EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 5
1063-1068
Year: 2018

A COMPARATIVE ANALYSIS OF ANTIBACTERIAL POTENTIAL OF DIFFERENT LEAF EXTRACTS OF INDIAN SACRED TREES

Amudha Selvi Muniyan[1] and Ananthan Anuswedha*[2]

[1]Post Graduate Department of Applied Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai, Tamil Nadu, India.
[2]Assistant Professor, Post Graduate Department of Applied Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai, Tamil Nadu, India.

***Corresponding Author: Ananthan Anuswedha**
Assistant Professor, Post Graduate Department of Applied Microbiology, Justice Basheer Ahmed Sayeed College for Women, Chennai, Tamil Nadu, India.

Article Received on 20/03/2018 **Article Revised on 10/04/2018** **Article Accepted on 01/05/2018**

ABSTRACT
Medicinal plants are precious and very important sources of natural products for the health of human beings. Natural products such as plant extracts either as pure compounds or as standardised extracts provide unlimited opportunities for new drug discoveries. In the present study, an attempt has been made to analyse and compare the antibacterial efficacy of solvent leaf crude extracts of Indian sacred trees viz., *Aegle marmelous Linn. Correa*., *Feronia elephantum Linn., Ficus bengalensis Linn., Ficus religiosa Linn., and Mimusops elengi Linn.,*. These leaf extracts were screened using agar disc diffusion method and the extracts which showed the highest activity were analysed using microbroth dilution assay to determine minimum inhibitory concentration. Leaf extracts of *Ficus bengalensis Linn.* showed good antibacterial activity against most of the strains tested at a concentration of 100mg/ml. Therefore, these medicinal trees should be conserved and explored for drug development properties.

KEYWORDS: medicinal plants, antibacterial, drug discovery.

INTRODUCTION
Natural products play an imperative role in the field of Pharmaceutical biology. Plants have been an important source of medicine for thousands of years. Currently, the World Health Organization estimates that up to 80 percent of people still rely mainly on traditional medicines. The fact that many of the current drugs either mimic naturally occurring molecules or have structures that are fully or in part derived from natural motifs holds true.[1]

In recent years, multiple drug/ chemical resistance in both human and plant pathogenic microorganisms has been developed due to indiscriminate use of synthetic drugs. This drives the need to screen medicinal plants for novel bioactive compounds as they are biodegradable, safe and have fewer side effects.[2]

Aegle marmelos (Linn.) Corr., belongs to the family Rutaceae, and is popularly known as Bael tree. The Bael tree is native to India and is distributed throughout South East Asia. In Hindu mythology, leaves and wood of *Aegle marmelos L.* are used to worship Lord Shiva and is one of the most sacred Indian tree. The root, leaf, fruit and bark have been shown to possess medicinal properties.[3] *Mimusops elengi Linn.* is considered as a sacred plant among Hindus and has obtained important place in religious texts as well as in ancient Sanskrit literature. The tree is native to India and the bark, leaves, flower, fruit, root and seeds are medicinally important. *M. elengi* is well documented for several medicinal properties like antinociceptive, diuretic effects, gastroprotective, antibacterial, antifungal, anticariogenic, free radical scavenging, antihyperglycemic etc.[4] *Feronia elephantum(Linn.,) Correa* commonly called as Wood apple is a tropical medicinal trees native to India and Sri Lanka. The fruit stem bark and leaf is known to possess ethnomedicinal properties. It is distributed in deciduous and arid landscapes of several countries in South Asia. The tree as a whole, or its parts such as unriped fruit, riped fruit, root, bark, trunk gum and leaves have a broad spectrum of traditionally established therapeutic properties including antimicrobial and antidiarrhoeal effects.[5] *Ficus religiosa Linn.* is a large perennial tree, found throughout the plains of India within 170m altitude in the Himalayas, largely planted as an avenue and roadside tree especially near temples. It is a popular bodhi tree and has got mythological, religious, and medicinal importance in Indian culture since times immemorial. The plants have been used in traditional Indian medicine for various ailments. Traditionally the bark is used as an antibacterial, antiprotozoal, antiviral,

astringent, antidiarrhoeal, in the treatment of gonorrhea, ulcers, and the leaves used for skin diseases.[6] *Ficus benghalensis Linn*. commonly known as banyan fig, is a sacred tree native to the Indian subcontinent. The tree is considered sacred in India and temples are often built beneath. The fruit, leaves, bark and latex obtained from this tree is widely used for its medicinal properties.[7]

