing antibacterial and antidiarrhoeal actions.

Keywords: Phytochemical Screening; Antimicrobial; Antidiarrhoeal; *Feronia limonia*

1. Introduction

Antibiotic resistance developed by bacteria has become a vital issue all over the world. A good number of antibiotics are found to be inactive in recent years largely due to resistance development through the inappropriate and injudicious uses of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases [1]. So to combat the problem of microbial resistance and for substitution with effective ones the developments of new antibacterial agents are necessary. Diarrhoea is another important health problem around the world, responsible for more than 5 million deaths annually [2,3]. The investigation of the antimicrobial and antidiarrhoeal properties of plants has brought attention to the opportunity of producing a safe, economical and easily available source that could replace the synthetic antimicrobial and antidiarrhoeal compounds [4,5].

Feronia limonia is a deciduous, slow-growing, erect tree belonging to family Rutaceae and subfamily Auran-

tioideae [6]. It is distributed in deciduous and arid landscapes of several countries in South Asia [7]. *Feronia limonia* as a whole, or its parts such as unripened fruit, ripened fruit, root, bark, trunk gum and leaves have a broad spectrum of traditionally established therapeutic properties including antimicrobial and antidiarrhoeal effects [8,9]. Literature survey revealed that leaf extract of the plant has antioxidant [10], hepatoprotective [10], larvicidal [11], antitumor [12], antidiabetic [13], and CNS depressant potentials [14]. In the present study, assessment of antibacterial and antidiarrhoeal activities of the leaves of *F. limonia* was conducted to find out medicinal properties and to justify their traditional use for ailments of diseases.

2. Materials and Methods

2.1. Collection and Preparation of the Plant Material

The plant material was collected from Khulna district,

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Bangladesh and identified by the taxonomist of Bangladesh National Herbarium, Dhaka (Accession No. DACB-34397). The voucher specimens of the plants have been deposited in the herbarium for future reference. The leaves of collected plant were sun-dried for one week. The plant parts were then converted into a coarse powder with a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

2.2. Preparation of Plant Extract

About 150 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 [15-17]. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material and also using Whatman filter paper. By using a rotary evaporator (Bibby RE200, Sterilin Ltd., UK) the resultant filtrate was concentrated to powder form through complete evaporation of the extraction solvent. The filtrate was then air dried at room temperature to evaporate the extra ethanol. It rendered concentrate of reddish color which was designated as crude extract of ethanol and stored in a refrigerator until further investigation.

2.3. Experimental Animal

Young Swiss-albino mice (average weight 20 - 25 gm) were purchased from the Animal Research Branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR) for assessing biological activity. The animals were kept in standard environmental conditions for one week for adaptation after their purchase and fed ICDDR formulated rodent food and water. All the experiments were conducted on an isolated and noiseless condition.

2.4. Phytochemical Screening

The preliminary phytochemical screening with various qualitative chemical tests was performed to detect the presence of various classes of phytoconstituents in 10% (w/v) solution of the plant extract. Phytoconstituents like saponins, tannins, alkaloids, steroids, flavonoids, glycosides were identified by characteristic color changes using different reagents following standard procedures [18,19].

2.5. Antimicrobial Test

The antimicrobial assay was performed by disc diffusion method [20-22]. Eight pathogenic bacteria (collected from the International Centre for Diarrhoeal Disease and Research, Bangladesh) were inoculated on 16 ml previ-

ously sterilized nutrient agar media, mixed thoroughly and transferred immediately to the sterile Petri dish in an aseptic condition using a sterile loop. Prepared plant extracts (250 µg/disc and 500 µg/disc) and standard kanamycin solutions (30 µg/disc) were applied to the corresponding Petri dish. The plates were incubated for 24 hours at 37°C. After proper incubation, clear zone of inhibition around the point of application of sample solution were measured and expressed in millimeter (mm).

2.6. Antidiarrhoeal Test

The experiment was conducted by previously reported castor-oil diarrhea model [23]. The mice were screened initially by giving 0.5 mL of castor oil and only those showing diarrhoea were selected for the experiment. The test animals fastened overnight were randomly allocated to four groups consisting of six mice in each group. The animals of group I (control) received vehicles only (distilled water containing 0.1% Tween-80). Group-II (positive control) received standard antimotility drug loperamide (50 mg/kg body weight) as oral suspension. The group-III and group-IV (test groups) were treated with suspension of leaves extract of *Feronia limonia* at the oral dose of 250 mg/kg-body weight and 500 mg/kg-body weight. After one hour treatment with distilled water, standard drug or plant extract, each animal was given 0.5 mL of castor oil by oral route. Individual animals of each group were then placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhoea every hour in five hours study after the castor oil administration. Number of stools or any fluid material that stained the adsorbent paper were counted at each successive hour during the 4-hour period and were noted for each mouse. The latent period of each mouse were also counted. At the beginning of each hour new papers were placed for the old ones.

