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Antibacterial, Cytotoxic and Analgesic Activities of the Ethanol Extract of Feronia Limonia Linn Fruit Pulp.

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Abstract: *The present study was undertaken to explore the antibacterial, cytotoxic and analgesic activities of the ethanolic extract (E_T) of Feronia limonia fruit pulp. The pulp of elephant apple was extracted with ethanol by cold extraction procedure. We found that the E_T contains active phytochemicals that showed potential antimicrobial, cytotoxic and analgesic activities. Briefly, E_T showed better activities on average against both and the highest activity was found against Sarcina lutea (in case of gram positive) and Shigella shiga (in case of gram negative) with significant MIC values. Additionally the E_T extract of Feronia limonia fruit pulp possesses cytotoxic activity and LC50 was found to be in the dose of 48.76143µg/ml. Furthermore, analgesic activity of the E_T was evaluated using acetic acid-induced writhing model of pain in mice. The crude extract at 200 mg/kg and 400 mg/kg body weight doses displayed significant ($p<0.05$) reduction in acetic acid induced writhing in mice with the effect of 50.94% and 68.87% respectively which is comparable to the standard diclofenac sodium (81.44%). Prelude phytochemical investigation of the E_T showed the presence of alkaloid, saponin, flavonoids and steroids. The results indicated that the E_T has moderate antibacterial, cytotoxic and analgesic activities.*
Keywords: *Analgesic, Antimicrobial, Cytotoxicity, Ethanol Extract And Katbel (Feronia Limonia).*

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I. Introduction

Since disease, decay and death have always co-existed with life, the study of diseases and their treatment must also have been contemporaneous with the dawn of the human intellect. The primitive man must have used as therapeutic agents and remedial measures those things which he was able to procure most easily. The plant kingdom has been serving as the principle basis of medicine for animal kingdom from ancient time of animal existence on this earth. Only proper management and research on medicinal plants can able to lower the cost of medicine to fulfill vast majority of demands in the subcontinent [1].

The plant *Feronia limonia*, locally known as "KATBEL", belongs to the Rutaceae family, is a moderate-sized tree is widely distributed throughout Bangladesh, India and many other countries [2, 3]. Different parts of this plant are used as medicine with unique properties in the treatment of a variety of human diseases [4]. The plant belonging to the Rutaceae family has wide folk medicinal use. It is evident that the genus Feronia is an important source of biologically active compounds such as acetogenins, alkaloids, terpenoids, steroids, etc. and these compounds possess potential cytotoxic, anticancer, pesticidal, antimicrobial, parasiticidal properties. The folk medicinal practitioners use these plants as such without having knowledge about their side effects and toxicity. If it is possible to exclude the poisonous components present in the plants, which are toxic to human and animal body, then we can properly use these plants for the treatment of various diseases. In order to do so, research on this plant is of great importance to identify and characterize the bioactive principles from this plant.

Several researchers reported the isolation of some important bioactive compounds from the different parts (root, stem bark, leaves, fruit, seeds, etc) of the plant Feronia limonia. Although till now, few bioactive principles having potent antibacterial, antitumor, analgesic activities have been isolated from this plant, our attention was concentrated on the pulp of Feronia limonia for more bioactive principles and to evaluate their antibacterial, cytotoxic, and analgesic effect, which ultimately led to the proper use of this important medicinal plant for better health care system. Therefore, the purpose of this study was to determine antimicrobial activity of the pulp extract against different strain of bacteria, cytotoxic effect against brine shrimp and identification of analgesic effect in vivo.

II. Materials and Methods

1. Animal Maintenance

Adult healthy (4 weeks of age) Swiss albino male mice with average body weight (20-25 g) were purchased from ICDDRB (International Centre for Diarrheal Disease Research, Bangladesh). The animals were randomly selected and housed in polycarbonate cages with steel wire tops and wood-cobe bedding (six mice per cage). Animals were maintained under standard environmental conditions temperature (24±1.0°C), relative humidity: 55-65%, 12h:12h dark light cycle and had free access to feed as well as water ad libitum. The animals were acclimatized to laboratory condition for one week prior to experiment. Ethical permission of this study was obtained from the Institute of Biological Sciences, University of Rajshahi, Bangladesh (21/320-IAMEBBC/IBSc).