MATERIALS AND METHODS

i) Collection and preparation of plant powder[8]

The medicinal trees included in the study were *Aegle marmelous* (vilvam), *Feronia elephantum* (wood apple), *Ficus benghalensis* (banyan), *Ficus religiosa* (peepal) and *Mimusops elengi* (Magilam).The leaves of the sacred trees were collected from different temples in and around Chennai. The leaves were separated from the twigs and washed twice with double distilled water and then surface sterilised using 70% ethanol. The leaves were shade dried for 1-2 weeks. The leaves were then ground into a coarse powder form using a mixer.

ii) Preparation of crude extracts[9]

The crude extracts were prepared by hot and cold method of extraction. In the hot method of extraction, about 1 gram of the powdered plant material was mixed with 10 ml of the solvent, incubated in a shaker at 37ºC for 4 hours at 250 rpm after which it was placed in a water bath at 60ºC for 2 hours. The supernatant was filtered and dried in air at room temperature. For the cold method of extraction, about 1 gram of the powdered plant material was mixed with 10 ml of the solvent, incubated in a shaker at 37ºC for 4 hours at 250 rpm. The supernatant filtered and then dried in air at room temperature. The solvents used were methanol, chloroform, ether and acetone. The residue obtained after drying was dissolved in the appropriate solvent and used for antibacterial activity evaluation.

iii) Microbial cultures used

The test organisms used for screening were *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Klebisella pneumoniae, Salmonella paratyphi A, Salmonella typhi, Salmonella typhimurium, Shigella dysenteriae, Shigella flexneri, Pseudomonas aeruginosa, Proteus mirabilis and Vibrio cholerae.* All the strains were laboratory isolates of the Department of Microbiology, J.B.A.S College for Women, Chennai.

iv) Preliminary Screening using Disc Diffusion Assay[10],[11]

Discs(6mm) prepared from Whatmann No.1 filter paper was sterilised and impregnated with 20µl of various crude solvent extracts (concentration:100mg/ml). Broth cultures of the bacteria were prepared in nutrient broth. The culture was checked for turbidity by comparing with McFarland Standard (0.5).A lawn culture of the organisms to be tested was made on Mueller Hinton agar media. The prepared discs were placed on the plate so that each disc was at least 20mm from one another. The plates were then incubated at 37°C for 24 hours. The inhibition zone around each disc both in the experiment and the control were measured. Standard antibiotics as per the organisms tested were included as positive control and respective solvents without the plant extracts were used as the negative control.

v) Determination of Minimum Inhibition Concentration/Minimum Bactericidal Concentration[12]

The Microbroth dilution was performed on a microtitre plate. Doubling dilutions of the crude extract were prepared in Mueller Hinton broth. Bacterial cultures of 10^6 cfu/ml dilution were prepared with McFarland standard (0.5) and 10µl were added to each well of the microtitre plate and mixed well. The microtitre plates were incubated at 37ºC overnight and a loopful of the culture was streaked on to Nutrient agar plates. The plates were incubated at the same temperature and time as before. The growth/no growth pattern of the organisms corresponded to the MIC /MBC of the crude extract.

RESULTS

Table- i: Agar Disc Diffusion Assay.

SAMPLE TESTED: Crude extract of the five medicinal plants,
SOLVENT EMPLOYED: methanol

Organisms tested	Aegle marmelous		Feronia elephantum		Ficus benghalensis		Ficus religiosa		Mimusops elengi	
	H (mm)	C (mm)	H (mm)	C (mm)	H (mm)	C (mm)	H (mm)	C (mm)	H (mm)	C (mm)
1.Staphloccus aureus	12	9	15	14	7	9	12	12	8	12
2.Bacillus cereus	8	15	12	12	9	12	16	14	18	12
3.Escherichia coli	11	8	9	9	8	9	19	8	12	9
4.Klebsiella pneumonia	11	15	13	12	11	15	15	13	11	12
5.Salmonella paratyphi A	12	9	14	11	11	9	16	14	14	9
6.Salmonella typhi	11	9	12	11	12	11	14	13	11	12

7.Salmonella typhimurium	13	9	12	10	12	13	12	11	13	9
8.Shigella dysenteriae	11	9	11	9	14	11	12	11	10	11
9.Shigella flexneri	13	12	14	12	12	11	13	13	12	9
10.Proteus mirabilis	8	11	9	9	9	9	11	11	12	12
11.Pseudomonas aeruginosa-1	13	13	7	9	9	9	21	9	14	11
12.Pseudomonas aeruginosa-2	10	10	14	11	9	14	11	9	19	11
13.Vibrio Cholerae	11	9	14	11	9	9	11	9	11	8

H-hot extract; C-cold extract

Table-ii: Agar disc diffusion assay.

