Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 1, 2014

Research Article

COMPARATIVE PHYTOCHEMICAL STUDIES AND ANTIMICROBIAL POTENTIAL OF FRUIT EXTRACTS OF *FERONIA LIMONIA* LINN.

JAYASHREE V H, RAMESH LONDONKAR*

Dept of Biotechnology, Gulbarga University, Gulbarga 585106, Karnataka, India. Email: londonkarramesh53@gmail.com

Received: 16 Nov **2013,** *Revised and Accepted: 20 Dec* **2013**

ABSTRACT

Objectives: To study the phytochemical screening of different extracts of *Feronia limonia* Linn (FL) fruit pulp for the presence of secondary metabolites and to screen their activity against microbes. Also, physico-chemical characters and fluorescence analysis, along with phytochemical screening of crude drug and extracts were carried out.

Methods: The antimicrobial activity of pulp extracts were tested against bacteria and fungi through well diffusion method.

Results: The results reveal that the petroleum ether, chloroform, methanolic and aqueous fruit pulp extract contain alkaloids, phenolics, flavonoids, steroids, tannins, triterpenoids, saponins and glycosides. Among all the extracts of pulp the highest sensitivity was recorded for methanolic extract followed by aqueous extract.

Conclusion: The study shows that the different extracts of *F. limonia* against various bacterial and fungal strains indicate that the plant has potent antibacterial and antifungal effects. The result of the antimicrobial assay is an evidence of the ethnomedicinal uses of the plant and can be used as a potential source for developing new antimicrobial agents.

Keywords: Phytochemicals, Fruit pulp, Fluorescence, Antifungal, *Feronia limonia.*

INTRODUCTION

Feronia limonia Linn is a deciduous, slow-growing, erect tree belonging to the family Rutaceae. Its leaves, bark and fruits have medicinal values and used as traditional medicines for centuries due to their antimicrobial [1], antifungal [2] and insulin secretogouge[3]activities. The fruits are round to oval, 5-12.5 cm wide, with a hard, woody, greyish-white, scurfy rind about 6 mm thick, pulp brown, mealy, odorous, resinous, astringent, acid or sweetish, with numerous small, white seeds scattered through it. The fruits are used in India as a liver and cardiac tonic, and when unripe, as an astringent means of halting diarrhea and dysentery and effective treatment for hiccough, sore throat and diseases of the gums. The pulp is poulticed onto bites and stings of venomous insects, and is a good antidote to snakebites.

Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to provide new remedies to mankind. Higher plants have been shown to be a potential source for new anti-microbial agents [4]. The plants secondary products may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential target sites. Therefore, random screening of plants for active chemicals is as important as the screening of ethno botanically targeted species. However, for screening pharmaceutical and therapeutic potential of these ayurvedic medicines various bioassays were conducted to detect and confirm the antipathogenic effects in animal model [5] and to establish a good correlation with pathogens [6]. For achieving this, the drug targeting and therapeutic simulations are highly essential.

There is an increasing trend in the emergence of resistance to antimicrobial agents, not only due to the poor quality drugs, patient non-compliance, and irrational use of antimicrobial agents, but also to spontaneous mutations within the microbial populations [7,8]. Therefore, measures should be adopted to control the use of antimicrobial agents, to understand the genetic mechanisms of bacterial resistance, and to continue studies to develop new drugs. Ultimately, this may greatly contribute to provision of more appropriate and efficient antimicrobial agents to the patient. Hence, this study intends to evaluate the phytochemical constituents and antimicrobial activity of fruit pulp of *Feronia limonia* Linn.

MATERIALS AND METHODS

Plant material

Fruits of *Feronia limonia* Linn were collected from local market Bellary, Karnataka, India during the month of March, 2012. It was authenticated by the Department of P.G studies and Research in Botany, Gulbarga University, Gulbarga.

Preparation of plant extract

Crude fruit pulp extract was prepared by Soxhlet extraction method. About 50gm of powdered plant material was packed in a thimble and extracted successively with 350ml of petroleum ether, chloroform, methanol and aqueous. The process of extraction is carried out until the solvent in siphon tube of an extractor become colorless. The extract was taken in a petriplate and kept in hot air oven and heated at 30-40ºC till the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC for future use.

