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EVALUATION OF ANTIMICROBIAL AND ANTICANCER PROPERTIES OF *STREPTOMYCES ROCHEI JA246*

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ABSTRACT

Introduction: Antibiotics are crucial drugs that hinder the synthesis of cell walls, depolarise the cell membrane, stop the synthesis of proteins, block the synthesis of nucleic acids, and prevent the metabolic processes of bacteria. Nevertheless, the increase in antibiotic resistance is an alarming health issue universally, necessitating the emergence of novel medications and approaches to treat these resistant illnesses. Drug resistance either innate or acquired presents a significant hurdle to addressing cancer. Drug permeability pump overexpression, epigenetic alteration, and genetic mutations are some causes of resistance.

Objectives: The study aims to identify bioactive secondary metabolites from *Streptomyces rochei*, evaluate their antibacterial activity against 12 clinical isolates, and evaluate anticancer activity against MDA-MDB-231 cell lines.

Method: Preliminary screening for antibacterial activity by the perpendicular streak method was performed and the organisms were then narrowed down for the disc-diffusion method. *In vitro,* anticancer activities were observed in triple-negative breast cancer cell lines (MDA-MB-231), and partial sequences of the 16s rRNA gene and phylogenetic tree construction were examined.

Results: The study analyzed a total of 35isolates out of which isolate JA3 was identified as *Streptomyces rochei* JA246; evinced a broad spectrum of antimicrobial activity against 12 clinical pathogens, with a maximum zone of inhibition against 4pathogens: *Enterococcus durans* (21.13±0.0577), *ESBL [K.pneumonia]* (24.76±0.0577), *Salmonella paratyphi* (24.76±0.0577), and

Vibrio parahaemolyticus (MTCC 451) (19.13±0.0577) at 5µg/ml. Anticancer activity against MDA-MDB-231 cell lines which is a prominent model for studying triple-negative breast cancer is the MDA-MB-231 cell line which is extremely aggressive and invasive with a high percentage (over 90%) of CD44+/CD24low/- profile, which is linked to a higher risk of tumor development yielded positive dose-dependent viable results and weak inhibitory activities against normal Vero cell lines. As the concentration (20, 40, 60, 80, 100 µg/ml) of the extract of isolate JA3 increases, there is a reduction in the percentage viability of the cancerous cell line with 15.64% at 100 µg/ml.

Conclusion: Multidrug resistance in pathogenic bacteria and cancer poses one of the biggest threats to the public and *Streptomyces rochei* JA246 acts as a powerful weapon against it.

Keywords: *Streptomyces rochei* JA246, Actinomycetes, antibiotic resistance, antimicrobial activity, clinical isolates, anti-cancer activity

INTRODUCTION

The discovery, widespread implementation, and monetization of antimicrobial agents for infections transformed the therapeutic paradigm and modern medicine. Antibiotics are, in fact, increasingly one of the most crucial medical interventions required for the development of sophisticated medical methodologies, notably advanced surgical techniques, and solid organ transplantation, among others. Regrettably, this therapeutic achievement is currently in peril due to the notable rise in antibiotic resistance among prevalent bacterial infections, endangering the effective outcomes of patients in critical condition **[1]**. One of the three biggest concerns to public health in the twenty-first century, according to the World Health Organisation, is antibiotic resistance, also the most life-threatening illnesses that affect people today is cancer, and breast cancer is the second most common cause of cancer-related bereavement among women **[2]**. Globally, the impact of cancer on public health is substantial. Furthermore, as populations age and some regions see an increase in cancer incidence rates, the disease's influence on public health is anticipated to increase. Many pharmaceutical compounds, used to treat infections and cancer are derived from microorganisms. One of the most consequential sources of novel bioactive substances, such as enzymes and antibiotics **[3]**, that have a variety of therapeutic applications against an extensive spectrum of pathogenic species, including bacteria, fungi, and parasites, is actinomycetes. Indeed, about 60% of medicines that are approved originate from nature **[4]**. Approximately half of the antibiotics that have been isolated come from microorganisms. Bioactive substances from actinomycetes obtained from unexplored habitats may be extracted and used as

effective anti-tumor agents and to impede; multi-drug-resistant organisms. In light of this study aimed to determine if actinomycetes possessed antimicrobial and anticancer properties against clinical pathogens and breast cancer cell lines (MDA-MB-231).

2. MATERIALS AND METHODS

2.1 Sample collection and isolation of actinomycetes

The soil samples were collected from various localities, near central and south India namely Clay soil, Red soil, Tulsi rhizosphere soil, Thoothuvalai rhizosphere soil, Siriyanangai rhizosphere soil, Nilgris mountain soil, Adyar eco-park Mangrove soil, Alsoor Deep lake soil, Forest soil, Vandaloor zoo soil, Organic farm soil, and dried for a week in sterile condition **[5]**. The samples were serially diluted and plated onto Starch casein agar plates suspended with actidione, rifampicin, and nystatin (30µg/ml) to inhibit fungal growth. The plates were incubated for 7-14 days at room temperature for the growth of actinomycetes.

2.2 Screening for Bioactive metabolite against antimicrobial and anti-cancer activity

35 strains were isolated from various localities of central and south India. These strains were grown in ISP2 medium, and incubated for 7-14 days at room temperature. The broth filtered and collected after 14 days of growth were tested for its antimicrobial activity. Perpendicular streak method was performed with 24-hour-old test cultures of *E.coli, Salmonella typhii, S.mutans, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas putida* and *Pseudomonas aeroginosa* . Based on the results, the filtrates were mixed with equal volumes of ethyl acetate and shaken for 48 hours in a rotatory shaker **[6]**. The organic layer was then separated using a separating funnel and evaporated to dryness. The test organisms were swabbed on nutrient agar plates. 6mm sterile discs were loaded with 1.25µg/ml, 2.5µg/ml, and 5µg/ml concentrations of the extract and placed on the swabbed plates. The plates were incubated for 24 hours at 37° C to estimate the zone of inhibition in mm **[7]**. The experiments were performed in triplicates to obtain concordant results.

