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Production of Antibiotics Isolated from Soil Bacteria from Rhizospheric and Non-Rhizospheric Region of Medicinal Plants

KEYWORDS: rhizosphere and non-rhizosphere, secondary metabolites, optimization, Rf value, chromatography

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ABSTRACT *The present study was carried out to explore the production of antibiotics from soil microbes of medicinal plants. These microbes were isolated from the rhizospheric and non-rhizospheric region of two medicinal plants-Oscimum tenuiflorum and Aloe barbadensis. The bacterial cultures present in these soil samples were isolated by primary screening and sub-culturing methods. The results of Antibiotic Sensitivity Test revealed the activity of isolates which further led to characterization of isolates through Gram's staining and Bergey's manual. The isolates obtained were Proteus vulgaris, Streptococcus epidermis, Lactobacillus fermentum, Bacillus cereus, N.mucosa, Sterptococcus equisimilis, Streptococcus faecalis and Bacillus subtilis. These isolates were cultured in optimized production media in suitable nutrient sources, temperature conditions and pH range. Glucose and sucrose as carbon sources and ammonium chloride and ammonium sulphate as nitrogen sources at 370C and pH-7 were suitable for growth of majority isolates. This further led to secondary screening by intracellular and extracellular metabolite extraction from the cultures obtained in production media. These antimicrobial properties of the metabolites were tested by AST which gave positive results. This led to determination of antimicrobial compounds or antibiotics in secondary metabolites of cultures by chromatographic techniques. The results i.e. Rf values obtained from the silica gel Thin Layer Chromatography proved the possible presence of beta-lactum antibiotics in the secondary metabolites extracted from the cultures of Lactobacillus fermentum, Bacillus cereus and N.mucosa.*

Introduction

The plants which have medicinal properties and have potential uses as antimicrobial, antipyretic, anti-cancerous agents etc., are termed as medicinal plants. The soil microorganisms comprise a very large reservoir of biological diversity around the world which is essential for the functioning of terrestrial ecosystems [1]. Rhizosphere is a narrow zone around and influenced by a plant root which is inhabited by a vast population of microbes affected by the chemicals which are released from the roots of plant. It is a unique biological niche with a diverse microflora comprising bacteria, fungi, protozoa and algae [2] .It is a dynamic region where various biological and chemical processes take place along with the variety of chemicals released by roots and mediated by the soil microbes. The rhizosphere is divided into three zones which on the basis of their relative proximity to, and thus influence from, the root. Endorhizosphere includes parts of the endodermis and cortex where microbes and cations can occupy the "free space" between cells (apoplastic space), rhizoplane is the medial zone which is directly adjacent to the root and includes the root epidermis and the mucilage and the outermost zone called the ectorhizosphere extends from the rhizoplane out into the bulk soil [3]. Non-rhizosphere is the area of the soil not around the plant roots (surface-level soil).

Populations of microbes can boom or reduce in the space in response to the changes in soil conditions i.e. moisture, temperature or substrates like carbon. The microbes gain advantage in the process, by releasing antibiotic substances to suppress the particular competitors [4]. There is prevalence of essential nutrients like and growth factors necessary for bacteria and readily available in the root exudates of rhizospheric region. The aerobic bacteria are comparatively less in the rhizosphere as there is reduced oxygen level due to respiration by root. The wide effect of rhizosphere is usually observed with bacteria than with the actinomycetes and fungi. The gram-negative, rod shaped and non-sporulating bacteria which respond to root exudates are dominant in the rhizosphere (Pseudomonas, Agrobacterium). Gram-positive, rods, cocci and aerobic spore forming (Bacillus, Clostridium) are comparatively rare in the rhizosphere. The common genera of bacteria i.e. Azotobacter, Micrococcus, Pseudomonas, Arthrobacter, Flavobacter, Mycobacterium, Agrobacterium, Alcaligenes, Cellulomonas and others have been found to be either abundant or less populated in the rhizosphere[5] .

Secondary metabolites are classically organic compounds produced from microorganisms during the alteration of primary metabolite synthesis. Secondary metabolites don't have a role in the growth and development of microbes and are usually formed in the stationary phase. Many among secondary metabolites have ecological functions; which include defense mechanisms, also function as antimicrobial agents or antibiotics and by producing various pigments [6]. Antibiotics are one of the most important and widely employed secondary metabolites produced by the bacteria. The soil microbes are a major source of antibiotics. Various bacterial strains are selected for antibiotic production as its isolation, culturing, maintenance and strain improvement is easy. Antibiotics such as erythromycin, which have been derived from the Saccharopolyspora erythraea, are a commonly used antibiotic with a wide antimicrobial spectrum. It has mass production and usually administered orally. Bacitracin, derived from organisms classified under Bacillus subtilis, is an antibiotic usually used as a contemporary drug. Bacitracin is naturally synthesized as a non-ribosomal peptide synthetase that can synthesize peptides and used as an antibiotic [7] .

