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Antimicrobial Potential and Chemical Characterization of *Streptomyces Fulvissimus* Isolated from Salt Pan Soils of Nagapattinam District, Tamil Nadu, India

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KEYWORDS

Salt pan soil, Actinomycetes, Streptomyces fulvissimus, Multidrug-resistant pathogens, GC–MS, FT-IR

ABSTRACT:
Since the emergence of multidrug-resistant (MDR) bacterial pathogens is becoming widespread, there is a need for the discovery of new antimicrobial agents from unexplored ecological niches. Salt pans are extreme environments that contain halophilic and halotolerant Actinomycetes with potential biosynthetic capabilities. In this study, Actinomycetes were isolated from salt pan soils of Nagapattinam District, Tamil Nadu, India, and were screened for antibacterial activity against MDR pathogens. Primary and secondary screening identified a potent isolate, H5, which showed broad-spectrum antibacterial activity. Morphological, physiological, and biochemical characterization suggested that the isolate belonged to the genus Streptomyces. 16S rRNA gene sequencing confirmed the isolate as Streptomyces fulvissimus. The ethyl acetate crude extract of the H5 strain showed maximum antibacterial activity against Escherichia coli, Enterococcus faecalis, and Pseudomonas aeruginosa. Gas chromatography–mass spectrometry (GC–MS) analysis revealed the presence of several bioactive compounds, including N-hexadecanoic acid, oleic acid, squalene, and purine derivatives. Fourier-transform infrared (FT-IR) spectroscopy confirmed the presence of functional groups linked to antimicrobial activity. The results suggest that Streptomyces fulvissimus isolated from salt pan soils could be a potential source of bioactive secondary metabolites that can be exploited in the treatment of MDR bacterial infections.

1.INTRODUCTION

The rapid emergence of MDR bacteria is one of the most urgent global public health challenges in the world today, as the incidence rates of MDR bacterial pathogens increase globally and compromise traditional antibiotics in modern medical treatments. These factors include inappropriate prescribing or overprescribing; excessive and repeated use; misuse and abuse; and inappropriate doses; all of which have contributed to increased antibiotic resistance to infections caused by Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, and Enterococcus faecalis in a world that is in desperate need of new antimicrobial compounds from renewable sources (1).

Filamentous, Gram-positive *Actinomycetes* are also known to be producers of bioactive secondary metabolites, especially members of the genera *Streptomyces* and *Nocardiopsis*, which account for nearly two-thirds of all clinically used antibiotics of natural origin, with the ability to produce antibacterial, antifungal, antiviral, antitumor and immunosuppressive compounds (2). Research has shown that soil and extreme environments, such as saline habitats, solar salterns and coastal ecosystems, are reservoirs for rare and halophilic *Actinomycetes* with high biotechnological potential; for example, Arul Jose and Jebakumar described several genera of *Actinomycetes* from Indian coastal solar salterns, including *Streptomyces*,

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Micromonospora, *Nocardia* and *Nocardiopsis*, which have potential to produce novel antimicrobials (3).

In fact, several research groups have isolated antibiotic-producing *Actinomycetes* from soil and saline environments that were effective against MDR pathogens, such as those reported by Bajagain and Limbu (4), who found that soil *Actinomycetes* displayed potent antibacterial activity against both Gram-positive and Gram-negative bacteria, and Daigham and Mahfouz (5), who isolated *Streptomyces* species from Egyptian soils and demonstrated that ethyl acetate extracts had strong antibacterial and cytotoxic activities with FT-IR confirming the presence of bioactive functional groups associated with antimicrobial activity.

Drug-resistance is an important global health concern as pathogenic bacteria have developed multiple mechanisms of resistance to the currently available antibiotics; hence, there is an urgent need to discover new antimicrobial compounds with novel mechanisms of action. *Actinomycetes* are known to produce secondary metabolites (most of which are clinically used antibiotics) and some species of *Streptomyces* are prolific producers. Salt pans are extreme environments with high salinity, intense solar radiation, and low nutrient availability; these environments are known to select for microbial populations that evolve new metabolic pathways, some of which lead to production of bioactive compounds (6). The diversity of *Actinomycetes* in salt pan soils is relatively unexplored, as are their antimicrobial properties, and so we isolated *Actinomycetes* from different types of soil samples from two types of salt pans in Nagapattinam District and tested for activity against MDR bacterial pathogens, with the active metabolites characterized by molecular techniques.

