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ANTIMICROBIAL ACTIVITY OF ENDOPHYTIC BACTERIA ISOLATED FROM FEW PLANTS OF MUTHATHI WILDLIFE SANCTUARY MANDYA, KARNATAKA

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ABSTRACT: The worldwide usage of medicinal plants have been rapidly improved due to their medicinal property. Endophytic microorganisms are recognized as a potential source of novel chemical molecules that might be useful in the treatment of various diseases. In the present study, the plant samples were collected from Muthathi Wild Life Sanctuary, Mandya. The purpose of this study was to isolate the endophytic bacteria and their antibacterial activity against few human pathogens. Isolation of endophytic bacteria was carried out on nutrient agar media. About 41 bacterial endophytes were obtained from 17 medicinal plants in the study area. Out of this, 5 bacterial endophytes inhibits the best antimicrobial activity against *Enterobacter aeruginosa*, *Klebsiella pneumonia*, *Lacto bacillus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus mutan*s.

INTRODUCTION: Medicinal plants have a lot of antimicrobial properties, and it acts as a therapeutic agent against plants and human pathogens. It has broad applications as a treatment for various diseases. Endophytes have proved to be a rich source of novel chemistry and biological molecules. These are chemical synthesizer inside plants [1]. Endophytes protect plants against herbivores, insect attacks or tissue invading pathogens and thus display mutualistic, parasitic and communalistic connection with its host [2]. They carry out resistance mechanisms to protect its host plant from pathogenic invasion by producing secondary metabolites having antimicrobial activity.

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With this, endophytic bacteria are considered a reservoir of active metabolites that can be used as indications in drug developments [3, 4]. Antimicrobial resistance has been a major health issue and still presents a hazard to the health care system worldwide [5]. Studies have shown that microbes have established resistance to antibiotics through various molecular mechanisms such as prevention of access to drug targets and modification of the drug [6]. Thus, this global problem has directed to the increase in researches featuring endophytic bacteria, particularly those isolated from medicinal plants for their potential as a source of new antibiotics [7, 8, 9]. Endophytic bacteria are those live symbiotically with the inside plant tissues without any representation of apparent infection or adverse effect on their host [10].

Plant endophytic bacteria have been broadly recognized as an economic resource of vital and novel biomolecules and enzymes having probable application in agriculture, pharmaceutical and food industry [11].

In addition to the production of usual secondary metabolites of plant importance, bacterial endophytes have revealed the ability to inhibit disease development in plants. To explore the biodiversity of endophytic strains for novel metabolites that would lead to the identification of new drugs for current treatment of diseases, the present study was to isolate the endophytic bacteria and to investigate the antimicrobial properties of their secondary metabolites against some human bacterial pathogens.

MATERIALS AND METHODS:

Collection of Plant Sample: Healthy and matured leaves from selected medicinal plants were collected from the study area Muthathi Wild Life Sanctuary and brought to the laboratory in the sterile polythene bag and used for further experimental purpose.

Isolation of Bacterial Endophytes: The isolation technique was followed from the standard protocol [12]. The fresh plant leaves were subjected to a surface sterilization procedure. The leaves were cut into small pieces (0.5-1.0 cm), and washed in running tap water and rinsed in 70% ethanol for 30 sec then rinsed in sodium hypochlorite (3%) for 3 min finally washed in sterile water and pat dried. Then it is placed on nutrient agar and incubated at 37 ºC for 24 h after an incubation period the plates were observed for the growth of endophytic bacteria.

Selected Test Organisms to Evaluate the Antimicrobial Activity: Three gram-positive bacteria: *Staphylococcus aureus*, *Streptococcus mutans*, and *Lacto bacillus*. Four gram-negative bacteria: *Pseudomonas aeruginosa*, *Enterobacter aeruginosa*, *Klebsiella pneumonia* and *Proteus mirabilis* were obtained from the Skanda Life Science Pvt. Ltd., Bengaluru and were maintained at nutrient agar slants and stored at 4 °C.

