Int J Pharm Sci Bio 2010;1(2):96-99

RESEARCH ARTICLE

ISSN 2229-3604

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOTECHNOLOGY

Available Online at http://www.ijpsb.com

ANTIBACTERIAL POTENCY OF FRUIT EXTRACTS OF *FLACOURTIA INERMIS* AGAINST MULTIDRUG RESISTANT STRAINS AND COMPARISON OF ITS ACTIVITY WITH THAT OF STANDARD ANTIBIOTICS

Shibumon George*, Benny PJ

Department of Zoology, St. Thomas College Palai, Kottayam, Kerala, India

ABSTRACT

Background and Purpose of the Study: In the current scenario of drug resistance, new, effective and non resistant drugs are necessary against microbial attack. In recent years, great attention is given to antimicrobial screening studies for identifying a plant with potential antimicrobial activity. In the present study, different extracts of fruits of *Flacourtia inermis* Roxb. were used for studying their antibacterial property against multidrug resistant bacterial strains. **Methods:** The antibacterial activity and antibiotic sensitivity comparison of fruit extracts were carried out by disc diffusion method. **Results:** The study revealed that the acetonic extract of *Flacourtia inermis* is very powerful to inhibit the growth of tested strains. Among the sensitive strains, *Serratia marcescens* showed highest susceptibility followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. Antibiotic sensitivity study revealed that the tested bacteria were completely sensitive to the acetonic extract of *Flacourtia inermis* even though they were multidrug resistant. **Conclusion:** The acetonic extract of *Flacourtia inermis* possesses a broad spectrum of antibacterial agent active against multidrug resistant bacterial strains.

Key Words: *Flacourtia inermis*, Loika, Lavalolikka, antibacterial, antimicrobial, multidrug resistant bacteria, antibiotic comparison, fruit extract.

INTRODUCTION

Simultaneous with population explosion, virulent strains of microorganisms become more common and their increased attack accounts for increased mortality in different parts of the world [1]. India, being a country with high density of population, infectious diseases becomes a great challenge in the health and economic sector. The other developing countries are also facing the same problem.

To prevent infectious diseases, large number of antibiotics of different chemical nature has been developed in the last few decades. Most of them are synthetic drugs. Now days, a good number of antibiotics are found to be ineffective due to a natural phenomenon called drug resistance by microorganisms. Inappropriate and injudicious uses of antibiotics or self-treatment practices are some of the major reasons for rapid and widespread drug resistance [2,3,4]. In recent years, hospital environments become a major site for the emergence of multiple drug resistant strains that make the situation more dangerous [5]. More over, theses synthetic antibiotics have many side effects including organ-damaging effects, histotoxicity, drug-to-drug interaction problems and so on.

In India, for centuries, the traditional systems of medicines like Ayurveda, uses plant materials, mainly in crude forms, to prevent various infectious diseases [6,7]. The problem of drug resistance is not yet been observed by any of the traditional practices. The main reason is that plant contains several biologically active chemical constituents called secondary metabolites; most of which are natural agents against microbial attack. Added advantages of plant-based drugs are, they are non-toxic, have no side effects and are having multiple health protecting effects [8].

In recent years, many of the research activities in India and around the world are in search for developing plant-based medicines [9]. This is because, natural products may serve as direct source of drug or as a starting material for synthesis of different kinds of drugs or as a prototype for developing new drugs [10,11].

India is a country with large genetic diversity of medicinal plants. Traditional medicine and modern medicine have already identified the antimicrobial and other medicinal values of most of these plants [12,13,14]. However, huge number of medicinal plants is yet remains unidentified. As a first step,

Corresponding Author: Dr. Benny PJ
Department of zoology, St.Thomas College, Palai Arunapuram, P.O., Kottayam District, Kerala State, India. Pin-686 574.
E mail: shibugeorgepala@gmail.com

screening of plants for their antimicrobial properties is helpful for identifying such potent plants. In the present study, *Flacourtia inermis* Roxb, a tree whose medicinal significances are still unknown, was taken for investigating its antibacterial property against multidrug resistant bacterial strains.

Flacourtia inermis Roxb. is belonging to Flacourtiaceae family. It is very common in village areas of Kerala State in India, where its fruits are commonly known as Loika or Lavalolikka. For centuries, people of Kerala use its fruits either fresh or cooked. A report showed that its fruit contains tremendous antioxidant properties [15]. However, its other medicinal and biological properties are still uncertain. Therefore, the antibacterial properties of its crude extracts are studied here.