2. Extraction and Plant Material Processing

The plant *Feronia limonia* L. was taxonomically identified by using standard taxonomical methods in the department of Botany, University of Rajshahi, Bangladesh. The fruit *Feronia limonia* were collected during the month of July-August, 2017 from the relevant area (Saheb Bazar) of Rajshahi District. After collection the fruit pulp of the plant *Feronia limonia* L was sun dried for 6-7 days and finally kept in an electric oven for 72 hours at 37^{o}C. Following complete drying, the dried samples were then pulverized into a coarse powder with the help of a mechanical grinder (FFC-15, China) and extracted with ethyl alcohol by using a Soxhlet extraction method. The extraction process was performed for 3 repeating cycles. After that, extract was filtered through Whatman No.1 filter paper. The filtrate was dried and concentrated with a rotary evaporator under reduced pressure at 50º-60ºC to afford crude ethanol extract (E_T).

3. Phytochemical Tests

The crude ethanolic extract of the fruit pulp of Feronia limonia was tested for its different chemical groups as tannins, saponins, steroids, alkaloids and flavonoids by the phytochemical analysis processes described by Evans [5].

4. Antimicrobial (Antibacterial) Activity Screening (*In Vitro*)

The ethanol extract of fruit pulp of the *Feronia limonia* L plant were tested for antibacterial activity by disc diffusion assay method [6, 7]. Eight pathogenic bacteria of which four gram positive (*Bacillus megaterium, Bacillus subtilis, Sarcina lutea & Staphylococcus aureus*) and four gram negative bacteria (*Shigella sonnei, Pseudomonas auringinosa, Escherichia coli & Shigella shiga*) were collected from the Institute of Biological Science (IBSC), University of Rajshahi, Bangladesh. Standard *Kanamycin* disc (30 µg/disc) and blank disc saturated with the respective solvent were used as positive and negative control respectively to study the antibacterial activity. The antibacterial activity of E_T, were tested against eight bacteria at concentrations of 200 µg/disc and 400 µg/disc.

5. Determination of Minimum Inhibitory Concentration (MIC)

The MIC values for E_T extract were determined by serial dilution technique [8, 9]. Eight pathogenic bacteria of which four gram positive (*Bacillus megaterium, Bacillus subtilis, Sarcina lutea & Staphylococcus aureus*) and four gram negative bacteria (*Shigella sonnei, Pseudomonas auringinosa, Escherichia coli & Shigella shiga*) were used to identify MIC values of E_T.

6. Brine Shrimp Lethality Bioassay (Cytotoxicity Test)

The Brine Shrimp Lethality Bioassay (Cytotoxicity test) was carried out using the method described by [10]. Briefly, Artemia salina leach or brine shrimp eggs was subjected to hatch and mature as nauplii (larvae) in seawater for 48 h at 25^{o}C. Serially diluted test solutions (80 µL in DMSO from a stock solution of 5 mg/mL of ET extract) were added to the seawater (5 mL), containing 10 nauplii. Following incubation at 25^{o}C for 24 h, the number of alive nauplii was counted. The LC50 (50% lethal concentration of ET µg/ml) value was calculated from triplicate experiments using probit analysis [8]. In this experiment Ampicillin trihydrate was used as a positive control.

7. Acetic Acid-Induced Writhing Test in Mice (Analgesic Activity)

In this experiment a total of 24 mice were divided into four groups (each group comprises six mice). Group I served as vehicle control mice received vehicles (1% tween 80 in saline), group II served as standard group and received diclofenac (10 mg/kg i.p) as standard analgesic drug, group III and group IV received orally 200 and 400 mg/kg of E_t extract of *feronia limonia* fruit pulp respectively. Writhing was induced in mice by intraperitoneal administration of 0.1 ml of 1% acetic acid. Extract and vehicle were administered orally 30

minutes before intraperitoneal administration of 1% acetic acid but diclofenac sodium was administered intraperitoneally 15 minutes before injection of acetic acid. After an interval of 5 minutes, the mice observed for specific contraction of body, referred to as "writhing" for the next 10 minutes [11-13].

