Exploration of bioactive compounds from marine derived *Streptomyces coelicolor* strain KR23 for insights into antimicrobial potential and secondary metabolite profiling

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Abstract

This research examines the antimicrobial efficacy of Actinomycetes procured from marine sediment specimens, with a particular emphasis on the Streptomyces coelicolor strain KR23. The phylogenetic assessment, predicated on 16S rRNA gene sequencing, indicated that the isolated strain exhibits a close phylogenetic relationship with S. coelicolor, a species renowned for its extensive biosynthetic and metabolic functionalities. The extraction of bioactive compounds was conducted utilizing ethyl acetate and antimicrobial evaluations revealed considerable activity against a variety of bacterial pathogens including Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pyogenes. The analysis via Gas Chromatography-Mass Spectrometry (GC-MS) discerned crucial bioactive compounds such as 2-phenylacetamide, succinic acid, plicamycin, uric acid, Gentamicin and l-tyrosine butyl ester.

These compounds, which demonstrate a spectrum of action mechanisms, underscore the potential of S. coelicolor strain KR23 as a significant resource for the innovation of novel antimicrobial agents. Additional investigations are warranted to elucidate the synergistic interactions of these compounds and their implications in addressing antibiotic-resistant bacterial strains.

Keywords: *S. coelicolor,* Bioactive compounds, Antimicrobial activity, GCMS.

Introduction

Streptomyces coelicolor is a prominent model organism in microbiological research, particularly known for its antibiotic production and complex developmental processes. This bacterium has been extensively studied for over 80 years, revealing its potential in producing a wide range of bioactive secondary metabolites including antibiotics, which are crucial in medicine and biotechnology.

S. coelicolor is renowned for synthesizing over 20 gene clusters responsible for various antibiotics such as undecylprodigiosin and actinorodine[12]. Its metabolites exhibit antimicrobial properties against resistant pathogens, making it a valuable resource for developing new therapeutic agents[1].

Research has also shown that *S. coelicolor* plays a vital role in understanding the genetic and biochemical pathways involved in anticancer drug discovery, paving the way for innovative treatments that target specific cancer cell mechanisms while minimizing damage to healthy tissues[3]. This unique bacterium not only contributes to antibiotic production but also serves as a model organism for studying secondary metabolite biosynthesis, enhancing our knowledge of how microorganisms can be harnessed in pharmaceutical applications. It also exhibits significant antioxidant properties, which are primarily attributed to its ability to produce various Bioactive compounds.

Present study isolation of *S. coelicolor* strains KR23 aims to explore their potential bioactive compounds through GCMS studies, further expanding the antimicrobial properties of these strains and their potential therapeutic applications in combating wound healing related application[17]. Antibiotic overuse and prescription have been identified as one of the mechanisms contributing to the bacterial population's resistance, which poses a serious public health risk since diseases that were previously manageable, are harder to control. This alarming trend underscores the urgent need for more judicious practices and public education on the appropriate use of antibiotics to preserve their effectiveness for future generations[6].

S. coelicolor is a notable species of soil-dwelling bacteria that has gained attention for its ability to produce a variety of bioactive compounds including antibiotics like actinomycin and streptomycin highlighting the importance of exploring natural sources in the fight against antibiotic resistance[3,8]. This comprehensive evaluation will not only enhance our understanding of the bioactive profile of *S. coelicolor* strains but also will pave the way for innovative approaches in nutraceutical development aimed at promoting longevity and disease prevention.

Material and Methods

Sample Collection: Mangrove soil originating from a specific site in India was procured for research purposes. The soil sample was collected from the Vellar estuary, Parangipettai, Tamil Nadu, India. The collection process involved obtaining soil specimens from a depth of 15

centimetres beneath the soil's surface, which were subsequently placed in aseptic containers for transportation to the laboratory. The process of isolating Actinomycetes from the mangrove soil entailed utilizing a starch casein agar (SCA) medium comprising of a mixture of 50% seawater and 50% distilled water.

Molecular Characterization: The bacterial genomic DNA purification kit (Himedia) was used to isolate the total genomic DNA. Primers of 16S rRNA 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492 R (5'-TACGGCTACCTTGTTACGACTT -3') were used to amplify the 1500bp gene. The following were the PCR (Prime96 – Himedia) conditions: Initial denaturation 94°C for 3min, followed by 30 cycle of denaturation at 94°C for 1min, annealing at 55oC for 1min, extension at 72°C for 1 min and final extension at 72°C for 3 min. The amplified product was confirmed with 1.2% of agarose gel.

