International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-4, 2014 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Methanolic Fruit Pericarp Extract of *Feronia limonia* against Microorganisms Involved in Pathology of Various Diseases

Swathi P[1*], Eswar Kumar K[1], Sastry N[2]

[1]*A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India.*

[2] *Department of Biochemistry, Institute of Science, GITAM University, Visakhapatnam, India.*

Manuscript No: IJPRS/V3/I4/00427, Received On: 08/11/2014, Accepted On: 10/11/2014

ABSTRACT

To evaluate the antimicrobial activity of *Feronia limonia* against various microorganisms. The qualitative phytochemical analysis was conducted to screen the presence of active compounds like alkaloids, glycosides, saponins, tannins flavonoids, volatile oils, unsaturated sterols, free fatty acids, reducing sugars and steroids. The antimicrobial activity of methanol pericarp extract of *Feronia limonia* L. (Rutaceae) was studied by agar well diffusion method *in vitro*. Methonolic pericarp extract of *Feronia limonia* showed presence of flavonoids, volatile oils, unsaturated sterols and free fatty acids except alkaloids, glycosides, saponins, tannins, reducing sugars and steroids. The pericarp of *Feronia limonia* was extracted with methanol. The effect of antimicrobial potential was examined against *Salmonella typhi, Vibrio cholerae, Shigella dysenteriae and Enterococcus faecalis.* The methanol extract of the fruit pericarp has showed consistently significant inhibitory activity on different bacterial species tested and found the significance of antimicrobial activity of *Feronia limonia.*

KEYWORDS

Feronia Limonia, Phytochemicals, Antimicrobial Activity

INTRODUCTION

Infectious diseases are the world's leading cause of premature deaths. The mortality rate was found to be more than 50,000 people every day. The pathogenic infections due to the variety of bacterial agents such as Escherichia coli, *Salmonella typhi, Vibrio cholerae, Shigella dysenteriae and Enterococcus faecalis* and *Stapylococcus aureus.* But in recent years reported that the microorganisms have been developing resistance against available antibiotics[1,2,3]. In addition to that the drugs associated with hypersensitivity as adverse effect. There are reports on the curative potentials or abilities of medicinal plants and their products in the treatment of a wide range of infectious ailments such as urinary tract, gastrointestinal tract, respiratory tract and wound infections[4,5,6,7]. The potential value of such plant derived products prompted investigators to study new flavonoids to improve the treatment of various diseases. Due to side effects and emerging antibiotic resistance, the need for developing new anti microbial compounds. Plants are the best source for the identification of new drug compounds.

The fruit pericarp extract of *Feronia limonia* (Rutaceae) consists of 2, 6-dimethoxybenzoquinone and osthenol and Three volatile flavour components like methyl hexanoate, ethyl 3-hydroxyhexanoate, and butanoic acid. Free fatty acids like palmitic, oleic, linoleic, linolenic acid, palmitoleic and

***Address for Correspondence:**
Swathi Putta
CSIR-SRF-extended,
A. U.College of Pharmaceutical Sciences,
Andhra University, Visakhapatnam, India.
E-Mail Id: swatidbm@gmail.com

stearic acids; β-sitosterol, β-amyrin[8], unsaponifiable matter like lupeol and stigmasterol[9].

Phytochemical techniques played a significant role in searching raw materials and resources for pharmaceutical industry. Preliminary Phytochemical tests are helpful in finding and locating chemical constituents which are source of pharmacologically active principles.

MATERIALS AND METHODS

Plant Materials

The ripened wood apple *(Feronia limonia*) was obtained from local market. The peels were manually separated. Peels were shade dried. The peels were powdered in a grinder to get 40-mesh size powder. The moisture content of peel powder was found to be 10.5%. The peel powder (25 g) was extracted by soxhlet extraction using methanol.

The residue was re-constituted with the same solvent. The extracts were pooled and concentrated under vacuum at 40°C. The extract was dissolved in water and was used in the experimental studies.

Phytochemical Analysis

The extracts were analyzed by the following procedures[10]. To test for the presence of the flavonoids, volatile oils, unsaturated sterols and or/ triterpenes, alkaloids, saponins, tannins, terpenoids, glycosides and reducing sugars

Flavonoids

4ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones.

Volatile Oils

2ml of extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl. A white precipitate is formed if volatile oils are present.