Preliminary Phytochemical screening

Preliminary Phytochemical screening was performed following the standard method of Kokate [9] and Harborne [10]. The metabolites screened included alkaloids, phenolics, flavonoids, steroids, tannins, triterpenoids, saponins, glycosides, gums & mucilage, carbohydrates, proteins & amino acids, lipids & fats.

Physicochemical analysis

The fruit pulp subjected for determination of physicochemical parameters like ash values and extractive values according to Indian pharmacopeia [11]; and fluorescence studies as described by Chase and Pratt [12].

Test microorganisms

The test organisms used in this experiment includes four bacteria strains namely *Salmonella typhimurium* (MTCC 98), *Klebsiella pneumonia* (MTCC 432), *Escherichia coli* (MTCC 45), *Pseudomonas aeruginosa* (MTCC 647), and two fungal strains *Aspergillus niger* (MTCC 282), *Aspergillus flavus* (MTCC 277) were procured from IMTECH, Chandigarh, India.

Culture media

Mueller-Hinton agar (MHA), Nutrient broth (NB), Potato dextrose agar (PDA) and Potato dextrose broth (PDB) media manufactured by HiMedia Laboratories Ltd., India.

Antimicrobial activity

The agar well diffusion method was used to test the plant extracts for antimicrobial activity. The bacterial isolates were first grown in a nutrient broth for 18 h before use and standardized to 0.5 McFarland standards (10^6 CFU/ml). The fungal isolates were allowed to grow on Potato dextrose agar (PDA) at 25°C until they are sporulated. The fungal spores were harvested and standardized to 0.1 at OD at 600nm. 20ml of melted and cooled nutrient agar and potato dextrose agar were added 1 in 100 dilutions of 0.2ml of bacterial and fungal cultures respectively in sterile Petri plates. After the agar in each plate solidified, wells of 6mm each were bored using a cork borer. 50 µl of plant extracts at concentration ($50\mu gml^{-1}$), as well as the standard antibiotic solution was loaded into the wells. Control experiments were set up using streptomycin and Nyastatin ($50\mu gml^{-1}$) for the bacterial and fungal assays respectively. The plates were incubated at 37^0C, 24h for bacteria and 25^0C, 48h for fungi. The transparent ruler was used to calculate the zone of inhibition.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of plant extracts was determined using the broth dilution method as described by Sahm and Washington [13]. In this method, 1ml of the extract solution at the concentration of $50mgml^{-1}$ was added to 1ml of nutrient broth and subsequently transferred to make solutions of varying concentrations (25, 12.5, 6.25, 3.12, 1.56 $mgml^{-1}$) in different test tubes. Then 1ml of bacterial and fungal suspensions and 0.1ml of plant extracts were added to each test tube and incubated at 37^0C, 24h for bacteria and 25^0C, 48h for fungi. The test tube with the concentration of plant extract at which no detectable growth was observed was considered as the MIC.

RESULTS

Phytochemical analysis

During the present investigations the highest extractive value was found in aqueous (14%) followed by methanol (10.7%), petroleum ether (6.1%) and chloroform (3.8) as shown in (Table 1). The results of phytochemical analysis of *F. limonia* fruit pulp are summarized in (Table 2). Phytochemical compounds such as alkaloids, phenolics, flavonoids, steroids, tannins, triterpenoids, saponins, glycosides, gums & mucilage, carbohydrates, proteins & amino acids, lipids & fats are found to be present in this plant.

Table 1: It shows extractive Value of *Feronia limonia* Linn fruit pulp extracts

Solvent	Extractive value (%)
Petroleum ether	6.1
Chloroform	3.8
Methanol	10.7
Aqueous	14.0

Physicochemical analysis

The powder of the fruit pulp was analyzed for various physicochemical parameters. Total ash, water-soluble ash, acid-insoluble ash, alcohol and water soluble extractive values of the fruit pulp powder were done and the results are tabulated in (Table 3). The powder is examined in daylight and ultraviolet light (UV), to detect the fluorescent compounds and the observations are given in (Fig. 1).