Materials and methods

Sample collection:The soil samples were collected from rhizospheric and non-rhizospheric region of medicinal plants. The plants were-Oscimum tenuiflorum(from alluvial soil of Lucknow) and Aloe barbadensis (from red soil of Hyderabad)

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Isolation of microbes from soil by serial dilution:Microbes are generally found in soil as mixed populations. The serial dilution is a method followed to get reduced number of colonies or less populated colonies from the sample. The microbes are successfully cultivable only in liquid media and are generally isolated by serial dilution method [8] .

Primary Screening:Primary screening allows the detection and isolation of potential microbes. It includes spreading and streaking methods.

Antibiotic Sensitivity Test: AST is used to check the sensitivity of antibiotic against various pathogens. Antibiotic sensitivity is the vulnerability of bacteria to antibiotics. In AST, broth cultures of isolates were used as antibiotics source to check the sensitivity of pathogens by Agar well diffusion method. Nutrient agar plates were prepared and spread with 20 µl of pathogen. The pathogens used are as E.coli, S.aureus, P.aeruginosa, B.subtilis and S.typhi. 50 µl of the broth cultures was pipetted in the wells. It was incubated at 370C for overnight and zone of inhibition was observed [9].

Characterization by Bergey's manual: The gram's staining is followed by performing various tests which confirmed various species in the cultures.The biochemical tests were endospore staining, mannitol fermentation test, catalase test, glucose fermentation, oxidase test, lactose fermentation test, indole test, fructose fermentation test, nitrate reduction test, glycerol test and MRVP test [10] .

Optimization of culture conditions: Optimization of culture conditions leads to better production of cultures in vitro and provides such ideal conditions for the growth of this microorganism, to evaluate their productivity and growth characteristics. The following parameters were considered for same: Carbon sources (conc. 1%) – glucose, sucrose, lactose, mannitol and galactose, Nitrogen sources (conc 1%) – urea, glycine, potassium nitrate, ammonium sulphate , ammonium chloride, pH range – 5, 7, 9, 11, Temperature – 40C, 370C, 550C, Metal ions (conc. 1%) – zinc nitrate, lead nitrate, magnesium sulphate.

Secondary screening: Secondary screening is required to test the microbial growth potential by Growth Kinetic studies. This further leads to the production and extraction of secondary metabolites. The production of metabolites is at the end of the log phase of microbial growth. Hence the extracellular and intracellular metabolites are extracted by organic solvents.

Solvent Extraction method: The screening of secondary metabolites from microbes is a determined method to identify novel biologically active molecules. The preparation of production media to obtain microbes provides conditions which promote the synthesis of secondary metabolites in the media. The culture produced in production media were taken in separate eppendorf tubes and centrifuged at 10000 rpm for 10 min. The supernatant (extracellular) was collected in different eppendorf tubes.The eppendorfs containing pellets (intracellular) was taken seperately.

Intracellular secondary metabolite extraction: The pellets were dissolved in 0.5 ml of the methanol and allowed to mix for 1 hr. The pellet dissolved in methanol was centrifuged at 10000 rpm for 10 min. The top layer containing metabolites with methanol were transferred to new eppendorf tube. It was air dried and the mixture was dissolved in 100mM Tris Cl (pH-8).

Extracellular secondary metabolite extraction: The supernatant obtained was dissolved in 0.5ml of ethyl acetate and was allowed to mix for an hr. The mixture was spin at 10000 rpm for 10 min.The top layer was discarded and bottom layer containing metabolites with chloroform was retained in the eppendorf tube. It was allowed to air dry and the mixture was dissolved in 100mM Tris Cl (pH-8) [1] .

Characterization of antibiotics: AST confirmed the presence of antimicrobial compounds in the metabolite extracts of microbes. Hence these compounds (antibiotics) were detected by ThinLayer Chromatography. TLC is a simple, quick, and inexpensive procedure which is used to separate many components are in a mixture. A TLC plate is a sheet made of glass, metal, or plastic, coated with a thin layer of a solid adsorbent (usually silica or alumina). This liquid, or the eluent, or the mobile phase, advance slowly on the TLC plate by capillary action in the developing chamber. As the solvent moves past the spot (analyte) on the plate, equilibrium is established for each component of the mixture [11]. Movement of analyte is measured by retardation factor (Rf) which is: Rf = distance moved by analyte from origin / distance moved by solvent from origin.

