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Evaluation of the Antimicrobial Activity of *Eugenia singampattiana* Bedd. Endangered Medicinal Plant leaves extract

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Abstract : The antimicrobial activity of *Eugenia singampattiana* was evaluated on bacteria and fungal strains like *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Bacillus subtilis*, *Staphyllococcus aureus, Aspergillus flavas* (moult), *Aspergillus niger* (moult), *Penicillium notatum* (moult) and *Candida albicans.* Aqueous and methanol were used for the extraction. The antimicrobial activity was performed by agar disc diffusion method. The antibacterial effect of methanol extract showed great activity against *Salmonella typhi* (24 mm) and moderated activity were reported against *Pseudomonas aeruginosa* (20 mm), *Escherichia coli* (19 mm) and *Bacillus subtilis* (19 mm). The same extract showed least activity against *Staphyllococcus aureus* (17 mm) followed by *Klebsiella pneumonia* (14 mm). The aqueous extract exhibited significant activity against *Escherichia coli* (21 mm), *Pseudomonas aeruginosa* (20 mm), *Staphyllococcus aureus* (20 mm) followed by *Salmonella typhi* (19 mm) and *Bacillus subtilis* (15 mm). The remaining bacterial pathogen found to be least activity. Antifungal activity of *Eugenia singampattiana* methanol leaf extract showed great activity against *Candida albicans* (37 mm) and moderated activity were reported against *Penicillium notatum* (20 mm). The same extract showed least activity against and *Aspergillus flavas* (17 mm) followed by *Aspergillus niger* (15 mm). The aqueous extract exhibited significant activity against *Candida albicans* (35 mm) followed by *Penicillium notatum* (19 mm) and *Aspergillus flavas* (16 mm). The remaining fungal pathogen found to be least activity. Results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) showed that *Staphylococcus aureus* had the highest MIC (20 mg/ml) and MBC (20 mg/ml), while the lowest MIC of 8 mg/ml was shown by *Bacillus subtilis*. *Salmonella typhi* had MIC and MBC values of 10 and 15 mg/ml respectively. Further studies are needed to evaluate active compounds and probable medicinal benefits in chemotherapy among humans.

Hence, the *Eugenia singampattiana* plant can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals for therapeutic needs.

Keywords: pathogenic microorganisms, antimicrobial activity, disk diffusion method, *Eugenia singampattiana,* natural products.

INTRODUCTION

Eugenia singampattiana Bedd. (Myrtaceae) is a small tree found in evergreen forests, locally known as 'Korandi' and "Jungle Guava" by Kanni tribes in Tirunelveli district, Tamil Nadu is one of the endemic, threatened and aromatic tree species of the southern Western Ghats in Peninsular India with medicinal value[1]. The species is categorized as Endangered or Possibly Extinct by the Botanical Survey of India. They were used as edible in ripe fruits.

A large portion of the world's population depends on the traditional system of medicine for a variety of diseases [2]. Indian flora and fauna consists of

more than 2200 species of medicinal and aromatic plants. The revival of interest in natural drugs started in last decade mainly because of the wide spread belief that green medicine is healthier than synthetic products[3].

In the Indian system of medicine, several genera are used medicinally mainly as herbal preparations in the indigenous system of medicine and are sources of very potent and powerful drugs. According to WHO, 80% of the world's population is dependent on the traditional medicine and a major part of the traditional therapies involves the use of plant extracts or their active constituents. With the continuous use of antibiotics, microorganisms have become resistant. This has created immense clinical problem in the treatment of infections diseases. Therefore there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. One approach is to screen local medicinal plants for possible antibacterial and antifungal properties.

There is no work done antimicrobial activity of whole plant. Therefore, the present study has investigated antimicrobial activity of *Eugenia singampattiana* leaf extracts against several pathogenic microorganisms.

MATERIALS AND METHODS

Collection and Identification of Plant

Fresh disease free leaves of *Eugenia singampattiana* Bedd has been collected in the Western Ghats area, Tirunelveli district, Tamil Nadu. With the help of local flora the plant material were identified. The air dried leaf samples were powdered and stored in screw cap bottles at room temperature for further analysis.

Preparation of plant extracts

The powdered plant material was extracted in soxhlet apparatus successively with methanol and aqueous respectively due to their nature of polarity. After extraction, the extracts were filtered through Whatman No.1 filter paper and then concentrated in a vacuum at 40°C using a rotary evaporator. Each extract was transferred to glass vials and kept at 4°C before use.