2.3 Morphological, Physiological, and Biochemical characterization

2.3.1 Morphological characterization of Actinomycetes

Macroscopically the isolates were differentiated by their colony shape, size, and colour.

2.3.2. Microscopic examination of Actinomycetes:

Lactophenol cotton blue staining (LPCB):

This technique stains and preserves the structure of the Actinomycetes hence it can be observed under the microscope. A drop of 70% ethanol was placed on a clean microscopic glass slide. The specimens were immersed in the drop of alcohol. One or at most two drops of LPCB was added before the alcohol dries out. The coverslip was held between the index finger and thumb, and one edge of the drop of the mount was touched with the coverslip edge and lowered gently to avoid air bubbles. This preparation was examined making the initial examination using low power objective. A more detailed examination of spores and other structures was examined by switching to high power (40X) **[8]**.

2.4 Cultural Characterization of Actinomycetes:

Cultural characteristics of Actinomycetes refer to the growth characteristics and morphology in various kinds of culture media. It is usually determined after incubation for 14 days at 28°C strictly according to the methods used in the *INTERNATIONAL STREPTOMYCES PROJECT* (ISP). The colors of the substrate and aerial mycelia and any soluble pigments produced were determined as well **[9]**. The standard culture media used were: ISP medium (yeast extract-malt extract agar); ISP medium (oatmeal agar); ISP medium 4 (inorganic salt starch agar); ISP medium 5 (glycerol asparagine agar) and ISP medium 7 (tyrosine agar).

2.5 Identification of the isolated strain

8 strains were found to show potent results for the antimicrobial activity performed. They were further screened for anticancer activity against the MDA-MB-231 cell line based on these results. Out of the 8 strains, isolate JA3 showed an increased zone of inhibition in terms of antimicrobial activity and increased cytotoxicity in terms of MTT assay. Further JA3 was studied taxonomically and was identified as *Streptomyces rochei JA246* **[10]**.

2.6 Anticancer activity:

2.6.1 Cell Culture Maintenance

Vero (African green monkey kidney normal cell line) and MDA-MB-231 (human breast cancer cell line) were obtained from the National Centre for Cell Sciences (NCCS), Pune, India. Cells were maintained in the logarithmic phase of growth in Dulbecco's modified eagle medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, 100µg/mL streptomycin. They were maintained at 37°C with 5% CO_2 in a 95% air-humidified incubator.

2.6.2 Cytotoxicity effect

The cytotoxicity effect of the sample was tested against Vero and MDA-MB-231 cell lines by MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay. The cells were seeded in 96-well

micro-plates (1 x 10^6 cells/well) and incubated at 37°C for 48 h in a 5% CO_2 incubator and allowed to grow 70-80% confluence. Then the medium was replaced and the cells were treated with different concentrations of sample (20, 40, 60, 80, 100µg/ml) and incubated for 24 h. The morphological changes of untreated (control) and treated cells were observed under a digital inverted microscope (20X magnification) after 24 h and photographed. The cells were then washed with phosphate-buffer saline (PBS, pH-7.4) and 20µL of (MTT) solution (5 mg/mL in PBS) was added to each well. The plates were then stood at 37ºC in the dark for 2 h. The formazan crystals were dissolved in 100µL DMSO and the absorbance was read spectrometrically at 570nm **[11]**. The percentage of cell viability was calculated using the formula,

$$\text{Cell viability (\%)} = \frac{\text{(Absorbance of the sample) X 100}}{\text{Absorbance of control}}$$

The graph was plotted using the cell viability (%) at the Y-axis and the concentration of the sample on the X-axis

The 96 well plates were incubated for 3 h in the dark and the developed color was measured with ELISA reader at 570 nm. Triplicates were maintained for each treatment. Inhibitory concentration (IC50) values were directly determined.

2.7 FTIR Analysis

The bioactive metabolite of isolate JA3 was analyzed using FT-IR Spectra recorded in Bruker-Alpha-Platinum FT-IR System (Sophisticated Analytical Instrument Facility, Indian Institute of Science, Madras), in the spectral range of 500-4000 cm^{-1} and plotted intensity versus wavenumber.

2.8 GCMS Analysis

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df), and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL sample was injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature of 240 °C; ion source temperature of 240 °C; and ionization mode electron impact at 70 eV, a scan time of 0.2 sec, and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of the spectrum of known components stored in the GC-MS NIST (2008) library.

3. RESULTS and DISCUSSION

3.1 Isolation of Actinomycetes

A total of 35 actinomycetes were isolated from different ecological habitats. The strains were grown in starch casein agar plates and the purified colonies were stored in 20% glycerol stock at -20℃ for future use.

3.2 Antimicrobial Assay

8isolates showed potent results against test isolates for antimicrobial activity **(Figure 1 (c))** and anti-cancer activity. Among the 8 isolates, isolate JA3 (*Streptomyces rochei* JA246) showed exceptionally good results. The isolate was grown in an ISP3 medium to study its cultural, morphological, biochemical, and physiological characterization **(Figure 1(a))**.

