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The Pharma Innovation

ISSN: 2277- 7695
TPI 2015; 3(11): 50-54

www.thepharmajournal.com
Received: 19-11-2014
Accepted: 06-12-2014

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Evaluation of antibacterial and antidiarrhoeal activity of ethanolic extract of *Feronia limonia* Leaves

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Abstract
In the present study crude ethanolic extract of the leaf of *Feronia limonia* was investigated for possible pharmacological activity. The biological interest of these compounds, coupled with the use of this plant in traditional medicine prompted us to check *Feronia limonia* for antibacterial and antidiarrhoeal activity. The antibacterial activity of the ethanolic extract was investigated against ten microorganisms and showed moderate antibacterial activity against *Staphylococcus saprophyticus, Staphylococcus pyogenes, Escherichia coli, Shigella boydii, Shigella dysentery and Shigella flexneri;* maximum zone of inhibition was found for *Shigella boydii* (9 mm), *Shigella dysentery* (9 mm) and *Shigella flexneri* (9 mm) at 500 µg/disc. *Feronia limonia* extract produced a moderate reduction in the severity & frequency of diarrhoea produced by castor oil in mice compared with the standard drug loperamide (25 mg/kg). The antidiarrhoeal activity was found statistically significant ($p<0.02$ and $p<0.001$). The study clearly supports the medicinal value of this plant.

Keywords: Antimicrobial activity, antidiarrhoeal activity, Ethanolic extracts, *Feronia limonia*.

1. Introduction
In the world, about 25% of prescribed drugs are of plant origin [1]. Approximately 80% people rely on traditional plant based medicines for their initial health care needs in developing countries [2]. The Medicinal plants may be an important source of new chemical substances as it has potential therapeutic effects (Gill *et al*., 2011a). The plants contains a number of substances, plant extracts and their primary and secondary metabolites have important therapeutic role in the treatment of many diseases of human being [3, 5]. Worldwide more than 21000 plants are being used in medicinal purpose [6]. Free radical production is actually a normal part of life [7]. Environmental agents initiate free radical generation which led to different complication in body [8]. Free radical causes depletion of immune system antioxidants, change in gene expression and induction of abnormal proteins [9, 10]. Recently synthetic antioxidants are widely restricted because of their toxic and carcinogenic effects so the interest in finding natural antioxidants, without any undesirable effect, has increased greatly [11]. It has been reported that the antioxidant activity of plants might be due to their phenolic, flavonoid and tannin compounds [9, 12]. Recently, due to the inappropriate and injudicious uses or self treatment practices a good number of antibiotics are found to be microbiology resistant [13]. So to combat the problem of microbial resistance and for substitution with ineffective ones the developments of new antibacterial agents are necessary. Moreover, for new antimicrobial therapies it is presumed that the broad spectrum effectiveness of plant species may provide a suitable basis [14]. In developing countries diarrhoea is the recognized and one of the most important health problems [15]. There were about 7.1 million deaths due to diarrhoea for the year 1998 as per WHO estimation [16]. Most dangerous symptom of gastrointestinal problems is secretory diarrhoea [17] and is associated with excessive defecation and stool outputs, abnormally loose consistency of the stool [18]. About 250 species out of 500 species of medicinal plants are used for the preparation of traditional medicines in Bangladesh, majority of these plants have not yet undergone chemical, Pharmacological and toxicological studies to investigate their bioactive compounds [19].
Feronia limonia is a plant under family Rutaceae and subfamily Aurantioideae (Dreyer *et al*, 1972). It as a whole parts of the plant, or its parts such as unriped fruit, riped fruit, root, bark, trunk gum and leaves have a broad spectrum of traditionally established therapeutic properties (Tiwari, 1959). Leaf extract of the plant has antioxidant (Manjusha *et al*, 2004), larvicidal (Rahuman *et al*, 2000), antidiabetic (Joshi et. al, 2009) and hepatoprotective (Manjusha *et al*,

2004) potentials. In this study, we reported antimicrobial, antidiarrhoeal potentiality of the leaves of *Feronia Limonia.*

2. Materials and Methods

2.1 Collection and Identification of Plant material

The plant, *Feronia limonia* was collected from Khulna, Bangladesh. The sample was identified by Bangladesh National Herbarium, Dhaka, where the voucher specimen has been deposited and its Accession No. is DACB 34397.

2.2 Chemicals and Reagents

All the chemicals used are of analytical reagent grade. Kanamycin (30 µg/disc) was collected from Opsonin Pharmaceuticals Ltd. and Loperamide was collected from Square Pharmaceuticals Ltd., Bangladesh.

