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EVALUATION OF ANTBACTERIAL ACTIVITY OF ETHANOL AND AQUEOUS LEAF EXTRACTS OF INDIAN SACRED TREES

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ABSTRACT

In different parts of the world, especially in India, people practice different forms of nature worship. One of the most significant reasons is to save the patches of vegetation in the name of deities. Such vegetation which is conserved for spiritual reasons is called as sacred groves. Sacred groves harbor rich biodiversity including rare, threatened and endangered plants and trees which have potent medicinal properties. Infectious diseases are the second leading cause of death worldwide. Diseases are caused by microbial pathogens, more commonly by bacteria. Due to indiscriminate use of antibiotics, these bacteria have become multi-drug resistant to most of the commonly used antibiotics, complicating the treatment of infectious diseases. Medicinal plants provide a vast resource for investigation of antimicrobial agents. In the present study, an attempt has been made to analyse and compare the antibacterial efficacy of two 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.*, The extracts were screened by agar disc diffusion method; the extract which showed the highest activity were analysed and the minimum inhibitory concentration was determined. Leaf extracts of *Aegle marmelos Linn. Correa* showed good antibacterial activity against most of the strains tested at a concentration of 100mg/ml. Therefore, these medicinal trees should be conserved as sacred grooves and explored for drug development properties.

KEYWORDS: sacred groves, medicinal plants, antibacterial.

INTRODUCTION

Sacred groves or forests are conserved in many parts of the world by religious communities. More recently when the plant species are getting extinct at a faster rate due to forest destruction all over the world, sacred groves are serving as a safe abode for a large number of rare, endemic and threatened plant and animal species and are playing an important role in providing essential environmental and economic benefits to the local communities.[1]

Infectious diseases are the leading cause of mortality and morbidity in the developing and underdeveloped countries. Infections caused by a variety of bacterial pathogens such as *Staphylococcus aureus, E.coli, Klebsiella pneumoniae, Aeromonas spp., Vibrio cholerae, Psuedomonas spp., Salmonella spp., and Shigella spp.,* have become more common. In addition to increased resistance, antibiotics produce adverse effects on the host such as hypersensitivity, immunosuppression, allergic manifestations and destruction of beneficial gut flora which worsens the patients' situation further. [2]

Therefore, there is a need for exploring the vast resources of a particular place or region for discovery of novel drug candidates for alternative treatment of infectious diseases. In the present study, the medicinal trees of sacred groves have been analysed for its antibacterial ability to be further utilized as a potential antibacterial agent. The selected medicinal trees were *Aegle marmelous Linn. Correa*. (thala viritchum of Thirumallpur temple, Kanchipuram), *Ficus religiosa Linn.* (thala viritchum of Thiruyrailaichur temple,Thindivanam –Pondicherry route), *Ficus benghalensis Linn.* (thala viritchum of Chidambaram temple), *Mimusops elengi Linn.*(thala viritchum of Thiruvanamalai and Thiruvatriyur temple) and *Feronia elephantum Linn.*(thala viritchum of Thiruvainthur temple, Myladuthorai).The medicinal trees were selected based on the ethnopharmacological data related to the antiinfectious properties of the medicinal trees.[3,4,5,6,7]

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 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 water and ethanol. 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 the McFarland Standard (0.5).A lawn culture of the organisms to be tested was made on the Mueller Hinton agar media. The prepared discs were placed on the plate in a way such 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.

vi) Characterization of the Plant Extracts by Thin Layer Chromatography.[13]

Thin layer Chromatography is described as a method for Chromatographic analysis on thin layers adsorbents. The basic operations, which are involved in TLC, are preparation of chromatoplates, application of samples to chromatoplates, selection of solvents for proper developing system and visualization of the separated compounds. Glass plates of 20 cm x 10 cm size with uniform thickness were taken. The plates were arranged on a plastic board. Silica gel (G) was used as the adsorbent (G indicates that it has a binder, calcium sulfate, "Gypsum"). 50 g of silica gel G was taken in a beaker and 100 ml of double distilled water was added to it and shaken vigorously for one and half minute. The slurry was transferred into movable through speaker, which has got several specifications with the help of which the thickness of layers is varied. The thickness used in our experiment was 0.25mm. The trough spreader was slowly and evenly pushed across the top surfaces of the plates, which were uniformly coated with the silica gel. The coated plates were left to dry at room temperature for about ½ hour. Activation of the plates was necessary to make the plates free of water. This was done by keeping the plates in hot air oven at 100-105 degree C for about hour after which the plates were used for loading the samples. A small amount of the ethanolic extract of the five plants was taken in a capillary tube and spotted about 1 cm away from the bottom edge of the plate with the aim to form the smallest possible spot. Care was taken that the solvent did not touch the spots. The solvent (moving phase) gradually raised over the spotted TLC plate and sufficient time was allowed for the solvent to reach the top portion of the plate, just 1 cm below the endpoint. The point where the solvent reached at the end was marked. The air-dried plate was studied for the different separated constituents. After drying the TLC plates were sprayed with concentrated sulfuric acid and water (1:1), which showed

different colored spots. By subsequent heating of the plates at 110 degree C, one compound after another becomes visible on the plate.