Antimicrobial Assay: Antimicrobial activity of isolated endophytic bacteria was tested based on the protocol of [13] with slight modifications. The metabolites of bacterial endophytes were extracted with methanol solvent, and the antimicrobial activities of the extracts were tested against human bacterial pathogens using agar disc diffusion method. Few human pathogens were used to evaluate the antimicrobial activity against *viz*., *S. aureus*, *S. mutans*, *L. bacillus*, *P. aeruginosa*, *E. aeruginosa*, *K. pneumonia*, and *P. mirabilis*. Selected bacterial isolates were inoculated for about 10 ml of nutrient broth and incubated for 24h at 37 ºC. After the incubation, the samples were centrifuged at 4000 rpm for 15 min, and the supernatant was transferred to the fresh tubes, and 1 ml of methanol was subjected to the pellet.

Soybean casein digest agar media was prepared and poured 20 ml into each sterile Petri plates after solidification, 100 µl of human bacterial pathogens fresh cultures were spread by a sterile cotton swab. Agar plates were perforated with a sterile cork borer and made wells of 5 mm in diameter, and 25 µl of each sample was loaded with micropipette from 1 mg/mL, for standard 2.5 µl of ciprofloxacin and 22.5 µl of distilled water was mixed thoroughly and loaded for control 25 µl of distilled water was loaded in the wells.

Ciprofloxacin is an antibiotic used regularly for all the samples as a standard in the concentration of 1mg/mL. The plates were incubated at 37 ºC for 24 h. The antimicrobial activities were assessed by the presence or absence of inhibition zones in millimeter.

RESULTS: The present study was attempted for isolation of endophytic bacteria from different medicinal plants of Muthathi Wild Life Sanctuary, Karnataka, India. In the present study, the plant leaves samples were collected from Muthathi, and selective pre-treatment is a prerequisite for the isolation of endophytic microbes. According to [14, 15] a total of 41 endophytic bacteria were isolated from 17 plants of Muthathi Wild Life Sanctuary.

In the present investigation, results were established on the evaluation of secondary metabolites produced in static condition as well as directly diffused through agar wells. The antimicrobial activity was accompanied to 41 bacterial endophytes against 7 clinically significant human pathogens *viz*., *E. aeruginosa*, *K. pneumoniae*, *L. bacillus*, *P. mirabilis*, *P. aeruginosa*, *S. aureus* and *S. mutans* using agar well diffusion assay. The extent of antimicrobial activity was expressed in diameter of inhibition zones (mm) shown in **Table 1**. The endophytic

bacterial cultures followed by C5, C13, C17, C23, and C39 showed maximum zone of inhibition against all 7 bacterial pathogens but best results were seen by C5 against *E. aeruginosa*, *K. pneumoniae*, *L. bacillus*, *P. mirabilis*, *P. aeruginosa*, *S. aureus* and *S. mutans*.

TABLE 1: ANTIMICROBIAL ACTIVITY OF ENDOPHYTIC BACTERIA AGAINST FEW CLINICAL HUMAN PATHOGENS SHOWING THE ZONE OF INHIBITION BY AGAR WELL DIFFUSION METHOD