Table 2: It shows qualitative analysis of phytochemicals of *Feronia limonia* Linn extracts

Metabolites	Extracts			
	PE	CE	ME	AE
Alkaloids	+	+	+	+
Carbohydrates	+	+	+	+
Tannins	-	-	-	-
Phenolics	-	-	+	+
Flavonoids	-	-	+	+
Glycosides	-	-	+	+
Steroids	+	-	+	-
Lipids/fats	+	-	-	-
Saponins	-	-	-	+
Triterpenoids	+	-	-	-
Gum & mucilage	-	-	-	+
Proteins & amino acids	+	+	+	+

"+" = Presence of compound; "-" = Absence of compound

PE- Petroleum ether extract, CE- Chloroform extract, ME- Methanolic extract, AE- Aqueous extract.

Table 3: It shows proximate analysis of *Feronia limonia* Linn fruit pulp

S. No.	Parameter	Percentage
1.	Acid insoluble ash	0.82%
2.	Water soluble ash	4.96%
3.	Total ash	7.79%
4.	Alcohol soluble extractive	11.17%
5.	Water soluble extractive	8.15%

Antimicrobial assay

The antimicrobial activities of *F. limonia* fruit pulp extracts against the tested microorganisms were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and MIC values. The results are given in (Table 4). Among the tested extracts, methanol exhibited the highest antimicrobial activity and the least by chloroform. The aqueous extract showed moderate activity. The extracts have shown a potent antimicrobial activity against Gram-positive, Gram-negative bacteria and fungal strains. The zone of inhibition and MIC values were in the range of 15–21 mm and 3.125-12.5 mg/ml for the methanolic extract, 12–18 mm and 3.125-12.5 mg/ml for the aqueous extract, 12.5–25 and 0.078- 2.5 mg/ml for the chloroform extract, respectively. The results were compared with standard Streptomycin (20–22mm) for bacteria and Nyastatin (26–28mm) for fungi. And in positive control test tubes, standard drug showed complete inhibition (i.e. no turbidity) against all the test organisms for MIC. Among all the three extracts, methanol extract has shown good activity against tested organisms in comparison to the standard drug. Tween 80 at 1% was used as a negative control which has shown no inhibitory effect against the tested organisms.

In the present investigation the fruit pulp extracts of *F. limonia* has shown a great potential for antimicrobial activity against the tested microorganisms. The maximum inhibition zone and MIC values, which were sensitive to the methanolic extract, were in the range of 15–21 mm and 3.125-12.5 mg/ml, respectively. On the basis of zone of inhibition and MIC values, the *Pseudomonas aeruginosa* was more sensitive to the methanolic extract than all other organisms with inhibition zone of 21 mm and MIC value of 6.25 mg/ml respectively.

Table 4: It shows antimicrobial activity of different extracts of *Feronia limonia* Linn against test micro-organisms

Test organism	Strains	Zone of inhibition			MIC		
		CE	ME	AE	CE	ME	AE
Salmonella typhimurium	MTCC 98	8	16	12	12.5	12.5	12.5
Escherichia coli	MTCC 45	12	19	16	12.5	3.125	6.25
Klebsiella pneumoniae	MTCC 432	-	15	-	-	6.25	-
Pseudomonas aeruginosa	MTCC 647	11	21	17	25	6.25	12.5
Aspergillus niger	MTCC 282	-	22	18	-	6.25	6.25
Aspergillus flavus	MTCC 277	-	19	16	-	6.25	1.25

PE- Petroleum ether extract, CE- Chloroform extract, ME- Methanolic extract, AE- Aqueous extract, Standards- Streptomycin and Nyastatin.