8. Statistical Analysis

The results were expressed as mean ± standard error of mean (sem). Statistical analysis was performed by one way anova by using graph pad prism software version 5.03. P values *p<0.05 and ***p<0.001 were considered as statistically significant. The LC50 (50% lethal concentration of E_t µg/ml) value was calculated from triplicate experiments using probit analysis.

III. Results

1. Phytochemical Screening Test Result in the Crude Ethanol Extract (E_t) of *Feronia Limonia* Fruit Pulp

Phytochemical investigations of the ethanolic fruit pulp extract (E_T) of *Feronia Limonia* results the presence of tannins, saponins, steroids, alkaloids and flavonoids Table 1.

2. Antibacterial Activity of Crude Ethanol Extract (E_t) of *Feronia Limonia* Fruit Pulp

The antibacterial activity of the crude ethanol extract (E_T) of *Feronia Limonia* fruit pulp was tested against both gram positive and gram negative bacteria (Table 2) at concentrations of 200 and 400 µg/disc. Standard antibiotic Doxycycline (DXT-30 µg/disc) was used for comparisons. The results obtained are shown in Fig 1.

The produced zone of inhibition for E_T against *Bacillus megaterium, Bacillus subtilis, Sarcina lutea, Staphylococcus aureus* were 14, 15, 20, 14 mm at 50 µg/disc dose, while it were 18, 17, 25, 18 mm in case of 100 µg/disc dose Fig 2. On the other hand in case of gram negative bacteria i.e., *Shigella sonnei, Pseudomonas auringinosa, Escherichia coli,* and *Shigella shiga* he produced zone of inhibition for E_T at 50 µg/disc dose were 14, 16, 15 and 15 mm, respectively whereas in case of 100 µg/disc dose it were 21, 20, 18 and 19 mm, respectively Fig 3. The above results indicated that E_T of elephant apple pulp is active against both gram positive and gram negative bacteria. Finally we can conclude that the elephant apple pulp might be useful as probiotics or as the source of antimicrobial agents.

3. The MIC Values of Crude Ethanol Extract (E_t) of *Feronia Limonia* Fruit Pulp

The MIC values of E_T were determined by serial dilution technique against four gram positive bacteria (*Bacillus megaterium, Bacillus subtilis, Sarcina lutea & Staphylococcus aureus*) and four gram negative bacteria (*Shigella sonnei, Pseudomonas auringinosa, Escherichia coli & Shigella shiga*). These values are shown in Table 3. For the crude E_T the growth was observed in the test tube containing 64 µg/ml of extract against *Staphylococcus aureus, Shigella sonnei, Pseudomonas auringinosa, & Escherichia coli*. On the other hand *Bacillus megaterium, Bacillus subtilis, Shigella shiga & Sarcina lutea* were found to grow in the test tube containing 128 µg/ml of E_T.

4. Brine Shrimp Lethality Bioassay

Ethanol extract (E_T) of *Feronia limonia* (Linn) fruit pulp 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 48.76143µg/ml where Ampicillin trihydrate showed LC50 at 12.267235µg/ml Table 4. The control group of brine shrimp nauplii with and without DMSO exhibited no mortality.

5. Analgesic Activity of Crude Ethanol Extract of *Feronia Limonia* Fruit Pulp (E_t) on Swiss Albino Mice

The analgesic activity of E_T at 200 mg/kg and 400 mg/kg dose level were reduced 50.94% and 68.87% writhing inhibitory response but diclofenac was observed 81.44%. So, $\mathbf{E_T}$ extract 200 and 400 mg/kg was showed significant (p<0.05) inhibitory responses to pain induced by acetic acid when compared to control group i.e., Group I Table 5.