The amplified products were sequenced with Sanger Sequencing DNA analyzer (Barcode BioSciences – Bengaluru). To determine the identification and degree of similarity with other species in the database, the 16S rRNA sequence was input into the NCBI nucleotide blast. The sequence alignment and generation of phylogenetic tree were used in MEGA 11 software.

Production and Extraction of Bioactive Compounds: All media utilized in this research were formulated using 50 mL of filtered seawater and 50 mL of distilled water. Consequently, the growth was maximized at this specific ratio. Spores from the prospective actinomycete strain were harvested from starch casein agar and subsequently inoculated into 50 mL of inoculation medium contained within a 250 mL conical flask, which was maintained in a rotary shaker at 120 rpm for 48 hours at a temperature of 28∘ C. Following this, 10% of the inoculum was transferred into 100 mL of production medium and incubated in a rotary shaker at 120 rpm for a duration of 7 days at 28°C.

Upon completion of fermentation, mycelium and supernatant were initially separated through filtration and subsequently via centrifugation at 10,000 rpm for 30 minutes at 4∘ C. The extracellular compounds from the culture supernatant were extracted employing a liquid-liquid extraction technique utilizing an equivalent volume of ethyl acetate and concentrated using rotary evaporation.

Antimicrobial Activity: The assay was conducted by agar well diffusion method[7]. Spreading method was done by using test organisms i.e. *E.coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes* on Muller Hinton agar plates. The suspension was used to inoculate 90mm diameter Petri plates. Wells were punched and filled with 50ul of extract and 10ug of rifampicin as a positive control. Plates were incubated at 37oC for 24 hrs. Antibacterial activity was evaluated by measuring the zone of inhibition in diameter.

Antioxidant Activity

Determination of the Total Phenolics: The quantification of total phenolic compounds in four fractions (bioactive compounds) was conducted using spectrophotometric analysis with the Folin-Ciocalteu reagent, following a slightly modified protocol established by Tan et al[16]. The extract was combined with Folin-Ciocalteu reagent in a 1:1 ratio, followed by the addition of 4 mL of 1 M sodium carbonate and the mixture was permitted to stand for 15 minutes. The absorbance was subsequently measured spectrophotometrically at a wavelength of 765 nm. A standard curve was established utilizing diverse concentration of gallic acid (100 –1000 μg/mL), facilitating the quantification of the total phenolic content of the extract expressed as µg gallic acid equivalents (GAE) per mg of extract. The assay was conducted in triplicate and the findings were averaged to enhance precision.

Total Antioxidant Activity: The comprehensive antioxidant activity of the fractions was evaluated using the protocol delineated by Prieto et al[9]. An aliquot of 0.3 mL from each fraction was amalgamated with 3.0 mL of the reagent solution, which comprised of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The reaction mixture was subsequently incubated at a temperature of 95°C for a period of 90 minutes in a regulated water bath. The absorbance of each sample mixture was measured at a wavelength of 695 nm. Ascorbic acid at a concentration of 100 µg/mL was utilized as the standard control.

Free Radical Scavenging Activity by DPPH: The free radical scavenging potential of each fraction was evaluated utilizing DPPH (1,1-diphenyl-2-picrylhydrazyl)[11]. A volume of 2 mL of DPPH solution (0.002% in methanol) was combined with 2 mL of various concentrations (5–200 μg/mL) of each fraction and the standard (ascorbic acid) in individual tubes. The tubes were allowed to incubate in the dark at room temperature for a duration of 30 minutes, after which the optical density was assessed at 517 nm employing a UV-Vis spectrophotometer. The absorbance of the DPPH control (lacking extract/standard) was recorded. The scavenging activity was determined using the equation:

$$\text{Scavenging activity (\%)} = [(A - B)/A] \times 100$$

where A represents the absorbance of the DPPH control and B denotes the absorbance of DPPH in the presence of the extract/standard.

Total Reducing Power: The total reducing capacity of the extracts was assessed in accordance with the methodology established by Subramaniam et al[13]. The fractions containing bioactive compounds (100 μg/mL) were combined with 1% potassium ferricyanide and subsequently incubated at 50∘ C for a duration of 20 minutes; thereafter, 2.5 mL of 10% trichloroacetic acid (TCA) was introduced to the mixture and centrifuged at 5000 rpm for 10 minutes. The supernatant (2.5

mL) was then blended with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride and the resultant color intensity was quantified at 700 nm. Ascorbic acid (100 μg/mL) served as the standard control.