2.7. Statistical Analysis

Results were expressed as mean \pm standard error of mean (SEM). Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnett's multiple comparisons. The results obtained were compared with the control group. P values < 0.05 were considered to be statistically significant.

3. Results and Discussion

3.1. Phytochemical Screening

The crude extract was subjected for chemical group tests to identify various types of important chemical constituents. Phytochemical studies showed that alkaloids, steroids, tannins and flavonoids are present in the ethanolic extract of *Feronia limonia* (Table 1). However, glyco-

side, gum, carbohydrate and saponin were absent in ethanol extract of the plant.

3.2. Antimicrobial Test

Antibacterial activity of the ethanol extract of *Feronia limonia* leaves (250 µg/disc and 500 µg/disc) was evaluated by determining zones of inhibition (mm) against four Gram positive and four Gram negative bacteria and compared with the standard antibiotic kanamycin (30 µg/disc) (Table 2). For both gram positive and gram negative bacteria kanamycin demonstrated almost similar actions which justified its use as standard in this study. The study showed the plant extract possessed moderate dose-dependent antibacterial activity against Gram positive *Staphylococcus saprophyticus* and *Staphylococcus pyogenes* and all tested Gram negative bacteria. However, the plant extract was ineffective against Gram positive

Enterococcus faecalis and *Streptococcus agalactiae*. Antimicrobial activity of the plant against both Gram positive and Gram negative bacteria may be due to the presence of broad spectrum antibiotic compounds [24,25] or the previously reported compounds like essential oil, rich in methyl chavicol [26].

3.3. Antidiarrhoeal Test

Castor oil (0.5 mL, p.o.) induced diarrhoea promptly within one hour in the animals and produced a considerable amount of stool. The time for diarrhoeal induction in mice was prolonged by administration of ethanol extract of leaves of *F. limonia* at the doses of 250 and 500 mg/kg (Table 3). The plant extract significantly reduced the total number of faeces as well as of diarrhoeic faeces at a higher dose of 500 mg/kg body weight.

Table 1. Phytochemical constituents of *Feronia limonia* leaves.

Extract	Alkaloid	Glycoside	Steroid	Gums	Carbohydrate	Tannins	Flavonoids	Saponins
Ethanol	+	-	+	-	-	+	+	-

Table 2. *In-vitro* antimicrobial activity of ethanolic extract of *F. limonia*.

Bacterial strains	Diameter of zone of inhibition (mm)		
	Kanamycin (30 µg/disc)	Ethanol extract (250 µg/disc)	Ethanol extract (500 µg/disc)
Gram positive (+)			
<i>Staphylococcus saprophyticus</i>	21	5	7
<i>Enterococcus faecalis</i>	20	0	0
<i>Staphylococcus pyogenes</i>	24	6	7
<i>Streptococcus agalactiae</i>	14	0	0
Gram negative (-)			
<i>Escherichia coli</i>	24	5	8
<i>Shigella boydii</i>	22	7	9
<i>Shigella dysenteriae</i>	16	7	9
<i>Shigella flexneri</i>	24	7	9

Table 3. Effect of *F. limonia* on castor oil-induced diarrhoea in mice.

Treatment	Latent period (hr)	Total number of faeces in 4 hr	Mean of defaecation in 4 hour.
Control	0.64 ± 0.27	46	9.2
Loperamide (50 mg/Kg)	2.57 ± 0.06	19	3.8
Extract (250 mg/kg)	0.80 ± 0.21	41	8.2*
Extract (500 mg/kg)	0.96 ± 0.17	32	6.4*

Values are expressed as mean ± SEM (n = 6), *P < 0.01 vs control.

It is well evident that castor oil produces diarrhoea due to its most active component ricinoleic acid which causes irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion [27]. Since the ethanol extract

of *F. limonia* successfully inhibited the castor oil-induced diarrhoea, the extract might have exerted its antidiarrhoeal action via antisecretory mechanism which was also evident from the reduction of total number of wet faeces in the test groups of the experiment.

Furthermore, the standard chemical tests performed in this study showed that the leaves of the plant species contain tannins, steroids and flavonoids. Tannins have been reported in several studies to have antidiarrhoeal effect [28,29]. In fact, tannins denature proteins and form protein tannate, which makes the intestinal mucosa more resistant and reduces intestinal secretion [30]. The antidiarrhoeal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretion [31,32]. Hence, the antidiarrhoeal activity of the plant may be due to its content of tannins and/or flavonoids. In addition, the antidiarrhoeal activity of the plant may be associated with its antimicrobial effect.

4. Conclusion

The results of the present study indicate that the ethanol extracts of *F. limonia* leaves possess significant antibacterial and antidiarrhoeal potentials in dose dependant manner. The present data justify the traditional uses of this plant for the treatment of various diseases. However, further studies are required for isolation and purification of the active principles of the plant responsible for these effects and to better understand the mechanism of such actions. As the leaves extracts possess tannin and flavonoids, which indicates the presence of antioxidant capacity, we have further plan to conduct study to investigate antioxidant property.

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