SAMPLE TESTED: Crude extract of the five medicinal plants
SOLVENT EMPLOYED: acetone

Organisms tested	**Aegle marmelous**		**Feronia elephantum**		**Ficus benghalensis**		**Ficus religiosa**		**Mimusops elengi**	
	H (mm)	**C (mm)**	**H (mm)**	**C (mm)**	**H (mm)**	**C (mm)**	**H (mm)**	**C (mm)**	**H (mm)**	**C (mm)**
1.Staphloccus aureus	15	9	14	7	11	12	17	9	12	16
2.Bacillus cereus	12	15	19	15	16	16	16	15	21	12
3.Escherichia coli	12	19	18	15	15	16	12	20	19	23
4.Klebsiella pneumonia	12	14	19	20	12	19	19	16	14	12
5.Salmonella paratyphi A	13	13	16	15	16	17	9	16	12	12
6.Salmonella typhi	15	15	15	12	14	15	13	14	15	15
7.Salmonella typhimurium	15	14	16	16	19	22	10	18	20	14
8.Shigella dysenteriae	18	14	15	14	15	19	14	15	13	14
9.Shigella flexneri	21	19	13	15	18	18	14	14	15	14
10.Proteus mirabilis	12	14	11	11	15	14	16	17	15	12
11.Pseudomonas aeruginosa-1	14	14	20	18	19	19	17	14	19	21
12.Pseudomonas aeruginosa-2	15	18	16	17	22	16	14	11	16	18
13.Vibrio Cholerae	14	11	14	17	14	15	12	14	22	15

Table-Iii: Agar Disc Diffusion Assay.

SAMPLE TESTED: Crude extract of the five medicinal plants
SOLVENT EMPLOYED: chloroform

Organisms tested	***Aegle marmelous***		***Feronia elephantum***		***Ficus benghalensis***		***Ficus religiosa***		***Mimusops elengi***	
	H (mm)	**C (mm)**	**H (mm)**	**C (mm)**	**H (mm)**	**C (mm)**	**H (mm)**	**C (mm)**	**H (mm)**	**C (mm)**
1.Staphloccus aureus	10	11	12	9	9	9	10	12	14	9
2.Bacillus cereus	8	9	10	12	8	11	9	14	8	11
3.Escherichia coli	11	11	9	13	13	12	10	9	9	11
4.Klebsiella pneumonia	11	14	12	11	9	9	12	12	8	16
5.Salmonella paratyphi A	9	9	12	13	12	9	12	11	11	11
6.Salmonella typhi	10	9	13	13	19	9	18	11	11	11
7.Salmonella typhimurium	9	11	18	12	13	11	13	11	12	11
8.Shigella dysenteriae	10	11	11	11	11	11	10	9	11	9
9.Shigella flexneri	9	12	16	16	11	22	13	14	12	11
10.Proteus mirabilis	11	11	11	11	9	9	11	12	11	13
11.Pseudomonas aeruginosa-1	12	13	12	7	12	9	12	20	9	11
12.Pseudomonas aeruginosa-2	9	12	16	8	12	12	11	11	11	11
13.Vibrio Cholerae	9	10	14	10	11	14	9	11	11	8

Table- Iv: Agar Disc Diffusion Assay.

SAMPLE TESTED: Crude extract of the five medicinal plants
SOLVENT EMPLOYED: Ether