3.3 Morphological, Biochemical and Physiological Characterization

The Lactophenol Cotton Blue stain of isolate JA3 shows that it is a Gram-positive aerobic organism with a fawn brown colony color **(Figure 1(b))**. Their growth in different ISP mediums is given in **Table 1**. The isolate JA3 showed excellent growth in all the mediums ISP2, ISP3, ISP4, ISP5, ISP6 and ISP7. The development parameters of the aerial mycelium, pigmentation dynamics **[12]**, and growth activity of all *Streptomyces rochei JA246* were examined in a morphological analysis based on using the substrate mycelium, aerial mycelium, sporulation, agar, following the procedures provided in Bergey's Manual of Systematic Bacteriology **[13]**.

In the present study, actinomycetes isolate identified as *Streptomyces rochei* JA246(OP422232) was found to develop an open spiral with a smooth surface as this is found to be the main characteristic feature of *Streptomyces.* The filamentous structure of *S. rochei* is typical of Streptomyces species and is typified by the production of mycelium **[14]**. Aerial mycelium showed a grey color in ISP2 medium, with a fawn brown colony color spore morphology of open spirals and smooth spore surface, faint brown pigment, and brown reversible pigment. The growth of aerial mycelium, which is essential for spore generation, results from the main or substrate mycelium's tip extension development **[15]**. Growth in ISP3 medium showed aerial mycelium to be white, and colony color to be fawn brown, followed by open spiral spore chain and warty spore surface, with faint brown pigment and brown reversible pigment. The growth medium can have an impact on the morphology; investigations show that colony development can result in both smooth and rough textures. Depending on the particular media used for growing, the substrate and aerial mycelium might have different colours depending on the cultural circumstances **[16]**. ISP4 again showed a faint grey aerial mycelium, with a sandy yellow colony color, spore morphology to be open spirals, and warty spore surface with brown reversible pigment. ISP5 and ISP6

showed grey aerial mycelium with pale brown colony color, open spirals, and spore morphology followed by a smooth spore surface for ISP5 and a smooth spore surface for ISP6, with brown as reversible pigment. Growth on ISP7 showed greyish-white aerial mycelium, fawn brown colony color, open spiral spore morphology and warty spore surface, and brown pigment production. These variations can include shades of white, yellow, or other pigments **[17]**.

The actinomycetes isolates were grown on different culture media like yeast extract malt extract agar, oatmeal agar, glycerol asparagine agar, tyrosine agar medium, and starch casein agar, starch casein agar was more suitable than the other media tested. It is also possible to evaluate the synthesis of soluble pigments, albeit certain strains might not produce colors in particular conditions **[18]**.

3.4 Antimicrobial activity

Based on the results performed for the perpendicular streak method, 8 isolates showed potential antimicrobial activity out of the total 35 isolates. These 8 isolates were further tested using their extracts by disc diffusion method **(Figure 2)**. The 8 isolates were used to perform the antimicrobial activity by disc diffusion method. Isolate JA3 showed potent activity against all the test organisms **(Figure 1(c))**. JA3, when tested against 24-hour-old clinical pathogens namely *ESBL Klebsiella (ICMR-6), ESBL E.coli (clinical pathogen), Staphylococus aureus [Methiciline Sensitive S. aureus],* MRSA *[Methiciline Resistant S. aureus]* ICMR – 5, *Enterococcus durans* (P 502), ICMR-19*Acetobacter baumanii (Carbapenem R), Enterococcus faecalis, ESBL [Klebsiella pneumonia], Salmonella paratyphi, Vibrio parahaemolyticus* (MTCC 451), *Yersinia enterocolitica* (MTCC 840), *Vibrio fischeri* (ATCC 1738) with Streptomycin as the standard. Streptomyces rochei was found to show potent antimicrobial activity against the 12 clinical pathogens except against *Vibrio parahaemolyticus* (MTCC 451) at 1.25 µg/ml. and 2.5 µg/ml respectively. The actinobacterium *Streptomyces rochei JA246* possesses antimicrobial against various pathogens and anticancer properties, making it a significant one. Effective antagonistic properties against Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa,* and *Salmonella enteritidis* are found to be displayed by *Streptomyces rochei* **[19]**. A maximum zone of inhibition was found against *ESBL [Klebsiella pneumonia]* (24.76±0.115mm at 5µg/ml) which was comparatively higher than the standard Streptomycin (21±0.115mm at 10µg/ml) **(Table 2)**. In a study published in the World Journal of Microbial Biotechnology, antagonistic activity carried

out by *Streptomyces rochei JA246* against *Klebsiella pneumonia* was found to be 21m±0.00mm less when compared to the results in the present study carried out **[20]**. The second highest zone of clearance was against *Vibrio parahaemolyticus* (MTCC 451) (22.16±0.115mm at 5µg/ml) higher than the standard (19.32±0.057mm at 10µg/ml). Literature quotes for a study where the isolate's maximal antibacterial effectiveness against *V. parahaemolyticus* was demonstrated by its ethyl acetate extract of *Streptomyces sp.* ECR377 (9.66 ± 0.94 mm) **[21]**. Moderate zone of inhibition was found against *Salmonella paratyphi* (19.13±0.057mm), *Yersinia enterolitica* (MTCC840) **(**14.96±0.057mm), and *Vibrio fischeri* (ATCC 1738) (13.13±0.057mm) at 5µg/ml. Standard streptomycin was found to show minimum inhibitory activity against *Salmonella paratyphi* (18.55±0.057mm)*, Yersinia enterolitica,* and *Vibrio fischeri* (11.22±0.057mm) respectively. Literature references show that a study carried out using crude extracts of *Streptomyces sp*. showed minimum inhibitory activity against *Salmonella paratyphi* (6.0±1.2mm) Different actinomycetes strains exhibited varying degrees of bioactivity profiles in their antagonistic activity, which may have resulted from variations in the bioactive secondary metabolites that the isolates produced **[22]**.

