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Optimization, Chromatography-based Purification and characterization of novel Antibacterial compound 4-formyl-2-hydroxy bicyclo [4.1.0] heptane-7-carboxylic acid produced by *Streptomyces rochei* (OM746935)

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ABSTRACT: Actinobacteria are the most affordable source of novel antibiotic production for the biotechnological and pharmaceutical industries. In the present work, *Streptomyces rochei* was isolated from Bargur hills and was investigated for their antimicrobial activity by agar well diffusion method, *Streptomyces rochei* was active against all the test organisms, and the maximum zone of inhibition was observed in *Staphylococcus aureus* lowest concentration of MIC 1.25 mg/ml. Ethyl acetate extract showed four fractions in TLC and the active fraction was identified by bioautography. This study investigates the optimization of the nutrient content of the fermentation medium by alteration in carbon and nitrogen source, medium PH, and incubation days are monitored. Then purification of bioactive compounds by column chromatography, 30 fractions were obtained 21, 23, and 24th fractions active against all the tested organisms. All these active fractions are again purified by preparative HPLC which contains C-18 column. Sixteen fractions were again investigated for antibacterial activity, the best activity was obtained in the 7th fraction. Significant peaks were visible in the HPLC chromatogram at the 11th and 16th minutes. Using a UV detector, the maximum absorbance was recorded in the UV range of 220 nm. The active fraction was further characterized by FTIR, GCMS, and NMR. Based on the NMR shift values structure and name of the compound are predicted as 4-formyl-2-hydroxy bicyclo [4.1.0] heptane-7-carboxylic acid. This compound is responsible for the action of antibacterial activity. Actinomycetes isolated from terrestrial sources can each produce a variety of secondary metabolites; in fact, the vast majority of antibiotics originate from these sources. However, a number of diseases are developing resistance to the widely prescribed antibiotics. The development of drugs to stop the spread of these infections is therefore urgently needed.

Keywords: Optimization, column chromatography, preparative HPLC, FTIR, GC-MS.

INTRODUCTION

Nature gives a variety of structurally different constituent and pharmacologically potential compounds that play a significant role in the development of novel drugs (Eskander *et al*., 2020). Actinobacteria are major producers of antibiotics and supply valuable components to the pharmaceutical industries. It can able to produce different secondary metabolites that are sterol, lactone, terpenoid, phthalate, fatty acid, steroidal glycoside, polysaccharide-protein, etc. Almost 75% of various antibiotics and active metabolites are produced by *Streptomyces* species and their potential effectiveness is still in investigation (Miyadoh *et al*., 1993). Some of these elements restrict microbial competitors in the environment to maintain their source of nutrition, which is crucial for ecological systems. *Streptomyces* species have the ability to produce such bioactive compounds indicating their extremely organized metabolic pathways, which enable them to rule over other ecosystem residents (Ilic-tomic *et al*., 2015, Chevrette *et al*., 2019). The development of novel compounds from actinomycetes is a goal of interest in drug discovery (Takahashi *et al*., 2018). since these compounds typically have complicated structures, are difficult to synthesize and have a variety of medical applications and bioactivities (Jakubiec *et al*., 2008; Law *et al*., 2020).

Actinomycetes, a subclass of bacteria, have an unheard-of capacity to produce secondary metabolites that have anticancer, antibacterial, antioxidant, and antiviral, properties. The majority of these are derived from

members of the *Streptomyces genus*, which includes various classes of antibiotics like aminoglycosides, macrolides, and beta-lactams (Lima *et al*., 2012). Daptomycin, linezolid, and streptogramin combo (quinupristin/dalfopristin) are newer therapeutic medicines that have recently made their way into the clinical setting to treat multidrug-resistant bacteria (Levy and Marshall 2004). Numerous secondary metabolites made by *Streptomyces* sp are being used effectively as antibiotics to treat illnesses that are resistant to therapy in both people and animals (Cho *et al*., 2012). Bioactive compound production is highly based on the species of microorganism, nutritional condition, and cultural characteristics (Wang *et al*., 2011; Jose *et al*., 2011). The microorganism's producing secondary metabolite is critical based on the medium constituents and other optimal levels. The discovery of antibiotics is needed much effort towards the optimization of medium components to increase the production rate of antibiotics.

Actinomycetes are a major contributor to the production of various new metabolites that are used in pharmaceutical research and other fields of industry. Actinomycetes, which can efficiently digest a wide variety of xenobiotic chemicals and can also change them into organic compounds of great commercial value, was the source of a wide variety of antibiotics that are currently available.