Results

1. Oscimum tenuiflorum(TULSI) SOIL SAMPLE

The soil sample was collected from Indira Nagar region of Lucknow.The mixed culture plates with few isolated colonies were obtained after spreading from diluted samples of rhizospheric soil with dilution of 10-3 and non-rhizospheric soil with dilution of 10-3

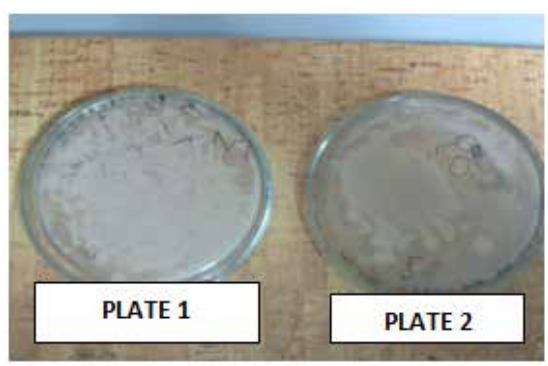

FIGURE 1: RHIZOSPHERIC SOIL SAMPLE- PLATE 1AND NON RHIZOSPHERIC SOIL SAMPLE- PLATE 2

• RESULTS OF SOLID TO SOLID PLATE CULTURING BY STREAKING METHOD

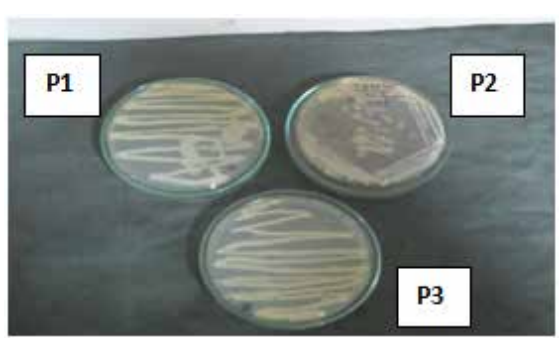

FIGURE 2: CULTURES OBTAINED IN P1, P2 AND P3 FROM PLATE 2(NON-RHIZOSPHERIC SOIL DILUTED SAMPLES)

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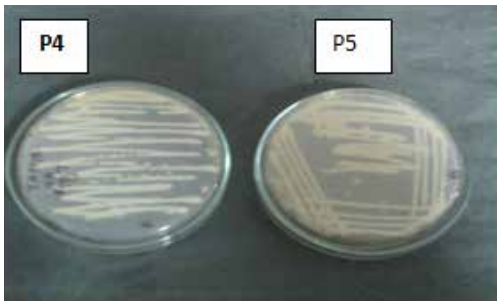

FIGURE 3: CULTURES OBTAINED IN P4 AND P5 BY FROM PLATE 1(RHIZOSPHERIC SOIL DILUTED SAMPLES)

2. Aloe barbadensis (ALOE VERA) SOIL SAMPLE

The soil sample was collected from red soil of Hyderabad. The mixed culture plates with isolated colonies were obtained after spreading from diluted samples of rhizospheric soil with dilution of 10-3 and non-rhizospheric soil with dilution of 10-3 of aloe.

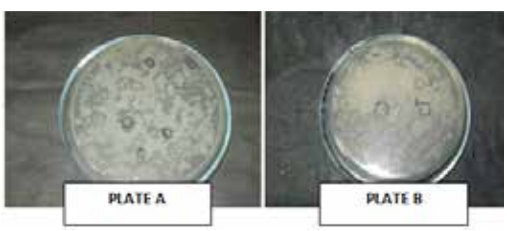

FIGURE 4: RHIZOSPHERIC SOIL SAMPLE- PLATE A AND NON-RHIZOSPHERIC SOIL SAMPLE- PLATE B

- **RESULTS OF SOLID TO SOLID PLATE CULTURING BY STREAKING METHODS**

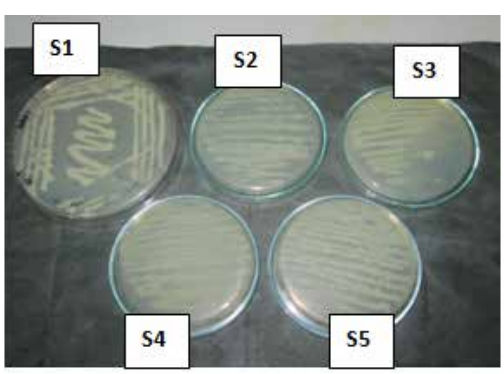

FIGURE 5: CULTURES OBTAINED IN S1, S2, S3, S4 AND S5 FROM PLATE A (RHIZOSPHERIC SOIL DILUTED SAMPLES)