Identification of Bioactive compounds with GCMS: The analysis of the active ethyl acetate extract was executed by Gas Chromatography Mass Spectrometry (GCMS). For component identification, the Shimadzu QP2010 ultra has been used. The appliance was made using fused silica capillaries from Elite-1. Helium gas (99.99%) was the carrier gas with a constant flow rate of 1.21 ml/min and split ratio:10. Temperature of injector was set on 160°C; Ion-source temperature was 200°C. The oven temperature was intended from 60°C with an increment of 280°C for around 22 min. Mass spectra were taken at 70eV at a scan interval of 0.5 seconds. The chemical composition of the extract was determined by measuring the peak area and the retention time by comparing the NIST 14 library[4,5].

Statistical Analysis: The experiment was conducted in triplicate. The outcome was presented as mean ± standard deviation (SD). The statistical package for the social sciences (SPSS) software was utilized to conduct the statistical analysis for the antimicrobial activity.

Results and Discussion

The current investigation was conducted utilizing marine sediment samples, wherein isolated Actinomycetes were characterized through the amplification and sequencing of the 16S rRNA gene and searched by NCBI nucleotide Blast to confirm as *Streptomyces coelicolor* Strain KR23. Sequence was aligned and deposited to NCBI GenBank with accession no: PQ606398. The phylogenetic affiliations with other *Streptomyces* species used MEGA 11.0 software[14]. The findings substantiated that *Streptomyces coelicolor* represents the closest relative of Neighbor Joining method[10,15]. Fig. 1 suggests that the isolated Actinomycetes exhibits substantial genetic congruence with this extensively researched species, renowned for its antibiotic biosynthesis and metabolic functions.

The *S. coelicolor* strain KR23 was employed to extract bioactive compounds utilizing ethyl acetate, recognized for its capacity to synthesize a diverse range of secondary metabolites, which demonstrated antimicrobial activity against *Escherichia coli* (10mm), *Bacillus subtilis* (12mm), *Pseudomonas aeruginosa* (8mm), *Staphylococcus aureus* (11mm) and *Streptococcus pyogenes* (10mm) as in fig. 2. The observed activity at significant levels underscores the potential of these compounds for development into novel antimicrobial agents, which could play a pivotal role in mitigating antibiotic resistance and enhancing therapeutic alternatives.

The antioxidant characteristics exhibited by *S.coelicolor* KR23 are illustrated in the figure 4, indicating total phenol at 21.75 ± 0.38, total antioxidant at 25.01 ± 0.29, free radical scavenging activity (DPPH) at 18.5 ± 0.53 and reducing power at 17.26 ± 0.44 in comparison to the standards of gallic acid and ascorbic acid. Further analysis of the extract was conducted via Gas Chromatography-Mass Spectrometry (GC-MS) to identify the specific bioactive compounds present, unveiling a heterogeneous assortment of substances that may contribute to its antimicrobial efficacy. The outcomes of the GC-MS analysis elucidated several crucial compounds as in fig. 3 and table 4, comprising of 2-phenylacetamide, succinic acid, plicamycin, uric acid, gentamicin and l-tyrosine butyl ester identified within the extract.

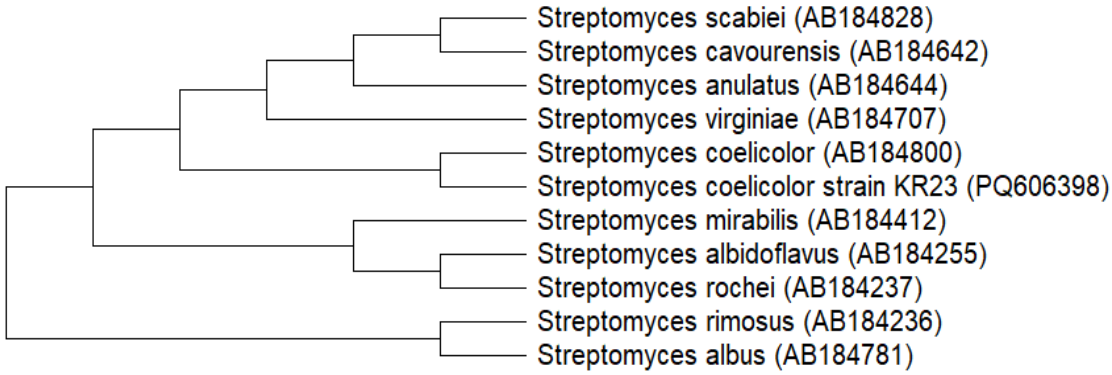

Figure 1: Neighbor Joining tree for *Streptomyces coelicolor* KR23 (PQ606398)

