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Evaluation of antimicrobial and cytotoxic activity of the plant extracts of *Feronia limonia* (Linn).

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Abstract

Feronia limonia L. belongs to family Rutaceae. All parts of this plant are prescribed in the indigenous system of medicine for treatment of various ailments. The aim of present study was designed to investigate antibacterial & cytotoxic activities of the fruit pulp of the plants *Feronia limonia* (Linn) by using in vitro techniques. The antibacterial activity was determined by using disc diffusion method where ten pathogenic bacteria were used as test organisms. The extract displayed the moderate antibacterial action in terms of the diameter of the zone of inhibition. The extract was also subjected to brine shrimp lethality bioassay for possible Cytotoxicity where a concentration dependent increment in percent mortality of brine shrimp naupili was produced by the extracts indicates the presence of cytotoxic principles. The maximum zone of inhibition was obtained for *Salmonella paratyphi* B and *Salmonella paratyphi* (15mm) at a concentration of 500µg/disc. The Ethyl acetic extract of *Feronia limonia* possesses cytotoxic activity. LC_{50} was found in the dose of 8.91µg/ml. Further investigation of the plant is required to confirm its pharmacological activity and thereby utilize it as a useful medicinal plant.

Keywords: Disc diffusion assay, Kanamycin, Brine Shrimp lethality bioassay, Percent mortality.

Introduction

Feronia limonia L. (Family Rutaceae) is a small deciduous tree commonly known as Wood Apple or Kaitha & is widely distributed in most tropical & subtropical countries. All parts of this plant are prescribed in the indigenous system of medicine for treatment of various ailments. This plant recently gained a great therapeutically relevance owing to their high coumarins & monoterpenoids content, which is explored for treatment of snake bite.[1] Fruits, leaves & stem bark of *Feronia limonia* L. have been studied for antitumor[2] and larvicidal[3] activity.

All parts of this plant are prescribed in the indigenous system of medicine for treatment of various ailments. The fruits of this plant are used in diarrhea and dysentery[4], tumors, asthma, wounds, cardiac debility and hepatitis.[5] Recently, the fruit pulp of this plant is studied to have anti-inflammatory, antipyretic and analgesic activities[6], antiulcer[7], hepatoprotective, wound healing and antioxidant activities.[8, 9] The fruit shells were reported to contain antifungal compounds, namely, psoralene, xanthotoxin, 2,6 dimethoxybenzoquinone and sterol.[10]

Plant-derived drugs remain an important resource, especially in developing countries, to combat serious diseases. Approximately 60–80% of the world's populations still rely

on traditional medicines for the treatment of common illnesses. Medicinal plants have a long-standing history in many locations in Bangladesh and continue to provide useful and applicable tools for treating ailments. Because natural products of the higher plants may give a new source of antimicrobial agents, as well as anticancer agents, there are many research groups that are now engaged in medicinal plant research. In present study focus in vitro evaluation of antimicrobial activity and Cytotoxicity of the ethyl acetate extract from the fruit pulp of *Feronia limonia* (Linn).

Materials and Methods

Plant Material

Using standard taxonomical methods, supplied by the Bangladesh Forest Research Institute (BFRI), Chittagong identified the plant part. The fruit pulp of the plant *Feronia limonia* (Linn) was collected from Dhaka.

Extraction and Preparation of the Plant sample

The fruit pulp of the plant *Feronia limonia* (Linn) was sun dried for 5 days. The dried samples were ground to a coarse powder with a mechanical grinder and extracted with petroleum ether and ethyl acetate by using a Soxhlet extraction method. The extract was filtered. The filtrate was dried at 50ºC to 60ºC and the yielded percentage was calculated.

Bacterial Media (Nutrient Agar Media)

36g of Nutrient Agar Media was mixed with distilled water and then sterilized in an autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into Petri dishes. The solidified plates were bored with 5mm diameter cork bearer. The plates with wells were used for the antibacterial studies.

Antimicrobial screening (in vitro)

The antimicrobial activity of the compounds Feronia limonia, were measured by disc diffusion method.[11, 12] The prepared culture plates were inoculated with different selected strains of bacteria and fungi using the streak plate method. Wells were made on the agar surface with 6mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37ºC+2ºC for 24 hours for bacterial and 25+2ºC for 48 hours for fungal activity. The plates were observed for the zone clearance around the wells.

The ethyl acetate extract was dissolved in sterile distilled water to form dilution such as 100µg, 150µg and 200µg. Each concentration of the plant extract was tested against different bacterial pathogens and fungal species .The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

Cytotoxicity test

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds.[13, 14] Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The eggs of the brine shrimp, *Artemia salina*, were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 hours to mature shrimp called Nauplii. The brine shrimp lethality bioassay was performed to predict the cytotoxic activity[13, 15] of the *Feronia limonia*. For an experiment, the test samples (extract) were prepared by dissolving them in DMSO (not more than 50 µl in 5 ml solution) plus sea water (3.8% NaCl in water) to attain concentrations – 5µg/ml, 10µg/ml, 20µg/ml, 40µg/ml, and 80µg/ml. A vial containing 50µl DMSO diluted to 5ml was used as a control. Standard Vincristine sulfate was used as positive control[16, 17] then matured shrimps were applied to each of all experimental vials and control vial. After 24 hrs, the vials were inspected using a magnifying glass and the number of surviving naupili in each vial was counted. The mortality end point of this bioassay was defined as the absence of control forward motion during 30s observation[18] from this data the percent of lethality of the brine shrimp naupili for each concentration and control was calculated. An approximate linear correlation was observed when the logarithm of concentration versus percentage of mortality[19] was plotted on the graph paper and the values of LC_{50} were calculated Using Microsoft excel 2007.