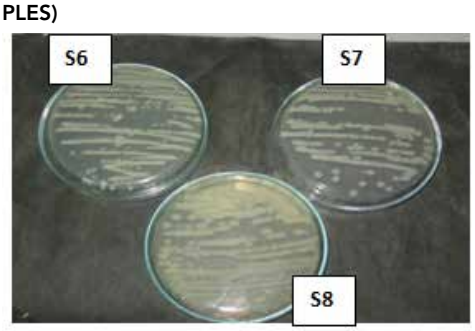

FIGURE 6: CULTURES OBTAINED IN S6, S7 AND S8 FROM PLATE B (NON-RHIZOSPHERIC SOIL DILUTED SAMPLES)

- **RESULTS OF CHARACTERIZATION**
- **GRAM'S STAINING**

The results of Gram's staining defined the bacteria as gram positive or negative. It also specified the shape of the bacteria.

1. Tulsi samples (P1-P5)

CULTURES	GRAM POSITIVE/ NEGATIVE	SHAPE
P1	NEGATIVE	RODS
P2	POSITIVE	COCCI
P3	POSITIVE	RODS
P4	POSITIVE	RODS
P5	NEGATIVE	COCCI

TABLE 1: RESULTS OF GRAM'S STAINING

2. Aloe samples (S1-S8)

CULTURES	GRAM POSITIVE/ NEGATIVE	SHAPE
S1	POSITIVE	COCCI
S2	NEGATIVE	COCCI
S3	POSITIVE	COCCI
S4	NEGATIVE	COCCI
S5	POSITIVE	COCCI
S6	POSITIVE	RODS
S7	POSITIVE	RODS
S8	POSITIVE	RODS

TABLE 2: RESULTS OF GRAM'S STAINING

- **BERGEY'S MANUAL**

The characterization by Bergey's manual defines the type of species present in the culture through a series of experiments for gram positive or gram negative, rods or cocci shape bacteria.

1. Tulsi soil cultures

TESTS	P1	P2	P3	P4	P5
Endospore	+	+	-	+	+
Catalase	NA	+	-	+	
Mannitol	NA	-	NA	-	NA
Glucose Fermentation	+	-	+	NA	+
Oxidase	-	NA	NA	NA	NA
Lactose	-	NA	NA	NA	NA
Indole	+	NA	NA	NA	NA
Fructose	NA	+	NA	NA	NA
Nitrate	NA	NA	NA	NA	+

TABLE 3: RESULTS OF BERGEY'S MANUAL : (+) – POSITIVE RESULTS, (-) – NEGATIVE RESULTS , (NA) – NOT

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ALLOWED

• The following bacterial species were found to be present in the 'tulsi' soil samples from the results of characterization :

P1- Proteus vulgaris, P2- Streptococcus epidermis,P3- Lactobacillus fermentum,
P4- Bacillus cereus, P5- N. mucosa

2. Aloe soil cultures

TESTS	S1	S2	S3	S4	S5	S6	S7	S8
Endospore	-	+	+	+	+	+	+	+
Catalase	-	NA	-	NA	-	-	-	+
Mannitol	NA	NA	NA	NA	NA	NA	NA	+
Glucose Fermentation	NA	+	NA	+	NA	+	+	-
Oxidase	NA	NA	NA	NA	NA	NA	NA	NA
Lactose	NA	NA	NA	NA	NA	NA	NA	NA
Indole	NA	NA	NA	NA	NA	NA	NA	NA
Fructose	NA	NA	NA	NA	NA	NA	NA	NA
Nitrate	NA	+	NA	+	NA	NA	NA	NA
MRVP	NA	NA	NA	NA	NA	NA	NA	+
Glycerol	+	NA	+	NA	NA	NA	NA	NA

TABLE 4: RESULTS OF BERGEY'S MANUAL, (+) – POSITIVE RESULTS, (-) – NEGATIVE RESULTS, (NA) – NOT ALLOWED

• The following bacterial species were found to be present in the 'aloe vera' soil samples from the results of characterization :S1- Streptococcus equisimilis, S2- N.mucosa ,S3- Streptococcus equisimilis ,S4- N.mucosa ,S5- Streptococcus faecalis ,S6 – Lactobacillus fermentum , S7 – Lactobacillus fermentum ,S8 – Bacillus subtilis