This research work revealed the structural prediction of bioactive compounds produced by the *Streptomyces rochei* BF3A strain. A total of 30 different actinomycetes were isolated from soil samples in 10 different forest zones of bargur hills. The antibacterial potential of the isolated actinomycetes was studied. *Staphylococcus aureus* was suppressed by strain BF3A, which was identified as *Streptomyces rochei*, at a lower dose of 1.25 mg/ml and exhibited antagonistic action towards all of the examined species (Kokila *et al*., 2023).

MATERIALS AND METHODS

Bacterial Strains. *Escherichia coli* (ATCC8739), *Klebsiella pneumoniae* (ATCC 9621) and *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29737), *Bacillus coagulans* (MTCC6735) used for antibacterial studies.

Optimization of Medium. The present study for culturing of *Streptomyces rochei* was inoculated in ISP2 medium, which contains yeast extract, malt extract, dextrose, and agar, exhibiting the best antibacterial action. The optimization studies were carried out by the modification of the carbon source, nitrogen source, medium pH, and production medium incubation days. The control medium was an ISP2 medium. The dextrose carbon supply was replaced by the addition of glucose, fructose, starch, galactose, and dextrose as a control. The nitrogen source of ISP2 medium yeast extract was alternated by peptone, Beef extract, sodium nitrate, and ammonium nitrate. The ISP2 medium contains 7.2 pH, and the optimization of pH was monitored by the changes in various pH such as 6.5 to 8.5. The test culture *Streptomyces rochei* was inoculated in an ISP2 broth medium and incubated for about 7 days for the extraction of bioactive metabolites. In order to check the proportion of bioactive compounds versus incubation days. The test organism was inoculated and incubated in the ISP2 medium the extraction of bioactive compounds was carried out on different days of incubation (i.e.) 5^{th}, 7^{th}, 9^{th}, 11^{th}, and 13^{th} day.

Extraction of Bioactive Compound. The culture filtrate was extracted using a different solvent system such as Methanol, Chloroform, Ethyl acetate, and Hexane. Following extraction, the crude extracts were separated using different chromatographic methods. The TLC sheet contains precoated silica gel that separates the chemical compounds usinga solvent system (ethyl acetate and hexane at a 6:4 ratio). The four spots found in TLC Fraction D have been reported for their antibacterial activity by bioautography.

Chromatography based Purification. The active TLC fractions were collected and pooled together to increase the concentration of the sample. TLC solvent systems were used for better separation in the silica gel column and the fractions are collected separately. The 24-28 min fraction, showed maximum antibacterial activity. By repeating this procedure, the active column fraction was concentrated. This active fraction was subjected to phytochemical analysis of Tannins, Alkaloids, Flavonoids, Terpenoids, and Phenol. The concentrated active column fraction was further purified by preparative HPLC. The samples are chromatograms with C-18 columns. The solvent system was water and methanol 40: 60 ratio and 0.01% TFA buffer. This program was conducted for 30 minutes with a flow rate of 1ml/mint, mobile phase A contained water and 100% TFA, and mobile phase B contain 40% of water and 60% of methanol. The UV range was set at 220 nm, 280 nm, 405 nm, and 600 nm. All the test samples were collected separately and rechecked for their antibacterial ability.

Characterization of Active Fraction. The active fraction of F-14 was carried out for structural analysis by FTIR, GC-MS. NMR. The active fraction of *Streptomyces rochei* was analyzed using FT-IR spectral (Palanichamy *et al*., 2018) in the Shimadzu FT-IR system in the spectrum range of 4000 to 500 cm^{-1}. By using a gas chromatograph interfaced with a mass spectrometer (GC-MS) Elgorban *et al.* (2019) and the Shimadzu TQ8040NX ultra, GCMS analysis was used to identify the composition of the active fraction 14. Fused silica capillary Elite-1 was utilized to construct the instrument. Helium is used for the carrier gas with a flow rate of 1.21ml/min. The desired oven temperature ranged from 60°C (continuous for 3 minutes) to 280°C (increment for 22 minutes) (Kumari *et al*., 2020). The active fraction was applied to structural analysis by NMR (Hughes *et al*., 2015) and the instrument of Bruker-3 Advance 500, 125.79 MHz for ^{13}C NMR, 500.23 MHz for ^{1}H NMR, 125.79MHz for DEPT 135, Temperature of the analysis is 298 k, Spectrum recorded with SWH = 10 kHz for ^{1}H NMR, SWH = 29 kHz for ^{13}C NMR, and SWH = 20 kHz for DEPT 135. The spectrum frequency of SF01 and SF02 is 500 for all ^{13}C, ^{1}H, and DEPT 135. C- SEARCH tool is used to

find the structural similarity based on chemical shift values of NMR reference data.