IV. Discussion

In the present study, the active phytochemicals of ethanolic extract (E_t) of *Feronia limonia* fruit pulp was studied table 1, additionally the antimicrobial, cytotoxic and analgesic activity of the fruit pulp extract was also tested to understand the potential activity. The antimicrobial activity of the plant extract was tested against eight potentially pathogenic microorganisms table 2 by using disc diffusion method at two different concentrations (200 and 400 µg/disc) of the extract to understand the antimicrobial activities. The E_t extract showed better activities on average against both gram-positive and gram-negative bacteria and the highest

activity was found against *Sarcina lutea* (in case of gram positive) and *Shigella shiga* (in case of gram negative). The E_t extract showed significant MIC values table 3.

The E_T extract of *Feronia limonia* fruit pulp possesses cytotoxic activity and LC50 was found in the dose of 48.76143µg/ml Table 4. The antimicrobial and cytotoxic activity of E_T makes us enthusiastic to initiate *in vivo* animal experiments. We found remarkable analgesic potential of E_T extract of *Feronia Limonia* fruit pulp **Table 5**. Acetic acid-induced writhing sensitizes localized inflammatory response that lead to the release of arachidonic acid from phospholipids [14]. More specifically, prostacyclin and prostaglandin-E reported to be responsible for pain sensation by exciting A-fibres. Activities in the A-fibres cause a sensation of sharp well localization pain. Any compounds can show analgesia predominantly by inhibition of prostacyclin and prostaglandin-E synthesis pathway [15].

Flavonoids being powerful antioxidants are reported to play a role in analgesic activity by targeting prostaglandins [16, 17]. Since, ET extract of Feronia Limonia fruit pulp contain flavonoids and might have inhibit the prostaglandin synthesis. The ET extract at 200 mg/kg and 400 mg/kg b.w doses displayed significant ($p<0.05$) reduction in acetic acid induced writhing in mice with the effect of 50.94% and 68.87% respectively which is comparable to the standard, diclofenac sodium (81.44%).

V. Conclusion

In conclusion, it can be suggested that the crude ethanolic extract of *Feronia limonia* Linn fruit pulp may possess antimicrobial, cytotoxic and analgesic activities, which correlate well with the traditional use of this plant. Therefore, further pharmacological and biochemical investigations of bioactivity guided *in vitro* and *in vivo* phytochemical studies are required to find out the specific compounds responsible for antimicrobial, cytotoxic and analgesic action of the ethanol extract of *Feronia limonia* Linn fruit pulp.

Acknowledgments

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Conflicts Of Interest

The authors declare no competing financial interest.