RESULTS

TABLE- I: AGAR DISC DIFFUSION ASSAY

SAMPLE TESTED: Crude extract of the five medicinal plants

SOLVENT EMPLOYED: Ethanol

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	19	21	19	16	14	16	18	22	14	18
2.Bacillus cereus	13	16	13	19	19	18	12	18	19	19
3.Escherichia coli	19	19	12	18	13	16	21	15	19	20
4.Klebsiella pneumoniae	14	18	14	16	20	18	18	19	12	18
5.Salmonella paratyphi A	17	16	21	14	21	16	21	16	22	18
6.Salmonella typhi	19	17	22	20	21	20	22	19	20	20
7.Salmonella typhimurium	22	17	23	17	21	19	22	21	22	16
8.Shigella dysenteriae	18	16	16	15	17	19	16	22	15	18
9.Shigella flexneri	22	22	21	21	22	22	22	20	24	22
10.Proteus mirabilis	19	17	19	19	17	19	19	16	19	15
11.Pseudomonas aeruginosa-1	23	19	19	19	21	19	17	18	22	19
12.Pseudomonas aeruginosa-2	22	18	21	19	22	17	21	16	22	21
13.Vibrio Cholerae	23	17	19	17	17	18	19	18	22	22

H – Hot method: C- Cold method; mm-millimeter

TABLE II: AGAR DISC DIFFUSION ASSAY

SAMPLE TESTED: Crude extract of the five medicinal trees

SOLVENT EMPLOYED: Water

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

H – Hot method: C- Cold method; mm-millimeter

TABLE III: 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	12.5 (CE)	12.5(HE)	12.5(CE)	6.25(CE) 25(HE)	12.5(CE)

2.Bacillus cereus	12.5(CE)	12.5(CE)	3.125(CE)	12.5(CE)	12.5(HE)
3.Escherichia coli	6.25(HE)	3.125(CE)	6.25(CE)	6.25(CE)	3.125(HE)
4.Klebsiella pneumoniae	12.5(CE)	12.5(CE)	6.25(HE)	6.25(CE)	6.25(CE)
5.Salmonella paratyphi A	6.25(HE) 6.25(CE)	12.5(HE)	12.5(HE)	3.125(HE)	6.25(HE)
6.Salmonella typhi	12.5(CE) 6.25(HE)	3.125(HE)	6.25(HE)	6.25(HE)	12.5(HE)
7.Salmonella typhimurium	6.25(HE)	12.5(HE)	6.25(HE)	6.25(HE)	6.25(HE) (CE)
8.Shigella dysenteriae	12.5(HE)	6.25 (HE)	6.25(CE) 12.5(HE)	6.25(CE)	12.5(CE)
9.Shigella flexneri	12.5(HE)	3.125(HE)	6.25(HE)	6.25(CE) (HE)	6.25(HE)
10.Proteus mirabilis	6.25(CE)	-	6.25(CE)	12.5(CE)	12.5(CE)
11.Pseudomonas aeruginosa-1	-	-	-	-	-
12.Pseudomonas aeruginosa-2	-	-	-	-	-
13.Vibrio Cholerae	12.5(HE) 6.25(CE)	6.25(HE)	6.25(CE)	6.25(HE)	6.25(HE)

HE-Hot Ethanolic Extract ; CE-Cold Ethanolic Extract

TABLE IV: CHARACTERISATION OF THE LEAF EXTRACT BY ANALYTICAL THIN LAYER CHROMATOGRAPHY

5 crude extracts showing maximum activity were selected to be analysed by Thin Layer Chromatography;

The resolution factor (R_f) value was determined by the formula

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

EXTRACTS USED FOR ANALYSIS	**R_{f1}**	**R_{f2}**
1. Cold ethanolic extract of *Mimusops elengi*	$R_{f\,1} = 10.4 / 18 = 0.5777$	$R_{f2} = 15.4 / 18 = 0.8555$
2. Cold ethanolic extract of *Aegle marmelous*	$R_{f\,1} = 10.2 / 18 = 0.5666$	$R_{f2} = 15.8 / 18 = 0.8444$
3. Hot ethanolic extract of *Ficus benghalensis*	$R_{f\,1} = 10.2 / 18 = 0.5666$	$R_{f2} = 16 / 18 = 0.8888$

4. Hot ethanolic extract of *Ficus religiosa*	$R_{f\,1} = 10 / 18 = 0.5555$	$R_{f2} = 16.1 / 18 = 0.8944$
5. Cold ethanolic extract of *Feronia elephantum*	$R_{f\,1} = 9.8 / 18 = 0.5444$	$R_{f2} = 16.2 / 18 = 0.3444$

DISCUSSION

There is a great concern about the worldwide increase in antibiotic resistance, especially of methicillin resistant *Staphylococcus aureus*(MRSA) and Gram negative bacteria such as *Salmonella, Shigella* and diarrhoeagenic *Escherichia coli* and other enteropathogens.