Unsaturated Sterols And Or Triterpenes

For testing the presence of unsaturated sterols and triterpenes, 1g of the air-dried powder of the studied plant was extracted with few ml of ethanol then filtrated and the filtrate was evaporated till dryness. The residue was dissolved in 10 ml chloroform, filtered and the filtrate was divided into two equal portions for proceeding the following tests.

A. Libermann-Burchard Test

To the first portion of chloroform filtrate 1 ml of acetic acid anhydride was added, followed by 2 ml of sulphuric acid down the wall of the test tube. The appearance of a reddish violet colour at the junction of the two layers and a bluish green colour in the acetic acid layer indicates the presence of unsaturated sterols and or/triterpenes.

B. Salkowiski's Test

To the second portion of chloroform filtrate an equal volume of sulphuric acid was added. The appearance of a red colour indicated the presence of unsaturated sterol and /or triterpenes.

Saponins

Saponins were detected using the froth test. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5 ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Tannins

To a portion of the extract diluted with water, 3-4 drops of 10% ferric chloride solution is added. A blue color is observed for gallic tannins and green color indicates for catecholic tannins.

Reducing Sugars

To 0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and

heated over water bath. Brick red precipitate indicates the presence of reducing sugars.

Glycosides

25ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10%NaOH, then 5ml of Fehling solution added. Glycosides are indicated by a brick red precipitate.

Alkaloids

2ml of extract was measured in a test tube to which picric acid solution was added. An orange coloration indicated the presence of alkaloids.

Terpenoids

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid.

Steriods

(Liebermann Burchard reaction: 200 mg plant extract in 10 ml chloroform, filtered), 2 ml filtrate + 2 ml acetic anhydride + conc. H_2SO_4. Blue green ring indicated the presence of steroids.

Anthroquinones

Borntreger's test was used for the detection of anthroquinones. 5 g of plant extract was shaken with 10 ml of Benzene. This was filtered and 5.0 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of violet colour in the ammonical (lower) phase indicated the presence of free hydroxyl anthroquinones.

Test Microorganisms

Salmonella typhi, Vibrio cholerae, Shigella dysenteriae and Enterococcus faecalis, are clinical isolates collected from King George Hospital, Visakhapatnam, India.

Anti Microbial Assay by Agar Well Diffusion Method

The bacteria were grown in Muller-Hinton media (HiMedia Pvt. Ltd., Mumbai, India) at 37 ^{0}C and maintained on nutrient agar slants at 4 ^{0}C and stored at -20 ^{0}C. Inoculum of bacteria was prepared by growing pure isolate in nutrient broth at 37 ^{0}C for overnight. The overnight broth bacterial cultures was sub-cultured in fresh nutrient broth and grown for 3hrs to obtain log phase culture. The diluted suspension which has the absorbance of 0.600 at 450nm determined spectroscopically (Electronics India) then it was used as inoculums for fungi. The agar plates were prepared by pour plate method using 20ml of agar medium. The sterile agar medium is cooled to 45^0 C and mixed thoroughly with 1ml of growth culture of concerned test organism (1 x 10^8 cells) and then poured into the sterile petri dishes and allowed to solidify. Wells of 6 mm size were made with sterile cork borer and test extracts were added. The agar plates were incubated at for 24 hours at 37 ^{0}C for bacteria. The diameter of inhibition zones was measured in mm using HiMedia zone reader. Ciprofloxacin (Antibiotic) was used as Standard while Solvent (DMSO) used for control[11,12].

RESULTS AND DISCUSSION

Phytochemical Analysis

Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes[13]. Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal science and are under investigation for Antibacterial, Antineoplastic and other Pharmaceutical functions[14]. Where is 15 no reference Volatile oils are complex of compounds with strong odour. And known to have antiseptic, bactericidal, virucidal and fungicidal activities[15]. Phytochemical tests of the above extracts were performed through chemical reagents as described by Harbone 1998[16]. Eleven chemical groups were screened for MPFL. The detailed investigations of phytochemicals in MPFL were shown in table 1. The MPFL shown to have positive results for presence of flavonoids, volatile oils, unsaturated sterols and free fatty acids. The alkaloids, glycosides, saponins, tannins, reducing sugars

and steroids were found to be absent in methonolic pericarp extract of *Feronia limonia*.

Table 1: Phytochemical analysis of methonolic pericarp extract of *Feronia limonia*.