Organisms tested	Aegle marmelous		Feronia elephantum		Ficus benghalensis		Ficus religiosa		Mimusops elengi	
	H (mm)	C (mm)	H (mm)	C (mm)	H (mm)	C (mm)	H (mm)	C (mm)	H (mm)	C (mm)
1.Staphloccus aureus	7	11	8	8	7	7	9	9	8	8
2.Bacillus cereus	9	7	11	10	8	11	10	12	8	10
3.Escherichia coli	12	11	12	12	8	13	12	10	9	9
4.Klebsiella pneumonia	11	12	12	10	11	13	12	10	9	9
5.Salmonella paratyphi A	13	10	12	9	9	9	15	12	11	9
6.Salmonella typhi	15	14	16	14	9	14	14	15	16	11
7.Salmonella typhimurium	13	11	11	12	11	9	13	9	12	10
8.Shigella dysenteriae	11	11	12	11	11	9	11	14	11	9
9.Shigella flexneri	17	12	15	15	9	14	17	11	16	14
10.Proteus mirabilis	9	10	12	9	8	8	12	11	13	12
11.Pseudomonas aeruginosa-1	8	11	14	12	12	9	13	9	8	11
12.Pseudomonas aeruginosa-2	9	11	10	9	11	9	11	10	11	12
13.Vibrio Cholerae	14	14	11	12	9	11	10	14	13	11

Table V: Determination of Minimum Inhibition Concentration.

The crude extracts, which showed a zone diameter of 15mm or more, were chosen for the MIC assay.

Organisms tested	Aegle marmelous	Feronia elephantum	Ficus benghalensis	Ficus religiosa	Mimusops elengi
	MIC	MIC	MIC	MIC	MIC
1.Staphloccus aureus				6.25(HA)	
2.Bacillus cereus		12.5(HA)	3.125(HA) 3.125 (CA)	12.5(HA)	3.125(HA)
3.Escherichia coli	3.125(CA)	3.125(HA)	6.25(CA)	6.25(CA)	3.125(CA)
4.Klebsiella pneumonia	-	12.5(HA) 12.5(CA)	6.25(CA)	6.25(HA) 3.125(CA)	12.5(CE)
5.Salmonella paratyphi A	6.25(HE) 6.25(CE)	12.5(HE)	3.125(HE)	3.125(HE)	12.5(HE)
6.Salmonella typhi		3.125(HE)	6.25(HC)	6.25(HC)	12.5(HE)
7.Salmonella typhimurium			6.25(HA) 3.125(CA)	6.25(CA)	
8.Shigella dysenteriae	12.5(HA)			6.25(HA)	
9.Shigella flexneri	12.5(HA) 3.125(CA)		3.125(HA) 3.125(CA)	6.25(HA)	
10.Proteus mirabilis		-		12.5(CA) 3.125(HA)	
11.Pseudomonas aeruginosa-1	-	-	-	-	-
12.Pseudomonas aeruginosa-2	-	-	-	-	-
13.Vibrio Cholerae		6.25(CA)			3.`125(HA)

HA- hot acetone extract; CA-cold acetone extract; CE- cold ether extract; HE-hot ether extract.

DISCUSSION

Antibiotic drug resistance is a serious public health issue especially in a developing country such as India because of easy availability and higher consumption of inappropriate antibiotics. The infectious disease burden is highest in India amongst all the countries of the world and recent report underlines the irrational use of antimicrobial agents against these diseases. Antimicrobial resistance will result in difficulty in

controlling the diseases in the community and ineffective delivery of the health care services.[13]

Meta analyses of the drug susceptibility results of various laboratories in India reveal an increasing trend of development of resistance to commonly used antimicrobials in pathogens like *Salmonella, Shigella, Vibrio cholerae, Staphylococcus aureus, Neisseria gonorrhoeae, N. meningitidis, Klebsiella, Mycobacterium tuberculosis*, HIV, plasmodium and others.[14,15]

Medicinal plants have been used from time immemorial to treat infectious diseases. Bacteria may find it difficult to develop drug resistance to plant metabolites as these are natural. Synergistic combinations of medicinal plant components with known antibiotics may slow down the development of drug resistance in multidrug resistant bacterial pathogens.

The present study revealed the potent antibacterial potentials of five Indian sacred trees against *Staphylococcus aureus* and other enteropathogens that are the major causative agents of diarrhoea and dysentery, which is a major problem that is highly prevailing in developing and underdeveloped countries especially in infants and children. Many plants that are conveniently available in India have been reported by early investigators to be effective against diarrhoea and dysentery as they are used by local people as traditional folklore medicine. These trees have been given special attention.[16]

In the preliminary screening using agar disc diffusion assay, *Ficus benghalensis, Ficus religiosa* showed good antibacterial activity against a wide spectrum of the pathogens tested, followed by *Feronia elephantum, Mimusops elengi and Aegle marmelous* showed antibacterial activity only against bacterial pathogens. The concentration of the crude extract was 100mg/ml.