2.3 Preparation of the Plant material

The collected plant parts (leaf) were separated from undesirable materials or plants or plant parts. They were sun-dried for one week. 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 commenced.

2.4 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. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate (ethanol extract) obtained was evaporated under ceiling fan until dried. It rendered concentrate of reddish color. The concentrate was designated as crude extract of ethanol.

2.5 Experimental animal

Young Swiss-albino mice aged 4-5 weeks, average weight 20-25 gm were used for the experiment. The mice were purchased from the Animal Research Branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDRB). They were kept standard environmental condition 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.

2.6 Antimicrobial Potential

There is no standardized method for expressing the result of anti-microbial screening [20]. Some investigators used the diameter of zone of inhibition and/or the minimum weight of extracts required to inhibit the growth of microorganisms. In our Study the antimicrobial assay was performed by using the disc diffusion method [21, 22]. Ten pathogenic bacteria were used as test organisms for antibacterial activity of sample extract. These organisms were collected from the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B), which are preserved in Microbiology Lab of Pharmacy Discipline, Khulna University, Khulna. 250 µg/disc and 500 µg/disc of the sample extracts were used to observe the antimicrobial activity of the plant extract and compared with the standard kanamycin (30 µg/disc). The test organisms were inoculated on 16 ml previously sterilized nutrient agar media, mixed thoroughly and transferred immediately to the sterile Petri dish in an aseptic condition using a sterile loop. Prepared sample and standard solutions were applied to the corresponding Petri dish. The plates were incubated for overnight at 37 ^{o}C. After proper incubation, clear zone of inhibition around the point of application of sample solution were measured which is expressed in millimeter (mm).

2.7 Antidiarrhoeal Potential

2.7.1 Castor oil induced diarrhoea

The experiment was conducted by castor oil model as described by (Yegnanarayan *et al*., 1982) [23]. The mice were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhoea were selected for the final experiment. The test animals were randomly chosen and divided into four groups having five mice in each. Of the experimental groups, group-I or the control received only distilled water containing 1% Tween-80. Group-II or the positive control received standard antimotility drug, loperamide at a dose of 50 mg/kg-body weight as oral suspension. The 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.

The mice were fed with the samples 1 hour prior to the oral administration of castor oil at a dose of 0.5 ml per mouse. Individual animals of each group were 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.

2.7.2 Statistical Analysis

Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the control group. P values <0.001 were considered to be statistically significant.

3. Results

3.1 Antimicrobial Potential

Antibacterial activities of the extract were tested against sixteen pathogenic bacteria and were compared with the standard antibiotic kanamycin by measuring the zone of inhibition diameter and expressed in millimeter (mm) showed in table 1.

The ethanol extract of the leaf of *Feronia limonia* showed activity against the test organisms except *Enterococcus faecalis, Streptococcus agalactiae, Salmonella typhi, & Shigella sonnei*. The plant is widely and effectively used by the traditional practitioner for the treatment of various diseases. The results of antibacterial screening support the basis of use of the different part of this plant in different ailments.

3.2 Antidiarrhoeal Potential

In the castor oil-induced diarrhoeal mice, the ethanol extract of the leaves *Feronia limonia* at the dose of 250 and 500 mg/kg, reduced the total number of faeces as well as of diarrhoeic faeces, and the result was statistically significant (Table 2 & 3).

Table 1: *In vitro* anti-microbial activity of ethanol extract of *Feronia limonia*

Bacterial strains	**Diameter of zone of inhibition in mm**		
Gram positive(+)	**Kanamycin (30 µg/disc)**	**Ethanol extract (250 µg/disc)**	**Ethanol extract (500 µg/disc)**
Staphylococcus saprophyticus	21	5	7
Enterococcus faecalis	20	0	0
Staphylococcus pyogenes	24	6	7
Streptococcus agalactiae	14	0	0
Gram negative(-)			
Salmonella typhi	17	0	0
Escherichia coli	24	5	8
Shigella boydii	22	7	9
Shigella sonnei	20	0	0
Shigella dysenteriae	16	7	9
Shigella flexneri	24	7	9

Table 2: Effect of *Feronia limonia* on the latent period of castor oil induced Diarrhoeal episode in mice.