Experimental results are calculated as Mean±standard deviation (SD). Statistical comparisons using one-way analysis of variance (ANOVA). The results showed statistically significant.

3.5 Anticancer activity of actinomycetes isolates

Ethyl acetate extracts of actinomycete isolate JA1-JA8 were tested for their anti-cancer and cytotoxic effects against cancer cell lines MDA-MDB-231 and their normal Vero cell line. The isolates JA8, JA7, and JA6 showed potent anti-cancer activity against MDA-MDB-231 **(Figure 3(A))** with IC_{50} values of 71.82, 90.94 and 94.03. JA3, JA5 and JA1 55.07, 63.55 and 70.12µg/ml. compared with its inhibitory activities in normal Vero cell lines respectively **(Figure 4 (A, B)**.

The extracts of isolate JA3 identified as *Streptomyces rochei* JA246 demonstrated positive anti-tumor activity and weak inhibitory activity against normal Vero cell lines. As the concentration (20, 40, 60, 80, 100 µg/ml) of the extract of isolate JA3 increases, there is a reduction in the percentage viability of the cancerous cell line with 15.64% cell viability at 100 µg/ml **(Figure 3(B))**. Studies have demonstrated that A549 lung carcinoma cell line is resistant to the anticancer effects of crude extracts of Streptomyces rochei. These extracts' 50% inhibitory concentration (IC50) was found to be 75 µl/ml, suggesting

that as extract concentrations increased, cell viability significantly decreased **[23]**. Actinobacterium *Streptomyces rochei JA246* possesses strong anticancer properties. It is well recognised that this bacterium produces bioactive substances with possible medical uses, especially in the treatment of cancer.

3.6 FTIR Analysis

The active fraction of the brown pigment of isolate JA3 was characterized by FT-IR Spectroscopy. The FT-IR spectrum shown in **Figure 5 (A)** shows the band at 3349.72cm^{-1} corresponds to N-H stretching, belonging to the compound class aliphatic primary amine, weak band at 2116.08cm^{-1} corresponds to C ΞC stretching, belonging to the compound class alkyne, medium band at 1631.49cm^{-1} corresponds to C=C stretching, belonging to the compound conjugated alkene, strong band at 1557.41cm^{-1} corresponds to N-O stretching, belonging to the compound nitro group, medium band at 1450.12cm^{-1} corresponds to C-H bending, belonging to the compound alkane, methyl group, medium band at 1397.56cm^{-1} corresponds to O-H bending, belonging to the compound, carboxylic group, medium band at 1312.52cm^{-1} corresponds to O-H bending, belonging to the compound, phenol group, strong band at 1080.09cm^{-1} corresponds to C-O stretching, belonging to the compound, primary alcohol, which corresponds with the results published in their study on bio-pigment from actinobacteria **[24]** trisubstituted group, strong band at 699.01cm^{-1} corresponds to C=C bending, belonging to the compound, disubstituted (*cis*) group, strong band at 610.91 cm^{-1} corresponds to C-I stretching, belonging to the compound, halo group and strong band at 528.52 cm^{-1} corresponds to C-Br stretching, belonging to the group of halo compound **(Table 3)**. These results detected, coincided with the published reference in their study on pigment produced from marine *Natrialba sp*. **[25]**.

3.7 GC-MS Analysis

The chromatogram of the characteristic signals, their molecular weight, molecular formula, and structure is given in **Table 4**. The extract of isolate JA3 showed 17 peaks in the chromatogram. The bioactive metabolite octanol revealed that antibacterial activity may be due to that compound. Bioactive metabolite Cyclopropane, 1-Heptyl-2-Methyl, revealed that anticancer activity may be due to that compound **(Figure 5 (B))**.

Table 1: Morphological and Biochemical Characterization of JA3

ISP Media	Growth	Aerial	Colony color	Spore chain morphology	Spore surface	Pigment	Reverse pigment
ISP2	Excellent	Grey	Fawn Brown	Open Spirals	Smooth	Faint brown	Brown
ISP3	Excellent	White	Fawn Brown	Open Spirals	Warty	Faint brown	Brown
ISP4	Excellent	Faint Grey	Sandy yellow	Open Spirals	Warty	Brown	Brown
ISP5	Excellent	Grey	Pale brown	Open Spirals	Warty	Brown	Brown
ISP6	Excellent	Grey	Pale brown	Open Spirals	Smooth	Faint brown	Brown
ISP7	Excellent	Greyish white	Fawn Brown	Open Spirals	Warty	Brown	Brown

ISP2 (yeast extract-malt extract agar); ISP3 (oatmeal agar); ISP4 (inorganic salt starch agar); ISP5 (glycerol asparagine agar), ISP6 (peptone yeast extract iron agar) and ISP7 (tyrosine agar).