➢ Eight distinct cultures were obtained by the characterization of cultures from both tulsi and aloe soil samples. They are-Proteus vulgaris, Streptococcus epidermis, Lactobacillus fermentum, Bacillus cereus, N.mucosa , Sterptococcus equisimilis Streptococcus faecalis, Bacillus subtilis

➢ **RESULTS OF OPTIMIZATION**

1. Carbon sources

SOURCES (CONC. 1%)	Proteus vulgaris (O.D AT 620nm)	Streptococcus epidermis (O.D AT 620nm)	Lactobacillus fermentum (O.D AT 620nm)	Bacillus cereus (O.D AT 620nm)
Glucose	0.6	0.6	0.59	0.53
Sucrose	0.54	0.38	0.47	0.53
Lactose	0.27	0.22	0.26	0.34
Mannitol	0.32	0.22	0.17	0.3
Galactose	0.32	0.19	0.6	0.16

TABLE 5: RESULTS OF CARBON SOURCES OPTIMIZATION WITH CULTURES

SOURCES (CONC. 1%)	N.mucosa (O.D AT 620nm)	Streptococcus equisimilis (O.D AT 620nm)	Streptococcus faecalis (O.D AT 620nm)	Bacillus subtilis (O.D AT 620nm)
Glucose	0.51	0.57	0.34	0.44
Sucrose	0.55	0.41	0.51	0.55
Lactose	0.35	0.14	0.31	0.22
Mannitol	0.35	0.16	0.28	0.23
Galactose	0.42	0.21	0.33	0.18

TABLE 6: RESULTS OF CARBON SOURCES OPTIMIZATION WITH CULTURES-The highlighted values in both the tables show the maximum growth of each isolate in the presence of the particular carbon source

2. Nitrogen sources

SOURCES (CONC. 1%)	Proteus vulgaris (O.D AT 620nm)	Streptococcus epidermis (O.D AT 620nm)	Lactobacillus fermentum (O.D AT 620nm)	Bacillus cereus (O.D AT 620nm)
Urea	0.3	0.2	0.18	0.24
Glycine	0.29	0.21	0.19	0.29
Potassium nitrate	0.36	0.17	0.21	0.34
Ammonium sulphate	0.42	0.21	0.24	0.32
Ammonium chloride	0.35	0.22	0.22	0.35

TABLE 7: RESULTS OF NITROGEN SOURCES OPTIMIZATION WITH CULTURES

SOURCES (CONC. 1%)	N.mucosa (O.D AT 620nm)	Streptococcus equisimilis (O.D AT 620nm)	Streptococcus faecalis (O.D AT 620nm)	Bacillus subtilis (O.D AT 620nm)
Urea	0.47	0.24	0.25	0.35
Glycine	0.46	0.16	0.38	0.33
Potassium nitrate	0.52	0.26	0.35	0.42
Ammonium sulphate	0.47	0.21	0.37	0.21
Ammonium chloride	0.51	0.34	0.36	0.25

TABLE 8: RESULTS OF NITROGEN SOURCES OPTIMIZATION WITH CULTURES-The highlighted values in both the tables show the maximum growth of each isolate in the presence of the particular nitrogen source

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3. pH values

pH VALUES	Proteus vulgaris (O.D AT 620nm)	Streptococcus epidermis (O.D AT 620nm)	Lactobacillus fermentum (O.D AT 620nm)	Bacillus cereus (O.D AT 620nm)
5	0.36	0.32	0.3	0.27
7	0.41	0.37	0.3	0.35
9	0.36	0.25	0.26	0.29
11	0.23	0.22	0.16	0.17

TABLE 9: RESULTS OF OPTIMIZATION WITH CULTURES AT DIFFERENT pH VALUES

pH VALUES	N.mucosa (O.D AT 620nm)	Streptococcus equisimilis (O.D AT 620nm)	Streptococcus faecalis (O.D AT 620nm)	Bacillus subtilis (O.D AT 620nm)
5	0.12	0.01	0.02	0.01
7	0.30	0.21	0.26	0.28
9	0.13	0.10	0.14	0.12
11	0.01	0.02	0.03	0.03

TABLE 10: RESULTS OF OPTIMIZATION WITH CULTURES AT DIFFERENT pH VALUES-The highlighted values in both the tables show the maximum growth of each isolate in the presence of the particular pH value

4. Temperature

TEMPERATURE (^{0}C)	Proteus vulgaris (O.D AT 620nm)	Streptococcus epidermis (O.D AT 620nm)	Lactobacillus fermentum (O.D AT 620nm)	Bacillus cereus (O.D AT 620nm)
4	0.14	0.1	0.12	0.13
37	0.32	0.23	0.17	0.27
55	0.18	0.16	0.01	0.01