RESULT AND DISCUSSION

In recent times, antibiotic resistance of pathogenic bacteria level is increased, and researcher has struggled to find novel antibacterial compounds in a natural environment. The forest ecosystem is the source of biodiversity of plants and microorganisms to produce potential novel bioactive metabolites (Tamilarasan *et al*., 2018; Baskaran *et al*., 2011). Our previous study explored the diversity of actinomycetes present in the Bargur hills and identified the potential compound-producing actinomycetes. Isolated Streptomyces are species-level identification by 16S rRNA sequencing. The physiological identification was done by various biochemical tests and growth patterns of potential strain. *Streptomyces rochei* produce antibacterial compounds against all the tested organisms. Antibacterial activity was done by agar well diffusion method. The lowest concentration of MIC was identified in *Staphylococcus aureus.* The active fraction of TLC was identified by bioautography and RF values of TLC are reported in our publication (Kokila *et al*., 2023).

Selection of medium based on the zone of inhibition. The generation of bioactive secondary metabolites is greatly influenced by the medium compositions. The ISP2 medium produces the largest zone of inhibition against all of the test bacteria compared to other mediums, among which, culture filtrate obtained from the ISP2 medium shows the largest zone of inhibition (22 mm) against *Staphylococcus aureus* (Table 1). Hence this medium is chosen for further optimization to boost *Streptomyces rochei* BF3A's bioactivity. ISP2 medium is selected for the fermentation of secondary metabolite based on the high zone of inhibition compared with other tested mediums. Hasnaa *et al*. (2018) reported ISP2 medium is suitable for sporulation and fermentation. Strain *Streptomyces rochei* BF3A did not need the changes in carbon supplementary, the ISP2 broth containing dextrose is the best source of carbon for the production of secondary metabolites, and a high production rate was observed in dextrose as a carbon source. The advantage of the isolate BF3A does not require any additional source for its growth and/or bioactive compound production.

Table 1: Selection of medium for optimization to enhance the production of secondary metabolites produced by *Streptomyces rochei* BF3A.

Sr. No	Name of the culture media	Zone of inhibition (mm)				
		E. coli	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus coagulans*
1.	ISP2	17	12	20	22	10
2.	ISP3	6	10	9	13	16
3.	ISP4	12	10	16	11	9
4.	Starch casein agar	8	0	0	7	8
5.	Starch nitrate agar	6	5	7	14	0

Effect of carbon and nitrogen source of production medium. In order to check the effect of carbon source on media optimization, the carbon source dextrose (4g/L). ISP2 media was altered with Glucose, Galactose, fructose, starch, and control-containing dextrose. The culture was inoculated in different carbon source media and tested for antibacterial activity. Of all these carbon sources dextrose gives the maximum zone (20mm) of inhibition around *Staphylococcus aureus* (Table 2), hence dextrose is the best carbon source to increase the production of bioactive compounds. Various nitrogen sources are applied for the production of bioactive compounds. ISP2 broth contains yeast extract as a nitrogen source. Instead of yeast extract, other nitrogen sources are used for the fermentation such as peptone, beef extract, sodium nitrate, and ammonium nitrate. Peptone (4g/L) containing production medium shows the largest zone of inhibition (24mm) against *Staphylococcus aureus* and is active against all the test bacteria. Fguira *et al*. (2005); Mehdi *et al*. (2006) reported the *Streptomyces* strain US80 and *Streptomyces* TN 97 require supplementation with glycerol and glucose or fructose for the production of antimicrobial compounds. The peptone was found to be the best source of nitrogen for increasing the production of antibacterial metabolites compared with other sources of nitrogen tested. Chattopadhyay and Sen (1997); Han *et al*. (2004) reported peptone is a good source of nitrogen for *Streptomyces rochei* G164 and *Streptomyces scabies*.

Table 2: Optimization of carbon source for the production of secondary metabolites produced by *Streptomyces rochei* BF3A.