Table 2: Antimicrobial activity of isolates JA3 by disc diffusion method

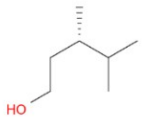
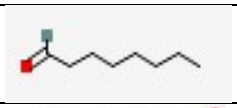
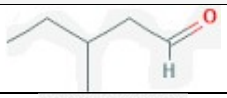
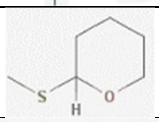
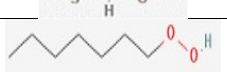
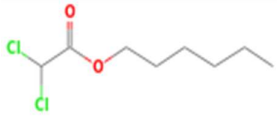
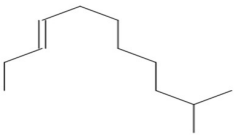
S. No.	Test Organisms	Zone of Inhibition (mm)			Streptomycin
		1.25µg/ml	2.5 µg/ml	5.0 µg/ml	10 µg/ml
1.	*ESBL Klebsiella (ICMR-6)*	9.366±0.0577	11.533±0.0577	13.133±0.0577	16.26±0.0577
2.	*ESBL E.coli (clinical pathogen)*	10.33±0.0577	12.633±0.0577	14.166±0.0577	16.6±0.0577
3.	*Staphylococus aureus [Methiciline Sensitive S. aureus],*	7.466±0.0577	9.551±0.173	10.066±0.115	18.06±0.115
4.	MRSA *[Methiciline Resistant S. aureus]* ICMR – 5	15.578±0.115	16.133±0.057	16.833±0.115	17.83±0.0577
5.	*Enterococcus durans* (P 502)	11.233±0.115	12.36±0.057	12.86±0.057	19.63±0.0577
6.	ICMR-19*Acetobacter baumanii (Carbapenem R)*	5.133±0.115	5.84±0.034	6.7±0.028	15.43±0.0577
7.	*Enterococcus faecalis*	15.95±0.046	17.33±0.115	18.066±0.057	21.9±0.0577
8.	*ESBL [Klebsiella pneumonia]*	20.166±0.115	21.86±0.057	24.76±0.115	21±0.115
9.	*Salmonella paratyphi*	15.95±0.057	17.33±0.057	19.13±0.057	18.55±0.057
10.	*Vibrio parahaemolyticus* (MTCC 451)	20.16±0.057	21.86±0.057	22.16±0.115	19.32±0.057
11.	*Yersinia enterocolitica* (MTCC 840)	10.06±0.057	11.96±0.057	14.96±0.057	11.22±0.057
12.	*Vibrio fischeri* (ATCC 1738)	0	0	13.13±0.057	11.22±0.057

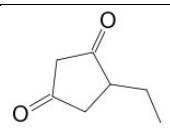
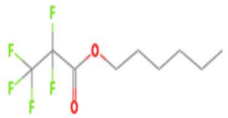
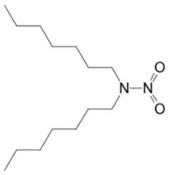
Each value represents the Mean ± SD of triplicateexperiments

Table 3: FTIR Analysis of isolate JA3

Peak Number	Group Frequency (cm^{-1})	Functional group assignment
1	3349.72	N-H (stretching, aliphatic primary amine)
2	2116.08	C ≡C (stretching alkyne)
3	1631.49	C=C (stretching conjugated alkene)
4	1557.41	N-O (stretching nitro group)
5	1450.12	C-H (bending, methyl group alkane)
6	1397.56	O-H (bending carboxylic group)
7	1312.52	O-H (bending phenolic group)
8	1080.09	C-O (stretching primary alcohol)
9	873.46	C-H (bending 1,2,4-trisubstituted group)
10	699.01	C=C (bending disubstituted (*cis*) group)
11	610.91	C-I (stretching halo group)
12	528.52	C-Br (stretching halo group)

Table 4: GC-MS Analysis of isolate JA3

HIT	Compound Name	Molecular Weight	Formula	Structure
1.	(S)-3,4-DIMETHYLPENTANOL	116	$C_7H_{16}O$	
2.	HEXANE, 1-(ETHENYLOXY)-	128	$C_8H_{16}O$	
3.	PENTANAL, 3-METHYL-	100	$C_6H_{12}O$	
4.	2H-PYRAN, TETRAHYDRO-2-(1-METHYLETHOXY)	144	$C_8H_{16}O_2$	
5.	HYDROPEROXIDE, HEPTYL	132	$C_7H_{16}O_2$	
6.	DICHLOROACETIC ACID, HEXYL ESTER	212	$C_8H_{14}O_2Cl_2$	
7.	OCTANAL	128	$C_8H_{16}O$	
8.	OCTANAL	128	$C_8H_{16}O$	
9.	OCTANAL	128	$C_8H_{16}O$	
10.	2-DECENE, 9-METHYL-, (Z)-	154	$C_{11}H_{22}$	
11.	3-UNDECENE, 10-METHYL-	168	$C_{12}H_{24}$	
12.	2-TRIDECENE, (Z)	182	$C_{13}H_{26}$	
13.	3-UNDECENE, (Z)-	154	$C_{11}H_{22}$	
14.	CYCLOPROPANE, 1-HEPTYL-2-METHYL	154	$C_{11}H_{22}$	

15.	**1,3-CYCLOPENTANEDIONE, 4-ETHYL**	**126**	**$C_7H_{10}O_2$**	
16.	**PENTAFLUOROPROPIONIC ACID, HEXYL ESTER**	**248**	**$C_9H_{13}O_2F_5$**	
17.	**1-HEPTANAMINE, N-HEPTYL-N-NITRO-**	**258**	**$C_{14}H_{30}O_2N_2$**	

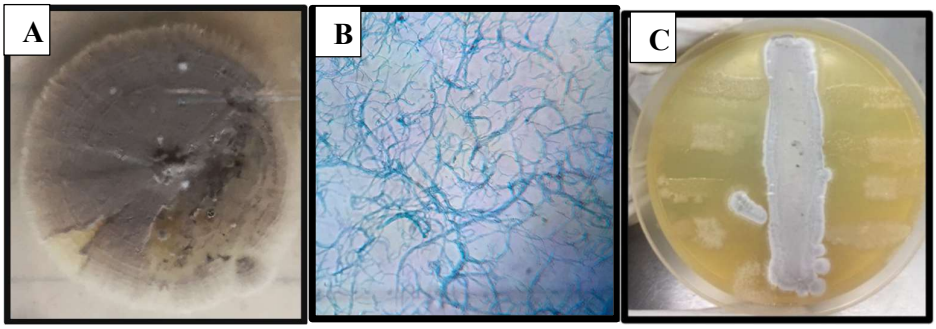


Figure 1: (A) Isolation of Actinomycetes JA3 (B) Microscopic view of JA3 (C) Perpendicular-streak method of JA3 against test organisms