In the present study, active *Actinomycetes* from soil were isolated, screened for antibacterial activity against MDR bacterial pathogens, and the bioactive secondary metabolites were identified using GC–MS and FT-IR analysis to identify the most promising isolate (A1 – *Nocardiopsis* sp. / H5 – *Streptomyces* flavidofuscus) as a potential source of novel antibacterial compounds for the treatment of antibiotic-resistant infections.

Materials and Methods

Study Area and Sample Collection

Samples of soil from different locations of the coastal salt pans in Nagapattinam District, Tamil Nadu, India were aseptically collected at 5–10 cm depth using sterile spatulas, transferred into sterile polyethylene bags, labeled and transported to the laboratory. The samples were air-dried at room temperature for 7 days to inhibit the growth of rapidly growing bacteria and fungi and to increase the population of *Actinomycetes*. (7)

Isolation of *Actinomycetes*

A dried soil sample (1 g) was suspended in 9 mL of sterile physiological saline and serially diluted up to 10^{-6}. 0.1 mL was spread from appropriate dilutions onto Starch Casein Agar (SCA) supplemented with nalidixic acid (25 µg/mL) and nystatin (50 µg/mL) to inhibit bacterial and fungal contaminants. Plates were incubated at 30 ± 2 °C for 7–10 days. Individual chalky, leathery colonies characteristic of *Actinomycetes* were isolated and repeatedly sub-cultured to obtain pure isolates.(8)

Test Pathogens

Multi drug resistant strains are *Pseudomonas aeruginosa, Escherichia coli, A.baumanii* and *Enterococcus faecalis* with known multidrug resistance were collected from a tertiary care hospital, Tamil Nadu. Cultures were maintained on nutrient agar slants at 4 °C and sub-cultured before use.

Primary Screening of Antagonistic Activity (Cross-Streak Method)

Primary screening was carried out by the cross-streak method. Actinomycete isolates were streaked as a single line on Mueller-Hinton Agar plates and incubated at 30 °C for 5 days. Test pathogens were streaked perpendicular to the actinomycete growth and incubated at 37 °C for 24 h. Inhibition was noted as no inhibition (–), moderate inhibition (+) and high inhibition (++). (9)

Secondary Screening Using Cell-Free Supernatant

Potent isolates were grown in Yeast Extract Malt Extract Broth (YEMB) at 30 °C, 150 rpm for 7 days. Cultures were centrifuged at 10,000 rpm for 10 min and the supernatant was collected. Antibacterial activity was evaluated by agar well diffusion method: wells (6 mm) were filled with 100 µL of supernatant and incubated at

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37 °C for 24 h, Zones of inhibition were measured in millimeters. (10)

Morphological, Physiological and Biochemical Characterization

Colony morphology, aerial mycelium, spore arrangement and pigmentation, Gram staining, motility tests, and biochemical tests (Indole, Methyl Red, Voges–Proskauer, Triple Sugar Iron, Urease, Nitrate reduction, Catalase and Oxidase tests) were carried out according to Bergey's Manual. (11)

Molecular Identification by 16S rRNA Sequencing

Genomic DNA was extracted using a commercial bacterial DNA isolation kit and the 16S rRNA gene was amplified using universal primers 27F and 1492R. PCR products were sequenced and compared with NCBI BLAST database, and a phylogenetic tree was constructed using MEGA software to confirm the identity of the isolate. (11)

Production and Extraction of Secondary Metabolites

An active isolate was cultivated in YEMB for 7 days, centrifuged, and extracted with ethyl acetate (1:1 v/v).

Antibacterial Activity of Ethyl Acetate Crude Extract

The organic layer was evaporated using a rotary evaporator to yield crude extract, dissolved in DMSO, and tested for antibacterial activity by the agar well diffusion method using different concentrations (25, 50, 75, and 100 µL) of crude extract. Zones of inhibition were measured and compared with positive (standard antibiotic) and negative (DMSO) controls. (9)

GC–MS Analysis

GC–MS analysis of the ethyl acetate extract was performed using a GC-MS system equipped with a DB-5 column, carrier gas (helium), and mass spectra matched to the NIST library to identify bioactive compounds. (10, 11)

FT-IR Analysis

Functional groups in the crude extract were determined using FT-IR analysis using Perkin Elmer FT-IR spectrophotometer in the range of 4000–600 cm^{-1} using KBr pellet method. 3.12 Statistical Analysis All experiments were conducted in triplicates. (11)

Statistical Analysis

Data were expressed as mean ± SD. Statistical significance was determined using one-way ANOVA, with $p < 0.05$ considered significant.