MATERIALS AND METHODS

Preparation of the crude extract

Fresh fruits of *Flacourtia inermis* were collected from the villages of Kerala state. Fruits were brought to the lab in fresh condition and washed, first with tap water and then with saline water followed by distilled water, wiped dry and cut into several small pieces, dried in Hot air oven at 60 °C and was powdered in a mixer grinder.

250 grams of the dried powder was serially extracted with 500 ml each of petroleum ether, acetone, methanol and water using a Soxhlet extractor. The extract was concentrated by vacuum evaporation using a rotary evaporator and the concentrate was stocked in a refrigerator at 4 °C and used for antibacterial assay. From the stock, aliquot was taken and redissolved in the respective solvent to get a final concentration equivalent to 20 mg of the dried fruit per disc.

Bacterial strains

Standard MTCC strains purchased from Institute of Microbial Technology, Chandigarh (IMTECH), India, were used for the study. They were
Staphylococcus aureus, sub species *aureus* (MTCC 96)
Escherichia coli (MTCC 443)
Pseudomonas aeruginosa (MTCC 741)
Serratia marcescens (MTCC 97)
Klebsiella pneumoniae, sub species *pneumoniae* (MTCC 109)

Antibiotic discs

Standard antibiotic discs purchased from Himedia Laboratories Pvt. Ltd, Mumbai, India were used for antibiotic sensitivity comparison. Following antibiotics were used for the study.

1. Chloramphenicol (30 mcg)
2. Streptomycin (10 mcg)
3. Erythromycin (15 mcg)
4. Gentamicin (10 mcg)
5. Penicillin G (10 u)
6. Ciprofloxacin (5 mcg)
7. Tetracycline (30 mcg)
8. Cloxacillin (1 mcg)
9. Cefotaxime (30 mcg)
10. Amikacin (30 mcg)

Culture media

The dehydrated Muller Hinton Agar (MHA) medium purchased from Hi-media Laboratories Pvt. Ltd. Mumbai, India was used. The medium was rehydrated, sterilized in an autoclave and was poured into sterilized petri dishes and allowed to set. The plates were stored at 4 - 10 °C in refrigerator. Before inoculation, the surface of the petriplates was dried in an incubator.

Antibacterial assay by Disc Diffusion Method

The antibacterial activity and antibiotic sensitivity were tested by Disc Diffusion Method [16]. The dried plates were inoculated by test strains uniformly over the surface using a sterile cotton swab. A sterile 6 mm Whatmann No.1 filter paper loaded with appropriate extract was placed on the surface of the inoculums and gently pressed by a sterile forceps. Control discs made up of each solvent like petroleum ether, acetone, methanol and water were also placed. The plates were incubated at 37 °C for 16 to 20 hrs. The zone of inhibition of bacterial growth around the disc was measured in millimeters. The experiment was repeated for three times and the average values were recorded.

Antibiotic sensitivity was compared with acetonic extract only because all other extracts were found to be insignificant towards the tested strains. Two acetonic extract discs, designated by letter ‘A’ and ‘B’ were loaded in the plates along with antibiotic discs, to confirm the activity. Antibiotic sensitivity was recorded as ‘S’ if sensitive or as ‘R’ if resistant.

RESULTS

The results of the activity of crude extracts of *Flacourtia inermis* fruit against standard strains is given in Table 1.

Control experiment did not show inhibitory zone. It revealed that the solvent alone is ineffective to produce antibacterial activity. However, the crude fruit extracts of *Flacourtia inermis* in acetone and methanol were active against the tested strains. Petroleum ether extracts and aqueous extracts are in ef-

fective with an exception of aqueous extract against *Escherichia coli*, where it showed a little activity, which is insignificant in antibacterial study.

Among the active extracts, methanolic extract showed only limited activity where as acetonic extract showed highest activity. On considering susceptibility, *Serratia marcescens* showed highest susceptibility followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. The results suggest that the acetonic extract of the dried fruit of *Flacourtia inermis* is an effective antibacterial agent. Therefore, the activity of acetonic extract was compared with standard antibiotics for assessing its antibacterial potency.

Table 1. Activity of crude extracts of *Flacourtia inermis* fruit against multidrug resistant strains

Bacterial Strain	Zone of inhibition of Crude Extracts in mm			
	Petroleum Ether Extract	Acetonic Extract	Methanolic Extract	Aqueous Extract
Staphylococcus aureus	0	20	8	0
Pseudomonas aeruginosa	0	19	10	0
Escherichia coli	0	17	9	0
Serratia marcescens	0	22	10	0
Klebsiella pneumoniae	0	18	9	0

Comparison of the activity of acetonic extract with standard antibiotics

The activity of acetonic extract was compared with commercially available, standard antibiotic discs by disc diffusion method. Results are given in Table 2.