Sr. No.	Name of the carbon source	Zone of inhibition (mm)				
		E. coli	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus coagulans*
1.	Glucose	6	8	10	12	8
2.	Fructose	0	2	4	6	9
3.	Starch	4	6	5	9	11
4.	Galactose	10	3	8	17	15
5.	Control (Dextrose)	13	11	10	20	16

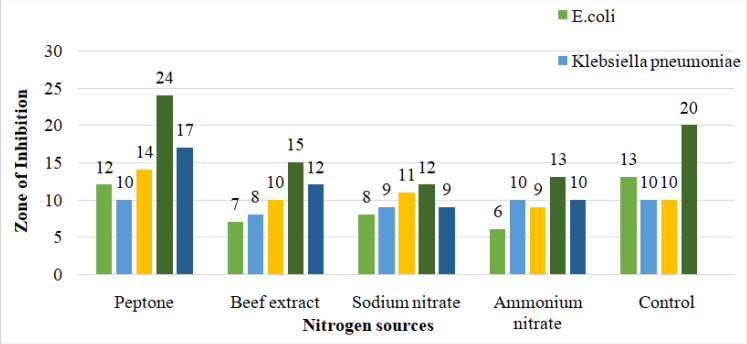


Fig. 1. Optimization of nitrogen source for the production of secondary metabolites produced by *Streptomyces rochei*.

Effect of ph and days of incubation on antibacterial activity. Effect of pH and incubation days to increase the production of secondary metabolites. The pH is one of the important factors to enhance the production of bioactive compounds. The graph shows (Fig. 2) that 7.5 pH is suitable for *Streptomyces rochei* and the zone of inhibition was increased from 7 to 7.5 pH. Hence this pH is better for the production of active metabolites. The Days of incubations were monitored to increase the antagonistic activity of *Streptomyces rochei* BF3A. The 7 days incubation (Fig. 3) period gives the maximum zone of inhibition (22 mm) against all the test organisms, particularly *Staphylococcus aureus* produces 22 mm of the zone of inhibition. The pH of the culture medium affects the growth of the culture as well as antibiotic production. Attimarad *et al*. (2012) reported the optimization of PH for the growth of ACT-A2 culture determining that PH level 7 is suitable to start the production of secondary metabolites. This study is similar to the PH 7 to 7.5 and is suitable for the growth and production of bioactive compounds from *Streptomyces rochei*. The stain started the log phase after 72h (3rd day) of incubation and show exponential growth up to 120 h (5th day) followed by a stationary phase extended up to 168 h (7th day). The crude obtained for the 7-day-old culture exhibited antibacterial activity against all tested organisms. Khattab *et al.* (2016) reported *Streptomyces* species of PS1 and PS28 to have antibacterial activity, and the zone of inhibition was high at5- 7 days of incubation. This product is similar to our work and the zone of inhibition has been increased from 5 to 7 days.

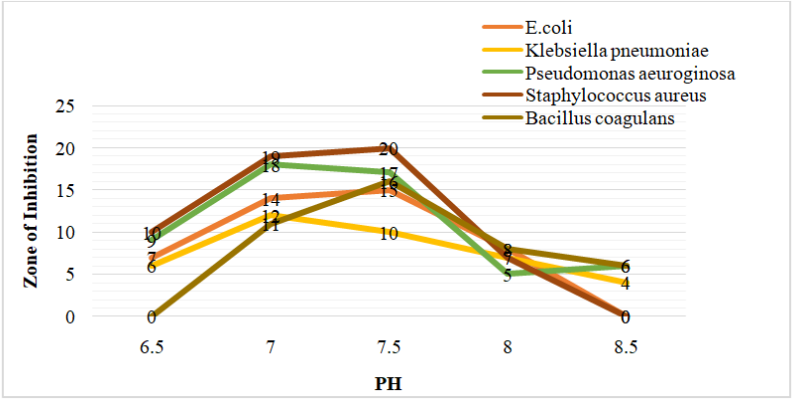


Fig. 2. Effect of pH for theproduction of secondary metabolites produced by *Streptomyces rochei*.

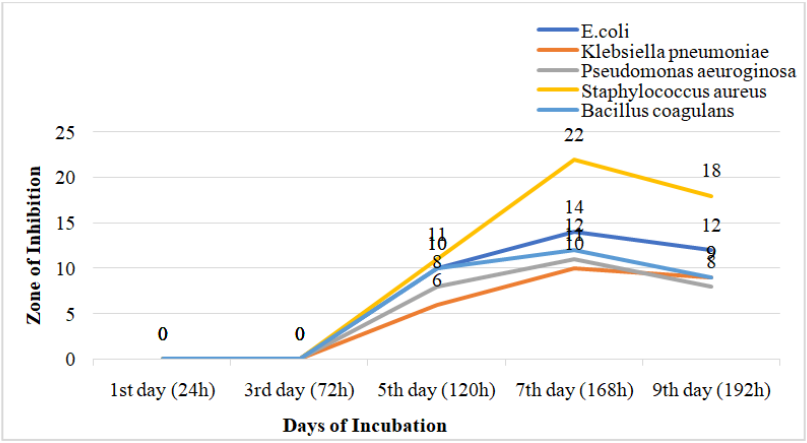


Fig. 3. Effect of days of incubation for the production of secondary metabolites produced by *Streptomyces rochei*.