Results

Sample Collection

Halophilic *Actinomycetes* were isolated from aseptically collected salt pan soil samples in Nagapattinam District, which were air-dried, serially diluted, and plated on selective media appropriate for *Actinomycetes* isolation; distinct colonies were subcultured repeatedly to obtain pure isolates. (Fig 1)

Fig 1: Saltpan Soil sample

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Primary Screening of Antagonistic Activity

The antagonistic potential of five halophilic/halotolerant bacterial isolates (HA3, HA5, HA7, HA8 and HA9) was evaluated against three multidrug-resistant (MDR) bacterial pathogens, *Pseudomonas aeruginosa, Escherichia coli* and *Enterococcus faecalis* using the cross-streak method.

Among the isolates, HA5 exhibited the strongest and broad-spectrum antagonistic activity, showing high inhibition (++ zones) against all three pathogens, whereas HA8 and HA9 showed moderate to high inhibition. In contrast, HA3 and HA7 showed limited or no activity against *P. aeruginosa*. This clearly demonstrates that HA5 is the most potent antagonistic isolate with promising antibacterial potential.

Table 1: Antagonistic activity against MDR Bacterial Pathogens

S.No	**Isolates**	*Pseudomonas aeuroginisa*	*Escherchia Coli*	*Enterococcus faecalis*	*Acienetobacter baumani*
1	HA3	-	-	+	-
2	**HA5**	+++	++	++	++
3	**HA8**	+	++	++	++
4	HA7	-	+	+	-
5	**HA9**	+	++	+	++

- : No inhibition, + : moderate inhibition, ++ : high inhibition

4.3 Secondary Screening Using Cell-Free Supernatant

Based on the primary screening, HA5, HA8 and HA9 were selected for secondary screening using cell-free supernatants. The well diffusion assay revealed that HA5 produced the largest zones of inhibition against all MDR pathogens, confirming that extracellular secondary metabolites were responsible for the antibacterial activity.

HA5 showed zones of 20 ± 1.00 mm against *P. aeruginosa*, 17 ± 0.58 mm against *E. coli* and 18 ± 1.53 mm against *E. faecalis*, which were significantly higher than HA8 and HA9. This indicates that HA5 secretes potent antibacterial compounds capable of inhibiting both Gram-negative and Gram-positive pathogens.

Table. 2. Secondary screening of cell-free supernatant of strains against MDR Pathogens

S.No	**Isolates**	*Pseudomonas aeuroginisa*	*Escherchia Coli*	*Acienetobacter baumanii*	*Enterococcus faecalis*
1	**HA5**	15±0.58	**17±0.58**	**20±1.00**	**18±1.53**
2	HA8	20±1.00	15±0.58	15±0.58	16±1.00
3	HA9	16±1.00	12±1.00	16±1.00	11±1.00

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Value represents mean ±SD; n=3, - No zone

Physiological, Cultural and Biochemical Characterization of H5

The potent isolate HA5 was subjected to detailed physiological and biochemical characterization. Morphological observations revealed circular colonies with white aerial mycelium and straight long chains of spores, typical of *Streptomyces* species. Gram staining showed Gram-positive filamentous morphology, confirming its actinomycetal nature.

Biochemical tests further supported this identification, as HA5 showed positive results for Indole, Methyl Red, Voges-Proskauer, TSI, Urease, Catalase and Oxidase tests, while being negative for nitrate reduction. Based on these phenotypic and biochemical traits, the isolate was tentatively identified as *Streptomyces* sp.

Table. 3. Physiological characteristics of H5 Strain

S.No	**Test**	**H5**
1	Gram staining	+
2	Motility	-
3	Colony form	Circular
4	Spores arrangement	Straight long chain of spores
5	Aerial mycelium and colour of the spore	White
6	Melanin pigment	-

-Absent; +Present;

Table. 4. Biochemical Identification of H5 Strain

S.No	Biochemical Test	H5	Tentative genera
1.	Indole	Positive	*Streptomyces* sp
2	Methyl- Red	Positive	*Streptomyces* sp
3	Voges-Proskaeur	Positive	*Streptomyces* sp
4	Triple Sugar Iron	Positive	*Streptomyces* sp
5	Urease	Positive	*Streptomyces* sp
6	Nitrate	Negative	*Streptomyces* sp
7	Catalase	Positive	*Streptomyces* sp
8	Oxidase	Positive	*Streptomyces* sp

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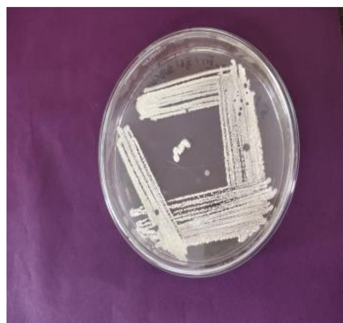