No	Phytochemicals	Methanolic pericarp extract of *Feronia limonia*
1	Flavonoids	+++
2	Volatile oils	+++
3	Unsaturated sterols and or/ triterpenes	+++
4	Saponins	---
5	Tannins	---
6	Reducing sugars	---
7	Glycosides	---
8	Alkaloids	---
9	Terpenoids	---
10	Steroids	---
11	Free fattyacids	+++

Antimicrobial Activity on Human Pathogens

Antimicrobial studies were carried out on human pathogenic bacteria *viz., Salmonella typhi, Vibrio cholerae, Enterococcus faecalis and Shigella dysenteriae.* The MPFL extract showed significant antibacterial activity (Table 2). The MPFL extract showed comparable antibacterial activity with Ciprofloxacin (antibiotic) (Fig.1). *S. aureus, E. faecalis* are Gram negative bacteria which were showed sensitive to extract. MPFL extract was not much effective against clinical isolates compared to non-clinical isolates.

Antimicrobial Activity of MPFL

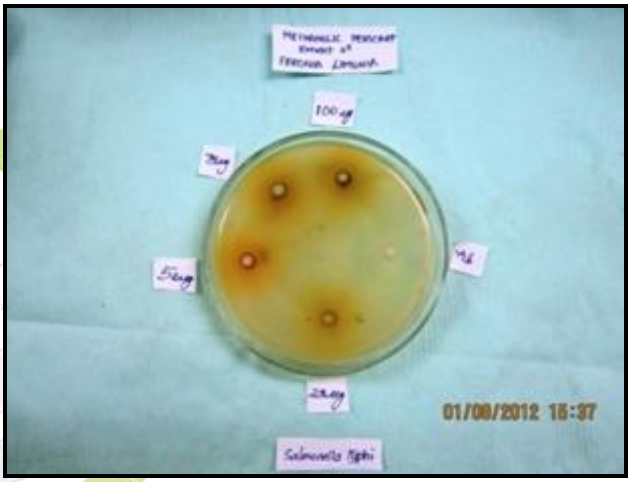

Figure 1(A): Salmonella typhi

Table 2: Anti Microbial activity of MPFL extract on human pathogens

S.No	Human pathogens	Causing Disease	Zone of inhibition of various diseases				
			25 µg	50 µg	75 µg	100 µg	Antibiotic (30 µg)
1	*Salmonella typhi* (G-ve)	Typhoid fever	5	6	7	8	10
2	*Vibrio cholera* (G-ve)	Cholera	4	6	8	10	10
3	*Shigella dysenteriae* (G-ve)	Dysentry	4	6	9	10	13
4	*Enterococcus faecalis* (G+ve)	Gastro intestinal infections	4	4	6	7	10

Figure 1(B): Vibrio cholera

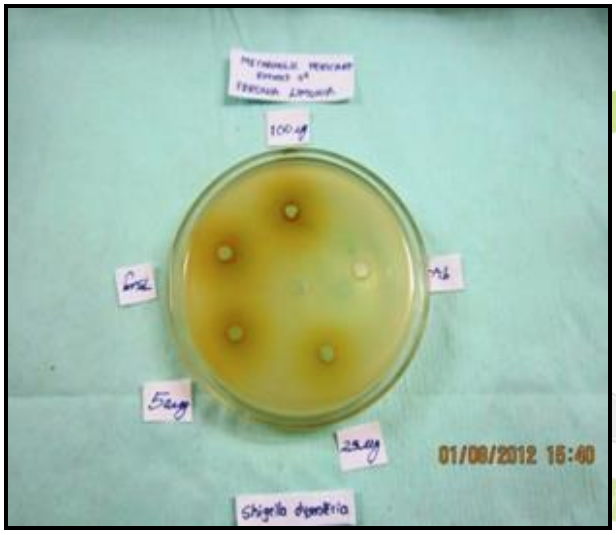

Figure 1(C): Shigella dysenteriae

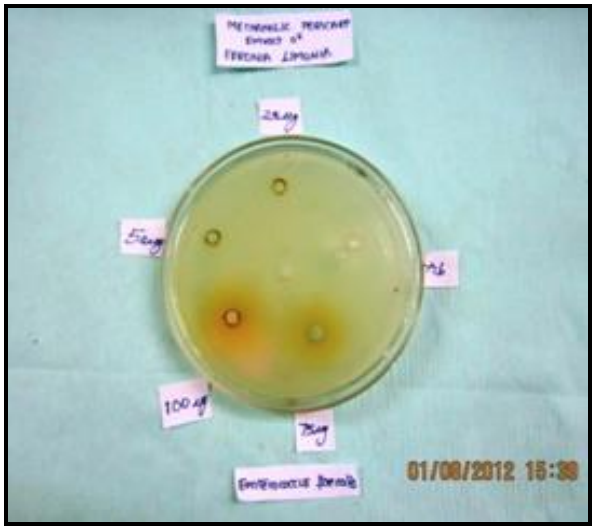

Figure 1(D): Enterococcus faecalis

CONCLUSION

In the present study was concluded that the presence of phytochemicals as flavonoids, volatile oils, unsaturated sterols and free fatty acids were present in MPFL. Antibacterial activity of MPFL was comparable with the commercially available antibiotic Ciprofloxacin.