Ten widely used antibiotic discs were included in the sensitivity test. It was found that each tested strains showed resistance against three or more tested antibiotics. Hence, they are multidrug resistant strains. However, none of the strains showed resistance to the acetonic extract. Among the strains, *Pseudomonas aeruginosa* was the one showing highest resistance. *Klebsiella pneumoniae* exhibited resistance against four antibiotics while all others showed resistance to three antibiotics.

The three antibiotics, Erythromycin, Penicillin G, and Cloxacillin were completely resisted by the tested organisms. Yet an interesting point is that these antibiotics are commonly prescribed in present day antimicrobial therapy. Therefore, instead of these 'outdated antibiotics' the active component of the fruit of *Flacourtia inermis*, can be considered as an effective alternative. More over, analysis of the result indicates that the acetonic fruit extract of *Flacourtia inermis* possess a broad spectrum of antibiotic activity as shown by Streptomycin, Gentamicin, Ciprofloxacin and Amikacin.

Table 2. Sensitivity comparison of crude acetonic extract and antibiotic discs against multidrug resistant strains

S No	Strains	Antibiotic disc										Total no of Resistant Bacteria
		1*	2*	3*	4*	5*	6*	7*	8*	9*	10*	
1	*Staphylococcus aureus*	S†	S	R‡	S	R	S	S	R	S	S	3
2	*Pseudomonas aeruginosa*	S	R	R	S	R	S	R	R	R	S	6
3	*Escherichia coli*	S	S	R	S	R	S	S	R	S	S	3
4	*Serratia marcescens*	S	S	R	S	R	S	S	R	S	S	3
5	*Klebsiella pneumoniae*	S	S	R	S	R	S	S	R	R	S	4

S† : Sensitivity, R‡: Resistance, 1*: Chloramphenicol , 2*: Streptomycin, 3*: Erythromycin, 4*:Gentamicin, 5*: Penicillin-G, 6*: Ciprofloxacin, 7*:Tetracycline, 8*:Cloxacillin, 9*: Cefotaxime, and 10*: Amikacin

DISCUSSION

In India, two *Flacourtia* species; *Flacourtia indica* (Burm.F.) Merr. and *Flacourtia jangomas* Raeusch. are extensively used in folk medicine. The roots of *Flacourtia indica* are used in treatment of diseases related with 'Vata and Pitta'. They are refrigerant and diuretic. Roots are also used for treating some skin disorders and rheumatism [17,18]. Leaves are used in the treatment of pruritus and scabies. Fruits are considered as an effective remedy for jaundice, gastropathy, splenomegaly and cholera [19,20].

Report suggests that *Flacourtia indica* possesses analgesic and anti-inflammatory activities [21].

Dried leaves of *Flacourtia jangomas* are widely used for treating bronchitis. Roots are impor-

tant remedy for relieving tooth ache. Fruits are used in treatment of liver related disorders [22].

Previous literatures are not available on the antimicrobial role of *Flacourtia inermis*. For centuries, the fruit of *Flacourtia inermis* has been used by the people of Kerala in fresh or cooked form. No toxic or side effect has been reported on it. Therefore, it can be assumed that its active antibacterial component is non toxic to man. Isolation and characterization of its active principle is necessary to suggest this compound for large scale preparation of new antibacterial drug. Recognition of the medicinal significance of *Flacourtia inermis* will be a turning point for increased cultivation and propagation of it in Kerala, which not only improves the economy of the country, but also means a new antibiotic for the whole world.

CONCLUSION

Screening of fruit extracts of *Flacourtia inermis* against multidrug resistant strains showed that the acetonic extract is the one showing highest antibacterial activity. All the tested microorganisms were perfectly sensitive to it, even though they were resistant to most of the commonly used antibiotics. The activity of fruit extract is similar to that of sensitive broad spectrum of antibiotics. In India, due to the lack of scientific knowledge, the fruit of *Flacourtia inermis* has not yet been used in traditional medicine. Based on the present study, its fruit extract in crude form can be considered as an effective antibacterial agent in folk medicine. After isolation of its active principle, the compound can be used as a prototype for developing a new broad-spectrum antibiotic.