Culture	Concentration µl (mg/mL)	Zone of inhibition in (mm)						
		Pm	Sa	Pa	Kp	Sm	Ea	Lb
C1		-	-	-	-	-	-	-
C2		+	-	+	-	+	+	-
C3		-	-	-	-	-	-	-
C4		++	+	-	-	++	-	++
C5		++	+	+++	++	+++	++	++
C6		-	-	-	-	-	-	-
C7		-	-	-	-	-	-	-
C8		-	-	-	-	-	-	-
C9		-	-	+	+	-	+	+
C10		-	+	+	+	-	++	-
C11		-	+	+	-	-	-	++
C12		-	+	+	-	+	+	++
C13		+	++	-	++	+++	+	++
C14		-	-	-	-	-	-	+
C15		-	-	-	-	-	-	-
C16		+	+	+	+	-	+	+
C17		++	+	+	+	++	+	++
C18		-	-	-	-	-	-	-
C19		-	-	-	-	-	-	-
C20		-	-	-	-	-	-	-
C21		++	-	+	+	-	+	+
C22		-	-	++	-	-	+	-
C23	25 µl/mL	++	-	+	++	++	+	++
C24		+	+	+	+	+	-	-
C25		-	-	-	-	-	-	-
C26		+	-	+	-	+	++	+
C27		++	-	-	-	+	++	++
C28		-	-	-	-	-	-	-
C29		-	-	-	-	-	-	-
C30		-	-	+	-	-	-	-
C31		-	-	-	+	-	-	-
C32		-	-	+	+	-	+	+
C33		-	-	-	-	-	-	-
C34		-	+	++	-	+	+	+
C35		+	-	-	-	-	-	-
C36		+	-	-	-	-	-	-
C37		-	-	-	-	-	-	+
C38		-	-	-	++	-	-	
C39		++	-	+++	+	+	++	+
C40		-	-	-	-	-	-	-
C41		-	-	-	-	-	-	-
Antibiotic ciprofloxacin		25	24	22	25	26	23	22

Inhibition Zone: - **:** No activity,

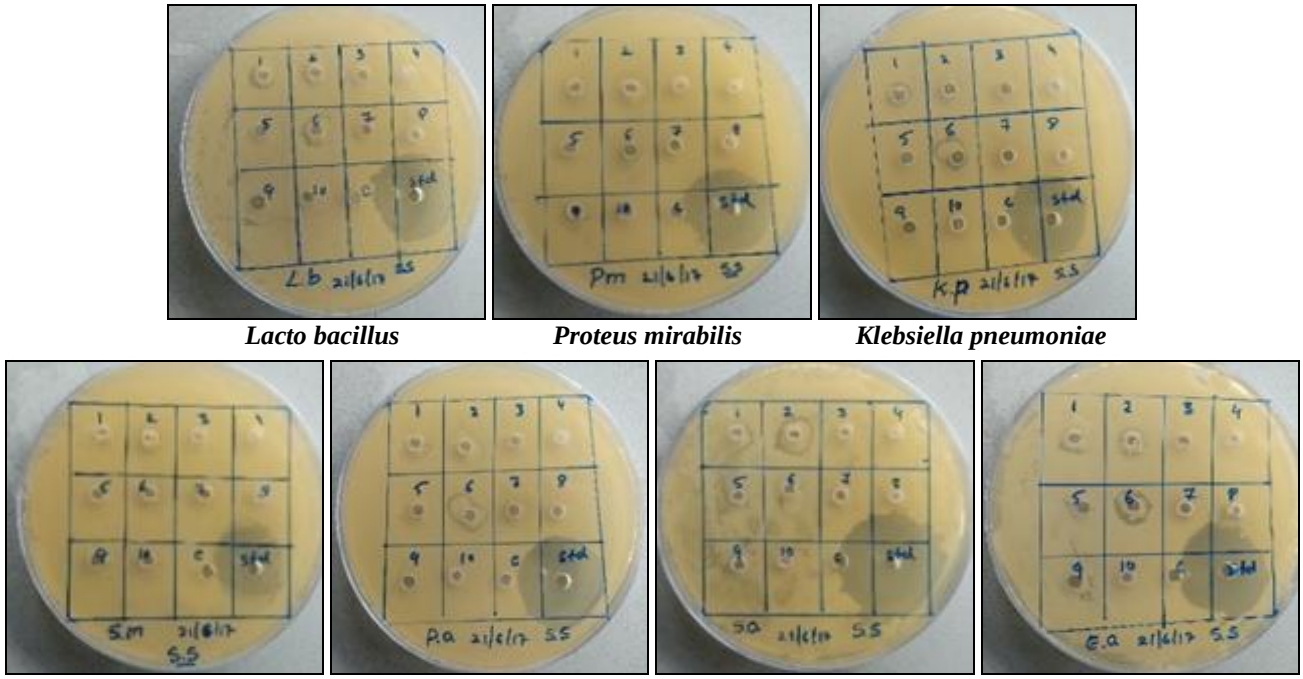
+**:** Weak activity indicates the clear zone 5~9mm,