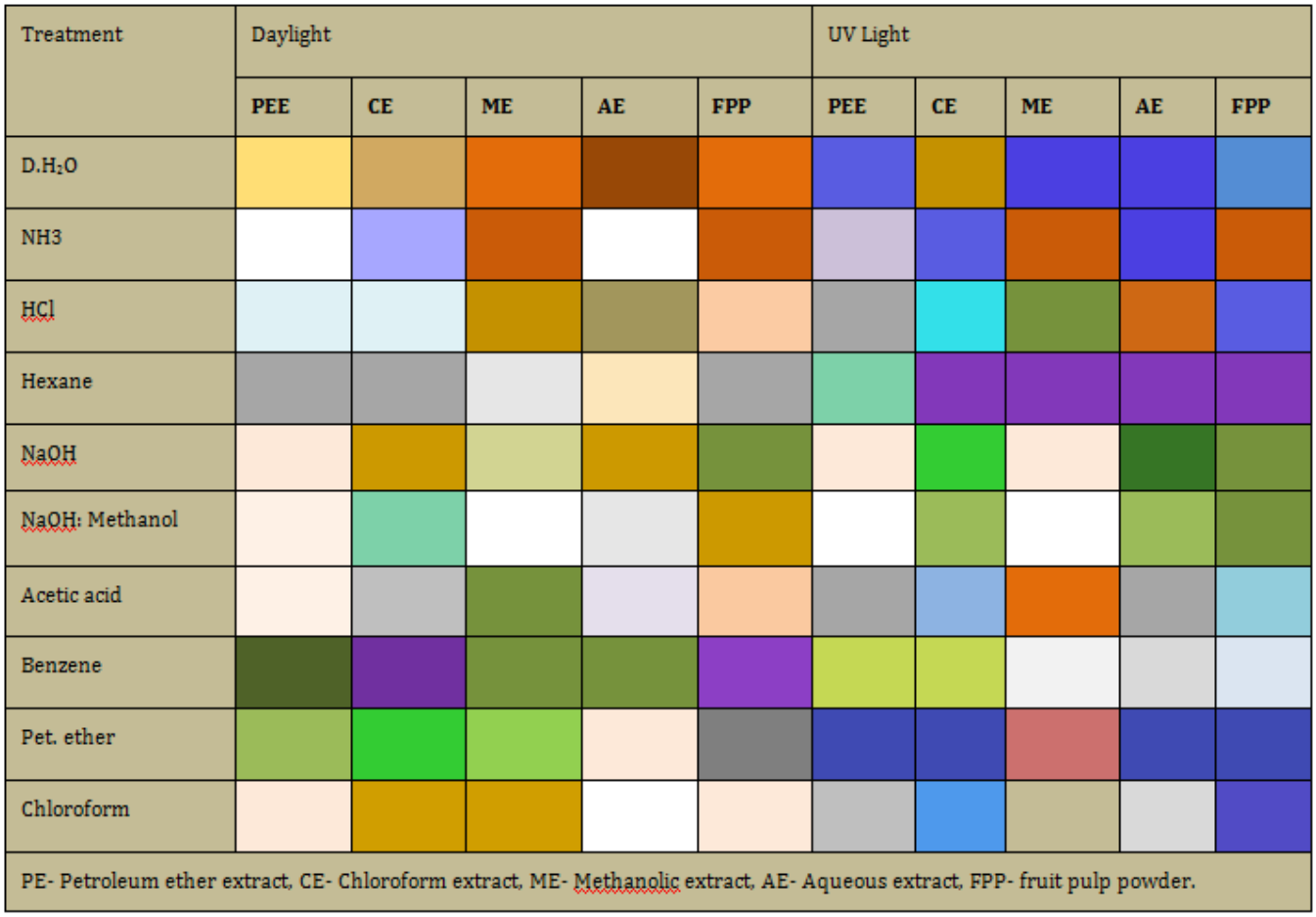

Fig. 1: It shows fluorescence chart of different extracts of *Feronia limonia* Linn fruit at daylight and UV light

DISCUSSION

The difference in the yield of extraction products may be due to the difference of polarity of solvents used for extraction, solubility of various ingredients, and type of extraction method [14]. In order to promote Indian herbal drugs, it is essential to evaluate the therapeutic potentials of the drugs as per WHO guidelines [15]. The bioactive compounds should be standardized on the basis of phytochemistry as stated by Kamboj [16]. The results of phytochemical analysis of *F. limonia* fruit pulp are summarized in (Table 2). Phytochemical compounds such as alkaloids, phenolics, flavonoids, steroids, tannins, triterpenoids, saponins, glycosides, gums & mucilage, carbohydrates, proteins & amino acids, lipids & fats are found to be present in this plant. Variation in the type of phytochemicals present in different solvents might be attributed to the ability of the solvents to dissolve specific type of phytochemicals in specific solution [17]. According to Cowan, a large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms *in vitro* [18]. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. The activity is probably due to the ability of extracellular and soluble proteins to form complex with bacterial cell walls [19].

Plant extracts are claimed to have a broad-spectrum antimicrobial activity and are considered as a main source for the search of lead compounds. The antimicrobial activity of *F. limonia* would be due to the presence of alkaloids, flavonoids and these compounds are most probably soluble in organic polar solvent. Thus, the variation in the antimicrobial activity of *F. limonia* extracts used in the present study might be attributed to the different compounds present in various solvents. Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antimicrobial activity.