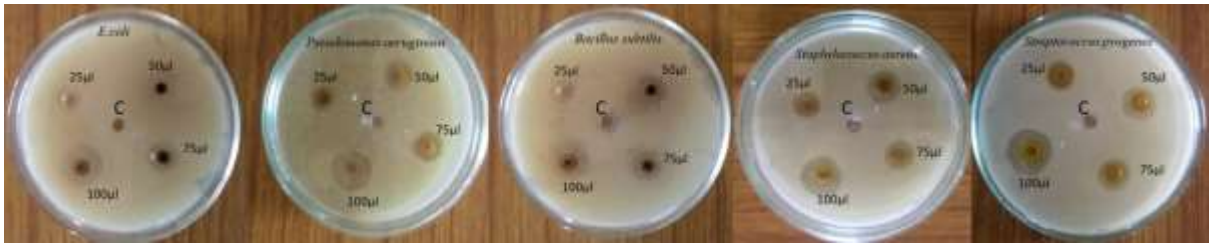

Figure 2: Antimicrobial activity of *Streptomyces coelicolor* KR23 against selected clinical organism

Table 1
Bioactive compounds identified through GCMS using *Streptomyces coelicolor* KR23 extract.

S.N.	RT	Name of the Compound	Formula	M.W	Biological Activity
1	5.161	Cyclohexanol	$C_6H_{12}O$	100.16	Insecticide
2	7.364	1-Methoxy-2-propyl acetate	$C_6H_{12}O_3$	132.16	Cosmetic use
3	7.742	Trimethylolpropane	$C_6H_{14}O_3$	134.17	Oil industry
4	8.033	Phenylacetaldehyde	C_8H_8O	120.15	Pesticide
5	8.188	(S)-Ethyl 2-aminopropanoate hydrochloride	$C_5H_{12}ClNO_2$	153.61	Cosmetic use
6	9.054	Phenethylamine	$C_8H_{11}N$	121.18	antidepressive agents
7	10.662	l-Valine, ethyl ester	$C_7H_{15}NO_2$	145.2	Antiviral agent
8	11.808	DL-Norleucine	$C_6H_{13}NO_2$	131.17	Anticancer drug
9	12.042	2-Phenylacetamide	C_8H_9NO	135.16	Anti Microbial agent
10	12.092	DL-Norleucine	$C_6H_{13}NO_2$	131.17	Anticancer drug
11	13.765	succinic acid	$C_4H_6O_4$		Antibiotic
12	13.867	3-Methylbutyraldehyde oxime	$C_5H_{11}NO$	101.15	Anti Skinning Agent
13	14.331	succinic acid	$C_4H_6O_4$	118.09	Antibiotic
14	15.662	Fumaric acid, ethyl 2-methylallyl ester	$C_{10}H_{14}O_4$	198.22	Unknown
15	16.348	Ethyl Alpha-D-Glucoside	$C_8H_{16}O_6$	208.21	Anti-fungal
16	16.408	5-Cyclopropylpyrrolidin-2-one	$C_7H_{11}NO$	125.17	Unknown
17	16.55	Methyl beta-D-glucopyranoside	$C_7H_{14}O_6$	194.18	Antidiabetic
18	16.985	Plicamycin	$C_{52}H_{76}O_{24}$	1085.1	Antimicrobial agent
19	17.335	Uric acid	$C_5H_4N_4O_3$	168.11	Antimicrobial, Anticancer
20	18.497	Gentamicin	$C_{21}H_{43}N_5O_7$	477.6	Antimicrobial agent
21	19.827	L-Ascorbyl 2,6-Dipalmitate	$C_{38}H_{68}O_8$	652.9	Cosmetic use
22	20.225	l-Tyrosine butyl ester	$C_{13}H_{19}NO_3$	237.29	Antimicrobial, Anticancer
23	21.575	Arachidonic acid	$C_{20}H_{32}O_2$	304.5	Anticancer Activity
24	23.152	9-octadecenoic acid	$C_{18}H_{34}O_2$	282.5	Anticancer Activity
25	24.235	Pimprinine	$C_{12}H_{10}N_2O$	198.22	Antifungal Agent