REFERENCES

1. Piddock, K. J. V., Wise R. (1989). Mechanisms of resistance to quinolones and clinical perspective. *Journal of Antimicrobial Chemotheraphy*, 23,475-483.
2. Singh, M., Chowdary, M. A., Yadava, J. N. S., Sanyl, S. C. (1992). The spectrum of antibiotic resistance in human veterinary isolates of Escherichia Coli collected from 1984-1986 in Northern India. *Journal of Antimicrobial Chemotheraphy*, 29,159-68.
3. Mulligen, M. E., Murry-Leisure, K. A., Ribner, B. S., Standiford, H. C. (1993). Methicillin resistance staphylococcus aureus. *American Journal of Medicine*, 94, 313-328.
4. Navarro, V., Villarreal, M. L., Rojas, G., Lozoya, X. (1996). Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. *Journal of Ethnopharmacology*, 53 (3), 143-147.
5. Eswar Kumar, K., Swathi, P., Nagendra sastry, Y., Kaladhar, D. S. V. G. K., Govinda Rao, D. (2013). In-vitro antimicrobial and antioxidant activities of aqueous pericarp extract of Punica granatum. *Journal of Applied Pharmaceutical Science*, 3(08), 107-112.
6. M07-A9., (2012). Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard – Ninth Edition, 32(2), 12-20.
7. Govinda Rao, D., Nagendra Sastry, Y., Kaladhar, D. S. V. G. K., Kamalakara Rao K and Krishna Chaitanya, K. (2011). Antibacterial Activity of Methanolic Seed Coat Extract of Borassus Flabellifer L.

International Journal of Pharmaceutical Sciences and Research, 2(9), 2435-2438.

8. Saima, Y., Das, A. K., Sarkar, K. K., SenSr, A. K., Sur, P. (2000). An antitumor pectic polysaccharide from Feronia limonia. *International Journal of Biological Macromolecules.* 27(5), 333-335
9. MacLeoad Alexander, J., Pieris, N. M. (1981). Volatile flavour components of wood apple (Feronia limonia) and a processed product. *Journal of Agricultural and Food Chemistry*, 29, 49-53.
10. Talukdar, A. D., Choudhary, M. D., Chakraborty, M., & Dutta, B. K. (2010). Phytochemical screening and TLC profiling of plant extracts Cyathea gigantea (Wall. Ex. Hook.) Haltt. and Cyathea brunoniana. Wall.ex.Hook. (Cl. & Bak.). Assam University Journal of Science & Technology: *Biological and Environmental Sciences*, 5(1), 70-74
11. Govinda, R. D. (2011). Antibacterial activity of Methanolic Seed Coat Extract of Borassus flabellifer L. *International Journal of Pharmaceutical Sciences and Research*, 2(9), 2435-2438.
12. Govinda Rao, D. Y., Nagendra Sastry, D. S. V. G. K., Kaladhar. (2011). Preliminary Studies on In Vitro Antimicrobial Activity and Phytochemical Analysis of Tender Seed Coat Aqueous Crude Extract of Borassus Flabellifer Linn. *Asian Journal of Biochemical and Pharmaceutical Research*, 3, 517-523.
13. Korkina, L. G., & Afanasev, I. B. (1997). Antioxidant and chelating properties of flavonoids. *Advances in Pharmacology*. 38, 151-63.
14. Yamunadevi, M., Wesely, E. G., & Johnson, M. (2011). Phytochemical studies on the terpenoids of medicinally important plant Aerva lanata L. using HPTLC. *Asian Pacific Journal of Tropical Biomedicine*, 220-225.
15. Bakkali, F., Averback, S., & Idaomar, M. (2008). Biological effects of essential oils. A review. *Food and chemical toxicology*, 446-475.
16. Harborne, J. B. (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. (3rd edition). Chapman and Hall Co., New York, 1-302.