TABLE 11: RESULTS OF OPTIMIZATION WITH CULTURES AT DIFFERENT TEMPERATURE

TEMPERATURE (^{0}C)	N.mucosa (O.D AT 620nm)	Streptococcus equisimilis (O.D AT 620nm)	Streptococcus faecalis (O.D AT 620nm)	Bacillus subtilis (O.D AT 620nm)
4	0.07	0.07	0.06	0.08
37	0.33	0.15	0.21	0.23
55	0.07	0.05	0.06	0.07

TABLE 12: RESULTS OF OPTIMIZATION WITH CULTURES AT DIFFERENT TEMPERATURE-The highlighted values in both the tables show the maximum growth of each isolate in the presence of the particular temperature value

5. Metal ions

METAL IONS (CONC. 1%)	Proteus vulgaris (O.D AT 620nm)	Streptococcus epidermis (O.D AT 620nm)	Lactobacillus fermentum (O.D AT 620nm)	Bacillus cereus (O.D AT 620nm)
$Zn(NO_3)_2$	0.20	0.18	0.16	0.17
$Pb(NO_3)_2$	0.07	0.06	0.07	0.04
$FeSO_4$	0.01	0.02	0.01	0.03

TABLE 13: RESULTS OF OPTIMIZATION WITH CULTURES WITH DIFFERENT METAL IONS

METAL IONS (CONC. 1%)	N.mucosa (O.D AT 620nm)	Streptococcus equisimilis (O.D AT 620nm)	Streptococcus faecalis (O.D AT 620nm)	Bacillus subtilis (O.D AT 620nm)
$Zn(NO_3)_2$	0.19	0.15	0.16	0.17
$Pb(NO_3)_2$	0.06	0.07	0.04	0.05
$FeSO_4$	0.02	0.01	0.03	0.02

TABLE 14: RESULTS OF OPTIMIZATION WITH CULTURES WITH DIFFERENT METAL IONS-The highlighted values shows the maximum growth of the isolate in presence of the particular metal ion

RESULTS OF AST PERFORMED WITH METABOLITES

- **Intracellular metabolites**

CULTURES	E.coli (ZONE OF INHIBITION-in mm)	S.aureus (ZONE OF INHIBITION-in mm)	P.aeruginosa (ZONE OF INHIBITION-in mm)	B.subtilis (ZONE OF INHIBITION-in mm)	S.typhi (ZONE OF INHIBITION-in mm)
Proteus vulgaris	17.5	10	13.5	12.5	14.5
Streptococcus epidermis	17.5	10.5	14.5	9.5	17
Lactobacillus fermentum	11	14.5	14	10	15
Bacillus cereus	16.5	12	15	Partial	18
N.mucosa	17.5	13	11.5	Partial	13.5
Streptococcus eqisimilis	17.5	11	15	14.5	14.5
Streptococcus faecalis	14	12.5	15	15	13
Bacillus subtilis	12.5	12.5	16.5	12.5	13

TABLE 15: ZONE OF INHIBITION OBSERVED WITH INTRACELLULAR METABOLITES LOADED IN THE WELLS

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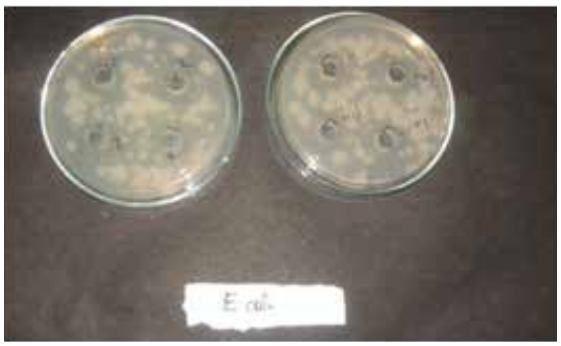
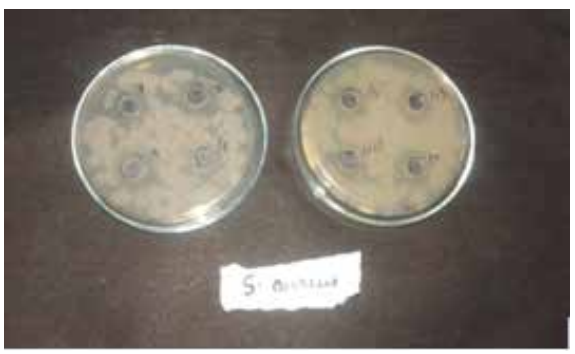
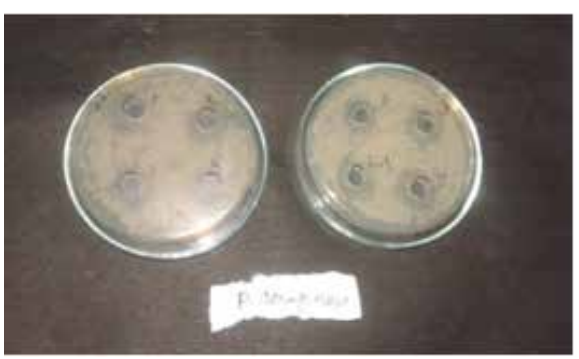
The highlighted values show the maximum zone of inhibition by the pathogen by the metabolite extract of that particular culture.