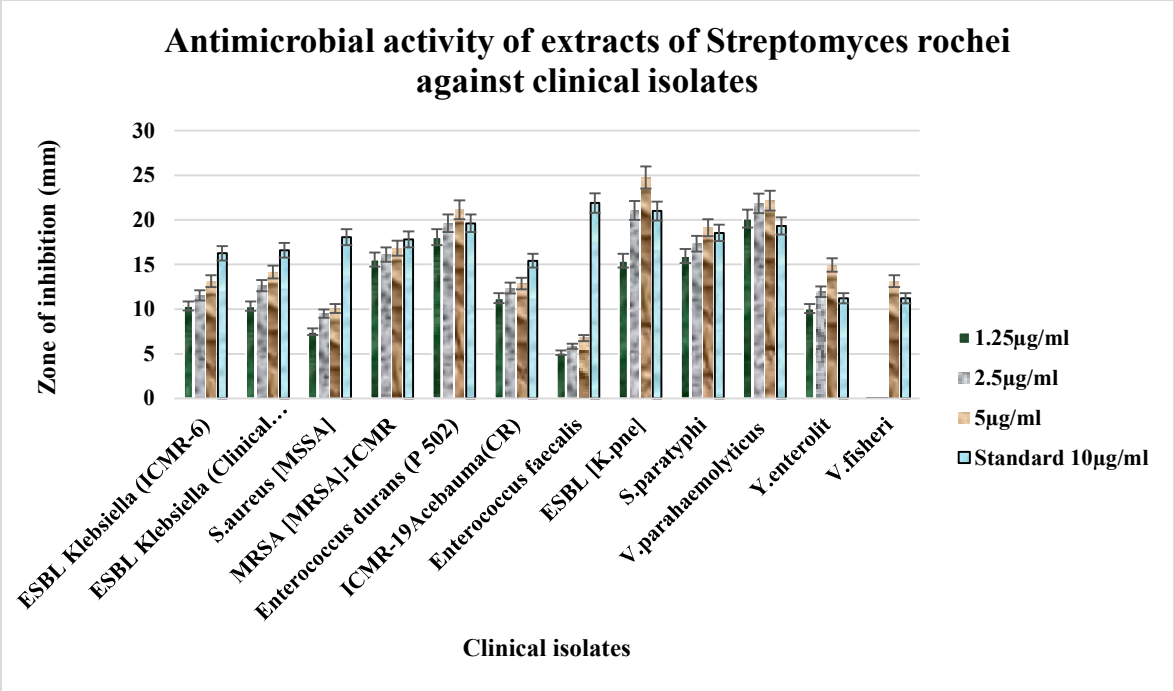


Figure 2: Graphical representation of antimicrobial activity of isolate JA3

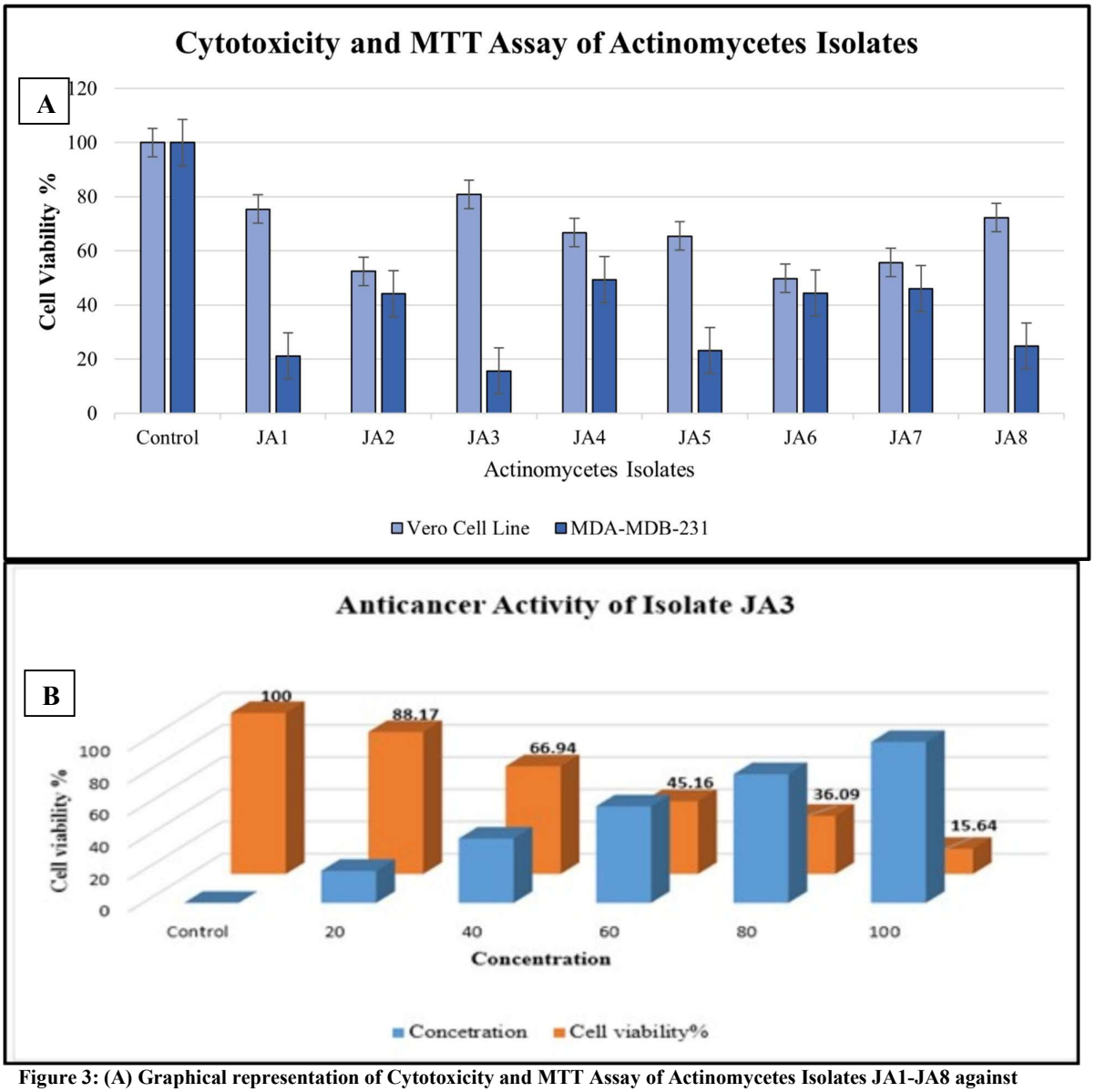


Figure 3: (A) Graphical representation of Cytotoxicity and MTT Assay of Actinomycetes Isolates JA1-JA8 against