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Antibacterial activity of endophytic bacteria from leaves of *Tamarindus indica*

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Abstract

The antibacterial activity of endophytic bacteria isolated from leaves of *Tamarindus indica* was evaluated against various gram positive and gram negative pathogenic bacteria namely *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhimurium*. Results were recorded for zone of inhibition around the disc. The endophytic bacteria isolated from *Tamarindus indica* shown antibacterial activity as 73.33 per cent isolates inhibited growth of *Staphylococcus aureus*, 86.67 percent isolates inhibited growth of *Escherichia coli* and 86.67 percent isolates inhibited growth of *Bacillus cereus*. The endophytic bacteria isolated from *Tamarindus indica* had not shown antibacterial activity against *Streptococcus pyogenes*, *Klebsiella pneumoniae* and *Salmonella typhimurium*.

Keywords: *Tamarindus indica*, endophytic bacteria, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*

Introduction

Antimicrobial resistance is one of the major public health problems especially in developing countries like India where relatively easy availability and inappropriate use of antibiotics leads to higher incidence of resistance. Global health problems due to drug resistance among pathogenic microorganisms have necessitates the urgent need for new and effective antimicrobial agents. Despite the existence of varied antibacterial and antifungal agents available in the market, there is a continuous need to search for novel drug compounds as the numbers of drug resistant microorganisms are continuously increasing (Pinner *et al.*, 1996) [9]. Over the last few years there has been increasing interest in the bioprospecting of endophytic bacteria and fungi.

Although previous searches for bioactive compounds focused on soil fungi and bacteria, the rate of discovery of interesting new compounds from the soil has diminished (Verpoorte, 1998) [14]. Research has turned to niches that have not yet been explored (Bills, 1995; Pelaez *et al.*, 1998) [3] for finding novel, pharmacologically active compounds for industrial screening programmes. One such niche is the healthy, green tissues of living plants, which are known to harbor a rich and diverse bacterial biota that is distinct from the soil mycobiota (Cannon and Simmons, 2002) [4]. In this context, endophytic bacteria isolated from indigenous plants are promising, because it is possible that they have acquired some of the genes from their host plants (Tan and Zou, 2001) [13]. To investigate endophytic bacteria isolates from indigenous plants as sources of bioactive secondary metabolites the present study was undertaken to evaluate the antimicrobial properties of their natural extracts.

Material and Methods

Location and place of work: The proposed work was conducted in the Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur (M.P.).

Collection of leaves and Sterilization of leaves: Fresh mature leaves of *Tamarindus indica* were procured from, three different places of Jabalpur *viz.* Jawaharlal Nehru Krishi Viswa Vidyalaya (J.N.K.V.V), Tropical Forest Research (T.F.R.I.), State Forest Research Institute (S.F.R.I), Jabalpur. Samples were immediately brought to laboratory and were used within 24 hrs and finally processed for isolation of endophytic bacteria. The sterilization of leaves and isolation of endophytic bacteria from the leaves was done according to Mahajan *et al.* (2014) [8], with some modifications.

Sterility check: To confirm that the surface of leaves were effectively sterilized, 1 ml of the sterile distilled water that was used in final rinse of surface sterilization procedures was plated on to nutrient agar media and incubated at 37 °C for 24 hrs. Then bacterial growths were observed.

Preparation and sterilization of media: King's B (KB) media (HiMedia), Mueller Hinton media (HiMedia) and BHI broth (HiMedia) were prepared by adding agar into the distilled water. Hot plate was used for the proper mixing of media and autoclaved at 121 °C for 15-20 minutes at 15 lbs.

Inoculation of leaves and isolation of endophytic bacteria: Media were poured into different autoclaved small petri plates and leaves of the plant were embedded in small petri plates. These plates were then incubated at 37 °C for 24 hrs.

Preparation of Antibacterial disc

For determination of antibacterial activity of endophytic bacteria, broths were centrifuged at 12000 rpm for 30 minutes at 4 °C Supernatant of each of these broths were taken, sterile disc were soaked in these broths in a sterile test tubes for 24 hrs and dried in laminar flow. After drying the discs were used immediately for disc impregnation in the inoculated plates as described by Kirubaharan *et al.* (1999) with slight modifications. Ciprofloxacin disc was used as control drug to compare the effect of treatment during *in vitro* study.

Antibacterial test

The prepared bacterial inoculums were evenly spread on a sterile Mueller Hinton agar plate as per method described by Bauer *et al.* (1969). The known antibiotic Ciprofloxacin (Ci) disc was simultaneously placed as a control for antibiotic sensitivity.