Group (dose)	**Numbering of mice**	**weight in gm**	**Latent period (hr)**	**Mean of latent period (hr)**	**Standard Deviation (SD)**	**Standard Error (SE)**	**t-test (P-value)**
I (Control)	1	21	0.25	0.64	0.42	0.27	-
	2	23	0.37				
	3	24	1.17				
	4	20	0.75				
	5	27	0.68				
II (Standard/positive control) Loperamide, 50 mg/Kg	1	21	2.52	2.57	0.21	0.06	6.9 *(P<0.01)*
	2	24	2.50				
	3	21	2.76				
	4	23	3.06				
	5	24	2.00				
III (Test group) 250 mg/kg, Ethanol extract of *Feronia limonia*	1	21	0.58	0.80	0.37	0.21	0.46 *(P<0.8)*
	2	26	0.71				
	3	23	0.41				
	4	24	1.38				
	5	21	0.92				
IV (Test group) 500 mg/kg, Ethanol extract of *Feronia limonia*	1	25	0.82	0.96	0.33	0.17	1.0 *(P<0.4)*
	2	25	1.00				
	3	23	1.33				
	4	25	1.26				
	5	20	0.42				

Table 3: Effect of *Feronia limonia* on castor oil induced diarrhoea in mice

Treatment	**Dose (mg/kg)**	**Total number of faeces in 4 hr.**	**Mean of defaecation in 4 hour**	**t-test (p value)**
Group I (Control)	10	46	9.2	-
Group II (Standard/positive control)	50	19	3.8	22.8 (P<0.01
Group III (Test group)	250	41	8.2	4.8 (p<0.01)
Group IV (Test group)	500	32	6.4	16.8 (P<0.01)

4. Discussion

It has been reported that the Polyphenolic compounds, like flavonoids, tannins and phenolic acids, commonly found in plants and have multiple biological effects, including antioxidant activity [24, 25, 26, 27]. The crude extract of *F. limonia* presence of alkaloids, steroid, tannins and flavonoids. The crude leaves extract of *F. limonia* produced 23.74% and 45.32% writhing inhibition in mice at oral doses of 250 mg/kg and 500 mg/kg body weights of mice respectively while the standard drug, Diclofenac-sodium showed 50.36% inhibition at a dose of 25 mg/kg body weight [28].

Antibacterial activities of extract (250 µg/disc and 500 µg/disc) were studied on ten pathogenic bacteria and were compared with the standard antibiotic kanamycin (30 µg/disc).

The present studies on Antimicrobial activities reveals that the ethanol extract of the leaf of *Feronia limonia* showed activity against the *Staphylococcus saprophyticus*, *Staphylococcus pyogenes, Escherichia coli, Shigella boydii, Shigella dysenteriae* & *Shigella flexneri*. However, demonstration of antimicrobial activity against both Gram-positive and Gram-negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds [29, 30].

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 [31]. Since the ethanol extract of *Feronia 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 in the experiment.

5. Conclusion

The observations found in the present study support this view that the medicinal plants hold a valuable source of potential antioxidants for the discovery of natural-product pharmaceuticals and to be used as preventive agents in the pathogenesis of various diseases. Further works on identification and isolation of active constituents in the extracts may be exploited by *in vivo* study to determine the underlying mechanism of the overall antioxidant activity. On the basis of the obtained results of antimicrobial potentials it can be suggested that, the plant extracts have antimicrobial activity against some microbial agents. The results of the present study also showed that the plant extract possess significant antidiarrhoeal potentials. These findings justify the traditional uses of this plant. Since the extract is reported to contain a range of compounds, it is difficult to ascribe these observed activities to any specific group of compounds. Hence, further studies are suggested to be undertaken to pin point the exact compound(s) and to better understand the mechanism of such actions of *Feronia limonia* scientifically.