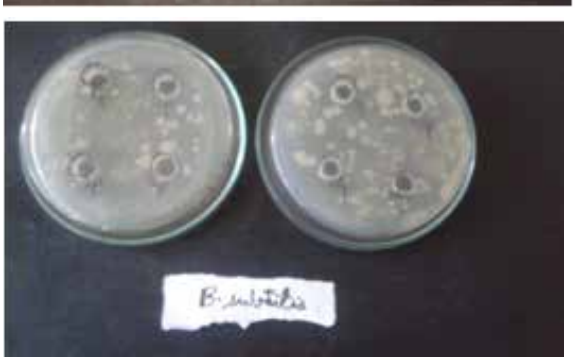


FIGURE 7: RESULTS OF AST WITH INTRACELLULAR METABOLITES LOADED IN WELLS SHOWING ZONE OF INHIBITION

- **Extracellular metabolites**

CULTURES	E.coli (ZONE OF INHIBITION-in mm)	S.aureus (ZONE OF INHIBITION-in mm)	P.aeruginosa (ZONE OF INHIBITION-in mm)	B.subtilis (ZONE OF INHIBITION-in mm)	S.typhi (ZONE OF INHIBITION-in mm)
Proteus vulgaris	10	10	Partial	15	10
Streptococcus epidermis	10	Partial	10	15	Partial
Lactobacillus fermentum	12.5	10	12	14	11
Bacillus cereus	Partial	Partial	12	Partial	10
N.mucosa	12	Partial	9	Partial	Partial
Streptococcus eqisimilis	10	10	Partial	12.5	Partial
Streptococcus faecalis	Partial	9	9	Partial	Partial
Bacillus subtilis	Partial	Partial	Partial	Partial	Partial

TABLE 16: ZONE OF INHIBITION OBSERVED WITH EXTRACELLULAR METABOLITES LOADED IN THE WELLS

The highlighted values show the maximum zone of inhibition by the pathogen by the metabolite extract of that particular culture.

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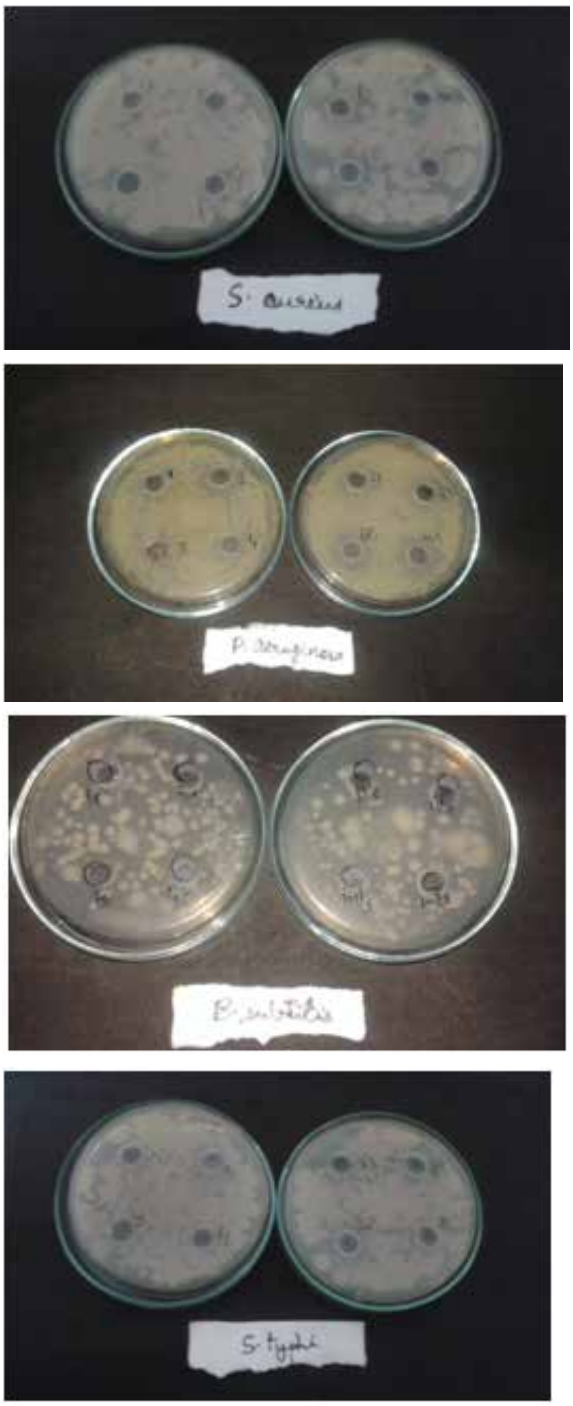

