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-tdioideae [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 unripeed fruit, riped 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,
Bangladesh and identified by the taxonomist of Bangla-
desh 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 air-
tight 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 occa-
sional 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 pa-
per. By using a rotary evaporator (Bibby RE200, Sterilin
Ltd., UK) the resultant filtrate was concentrated to pow-
der form through complete evaporation of the extraction
solvent. The filtrate was then air dried at room tempera-
ture to evaporate the extra ethanol. It rendered concen-
trate of reddish color which was designated as crude ex-
tract 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 (ICDDRB) for assessing biological activity.
The animals were kept in standard environmental condi-
tions for one week for adaptation after their purchase and
fed ICDDRB 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, gly-
cosides 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 ex-
tracts (250 µg/disc and 500 µg/disc) and standard kana-
mycin solutions (30 µg/disc) were applied to the corre-
sponding Petri dish. The plates were incubated for 24
hours at 37°C. After proper incubation, clear zone of in-
hibition around the point of application of sample solu-
tion 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 (dist-
tilled water containing 0.1% Tween-80). Group-II (posi-
tive control) received standard antimotility drug lopera-
mide (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 wa-
ter, 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 cas-
tor oil administration. Number of stools or any fluid ma-
terial 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 ± standard error of mean
(SEM). Statistical analysis for animal experiment was
carried out using one-way ANOVA followed by Dun-
ett’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 constitu-
ents. Phytochemical studies showed that alkaloids, ster-
oids, 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 facealis 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>
<thead>
<tr>
<th>Extract</th>
<th>Alkaloid</th>
<th>Glycoside</th>
<th>Steroid</th>
<th>Gums</th>
<th>Carbohydrate</th>
<th>Tannins</th>
<th>Flavonoids</th>
<th>Saponins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Kanamycin (30 μg/disc)</th>
<th>Ethanol extract (250 μg/disc)</th>
<th>Ethanol extract (500 μg/disc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram positive (+)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus saprophyticus</td>
<td>21</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Enterococcus facealis</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus pyogenes</td>
<td>24</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gram negative (-)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>24</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Shigella boydii</td>
<td>22</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Shigella dysenteriae</td>
<td>16</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>24</td>
<td>7</td>
<td>9</td>
</tr>
</tbody>
</table>

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

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 recinoleic 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]. Hence, the antidiarrhoeal activity of the plant may be due to its content of tannins and/or flavonoids. Additionally, the antidiarrhoeal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretion [31,32]. Therefore, 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.

REFERENCES


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