In the microbroth dilution assay to determine the minimum inhibitory concentration of the crude extract *Ficus benghalensis*, showed an MIC value of 3.125mg/ml against most of the pathogens tested, *Ficus religiosa, Aegle marmelous and Feronia elephantum*, showed an MIC value of 6.25 mg/ml and *Mimusops elengi* showed MIC value of 12.5mg/ml against most of the pathogens tested.

Among the solvents used, acetone crude extracts showed maximum antibacterial activity against most of the pathogens tested followed by ether, chloroform and methanol. In the two extraction procedures used, the hot method was most effective in extracting the antibacterial components when compared to the cold method.

CONCLUSION

The present study confirms the presence of antibacterial components from the crude extracts of the Indian sacred trees which can be harnessed for further drug discovery and development especially in the current scenario of alarming antibiotic resistant infections. The significance of these trees should be taken into account for their conservation. Sacred groves consisting of many such trees should be protected and not destroyed to ensure maximum utilisation of nature's resources to the utmost potential.

REFERENCES

1. Geneva. World Health Organization. The world health report, 1996.
2. Prusti A, Mishra SR, Sahoo S and Mishra SK. Antibacterial Activity of Some Indian Medicinal Plants. Ethnobotanical Leaflets, 2008; 12: 227-230.
3. Reena Nair and Swati Barche.Medicinal value of Bael, Aegle marmelos. International journal of farm sciences, 2016; 307-320.
4. Mohammad Abdul Motalib Momin, Mizanur Rahaman Khan, Johir Rayhan, Afrina Afrose, Sohel Rana, Anjuman Ara Begum. Evaluation of Antibacterial and Antidiarrhoeal Activities of Feronia limonia Leaf Extract. American Journal of Plant Sciences, 2013; 4: 2181-2185.
5. Bharat Gami, Smita Pathak, Minoo Parabia. Ethnobotanical, phytochemical and pharmacological review of Mimusops elengi Linn. Asian Pacific Journal of Tropical Biomedicine, 2012; 2(9): 743-748.
6. OP Ogunlowo, BD Arimah, MA Adebayo. Phytochemical analysis and comparison of in-vitro antimicrobial activities of the leaf, stem bark and root bark of Ficus benghalensis. IOSR Journal of Pharmacy, 2013; 3(4): 33-38.
7. S.B. Chandrasekar, M. Bhanumathy, AT Pawar, T Somasundaram. Phytopharmacology of Ficus religiosa. Pharmacogn Rev., Jul-Dec, 2010; 4(8): 195–199.
8. Nayan R. Bhalodia and V. J. Shukla. Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant. J. Adv. Pharm. Technol. Res, 2011; 2(2): 104-109.
9. J.N. Eloff. Which extractant should be used for the screening and isolation of antimicrobial components from plants?. Journal of Ethanopharmacology, 1998; 60(1): 1-8.
10. Navarro V, Villarreal M.L, Rojas G and Lozoya X. Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. Journal of Ethanopharmacology, 1996; 53: 143-147.
11. Bauer A.W, Kirby W.M.M, Sherris J.C and Turck M. Antibiotic suspectibility testing by a standardised single disk method. American Journal of Clinical Pathology, 1966; 45: 493-6.
12. Nostro, A., M.P. Germano, V. D'Angelo, A. Marino and M.A. Cannatelli. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Applied Microbial, 2000; 30: 379-384.

13. World Health Organization. Prevention and Containment of Antimicrobial resistance. [Last accessed on 2012 Mar 18]. Available from: http://www.ino.searo.who.int/LinkFiles/Other_Content_WHD11-Seminar_Presentation-WRpdf.
14. World Health Organization. Prevention and containment of antimicrobial resistance. Report of a regional meeting Chiang Mai, Thailand, 8th to 11th of June 2010. [Last accessed on 2011 Apr 12]. Available from:http://www.searo.who.int/LinkFiles/BCT_Reports_SEA-HLM-408.pdf.
15. Ganguly NK, Arora NK, Chandy SJ, Fairoze MN, Gill JP, Gupta U, et al. GARP-India working group. Rationalizing antibiotic use to limit antibiotic resistance in India. Indian J Med Res., 2011; 134: 281–94.
16. Satyavati GV and Gupta AK. Medicinal plants of India, Volume 2, Indian Council of Medical Research, New Delhi, 1987.