FIGURE 8: RESULTS OF AST WITH EXTRACELLULAR METABOLITES LOADED IN WELLS SHOWING ZONE OF INHIBITION

➢ **RESULTS OF CHROMATOGRAPHIC TECHNIQUES**

• **Thin Layer Chromatography**

S.NO	SAMPLES (EXTRACTS OF CULTURES)	MOVEMENT OF SOLVENT (cms)	MOVEMENT OF ANALYTE (cms)
1.	Lactobacillus fermentum (EXTRACELLULAR)	10.5	5.2
2.	Lactobacillus fermentum (INTRACELLULAR)	10.5	5.8
3.	Bacillus cereus (EXTRACELLULAR)	10.5	7.5
4.	Bacillus cereus (INTRACELLULAR)	10.5	8.5
5.	N.mucosa (EXTRACELLULAR)	10.5	8.4
6.	N.mucosa (INTRACELLULAR)	10.5	9

TABLE 17: RESULTS OF MOVEMENT OF ANALYTES AND SOLVENT ON TLC PLATE

S.NO.	Rf values
1.	0.49
2.	0.55
3.	0.71
4.	0.8
5.	0.8
6.	0.85

TABLE 18: Rf VALUE OF METABOLITES

Discussion

In the present study, the bacterial cultures were isolated from rhizospheric and non-rhizospheric region of medicinal plants- Oscimum tenuiflorum and Aloe barbadensis. They were characterized and identified by Bergey's manual and the eight distinct cultures obtained were - Proteus vulgaris, Streptococcus epidermis, Lactobacillus fermentum, Bacillus cereus, N.mucosa, Sterptococcus equisimilis, Streptococcus faecalis and Bacillus subtilis. These cutures were further subjected to growth kinetic studies followed by optimized media production for each culture. It further led to extraction of intracellular and extracellular metabolites by solvent extraction method by methanol and ethyl acetate respectively. The metabolites were tested for their antimicrobial activity with the pathogens E.coli, S.aureus, P.aeruginosa, B.subtilis and S.typhi. The positive results obtained from AST of metabolites led to the determination of antibiotics in the metabolites from cultures by Chromatographic techniques by Thin Layer Chromatography.

The Rf values obtained from silica gel coated Thin Layer Chromatographic technique with methanol and water as solvent, presented the information about the possible antibiotics in the extracts. The Rf values 0.49, 0.80 and 0.85 along with the development of pale red colour after ninhydrin reaction indicated the promising presence of antibiotics like ampicillin, penicillin and oxacillin respectively. The Rf values were obtained from chromatography of - extracellular metabolite extracts of Lactobacillus fermentum(0.49), intracellular extracts of Bacillus cereus(0.80), extracellular extracts of N.mucosa (0.80) and intracellular extracts of N.mucosa (0.85). These results were compared with the TLC for separations of antibiotics from a complex mixture of antibiotics to obtain beta lactum derivatives-ethyl acetate, water and acetic acid(60:20:20) for ampicillin ; acetone, benzene, water and acetic acid(65:14:14:7) for penicillin and butanol, water and acetic acid(60:20:20) for oxacillin. The β-lactam antibiotics produce pale red or reddish colour with ninhydrin reaction on TLC plates. The most widely used stationary phase for the analysis of beta-lactam is silica gel in which Si-OH groups are capable of hydrogen bonding with polar substances [12] .Ampicillin upon forced or oxidative degradation showed spots on TLC plates with Rf value of 0.49 using ethanol and water as solvent [13].Mixtures of penicillins were run on stannic arsenate-cellulose layers with solvents butanol, acetic acid and carbon tetrachloride in the ratio of 1:1:6 and Rf value of 0.80 was obtained [14] .

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