Vero Cell Line and MDA-MDB-231 (B) Graphical representation of Anticancer activity of *Streptomyces rochei*

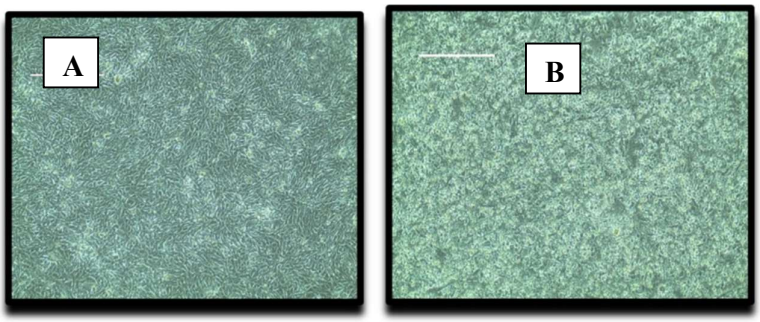
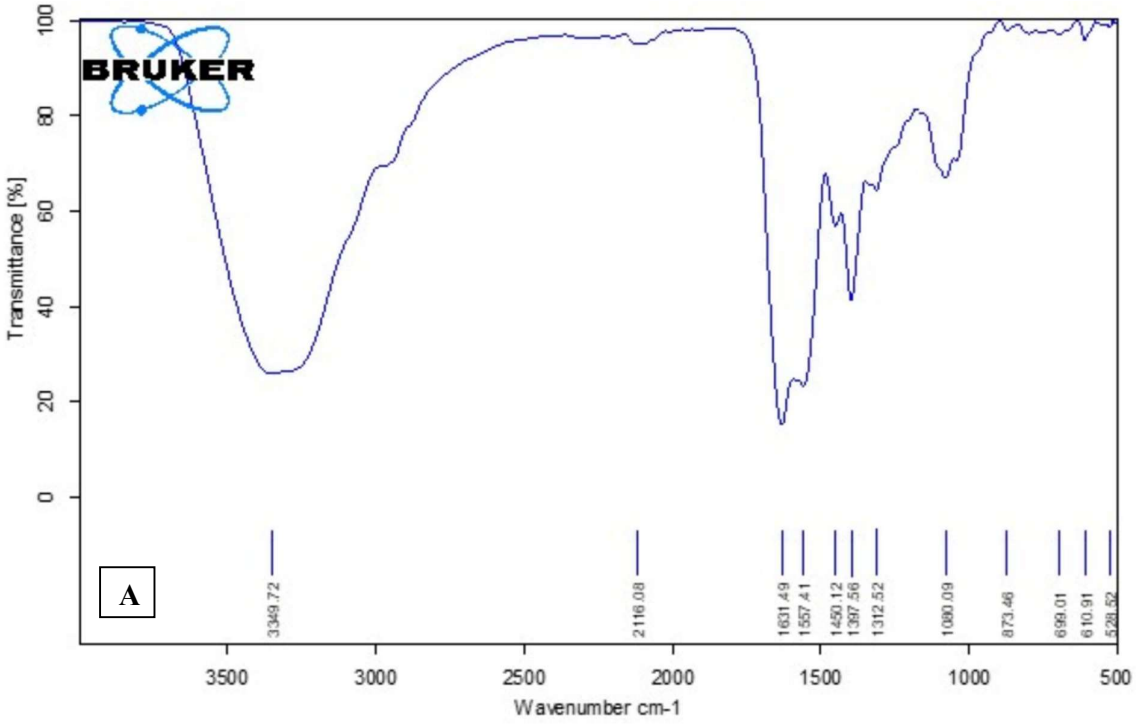


Figure 4: (A) Control (Vero Cell Line) (B) Toxicity of JA3 (MDA-MDB-231)