REFERENCES

1. Manisha V, Neha S, Satish S. Antimicrobial Activity of Stem Bark Extracts of Nyctanthes arbortristis Linn. (Oleaceae). Int J Pharm Phytochem Res 2009;1(1):12-14.
2. Alanis A J. Resistance to Antibiotics: Are We in the Post-Antibiotic Era? Arch Med Res 2005;36:697–05.
3. Zakaria ZA, Zaiton H, Henie EFP , Mat Jais AM, Engku Zainuddin ENH. In vitro Antibacterial Activity of Averrhoa bilimbi L. Leaves and Fruits Extracts. Int J Tropic Med 2007; 2(3):96-00.
4. Rath SK, Mohapatra N, Dubey D, Panda SK, Thatoi H N, Dutta SK. Antimicrobial Activity of Diospyros melanoxylon Bark from Similipal Biosphere Reserve, Orissa, India. African J Biotech 2009;8(9):1924-28.
5. Quadri LEN. Strategic Paradigm Shifts in the Antimicrobial Drug Discovery Process of the 21st Century. Infectious Disorders - Drug Targets 2007;7:230-37.
6. Amrita V, Sonal D, Shalini R. Antibacterial Effect of Herbs and Spices Eextract on Escherichia coli. Electronic J Biol 2009;5(2):40-44.
7. Khond M, Bhosale JD, Arif T, Mandal TK, Padhi MM, Dabur R. Screening of Some Selected Medicinal Plants Extracts for In-vitro Antimicrobial Activity. Middle East J Scientific Res 2009;4(4):271-78.
8. Parekh J, Jadeja D, Chanda S. Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. Turk J Biol 2005;29:203-10.
9. Parekh J, Chanda S. In vitro Antibacterial Activity of the Crude Methanol Extract of Woodfordia fruticosa Kurz. Flower (Lythraceae). Braz J Microbiol. 2007;38:204-07.
10. Clark AM. Natural Products as a Resource for New Drugs. Pharm Res 1996;13(8):1133-41
11. Newman DJ, Cragg GM, Snader KM. Natural Products as Sources of New Drugs over the Period 1981-2002. J Nat Prod 2003;66:1022-37.
12. Sharma A, Verma R, Ramteke P. Antibacterial Activity of Some Medicinal Plants Used by Tribals against UTI Causing Pathogens. World Appl Scien J 2009;7(3):332-39.
13. Harishkumar K, Hullatti K K, Sharanappa P, Sharma P (2010). Comparative Antimicrobial Activity and TLC Bio-autographic Analysis of Root and Aerial Parts of Andrographis serpyllifolia. Int J Pharmacy & Pharm Sci 2010;2(1):52-54.
14. Preethi R, Devanathan VV, Loganathan M. Antimicrobial and Antioxidant Efficacy of Some Medicinal Plants Against Food Borne Pathogens. Advances in Biological Research 2010;4(2):122-25.
15. Lakdusinghe M, Jayasinghe ULB. Antioxidant Phenolic Constituents from the Fruit Juice of Flacourtia inermis. Sri Lanka Association for the Advancement of Science Proceedings of the 63rd Annual Sessions, Part I – Abstracts 2007;129-30.
16. Kirby MDK, Bauer AW, Sherris JC, Turck M. Antibiotic Susceptibility Testing by Standardized Single Disc Diffusion Method. Am J Clin Pathol 1996;45:493-96.
17. Chatterjee TK. Herbal Options. Books and Allied (P) Ltd. Calcutta, India. 2000:203-206.
18. Prajapati N D, Kumar U. Agro's Dictionary of Medicinal Plants. Agrobios (India). 2003:136-137.
19. Nadkarni AK. Indian Material Medica. Popular Book Depot, Bombay. 1976;Vol.1. 555-556.
20. Chopra RN, Nayar SI. Glossary of Indian Medicinal Plants, 3rd Edtion. New Delhi; Council of Scientific and Industrial Research, 2004:94-95.
21. Gurav S, Gulkari V, Duragkar N, Sakharwade S, Itankar P, Patil A. Analgesic and Anti-inflammatory Activity of Flacourtia ramontchi L. Herit. Pharmacology online 2007;2:20-31.
22. Srivastava D, Prabhuji SK, Rao GP. Taxonomic and Ethno-biological Status of Flacourtia jangomas (Lour.) Raeus. : An Endemic Nutraceutical Plant of Eastern U.P. Medicinal Plants 2009;1(1): 49-53.

Acknowledgments

Authors are thankful to Dr.N.Sasidharan, Scientist, Kerala Forest Research Institute, Peechi, Trissure and Dr.Jomy Augustine, Head of the Department of Botany, St.Thomas College, Palai, for their valuable help given for identifying the taxonomic position of *Flacourtia inermis*. Authors are also grateful to Mr. Joy Nellikkunnel, Manager, S.B.T., Kakkoor, Ernakulam District, Kerala, for going through the manuscript.