References

[1] A Ghani, Medicinal Plants Of Bangladesh, Second Edition (Asiatic Society Of Bangladesh 2003).
[2] M Yusuf, Ju Chowdhury, Ma Wahab And J Begum, Medicinal Plants Of Bangladesh, Dhaka-1205: Bangladesh Council Of Scientific And Industrial Research 1994, 112-114.
[3] Kr Kirtikar, Bd Basu, Indian Medicinal Plants, Vol. I. (Bishen Singh Mahendra Pal Singh, India. 1993, 496-498).
[4] Y Saima, Ak Das, Kk Sarkar, Ak Sen And P Sur, An Antitumor Pectic Polysaccharide From Feronia Limonia, International Journal Of Biological Macromolecules, 27, 2000, 333-335.
[5] Wc Evans, Trease And Evan's Textbook Of Pharmacognosy. 13th Ed, (Cambridge University Press, London, 1989).
[6] A L Barry, Procedure For Testing Antimicrobial Agent In Agar Media, In: V. L. Corian (Ed) Antibiotica In Laboratory Medicines, (Willims And Wilkins Co. Baltimore, 1980).
[7] Bda Vander, Vlietnck, Screening Methods For Antibacterial And Antiviral Agents From Higher Plants. In: Assay For Bioactivity, (K. Hostiettman Academic Press, London, 1991).
[8] [8]. Dj Finney, Probit Analysis, 3rd Ed, (University Press. Cambridge, Uk, 1971).
[9] 9. R Reiner, Detection Of Antibiotic Activity, In: Antibiotics-An Introduction, (Roche Scientific Services, Switzerland, 1982).
[10] [Jl Malaughlin, Cj Chang And Dl Smith, Bench Top Bioassay For The Discovery Of Bioactive Natural Products, Natural Products Chemistry. 9, 1991, 388-409.
[11] W Ridtitid, C Sae-Wong, W Reanmongkol And M Wongnawa, Antinociceptive Activity Of The Methaolic Extract Of Kaempferia Galangal Linn. In Experimental Animals, Journal Of Ethanopharmacology 118, 2008, 225-30.
[12] M G Magaji, J A Anuka, I Abdu-Aguye, A H Yaro And I M Hussaini, Preliminary Studies On Anti-Inflammatory And Analgesic Activities Of Securinega Virosa (Euphorbiaceae) In Experimental Animal Models, Journal Of Medicinal Plants Research 2(2), 2008, 39-44.
[13] F Ahmed, Mst Selim, Ak Das, Msk Choudhuri, Anti-Inflammatory And Antinociceptive Activities Of Lippanodiflora Linn, Pharmazie, 59, 2004, 329-333.
[14] Je Brown And Car Evans, Luteolin Rich Artichoke Extract Protects Low Density Lipoprotein From Oxidation In Vitro, Free Radical Research, 29, 1998, 24255.
[15] N Voilley, Acids-Sensing Ion Channels (Asics): New Target For The Analgesic Effects Of Non Steroidal Anti-Inflammatory Drugs (Nsaids), Current Drug Targets. Inflammation And Allergy, 3, 2004, 71-79.
[16] Mm Hossain, Ms Ali, A Saha And M Alimuzzaman. Antinocicceptive Activity Of Whole Plant Extracts Of Paederiafoetida, Dhaka University Journal Of Pharmaceutical Sciences, 5, 2006, 67-69.
[17] Kms Rajanarayana, Mr Reddy, Chaluvadi And Dr Krishna, Biflavonoids Classification, Pharmacological, Biochemical Effects And Therapeutic Potential, Indian Journal Of Pharmacology, 33, 2001, 2-16.