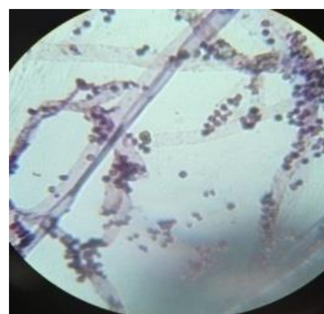

Fig.2. H5 Strain **Fig.3. Gram's staining (100 X)**

4.4 Molecular Identification

To confirm the identity, the 16S rRNA gene of H5 was sequenced and analyzed. Phylogenetic analysis showed a close relationship with *Streptomyces flavidofuscus*, confirming the isolate as *Streptomyces flavidofuscus*. This molecular confirmation validates the biochemical identification and highlights the strain as a promising antibiotic-producing actinomycete **(Fig.4).**

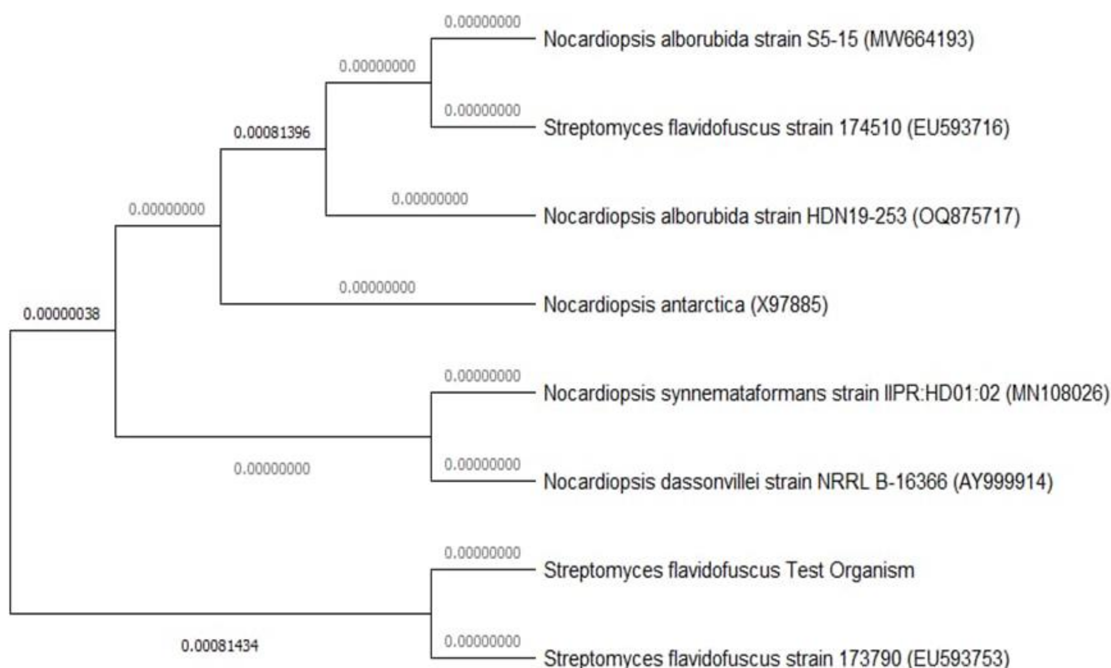

Fig.4. Phylogenetic tree of H5 Strain (*Streptomyces flavidofuscus*)

Antibacterial Activity of Ethyl Acetate Crude Extract

The antibacterial efficacy of ethyl acetate crude extract of H5 was evaluated at different concentrations (25, 50, 75 and 100 µl). A clear dose-dependent increase in antibacterial activity was observed. At 100 µl, the extract showed maximum inhibition against *A.baumanii* (26 mm), followed by *E. coli* (24 mm), *E. faecalis* (23 mm) and *P. aeruginosa* (18 mm). The activity was comparable with the positive control, demonstrating that the secondary metabolites of *Streptomyces flavidofuscus* possess strong antibacterial properties effective against MDR pathogens.