Test organisms used

Six bacterial strains were used namely *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Bacillus subtilis* and *Staphyllococcus aureus*. Four Fungal Strains were used namely *Aspergillus flavas* (moult), *Aspergillus niger* (moult), *Penicillium notatum* (moult) and *Candida albicans*.

Disc diffusion method

Antimicrobial activity of the leaf extracts was tested using the disc diffusion method[4]. Sterile nutrient agar plates were prepared for bacterial strains and Sterile Sabouraud's dextrose agar (SDA) were prepared for fungal strains inoculated by a spread plate method under aseptic conditions. The filter paper disc of 5 mm diameter (Whatman's No. 1 filter paper) was prepared and sterilized. The leaf extracts to be tested were prepared various concentrations of 25 µl, 50 µl, 75 µl, and 100µl and were added to each disc of holding capacity 10 microlitres. The sterile impregnated disc with plant extracts were placed on the agar surface with framed forceps and gently pressed down to ensure complete contact 2/4/2011of the disc with the agar surface. Filter paper discs soaked in solvent were used for negative controls. The bacterial plates were incubated at 37°C for 24 hours and fungal plates were incubated at 37°C for 72 h. After incubation, the size (diameter) of the inhibition zones was measured.

Determination of MIC and MBC

The minimum inhibitory concentration (MIC) of the extracts was estimated for each of the test organisms in triplicates. To 0.5ml of varying concentrations of the extracts (20.0, 18.0, 15.0, 10.0, 8.0, 5.0, 1.0 0.5, 0.05 and 0.005mg/ml), 2ml of nutrient broth was added and then a loopful of the test organism previously diluted to 0.5 McFarland turbidity standard (0.5 McFarland standard is prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate, with 9.95 mL of 1% sulfuric acid) for (bacterial isolates) and 106 cfu /ml (for fungal isolates) was introduced to the tubes. The procedure was repeated on the test organisms using the standard antibiotics (ciprofloxacin and cotrimoxazole for bacteria and nystatin and amphoteracin B for fungal isolates). A tube containing nutrient broth only was seeded with the test organisms as described above to serve as control. Tubes containing bacterial cultures were then incubated at 37°C for 24 h while tubes containing fungal spore cultures were incubated for 48 h at room temperature (30 – 32°C). After incubation the tubes were then examined for microbial growth by observing for turbidity.

To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient agar (for bacteria) and saboraud dextrose agar (for fungi) by streaking. Nutrient agar and saboraud agar only were streaked with the test organisms respectively to serve as control. Plates inoculated with bacteria were then incubated at 37°C for 24 hours while those inoculated with fungi were incubated at room temperature (30 –

32°C) for 48 h. After incubation the concentration at which no visible growth was seen was noted as the minimum bactericidal concentration.

RESULTS

The increase of antibiotic resistance of microorganism to conventional drugs has necessitated the search for new efficient and cost effective ways for the control of infectious diseases, the result of different studies provide evidence that some medicinal plants might indeed be potential source of new antibacterial agents [5]. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents, the first step towards this goal is in vitro antibacterial activity [6]. The extracts of higher plant can be very good source of antibiotics against various bacterial pathogen[7]. Plant based antimicrobial compounds have enormous therapeutics potential as they can serve the purpose without any side effects that are often associated with synthetic antibacterial compounds.

In the present study, the antimicrobial activities were performed with methanol and aqueous extracts of the leaf of *Eugenia singampattiana*. The study was made against six pathogenic bacteria and four fungal strains using the standard disc diffusion method. Antimicrobial activity of the extracts of *Eugenia singampattiana* was first time investigated against *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Bacillus subtilis*, *Staphyllococcus aureus, Aspergillus flavas* (moult), *Aspergillus niger* (moult), *Penicillium notatum* (moult) and *Candida albicans.*

All the extracts were inhibited growth of almost all the selected bacteria in the range of 7-24 mm and selected fungi in the range 0f 7-37 mm. Among them methanol extract showed great activity against *Salmonella typhi* (24 mm) and moderated activity were reported against *Pseudomonas aeruginosa* (20 mm), *Escherichia coli* (19 mm) and *Bacillus subtilis* (19 mm). The same extract showed least activity against *Staphyllococcus aureus* (17 mm) followed by *Klebsiella pneumonia* (14 mm). The aqueous extract exhibited significant activity against *Escherichia coli* (21 mm), *Pseudomonas aeruginosa* (20 mm), *Staphyllococcus aureus* (20 mm) followed by *Salmonella typhi* (19 mm) and *Bacillus subtilis* (15 mm). The remaining bacterial pathogen found to be least activity.