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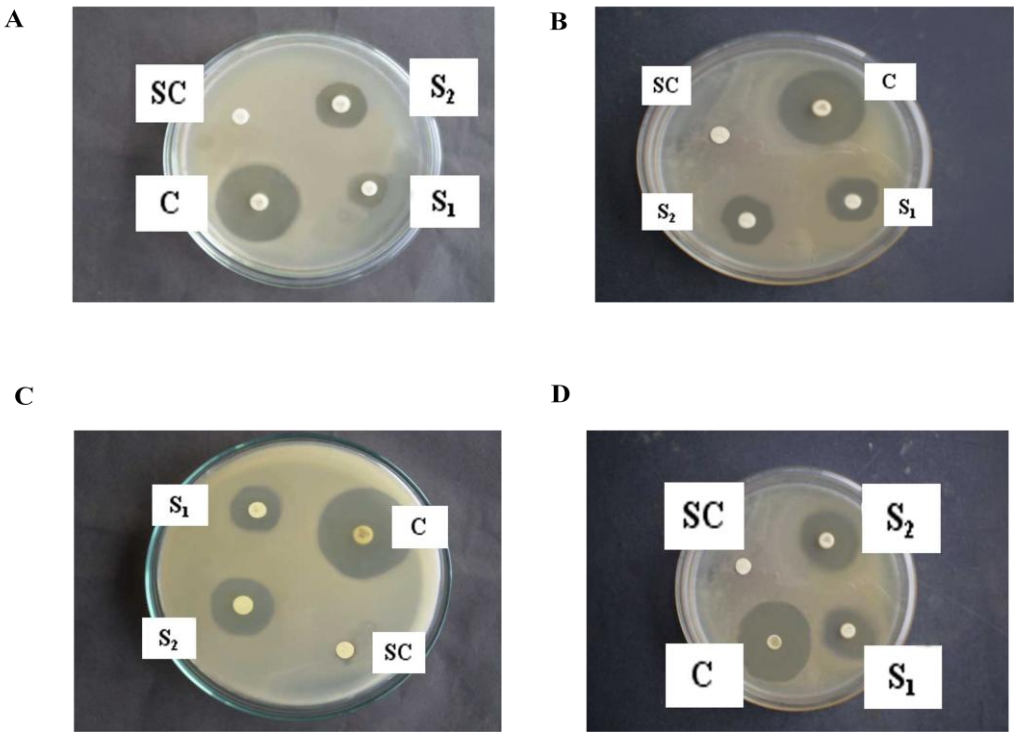
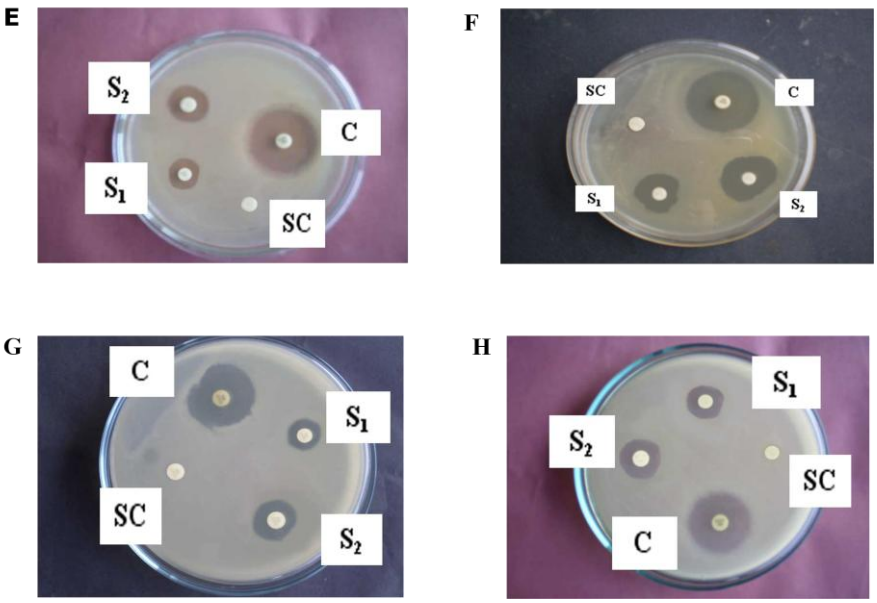
Figure 1



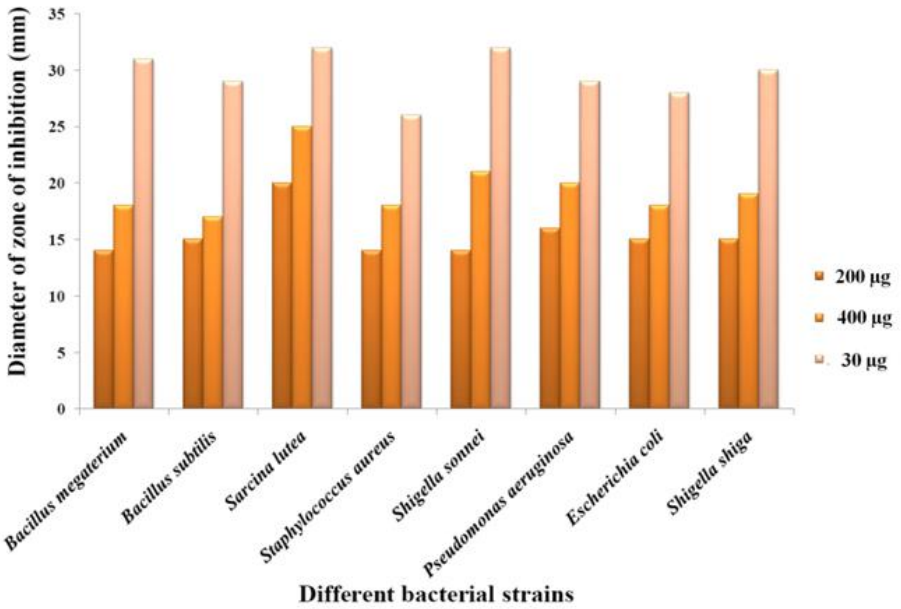
Antibacterial Activity Of E_t Against Different Gram Positive Bacterial Strains.
A. *Bacillus Megaterium* **B.** *Bacillus Subtilis* **C.** *Sarcina Lutea* **D.** *Staphylococcus Aureus*.
Here, Sc = Standard Control ; C = Control; S_1 = 200µg E_t; And S_2 = 400µg E_t.