However, the high genetic variability of bacteria enables them to neutralize the action of antibiotics by developing antibiotic resistance. Thus there has been a rapid search for new and potent antibiotics. In our investigation on different extracts of *F. limonia* set to be a good candidate against bacterial and fungal strains.

CONCLUSION

Based on the results obtained in this study, it reveals that the different extracts of *F. limonia* against various bacterial and fungal strains indicate that this plant is having potent antibacterial and antifungal effects. It is important to mention that the methanolic extract gave the best all-round results. Further purification and identification of bioactive components will enhance our comprehension on nature of the compounds present in the extract.

CONFLICT OF INTEREST

The authors declare that they have no competing interest.

REFERENCES

1. Khewkhom N, Greger H, Shangchote S. Antimicrobial activity of 16 plant extracts of the Rutaceae family against phytopathogenic fungi. Agricultural Sci. J 2008; 39:119-123.
2. Adikaram NKB, Abhayawardhane Y, Gunatilaka AAL, Bandara BMR, Wijeratne EMK. Antifungal activity, acid and sugar content in the wood apple (*Limonia acidissima*) and their relation to fungal development. Plant Pathology 2007; 38:258-265.

3. Gupta R, Johri S, Saxena AM. Effect of ethanolic extract of *Feronia elephantum* Correa fruits on blood glucose level in normal and streptozotocin-induced diabetic rats. Natural Product Radiance 2009; 8:32-36.
4. Mitscher LA, Drake S, Gollopudi SR, Okwute SK. A modern look at folkloric use of anti-infective agents. Journal of Natural Products 1987; 50:1025-1040.
5. Hostettmann K. Assays for Bioactivity. Methods in Plant Biochemistry. San Diego, Academic Press; 1991; 6:71-134.
6. McLaughlin JL, Rogers LL, Anderson JE. The use of biological assays to evaluate botanicals. Drug Inf. J 1998; 32:513-524.
7. Denyer SP, Hodges NA, Gorman SP. Bacterial resistance to antibiotics and Clinical uses of antimicrobial drugs. In Hugo & Russell's Pharmaceutical Microbiology. 5th ed. Blackwell Publishing; 2004; Chapt (13-14):220-250.
8. Health Protection Agency. Infection with organisms carrying extended spectrum beta-lactamase in the community 1st report. CDR Wkly 2003; 13(32):3.
9. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 34th ed. Pune: Nirali Prakashan; 2006; 246-268.
10. Harborne JB, Chapman. Phytochemical methods. In: Hall. A guide to modern techniques of plant analysis. London; 1973; p 279.
11. Indian Pharmacopoeia. Government of India, Ministry of Health and Welfare. 4th ed. New Delhi: Controller of Publications; 1996; 2:A53-54.
12. Chase CR, Pratt RJ. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J Am Pharmacol Assoc 1949; 38:324-333.
13. Sahm DF, JA Washington. Antibacterial susceptibility Test Dilutions Methods. In: Lennette EH. Manuals of Clinical Microbiology. 5th ed. Washington DC: Am. Soc. Microbiol; 1990; 1105-1116.
14. Mahour K, Mishra A, Kumar A, Vihan VS. Preliminary pharmacognostical and phytochemical investigation on *Feronia elephantum* corr. Fruit. J. Pharm Res 2008; 1:45-48.
15. World Health Organization. General guidelines for methodologies on research and evaluation of traditional medicine. Geneva 2000; 3-18.
16. Kamboj VP. Herbal medicine. Curr. Sci 2000; 78:35-39.
17. Yusha'u M, Olonitola SO, Aliyu BS. Sensitivity of Extended – spectrum β lactamases produding Enterobacteriaceae to *Acalypha maccafeana* extracts. Bayero Journal of Pure and Applied Sciences 2008; 1(1):15.
18. Cowan MM. Plant products as antimicrobial agents. Clin. Microbiol. Rev 1999; 564-582.
19. Moghadam MS, Maleki S, Darabpour E, Motamedi H, Mansour S, Nejad S. Antimicrobial activity of eight Iranian plant extracts against methicillin and cefixime restistant *Staphylococcous aureus* strains. Asian Pacific Journal Tropical Medicine 2010; 3:262-265.