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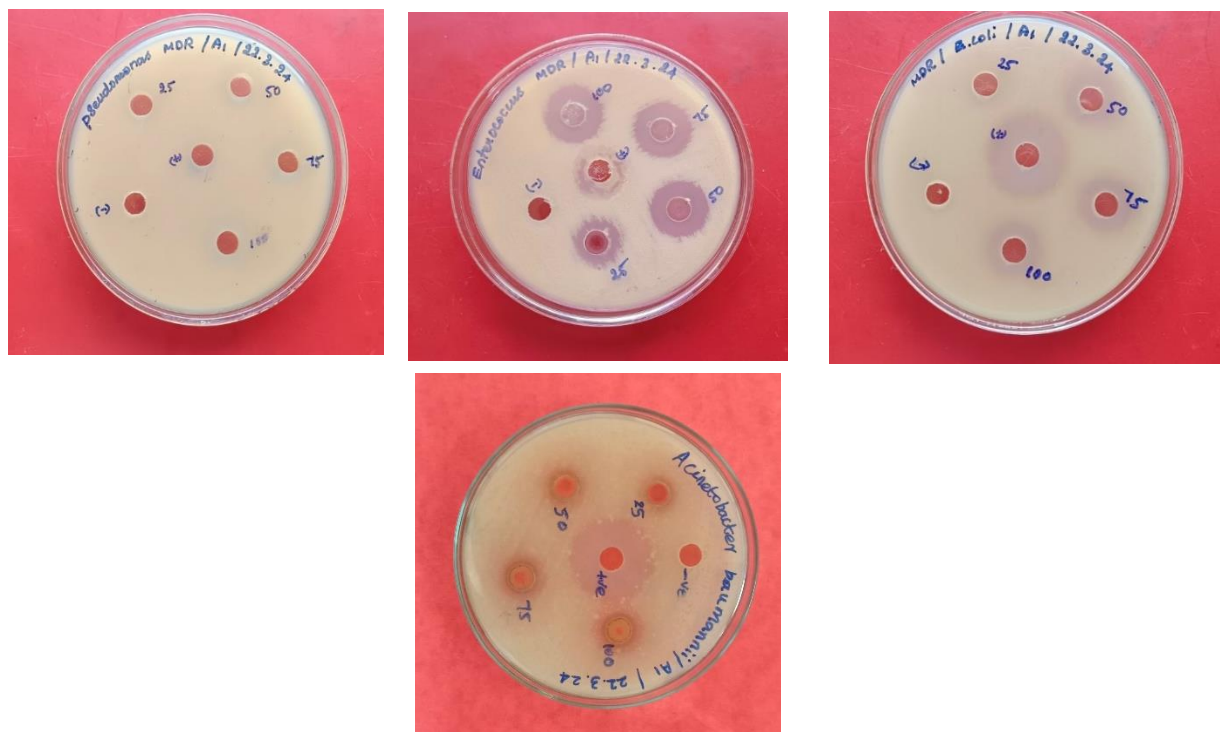

Fig.5. Antibacterial activity Plates of H5 ethyl acetate extract

Table.5: Antibacterial activity of ethyl acetate extract of H5 strain

Bacterial pathogens	Zone of inhibition mm					
	25 µl	50 µl	75 µl	100 µl	Positive	Negative
Pseudomonas aeuroginisa,	-	-	12±1.00	16±1.00	18±1.00	-
Escherchia coli	16±1.53	17±1.53	20±1.00	22±0.58	24±1.00	-
Enterococcus faecalis	16±1.00	19±1.00	20±0.58	22±1.15	23±0.58	-
Acienetobacter baumanii	**16±1.00**	**18±1.00**	**22±1.00**	**25±1.15**	**26±1.15**	

Value represents mean ±SD; n=3, - No zone

4.6 GC-MS Analysis of H5 Crude Extract

The ethyl acetate crude extract of the potent actinomycete isolate H5 was subjected to gas chromatography–mass spectrometry (GC–MS) analysis to identify the bioactive secondary metabolites present, and the chromatogram resulted in 30 compounds that were identified by comparing the mass spectra with the NIST library database; the detected compounds with their retention time, molecular weight, molecular formula, and peak area percentage are listed in Table 6. The most abundant compound identified among the metabolites was n-Hexadecanoic acid (16.56%) followed by 9-Octadecenamide (Z)-oleamide (14.19%), 5(4H)-Oxazolone, 4-methyl-2-phenyl- (10.47%), Undecanoic

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acid (8.63%), and 6-Octadecenoic acid (Z) (7.18%); other compounds were also detected in lower proportions.

The predominance of long-chain fatty acids, fatty acid amides and oxygenated hydrocarbons suggests that lipid-derived bioactive metabolites, known for their membrane-disrupting and antimicrobial activity, are primarily responsible for the antibacterial activity of H5, consistent with the broad-spectrum inhibitory activity of H5 against MDR pathogens observed in the bioassays. Specifically, n-hexadecanoic acid, oleamide and undecanoic acid are known to have antibacterial and antifungal activity, in agreement with the strong inhibitory activity of H5 against MDR pathogens in the bioassays.