Results

In Vitro Antibacterial Test

The results of the antimicrobial assay of the methanol extract of *Feronia limonia* indicated that the plant exhibited antimicrobial activity against the tested microorganisms at the concentration of 500µg/disc. The potential sensitivity of the extract was obtained against all the micro organisms tested and the zone of inhibition was recorded and presented in Table 1.

Table 1: In vitro antibacterial activity of the ethyl acetate extract of *Feronia limonia* (Linn)

Name of Bacteria	Zone of inhibition (mm)	
	***Feronia limonia* (Linn)**	**Kanamycin (Standard)**
	500µg/disc	30µg/disc
Salmonella paratyphi A	12	30
Vibrio mimicus	14	29
Staphylococcus aureus	14	25
Salmonella paratyphi	15	29
Shigella butea	14	30
Bacillus subtilis	13	30
Shigella bordetella	14	27
Bacillus megaterium	11	30
Salmonella paratyphi B	15	30
Shigella sonnei	13	24

Brine Shrimp Lethality Bioassay

Ethyl acetate extract of *Feronia limonia* (Linn) was tested for Brine shrimp lethality bioassay using brine shrimp nauplii and DMSO as a solvent. The extract showed positive results on brine shrimp lethality bioassay with high concentration. LC50 was found 8.91µg/ml where Vincristine sulfate showed LC50 at 0.52µg/ml (Table-2 and Figure 1). The Control was used to see whether DMSO had any effect on brine shrimp lethality. The control group of brine shrimp nauplii with and without DMSO exhibited no mortality.

Table 2: Brine Shrimp Lethality Bioassay for the ethyl acetate extract of *Feronia limonia* (Linn)

***Feronia limonia* (Linn)**				**Vincristine sulfate**			
Concentration in µg/ml	% Mortality	Log C	LC50 (µg/ml)	Conc.	% Mortality	Log C	LC50 (µg/ml)
400 µg/ml	100	2.602	8.91	40	100	1.602	0.52
200 µg/ml	100	2.301		20	100	1.301	
100 µg/ml	100	2.000		10	100	1.000	
50 µg/ml	90	1.698		5	90	0.699	
25 µg/ml	70	1.397		2.50	80	0.398	
12.5 µg/ml	60	1.096		1.25	60	0.097	
6.25 µg/ml	40	0.790		0.63	50	0.201	
3.125	40	0.490		0.31	40	-0.509	

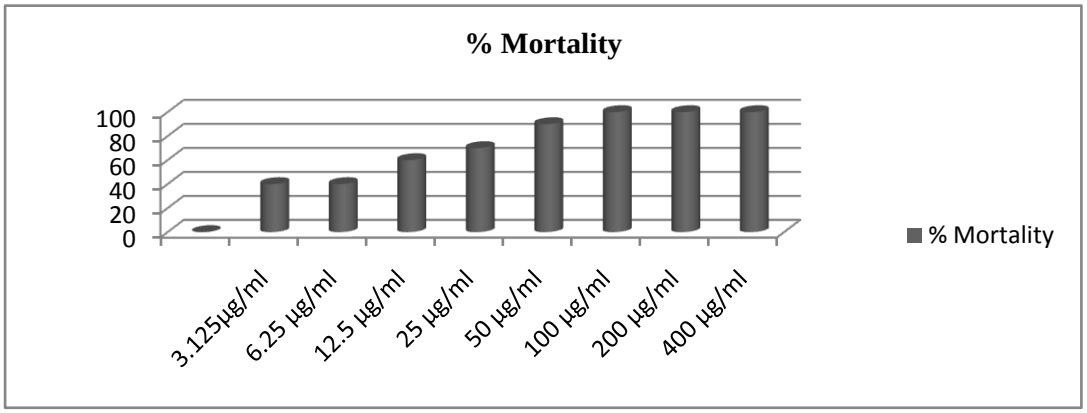

Figure 1: Plot of Log Concentration versus percent mortality

Discussion

In the present investigation, the active phytocomponents of *Feronia limonia* was studied and further the antimicrobial activity and Cytotoxicity of the plant extract was also tested to understand the most effective activity. The antimicrobial activity of the plant extract was tested against ten potentially pathogenic microorganisms by using disc diffusion method at different concentrations of the extract to understand the most effective activity. The maximum zone of inhibition was obtained for Salmonella paratyphi B and Salmonella paratyphi (15mm) at a concentration of 500µg/disc. The Ethyl acetic extract of *Feronia limonia* possesses cytotoxic activity. LC_{50} was found in the dose of 8.91µg/ml. The antimicrobial and cytotoxic activity might be due to the presence of various phytocomponents.

Conclusion

From the above studies, it is concluded that the traditional plants may represent new sources of anti-microbial with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. These local ethno medical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, Phytochemistry, ethno botany and other biological actions for drug discovery.

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