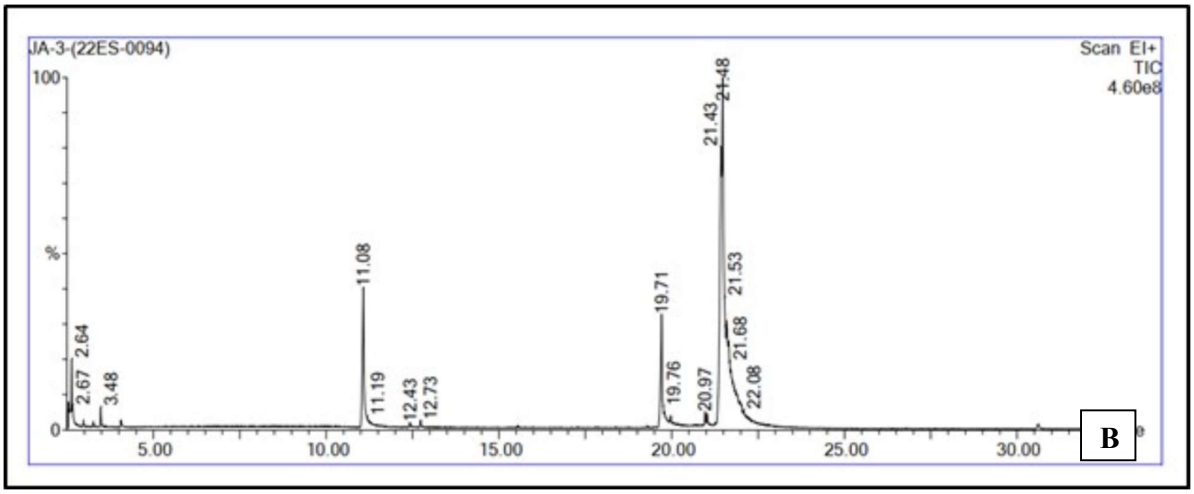


Figure 5: (A) FT-IR Analysis of Isolate JA3 (B) GC-MS Analysis of isolate JA3

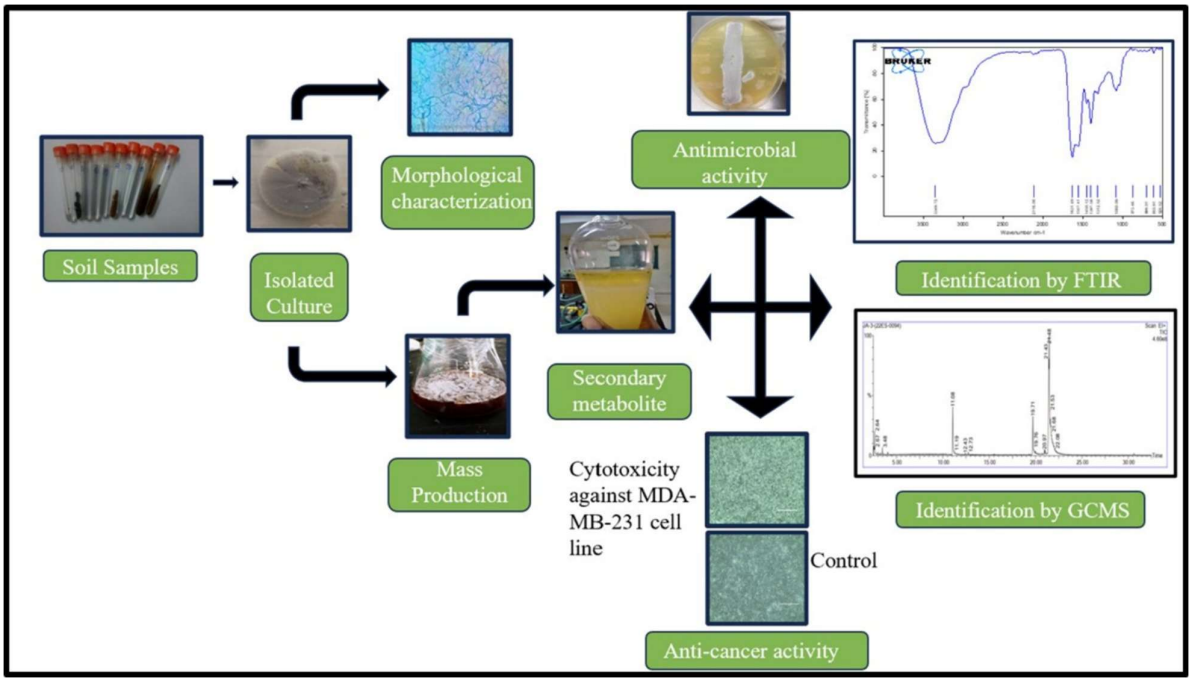


Figure 6: Graphical abstract of Antimicrobial and Anticancer Properties of *Streptomyces rochei* JA246

4. CONCLUSION

The study delineates the isolation of actinomycetes isolates, from central and south India. 35 strains of actinomycetes were tested for their antimicrobial activity by the perpendicular streak method against test isolates. 8 isolates showed potent antimicrobial activity, among which isolate JA3 showed substantial antimicrobial against test isolates, marking a maximum zone of inhibition against *ESBL [Klebsiella pneumonia]* (24.76±0.115mm at 5µg/ml) which was comparatively higher than the standard Streptomycin (21±0.115mm at 10µg/ml). The second highest zone of clearance was against *Vibrio parahaemolyticus* (MTCC 451) (22.16±0.115mm at 5µg/ml). Anticancer activity against MDA-MDB-231 cell line showed a dose-dependent reduction in the percentage viability of cell line. The ethyl acetate extracts of isolate JA3 were extracted and characterized by FT-IR and GC-MS Analysis. The major compound was identified to be Cyclopropane, 1-Heptyl-2-Methyl. The potent *Kitasatosporales* was identified as *Streptomyces rochei* JA246, NCBI ID: OP422232). Further investigations are required to analyze the bioactive metabolites of isolate JA3 for isolation and structural purification of antimicrobial and anticancer drugs **(Figure 6)**.

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