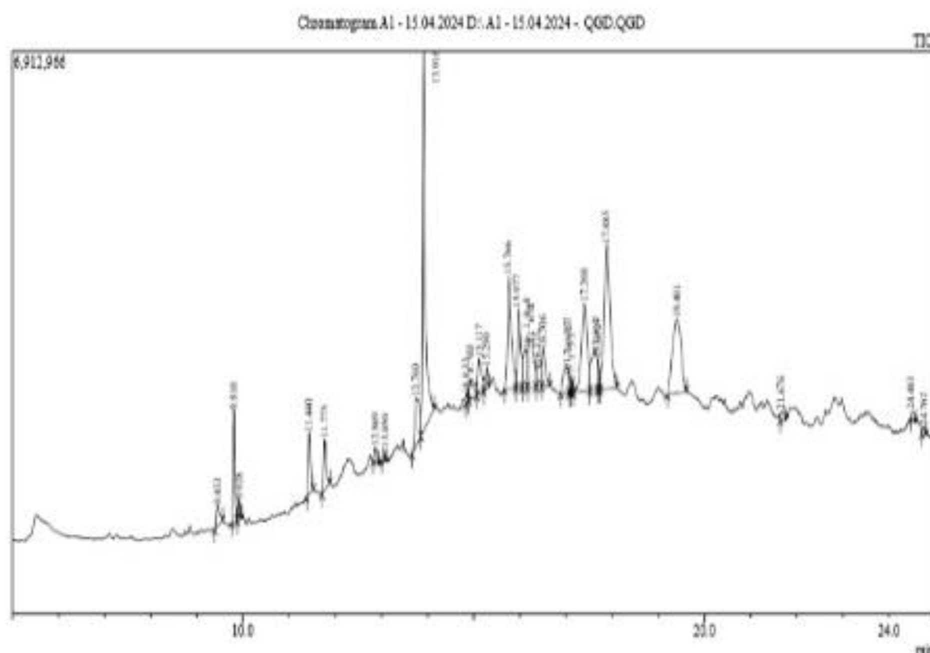

Fig.6.GCMS Spectrum of A1 Ethyl acetate extract

Table.6. Chemical compositions of A1 Ethyl acetate crude extract

S.No	Compound Name	Area %	Retention Time	Molecular Weight	Molecular Formula
1	Dodecanoic acid (CAS) Lauric acid	1.09	9.453	200.32	$C_{12}H_{24}O_2$
2	Davanone	3.15	9.810	236.35	C15H24O2
3	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7	0.51	9.928	220.3505	$C_{15}H_{24}O$
4	Lilac alcohol B	2.28	11.440	170.25	$C_{10}H_{18}O_2$
5	Tetradecanoic acid	2.20	11.775	228.3709	$C_{14}H_{28}O_2$

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6	Pentadecanoic acid	0.58	12.869	242.40	$C_{15}H_{30}O_2$
7	1-Hexadecanol (CAS) Cetal	0.36	13.050	242.44	$C_{16}H_{34}O$
8	Cyclopentadecanone, 2-hydroxy-	3.63	13.760	240.3816	$C_{15}H_{28}O_2$
9	n-Hexadecanoic acid	16.56	13.916	256.4241	$C_{16}H_{32}O_2$
10	9H-Purine, 9-(trimethylsilyl)-2,6-bis[(trimethy	0.47	14.833	280.47	$C_{11}H_{20}N_4OSi_2$
11	Heptadecanoic acid (CAS) Margaric acid	1.56	14.933	270.5	$C_{17}H_{34}O_2$
12	n-Nonadecanol-1	2.17	15.117	284.5203	$C_{19}H_{40}O$
13	9-Octadecenoic acid (Z)-, methyl ester (CAS)	0.86	15.290	296.4879	$C_{19}H_{36}O_2$
14	6-Octadecenoic acid, (Z)-	7.18	15.766	282.4614	$C_{18}H_{34}O_2$
15	Octadecanoic acid	4.18	15.977	284.5	$C_{18}H_{36}O_2$
16	2-{[2'-(3"-isopropenyl-1"-cyclopropenyl)-2'-m	2.67	16.108	124.18	$C_8H_{12}O$
17	1,3-Dioxolane-4-methanol, 2-pentadecyl-, acet	4.48	16.216	356.5	$C_{21}H_{40}O_4$
18	Hexadecanamide	1.79	16.375	255.44	$C_{16}H_{33}NO$
19	5-N-PENTADECYL-1,2,3,4-TETRAHYDRO	1.99	16.506	342.6010	$C_{25}H_{42}$
20	6-N-DECYL-1,2,3,4-TETRAHYDRONAPHT	2.32	17.022	272.5	$C_{20}H_{32}$
21	(-)-Caryophyllene oxide	0.41	17.092	220.35	$C_{15}H_{24}O$
22	DESACYL-KONDURANGOGENINS A	0.27	17.133	1149.3	$C_{59}H_{88}O_{22}$
23	Undecanoic acid (CAS) Undecylic acid	8.63	17.398	186.29	$C_{11}H_{22}O_2$
24	1,5-Hexadiene, 3,4-diethyl-1,6-diphenyl-, (E,E	3.61	17.642	290.45	C22H26
25	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-eth	1.08	17.692	568.9	C35H68O5