6. References

1. Rates SMK. Plants as source of drugs. Toxicon 2001; 39(5):603-613.
2. Food and Agriculture Organization. Trade in medicinal plants. Rome: Economic and Social Department, Food and Agriculture Organization of the United Nations; 2004. http://www.fao.org/docrep/008/af285e/af285e00.HTM, 23 April, 2014.
3. Gill NS, Bajwa JK, Dhiman P, Sharma and S. Sood *et al*. Evaluation of therapeutic potential of traditionally consumed Cucumis melo seeds. Asian J Plant Sci 2011; 10:86-91.
4. Gill NS, Bajwa JP, Sharma KD, Sood S *et al.* Evaluation of antioxidant and antiulcer activity of traditionally consumed Cucumis melo seeds. J Pharmacol Toxicol 2011; 6:82-89.
5. Sood SS, Bansal A, Muthuraman NS, Gill, Bali M. Therapeutic potential of *Citrus medica* L. peel extract in carrageenan induced inflammatory pain in rat. Res J Med Plant 2009; 3:123-133.
6. Karim A, Sohail MN, Munir S, Sattar S. Pharmacology and phytochemistry of Pakistani herbs and herbal drugs used for treatment of diabetes. Int J Pharmacol 2011; 7:419-439.
7. Gavani U, Paarakh PM. Antioxidant activity of *Hyptis suaveolens* poit. Int J Pharmacol 2008; 4:227-229.
8. Londonkar R, Kamble A. Evaluation of free radical scavenging activity of *Pandanus odoratissimus*. Int J Pharmacol 2009; 5:377-380.
9. El-Hela A, Abdullah A. Antioxidant and antimicrobial activities of methanol extracts of some Verbena species: *In vitro* evaluation of antioxidant and antimicrobial activity in relation to polyphenolic content. J Applied Sci Res 2010; 6:683-689.
10. Kumpulainen JT, Salonen JT. Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease. Royal Society of Chemistry, UK, 1999, 178-187.
11. Rechner AR, Kuhnle G, Bremner P, Hubbard GP, Moore KP, Rice-Evans CA. The metabolic fate of dietary polyphenols in humans. Free Radic Biol Med 2002; 33:220-235.
12. Frankle EN, Meyer AS. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. J Sci Food Agric 2000; 80:1925-1941.
13. Alanis AJ. Resistance to Antibiotics: Are We in the Post-Antibiotics Era? Arch Med Res 2005; 36:697-705.
14. Kaushikv PH. (Turmeric): antibacterial potentials. Chowkhamba Sanskrit Series office, Varanasi, 2003, 16.
15. Snyder JD, Merson MH. The magnitude of the global problem of acute diarrhea disease: A review of active surveillance of data. Bull WHO 1982; 60:605-613.
16. Park K. Park's text book of Preventive and Social Medicine. Banarsidas Bharat: Publishers: Jabalpur, 2000, 122-175.
17. Fontaine O. Diarrhea and treatment. Lancet 1988; 28:1234-1235.
18. Aranda MJ, Gianella RA. Acute diarrhea: A practical review. AM J Med Sci 1999; 106:670-676.
19. Ghani A. Medicinal Plants of Bangladesh. The Asiatic Society of Bangladesh, Dhaka, Bangladesh, Edn 2, 2003, 603.
20. Ayafor JF, Sondengam BL, Ngadjui BT. Veprisinium salt, anovel antibacterial quaternary alkaloid from Vepris Louisii. Planta Med 1982; 44(3):139-142
21. Bauer AW, Kirbey WMM, Sherries JC, Truck M. Antibiotic susceptibility testing by standardized single disc method. Am J Clin Pathol 1996; 45:493-496.
22. Barry AL. Procedures for testing antimicrobial agents in agar media. Antibiotics in laboratory medicine; (V. Lorian Ed.); Willams and Wilkins Company; Baltimore; USA, 1980, 1-23.
23. Yegnanarayan R, Shostri DS. Comparison of antidiarrhoeal activity of sons drugs in experimental diarrhea. Indian J Pharmacol 1982; 14(4):293-299.
24. Brown JE, Rice-Evans CA. Luteolin rich artichoke extract protects low-density lipoprotein from oxidation *in vitro*. Free Radical Res 1998; 29:247-255.
25. Vinson JA, Dabbagh YA, Serry MM, Jang J. Plant flavonoids, especially tea flavonols, are powerful antioxidants using an *in vitro* oxidation model for heart disease. J Agric Food Chem 1995; 43:2800-2802.
26. Gil MI, Ferreres FFA. Tomas-Barberan, Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. J Agric Food Chem 1999; 47:2213-2217.
27. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS *et al.* Antioxidant activity of plant extracts

containing phenolic compounds. J Agric Food Chem 1999; 47: 3954-3962.
28. Mohammad AMM, Faysal SB, Khan MR, Md. Iqubal HR, Md. Mustafizur R, Anjuman AB. Phytopharmacological evaluation of ethanolic extract of *Feronia limonia* leaves. Am J Sci Ind Res 2013; 4(5):468-472.
29. Cichewicz RH, Thorpe PA. The antimicrobial properties of Chile peppers (*Capsicum* species) and their uses in Mayan medicine. J Ethnopharm 1996; 52(2):61–70.
30. Srinivasan D, Perumalsamy LP, Nathan S, Sures T. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J Ethnopharm 2001; 49:217–222.
31. Gaginella TS, Stewart JJ, Olsen WA, Bass P. Action of ricinoleic acid and structurally related fatty acid on the gastrointestinal tract. II. Effect on water and electrolyte absorption *in vitro*. Journal Pharmacology and Experimental Therapeutics 1975; 195:355-356.