Figure 2



Antibacterial Activities Of E_t Against Different Gram Negative Bacterial Strains.
E. *Shigella Sonnei*, **F.** *Pseudomonas Aeruginosa*, **G.** *Escherichia Coli*, **H.** *Shigella Shiga.*
Here, Sc = Standard Control; C = Control; S_1 = 200µg E_t; And S_2 = 400µg E_t.

Figure 3
Antibacterial Activity of Ethanol Extracts (Et) of Feronia Limonia (Linn) Fruit Pulp Against Some Gram Positive and Gram Negative Bacteria.



List Of Tables

Table 1. Presence Of Phytochemicals In The Crude Ethanol Extract (E_t) Of *Feronia Limonia* Fruit Pulp

Plant Extract	Alkaloids	Saponins	Flavonoids	Tanins	Steroids
Ethanolic Extract	+	+	+	+	+

+ = Presence, - =Absence

Table 2. List of the Test Pathogenic Bacteria

Serial No.	Name Of Test Organism	Strain Number
	Gram Positive	
1	*Bacillus Megaterium*	Ql-38
2	*Bacillus Subtilis*	Ql-40
3	*Sarcina Lutea*	Ql-166
4	*Staphylococcus Aureus*	Atcc-259233
	Gram Negative	
5	*Shigella Sonnei*	Aj-8992
6	*Pseudomonas Aeruginosa*	Crl
7	*Escherichia Coli*	Fpfc-1407
8	*Shigella Shiga*	Atcc-26107

Table 3. MICs of Crude Ethanol Extract (E_t) of *Feronia Limonia* Fruit Pulp

Bacterial Strain (Gram Positive)	Ethanol Extract (Et) (µg/ml)	Bacterial Strain (Gram Negative)	Ethanol Extract (Et) (µg/ml)
Bacillus Megaterium	128	*Shigella Sonnei*	64
Bacillus Subtilis	128	*Pseudomonas Auringinosa*	64
Sarcina Lutea	128	*Escherichia Coli*	64
Staphylococcus Aureus	64	*Shigella Shiga*	128

Table 4. Brine Shrimp Lethality Bioassay for the E_t Extract of *Feronia Limonia* (Linn) Fruit Pulp

***Feronia Limonia* (Linn)**			**Ampicillin Trihydrate**		
Concentration In **µg/ml**	% Mortality	Lc50 (**µg/ml**)	Concentration In **µg/ml**	% Mortality	Lc50 (**µg/ml**)
5	13.3	48.76143	5	30	12.267235
10	26.6		10	46	
20	33.3		20	60	
40	46.6		40	73.3	
80	56.6		80	86.6	

Table 5. Analgesic Effect of E_t on Swiss Albino Mice

Groups	**Dose (mg/kg)**	**No Of Writhing**	**% Of Protection**
Group-L	Vehicle	31.8±.750	-
Group-Ll	10	5.9±0.423*	81.44
Group-Lll	200	15.6±0.59*	50.94
Group-LV	400	9.9±0.49*	68.87

Values were expressed in mean ± sem, (n=6). Group-L received 1% Tween 80 in saline and Group-Ll received 10 mg/kg Diclofenac-Na, Group-Lll and Group-LV received E_t extract 200 and 400 mg/kg body weight. *P<0.05 indicate significance compared with control group.

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