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26	9-Octadecenamide, (Z)- (CAS) OLEOAMIDE	14.19	17.885	281.4766	$C_{18}H_{35}NO$
27	5(4H)-Oxazolone, 4-methyl-2-phenyl- (CAS)	10.47	19.401	295.33	$C_{18}H_{17}NO_3$
28	1,5-UNDECADIENE, 6,7,7,8,8,9,9,10,10,11,	0.57	21.676	152.28	$C_{11}H_{20}$
29	5-Hepten-3-one, 2-(5-ethenyltetrahydro-5-met	0.45	24.483	236.3499	$C_{15}H_{24}O_2$
30	Scillarenin	0.30	24.762	384.5	$C_{24}H_{32}O_4$

FT-IR Characterization

FT-IR analysis confirmed the presence of functional groups responsible for bioactivity. Major absorption peaks at 3417 cm^{-1} (O–H stretching), 2032 cm^{-1} (C–H), 2131 cm^{-1} (N=C=N) and 1245 cm^{-1} (C–N stretching) indicated the presence of alcohols, hydrocarbons, amines and nitrile functional groups.

These functional groups are associated with antimicrobial activity, further supporting the GC-MS findings and validating the therapeutic potential of *Streptomyces flavidofuscus* H5 secondary metabolites.

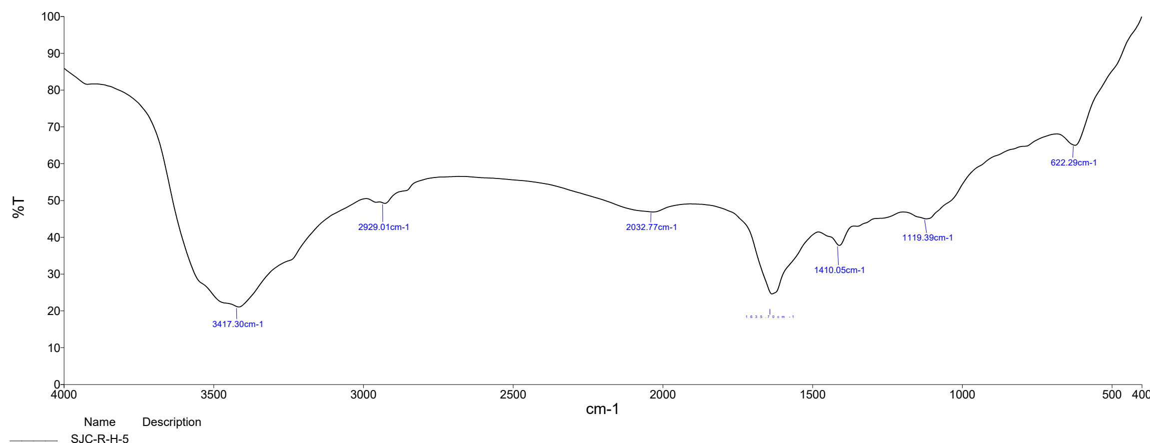

Fig.7: FT-IR analysis of functional groups for H5 crude extract

DISCUSSION

The results of the present study clearly show that *Actinomycetes* isolated from soil are a potential source of effective antibacterial compounds against MDR (multidrug-resistant) bacterial pathogens. Among the isolates, H5 was the most active isolate and was characterized as *Streptomyces flavidofuscus* by morphological, biochemical and molecular analyses, which further substantiates the potential of *Actinomycetes* to produce antibiotics from terrestrial as well as extreme environments.