Antifungal activity of *Eugenia singampattiana* methanol leaf extract showed great activity against *Candida albicans* (37 mm) and moderated activity were reported against *Penicillium notatum* (20 mm). The same extract showed least activity against and *Aspergillus flavas* (17 mm) followed by *Aspergillus niger* (15 mm). The aqueous extract exhibited significant activity against *Candida albicans* (35 mm) followed by *Penicillium notatum* (19 mm) and *Aspergillus flavas* (16 mm). The remaining fungal pathogen found to be least activity.

Results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are shown in Table 3. The result showed that *Staphylococcus aureus* had the highest MIC (20 mg/ml) and MBC (20 mg/ml), while the lowest MIC of 8 mg/ml was shown by *Bacillus subtilis*. *Salmonella typhi* had MIC and MBC values of 10 and 15 mg/ml respectively.

Table - 1. Antibacterial activity of *Eugenia singampattiana* leaves using Disc diffusion method

Extracts	**Study of indicator Test bacteria**	**Antibacterial Activity of plant Extracts (µl)***				
		Control	25	50	75	100
Methanol extracts	*Escherichia coli*	5	8	14	16	19
	Salmonella typhi	5	12	17	20	24
	Klebsiella pneumoniae	5	7	9	12	14
	Pseudomonas aeruginosa	5	9	13	17	20
	Bacillus subtilis	5	8	13	16	19
	Staphyllococcus aureus	5	7	10	13	17
Aqueous extracts						
	Escherichia coli	5	10	13	16	21
	Salmonella typhi	5	7	15	16	19
	Klebsiella pneumoniae	5	7	10	12	13
	Pseudomonas aeruginosa	5	8	14	16	20
	Bacillus subtilis	5	6	7	7	15
	Staphyllococcus aureus	7	12	15	17	20

* Each experiment was repeated thrice

Table - 2. Antibacterial activity of *Eugenia singampattiana* leaves using Disc diffusion method

Extracts	Study of indicator Test fungi	Antibacterial Activity of plant Extracts (µl)*				
		Control	25	50	75	100
Methanol extracts	*Aspergillus flavas* (moult)	5	8	13	15	17
	Aspergillus niger (moult)	5	7	11	13	15
	Penicillium notatum (moult)	5	12	14	16	20
	Candida albicans	5	11	16	25	37
Aqueous extracts						
	Aspergillus flavas (moult)	5	7	11	13	16
	Aspergillus niger (moult)	5	7	8	10	13
	Penicillium notatum (moult)	5	9	12	16	19
	Candida albicans	5	15	19	26	35

* Each experiment was repeated thrice

Table - 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Methanol extracts of *Eugenia singampattiana*

	Organism	**Leaf extracts**	
	MIC (mg/ml)	**MIC (mg/ml)**	**MBC (mg/ml)**
Escherichia coli	15.5	15	18
Salmonella typhi	10	10	15
Klebsiella pneumoniae	10	10	15
Pseudomonas aeruginosa	14	14	20
Bacillus subtilis	8	8	18
Staphyllococcus aureus	8	20	20
Aspergillus flavas (moult)	-	-	-
Aspergillus niger (moult)	-	-	-
Penicillium notatum (moult)	-	-	-
Candida albicans	-	-	-

DISCUSSION

The use of medicinal plants play a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms [8]. The results of present investigation showed broad spectrum antibacterial, anti fungal activity against the tested bacteria and fungi. Generally, antimicrobials provide the main basis for the therapy of microbial infections, and their effectiveness depends largely on the ability of such antimicrobial compound to stop or inhibit the growth of any microorganism in the body system they infect. However the high genetic variability of microorganisms enables them to rapidly evade the action of antimicrobials by developing resistance [9].

Oliveira, et al.,[10] have been studied antimicrobial activity of *Syzygium cumini* leaves extracts belonging to the family Myrtaceae. The leaves extract of *Syzygium cumini* showed highest antibacterial activity among the extracts tested. The present observation reveals that the aqueous leaves extract of *Eugenia singampattiana* showed the maximum antibacterial properties against all the tested bacteria. The similar observations were also studied by the4,[11 12].

There are several investigators have proved the antimicrobial potentiality of many plants. Mishra *et al.,* [13] studied extracts of Carambola, Guava. Kiwi, Papaya and Strawberry were tested against *Escherichia coli* by the agar well diffusion method. Likewise, Panda *et al.,*[14] have reported the antibacterial activity of various extracts of *V. negundo* plant and showed zone of inhibitions were maximum for *E. coli* followed by *Staphylococcus epidermidis* followed by *Pseudomonas aeruginosa*.