Results And Discussion

Isolation of endophytic bacteria

The sterilized leaves of *Tamarindus indica* were placed on the King's B media and incubated at 37 °C for 24 hrs. The morphological characterization of endophytic bacterial isolates exhibited diverse colonies, texture, shape and margin including round to irregular colonies which were white in colour, mucoid, soft with wavy and irregular margin.

The population density of endophytic bacteria vary from 10^2 to 10^9 and depends on many factors, including the plant being studied, the part under analysis, developmental stage of the plant cultivar (genotype) and the interaction with other organisms, as well as other environment related factors (Costa *et al.*, 2012).

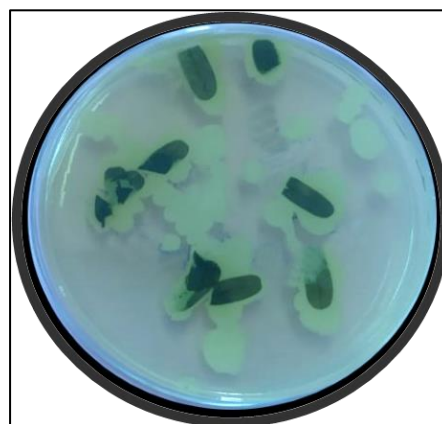


Plate 1: Growth of Endophytic bacteria from leaves of *Tamarindus indica*

Antibacterial sensitivity

The antibacterial activity of endophytic bacteria was evaluated against various gram positive and gram negative pathogenic bacteria namely *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhimurium*. Results were recorded for zone of inhibition around the disc. The inhibitory zone around the disc indicated absence of bacterial growth reported as sensitive and absence of zone reported as resistant. The *in vitro* antibacterial activity shown on plate no.05,06, and 07. The endophytic bacteria isolated from *Tamarindus indica* shown antibacterial activity as 73.33 per cent isolates inhibited growth of *Staphylococcus aureus*, 86.67 percent isolates inhibited growth of *Escherichia coli* and 86.67 percent isolates inhibited growth of *Bacillus cereus*. The endophytic bacteria isolated from *Tamarindus indica* had not shown antibacterial activity against *Streptococcus pyogenes*, *Klebsiella pneumoniae* and *Salmonella typhimurium*.

Table 1: *In vitro* antibacterial activity of endophytic bacteria isolated from leaves of *Tamarindus indica* against gram positive and gram negative bacteria:

S. No.	Isolate No.	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Bacillus cereus</i>
1	JN-1a	S	S	S
2	JN-1b	S	S	S
3	JN-1c	S	S	R
4	JN-1d	R	S	S
5	JN-1e	S	R	S
6	TF-2a	S	S	S
7	TF-2b	S	S	R
8	TF-2c	R	S	S
9	TF-2d	S	S	S
10	TF-2e	S	S	S
11	SF-3a	R	R	S
12	SF-3b	S	S	S
13	SF-3c	S	S	S
14	SF-3d	S	S	S
15	SF-3e	R	S	S

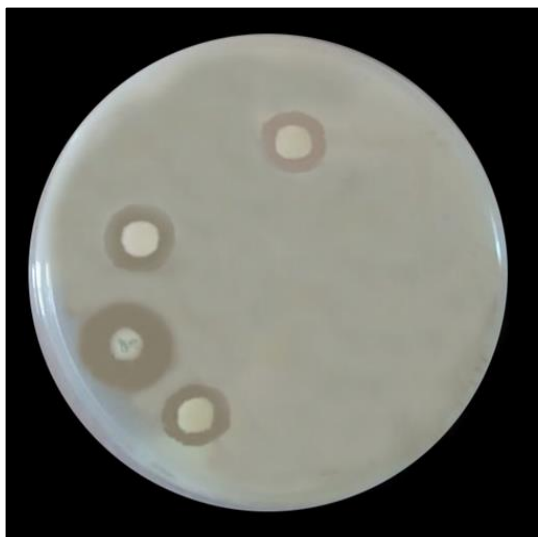


Plate 2: *In vitro* antibacterial activity of Endophytic bacteria isolated from leaves of *Tamarindus indica* against *Escherichia coli*

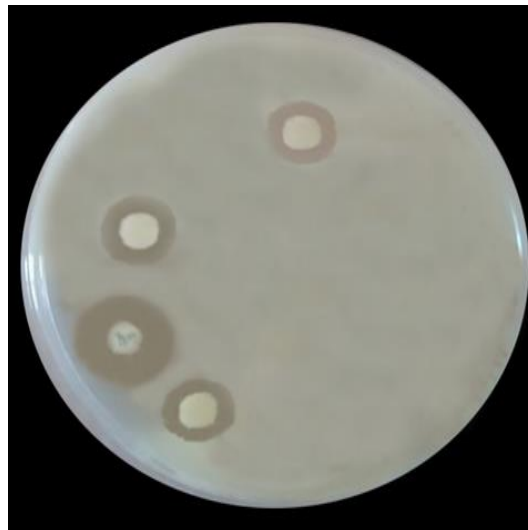


Plate 3: *In vitro* antibacterial activity of Endophytic bacteria isolated from leaves of *Tamarindus indica* against *Bacillus cereus*