++ **:** Moderate activity indicates the clear zone 10~ 12 mm,

++ +**:** High activity indicates the clear zone 13~16 mm and indicates the clear zone >16 mm.

a. C1 to C41 is the bacterial cultures isolated from selected medicinal plants.

b. Control: Dist. water



Lacto bacillus ***Proteus mirabilis*** ***Klebsiella pneumoniae***

Streptococcus mutans ***Pseudomonas aeruginosa*** ***Staphylococcus aureus*** ***Enterobacteria eruginosa***

FIG. 1: ANTIMICROBIAL ACTIVITY BY ENDOPHYTIC BACTERIA SHOWING INHIBITION ZONE AGAINST HUMAN PATHOGENS

DISCUSSION: Agar well diffusion method was adopted to determine the antimicrobial activities of the different clinical human pathogens. The present study for evaluating and screening of antimicrobial activity of endophytic bacteria was tested by agar well method. Based on the morphological characteristics a total of 41 culturable isolates were taken for the determination of antimicrobial activity. Out of which 5 isolates showed a broad spectrum of contrasting anti-microbial activity against the test pathogens by forming the highest zone of inhibition observed against test organisms and standard antibiotic are summarized in **Table 1**.

The isolates from the plants *Centella asiatica*, *Justicia adhathoda*, *Madhuca longifolia*, *Plectranthus ambionicus,* and *Tinospora cardifolia* showed the highest antimicrobial activity against all the test organisms with a zone of inhibition of 14 mm, 12 mm, 16 mm, 12 mm and 16 mm respectively. Overall this result suggests that 5 isolates have very good anti-microbial activity against *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Entarobacter aeruginosa*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Staphylococcus aureus,* and *Lactobacillus*. In the present study, *Centella asiatica* and *Tinospora cardifolia* isolates showed antibacterial activity against all the pathogens.

The primary identification of the bacterial isolates was done based on numerous morphological features of isolated endophytic bacteria. The colony characteristics of endophytic bacteria isolated from different medicinal plants were having irregular in shape while flat or raised elevation on Petri plate, edges of the colonies were undulated; the texture of the growth was smooth and rough, opaque and white. The microscopic examination of endophytic bacteria has shown that among the endophytic bacteria isolated from different medicinal plants were cocci.

The overall antimicrobial results showed that maximum sensitivity was observed against *P. aeruginosa* and *S. mutans*. Few isolates from different medicinal plants had shown antimicrobial activity against both gram-positive (*S. aureus,* S. *mutans,* and *L. bacillus*) and gram-negative bacteria (*P. aeruginosa*, *E. aeruginosa, K. pneumonia* and *P. mirabilis*). The antibacterial activity observed by Verma *et al*., (2009) the antimicrobial activity of endophytic actinomycetes from *A. indica* against *E. coli* [16], the leaves of *Hypericum scabrum* against *S. aureus* and Ebrahimia *et al*., (2010) [17], roots of *Aloe vera* possess strong antibacterial activity against *S. typhi* in dual culture assay [18] and a medicinal plant of *Vinca rosea* produced potential antimicrobial activity against *B. cereus, K.*

pneumonia and *E. coli* [19]. This study is almost similar to the above authors.

CONCLUSION: In the present study, a total of 41 bacterial isolates were obtained from 17 medicinal plants of Muthathi Wild Life Sanctuary Mandya, Karnataka. Only 5 bacterial endophytes, showed the capable antimicrobial activity against 7 different human bacterial pathogens. Thus, it can be concluded from the present investigation that, endophytic bacteria isolated from a few medicinal plants have antibacterial properties against few human pathogens. Hence, they pharmacologically important.

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CONFLICT OF INTEREST: Nil

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