The discovery of a potent remedy from plant origin will be a great advancement in bacterial infection therapies. The result of present investigation highlights that the antibacterial, antifungal potentiality of the extracts of *Eugenia singampattiana.* This study encourages the use of herbal extracts demonstrated that folk medicine can be used as effective modern medicine to combat pathogenic microorganisms.

This study is extendable with other major pathogenic bacteria to develop a novel broad spectrum antibacterial formulation in future. Now, our research will be focused to develop a broad spectrum antimicrobial combined herbal formulation with these plants.

REFERENCES

1. Pavendan P. Sebastian rajasekaran C. and Anand Gideon V., Pharmacognostic standardisation and Physico-chemical evaluations of leaves of *Eugenia singampattiana* Beddome endangered species, International Journal of Pharma and Bio Sciences, 2011, 2, 236 - 241.
2. Abhishek Mathur, Satish K. Verma, Sajad Yousuf, Santosh K. Singh, G.B.K.S. Prasad and Dua V. K., Antimicrobial potential of roots of *Riccinus communis* against pathogenic microorganisms, International J. Pharma and Biosciences, 2011, 2(1), 545-548.
3. Paarakh, P.M., *Terminalia arjuna* (Roxb.) Wt. and Arn. : A review. International Journal of Pharmacology, 2010, 6, 515-534.
4. Shyamala Gowri S. and Vasantha K., Phytochemical Screening and Antibacterial Activity of *Syzygium cumini* (L.) (Myrtaceae) Leaves Extracts, International Journal of PharmTech Research 2010, 2(2), 1569-1573.
5. National Committee for Clinical Laboratory Standards (NCCLS). Performance standard for antimicrobial disk susceptibility test, Approved standard NCCLS document M2-A7. Wayne Pa .2000.
6. Tona L. Kambu K. Ngimbi N. Cimanga K. and Vlietinck A.J., Antiamoebic and phytochemical screening of some Congolese medicinal plants. Journal of Ethnopharmacology, 1998, 61, 57-65.
7. Kone W.M. Atindehou K.K. Terreaux C. Hostettmann K. Traore D. and Dosso M., Traditional medicine in north Cote – Dlovoire screening of 50 medicinal plants for antibacterial activity, Journal of Ethno pharmacology, 2004, 93, 43-49.
8. Natarajan D. Shivakumar M.S. and Srinivasan R., Antibacterial activity of leaf extracts of Biophytum sensitivum (L.) DC. Journal of Pharmaceutical Sciences and Research, 2010, 2(11), 717-720.
9. Stephen A. Enabulele and Ehiagbonare J.E., Antimicrobial, Nutritional and Phytochemical Properties of *Perinari excelsa* Seeds, International Journal of Pharma and Bio Sciences, 2011, 2(3), 459 -470.
10. Oliveira, G.F. Furtado N.A.J.C. Filho A.A.S. Martins C.H.G. Bastos J.K. Cunha W.R. and Silva M.L.A., Antimicrobial activity of *Syzygium cumini* (Myrtaceae) leaves extract, Brazilian Journal of Microbiology 2007, 38, 381-384.
11. Karou D. Savadogo A. Canini A. Yamoog, S. Moniesano C. Simpore J. Golizi, V. and Traore A. S., Antibacterial activity of alkaloids from Cida acuta., African Journal of Biotechnology, 2006, 5(2): 195-200,
12. Ugbabe G.E. Ezeunala M.N. Edmond I.N. Apev J. and Salawu O.A., Preliminary Phytochemical, Antimicrobial and Acute Toxicity Studies of the Stem, bark and the Leaves of a cultivated *Syzygium cumini* Linn. (Family: Myrtaceae) in Nigeria, African Journal of Biotechnology, 2010, 9(41), 6943-6747.
13. Mishra N. Akhilesh Dubey A. Singh N. and Gupta P., Antimicrobial, Antioxidant and Chemopreventive Potential of vitamin C rich fruits, International Journal of Applied Biology and Pharmaceutical Technology, 2010, I(3), 915 - 920.
14. Panda S. K. Thatoi H. N. and Dutta S. K., Antibacterial activity and phytochemical screening of leaf and bark extracts of *Vitex negundo* from similipal biosphere reserve, Orissa, Journal of Medicinal Plants Research, 2009, 3(4), 294-300.

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