Table 2: *In vitro* antibacterial activity of endophytic bacteria isolated from leaves of *Tamarindus indica* against gram positive bacteria and gram negative bacteria

S. No.	Isolate No.	<i>Klebsilla pnunoniae</i>	<i>Streptococcus pyogenes</i>	<i>Salmonella typhimurium</i>
1	JN-1a	R	R	R
2	JN-1b	R	R	R
3	JN-1c	R	R	R
4	JN-1d	R	R	R
5	JN-1e	R	R	R
6	TF-2a	R	R	R
7	TF-2b	R	R	R
8	TF-2c	R	R	R
9	TF-2d	R	R	R
10	TF-2e	R	R	R
11	SF-3a	R	R	R
12	SF-3b	R	R	R
13	SF-3c	R	R	R
14	SF-3d	R	R	R
15	SF-3e	R	R	R

Overall 1 *in vitro* antibacterial activity of endophytic bacterial isolates: Total 15 endophytic bacterial isolates from *Tamarindus indica* were tested for antibacterial activity. Over all screening report is presented in Table 03 Out of 15 isolates from Jawaharlal Nehru Krishi Viswa Vidyalaya (J.N.K.V.V),

Tropical Forest Research Institute (T.F.R.I.), and State Forest Research Institute (S.F.R.I) Jabalpur, 11 isolates were effective against *Staphylococcus aureus*, 13 against *Escherichia coli*, and 13 against *Bacillus cereus* (Table 03).

Table 3: Over all *in vitro* antibacterial activity of endophytic bacteria isolated from *Tamarindus indica*.

S. No	Bacteria	No. of isolates from J.N.K.V.V.	No. of isolates from T.F.R.I.	No. of isolates from S.F.R.I.	Total no. of isolates which showed sensitivity
1	<i>Staphylococcus aureus</i>	4	4	3	11
2	<i>Streptococcus pyogenes</i>	0	0	0	0
3	<i>Bacillus cereus</i>	4	5	4	13
4	<i>Salmonella typhimurium</i>	0	0	0	0
5	<i>Klebsiella pneumonia</i>	0	0	0	0
6	<i>Escherichia coli</i>	4	4	5	13



Plate 4: *In vitro* antibacterial activity of Endophytic bacteria isolated from leaves of *Tamarindus indica* against *Staphylococcus aureus*

Guler *et al.* (2006) ^[6] reported that *Asparagus racemosus* possess antibacterial activity against gram positive and gram negative bacteria. Arunachalam and Gayathri (2010) ^[2] had conducted a study to determine the presence of antibacterial activity in the extract of endophytes isolated from the medicinal plant *Andrographis paniculata* by agar gel diffusion method. In the study, leaves were used for isolation of endophytic bacteria and extract were collected and subjected for screening against six human pathogenic bacteria and two pathogenic bacteria of fish. Roy and Banerjee (2010) ^[10] had isolated antimicrobial compound of endophytic bacteria from *Vinca rosea*. One of the isolated endophytes produced potential antimicrobial activity against some selected human pathogenic bacteria like *Bacillus cereus*, *Klebsiella pneumoniae*, *Vibrio cholerae*, *Escherichia coli* and yeast *Candida albicans*. Islam *et al.* (2018) ^[7] studied antibacterial activities of endophytic bacteria isolated from *Taxus brevifolia* against foodborne pathogenic bacteria: *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella typhimurium*. Acharavadee and Patcharee (2018) ^[1] studied that the ethyl acetate extract of the *Arthrimum* sp. MFLUCC16-1053 showed activity against both gram-positive and gram-negative bacteria. Specifically, the minimum inhibitory concentration against *Staphylococcus aureus* and *Escherichia coli* was 31.25 and 7.81 µg/mL, respectively.

Conclusion

Endophytic bacteria were present in the leaves of *Tamarindus indica* collected from J. N. K. V. V., T. F. R. I and S. F. R. I. Endophytic bacteria from leaves of *Tamarindus indica* had shown antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*. However, *Streptococcus pyogenes*, *Klebsiella pneumoniae* and *Salmonella typhimurium* were found to be resistant.

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