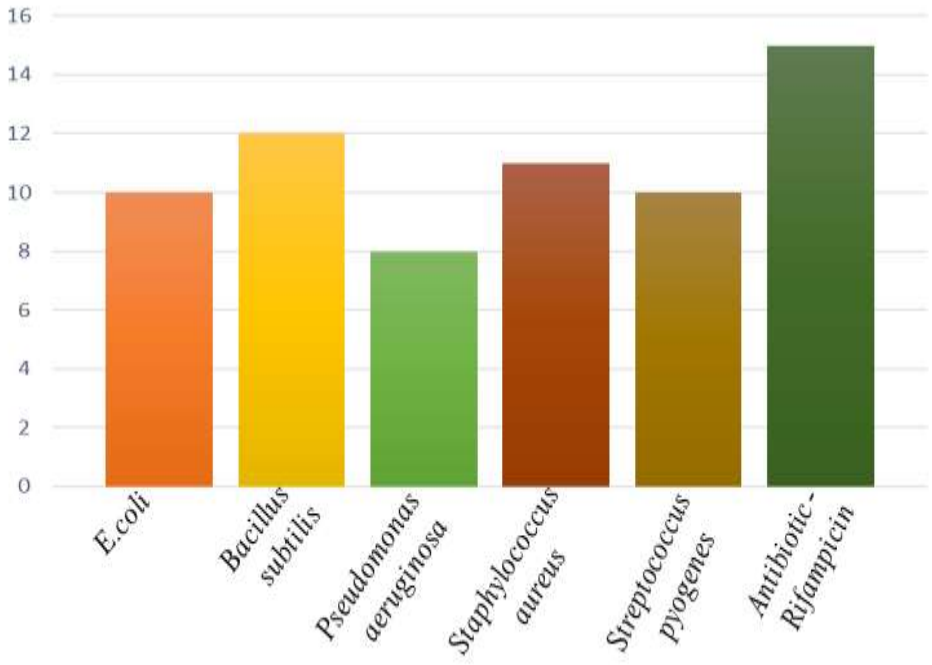

Figure 3: Antimicrobial activity of *Streptomyces coelicolor* KR23 against selected clinical organism.