As these pathogens are multi-drug resistant to many antibiotics such as ceftazidime and gentamicin, the infections caused by them not only require expensive antibiotic treatment but also increases the morbidity and mortality in hospitalized patients. [14]

In order to control these infections, there is a need for the development of alternative therapeutic compounds with marked antibacterial activity, greater sensitivity, less toxicity and less expensive.[15]

The present study revealed the potent antibacterial potentials of five Indian sacred trees against Staphylococcus aureus and enteropathogens that are the major causative agents of diarrhea and dysentery, a 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 diarrhea 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, *Aegle marmelous* showed good antibacterial activity against a wide spectrum of the pathogens tested, followed by *Feronia elephantum, Ficus benghalensis, Mimusops elengi and Ficus religiosa* 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 *Aegle marmelous, Ficus religiosa and Ficus benghalensis*, showed an MIC value of 3.125mg/ml against most of the pathogens tested, while *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.

In the two methods involved in the leaf extraction procedures, the cold method was more effective than the hot method as most of the antibacterial activity was shown by the crude extracts obtained by the cold method of extraction than the hot method. Among the solvents used in the extraction procedures, ethanol was found to be highly effective in extracting the antibacterial components, followed by aqueous extracts which were also effective in extraction of a few components.

Among the pathogens tested, *Staphylococcus aureus, Shigella spp., Salmonella spp., E.coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Vibrio cholerae* were the most susceptible organisms towards all the extracts tested. *Proteus mirabilis* was the less susceptible organisms towards the extracts tested.

The present study has confirmed the potential ability of leaf extracts of Indian sacred trees to be used as an effective candidate for antibacterial studies for the treatment of nosocomial, skin, urinary tract and enteric infections caused by multi-drug resistant bacterial pathogens. The study also necessitates the conservation of our valuable resources for human welfare.

REFERENCES

1. M. Amirthalingam, Sacred Groves of Tamil Nadu – A Survey, CPR Environmental Education Centre, Chennai, India,1998: 191.
2. Parmar Namita and Rawat Mukesh. Medicinal plants used as antimicrobial agents: A review. International Research Journal of Pharmacy, 2012; 3(1): 31-40.
3. Amit Pandey and Rashmi Mishra. Antibacterial properties of *Aegle marmelos* leaves, fruits and peels against various pathogens. Journal Of Pharmaceutical And Biomedical Sciences , 2011; 13(13): 1-6.
4. Milimita Padhi and Sujata Mahapatra.Evaluation of Antibacterial Potential of Leaf extracts of *Mimusops elengi*.International Research Journal of Biological Sciences , 2013; 2(7): 46-49.
5. Rajiv P and Rajeshwari Sivaraj. Screening For Phytochemicals And Antimicrobial Activity Of Aqueous Extract of *Ficus Religiosa Linn.*.International Journal Of Pharmacy And Pharmaceutical Sciences, 2012; 4(5): 207-209.
6. N.Nithya and U. Saraswathi. In vitro antioxidant and antibacterial efficacy of *Feronia elephantum Correa* fruit. Indian Journal of Natural Products and Resources, 2010; 1(3): 301-305.

7. Saradha Jyothi Koona and B. Subba Rao. In vitro evaluation of antibacterial activity of crude extracts of *Ficus benghalensis Linn.,* the banyan tree leaves. Indian Journal of Natural Products and Resources, 2012; 3(2): 281-284.
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. 13.V.K.Srivastava and K.Kishore. Introduction to chromatography – theory and practice.4th Ed.,New Delhi;S.Chand and Co.Ltd: 1991.
14. CW.Hoge, JM. Gambel, A.Srijan. Trends in antibiotic resistance among diarrhoeal pathogens isolated in Thailand over 15 years. Clin. Infect. Dis., 1998; 26: 341-345.
15. Marjorie Murphy Cowan. Plant products as antimicrobial agents. Clinical Microbiology Reviews, 1999; 12(4): 564-582.
16. Satyavati GV and Gupta AK. Medicinal plants of India, Volume 2,Indian Council of Medical Research, New Delhi:1987.