Primary screening identified H5 as having broad-spectrum antagonistic activity against *A.baumanii, P. aeruginosa*, *E. coli* and *E. faecalis*, with high inhibition zones compared with other isolates; secondary screening using cell-free supernatants confirmed that H5 produced the largest zones of *A.baumanii* (26 mm), followed by *E. coli* (24 mm), *E. faecalis* (23 mm) and *P. aeruginosa* (18 mm). suggesting that the antibacterial activity was due to extracellular secondary metabolites and not simple competition for nutrients. This is supported by the work of Bajagain and Limbu, who reported that solvent

extracts of *Actinomycetes* exhibited significant antibacterial activity, which again stresses the importance of submerged fermentation and solvent extraction for recovery of bioactive compounds (4).

The crude ethyl acetate extracts of H5 showed a dose-dependent antibacterial effect with the maximum inhibition observed at 100 µl, with the highest activity against *E. coli* (26 mm), followed by *E. faecalis* (25 mm) and *P. aeruginosa* (16 mm). This agrees with the results of Daigham and Mahfouz that ethyl acetate is an effective solvent for the extraction of antimicrobial metabolites from *Streptomyces* species and that the extracts showed significant activity against both Gram-positive and Gram-negative pathogens. The comparatively higher sensitivity of Gram-positive bacteria may be due to the lack of an outer membrane barrier that can interfere with the penetration of bioactive compounds (5).

GC-MS analysis revealed that the long-chain fatty acids, fatty acid amides and oxygenated hydrocarbons like n-hexadecanoic acid, oleamide, undecanoic acid, octadecenoic acid derivatives, squalene and oleic acid are responsible for the antibacterial activity of H5. These compounds are known to be membrane-disruptive, antimicrobial and anti-inflammatory, and their broad-spectrum activity against MDR pathogens can be attributed to their fatty acid composition. Previous studies have also identified fatty acids from *Actinomycetes* through GC-MS analysis as major contributors to the antibacterial activity.

Further confirmation of the chemical nature of the bioactive metabolites was obtained through FT-IR analysis, which showed characteristic absorption bands corresponding to O–H, C–H, C–N, N=C=N and C–O functional groups, characteristic of alcohols, amines, nitriles and esters, which are commonly found in antimicrobial molecules and corroborate the GC–MS findings. Similar FT-IR functional groups were reported by Daigham and Mahfouz in *Streptomyces* extracts, for which they correlated them with strong antibacterial and cytotoxic activities (5).

The isolation of *Nocardiopsis* sp. and *Streptomyces flavidofuscus* in this study confirms previous studies that saline and soil environments contain rare and industrially significant *Actinomycetes* with high pharmaceutical value; moreover, Arul Jose and Jebakumar (3) showed that solar salterns are hotspots of a diverse range of *Actinomycetes* capable of producing novel bioactive compounds, highlighting the importance of exploring under-explored ecosystems for antibiotic discovery.

Taken together, the results of this study indicate that the actinomycete isolates are potential sources of novel antimicrobial agents, and that further purification, structural elucidation, mechanism-of-action studies and in-vivo evaluation are needed to progress these bioactive compounds toward therapeutic applications to combat antimicrobial resistance worldwide.

CONCLUSION

The results of the present study reveal that salt pan soils of Nagapattinam District are a potential source of bioactive *Actinomycetes* with potent antimicrobial potential and *Streptomyces flavidofuscus* the most active isolate based on morphological, biochemical and 16S rRNA gene sequence analyses, which produced broad-spectrum antibacterial activity against clinically important multidrug-resistant pathogens including Pseudomonas aeruginosa, Escherichia coli and Enterococcus faecalis. Primary and secondary screening confirmed that the antibacterial activity was mediated by extracellular secondary metabolites, and the ethyl acetate crude extract showed strong, dose-dependent inhibition, especially against *E. coli* and *E. faecalis*. These findings have therapeutic significance and validate extreme saline ecosystems as valuable sources of novel antibiotic producers. Antibacterial efficacy of *Streptomyces flavidofuscus* H5 was found to be due to a mixture of pharmacologically active metabolites, including n-hexadecanoic acid, oleamide, undecanoic acid, octadecenoic acid derivatives, squalene and purine-based compounds, and functional groups responsible for antimicrobial activity, such as amide, ester, and carboxylic acid moieties, which may act synergistically to produce the strong and broad-spectrum antibacterial effect against MDR pathogens. In conclusion, this study confirms *Streptomyces flavidofuscus* H5 as a potential source of antibiotic discovery, and further purification, structural elucidation, mechanism-of-action and in-vivo studies are required to develop these bioactive metabolites for clinical applications in the combat against antimicrobial resistance.

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