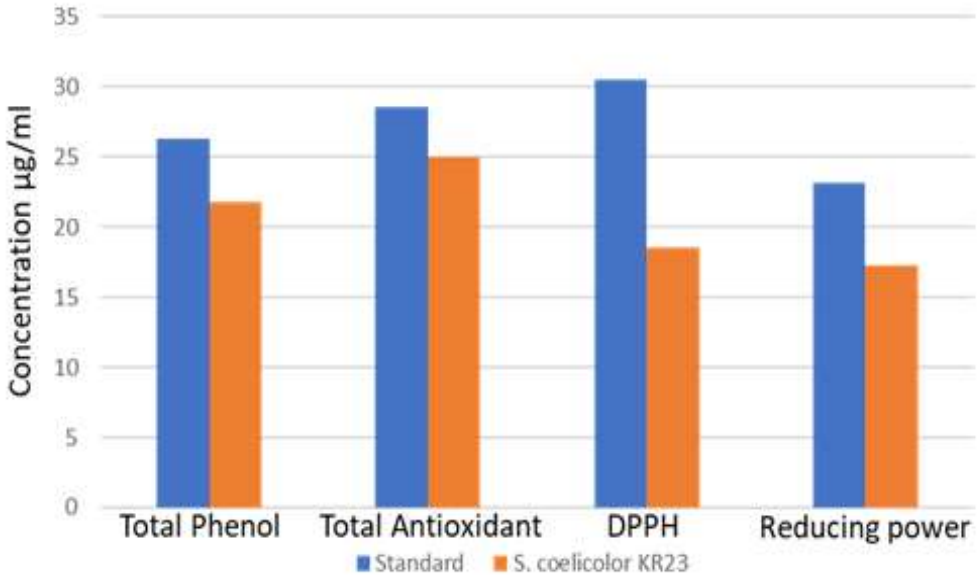

Figure 4: Antioxidant Properties Derived *Streptomyces coelicolor* KR23

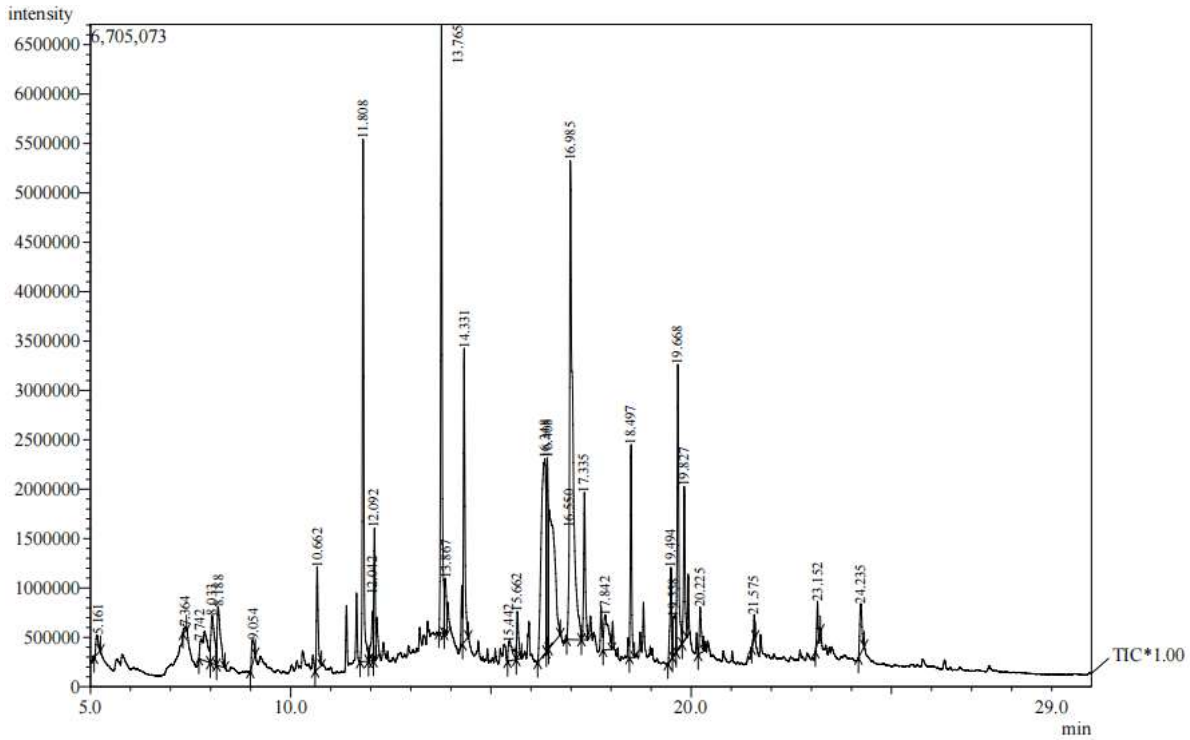

Figure 5: Chromatogram of GCMS for *Streptomyces coelicolor* KR23

The compounds, each characterized by distinct mechanisms of action, imply a multifaceted strategy in combating bacterial infections and necessitate further exploration into their individual contributions to antimicrobial effectiveness. Further studies are essential to determine the synergistic effects of these compounds and how they can be optimized for potential therapeutic applications in treating resistant bacterial strains. To find the activity against cancer, researchers are now investigating the potential of these substances not only for their antibacterial properties but also for their roles in inhibiting tumor growth and promoting apoptosis in cancer cells.

Conclusion

In conclusion, this study highlights the significant potential of Actinomycetes isolated from marine sediment, particularly *Streptomyces coelicolor* strain KR23 as a prolific source of bioactive compounds. The observed antimicrobial activity against multiple pathogenic bacteria underscores its relevance in addressing the escalating challenge of antibiotic resistance and also has a potential enhancement of antioxidant properties. Additionally, GCMS analysis revealed a diverse array of compounds with promising therapeutic applications, suggesting a multifaceted approach to combating bacterial infections.

Further investigations into the synergistic effects of these compounds and their potential role in cancer treatment, including tumor inhibition and apoptosis promotion, could pave the way for novel advancements in antimicrobial and anticancer therapies.

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