Purification of an Active Fraction by Chromatography. Optimized medium of ISP2 containing the composition of 4g of peptone, 10g of Malt extract, 4g of Dextrose, 1 liter of distilled water, pH 7.5, and an incubation period of 7 days shows the highest production of bioactive compounds. Previous work reported ethyl acetate extraction is best for the extraction of bioactive compounds from the culture medium. Active fraction TLC was subjected to column chromatography (Goutam *et al*., 2016), a total of 30 different fractions were obtained, and each fraction was separately screened for antibacterial activity by agar well diffusion method. The fractions 21, 23 and 24 th showed efficient antagonistic activity against testing bacteria of gram positives and gram negatives. The largest zone of inhibition was observed in the 21stfraction among other fractions of the 23rd and 24th. All the active fractions from column chromatography were mixed together, to make it concentrate and subjected to ultra-purification by Preparative HPLC (Barbosa *et al*., 2016). The sixteen fractions were collected individually and screened for anti-bacterial activity. Significant peaks were visible in the HPLC chromatogram at the 11th and 16th minutes. Using a UV detector, the maximum absorbance in the UV range is 220 nm. The antibacterial screening revealed that active fraction 7 produced the maximum zone of inhibition (22mm) against *Staphylococcus aureus* among other bacteria. Phytochemical analysis (Aziz, 2015) of the active fraction is compared with the crude extract. The crude extract is alkaloids and terpenoids positive, and the active fraction shows terpenoid positive. Motohashi *et al*. (2008) reported two terpenoid compounds produced by marine *Streptomyces* sp. MS239. Isolated terpenoid compounds are 5-dimethylallylindole-3-carboxylic acid and A80915G-8", these compounds were structurally confirmed by NMR. Hence this result *Streptomyces rochei* produce terpenoids is the major group that presents in the purified fraction and plays an important role in the antagonistic activity of *Staphylococcus aureus*. GC-MS, FTIR, and NMR were used to further identify the structural characteristics of the active fraction.

Fourier Transmission Infrared (FT-IR) Spectrum Analysis. The active fraction of BF3A was characterized by FT-IR spectroscopy. In the FTIR spectrum shown, the band 3447.52 corresponds to O-H stretching vibration, and the strong band at 2361.67 cm-1, 2101.30 corresponds to the C=C. The band at 1635.52, corresponds to the CH, strong band at 1355.86, 1268.11, and 1198.68 is the C-H group. The 1079.10 corresponds to the C-O group, and the last peak at 941.20 corresponds to the CH. These FTIR peaks showed the functional group of the active compounds. FTIR characterization of active groups such as alcohols, alkanes, amines, ketones, aromatic amines, aldehydes, esters, and aromatic compounds. Vineeta *et al*. (2018) reported the antibacterial activity of 2,6- distributed chromosome derivatives produced by *Streptomyces levis*, IR spectrum of the compound shows hydroxyl and carbonyl absorption bands at 3425 and 1648 cm^{-1} strong band, absorption bands at 3020 and 2927 cm^{-1} corresponded to CH and alkyl CH stretching, 1602 and 1504 cm-1 corresponds to C= C stretching. These CH, C=C functional group of the compounds is similarly found in the IR spectrum of *Streptomyces rochei* BF3A (Fig. 4).

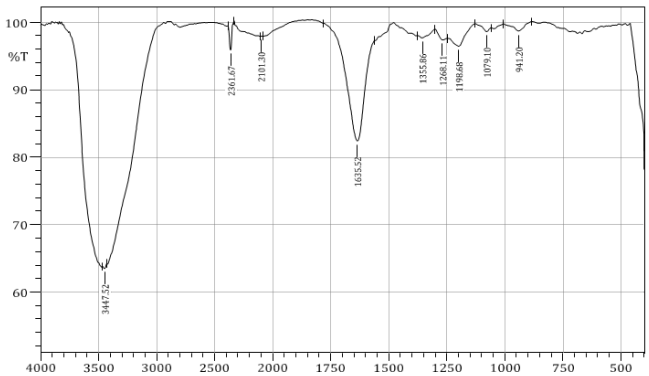


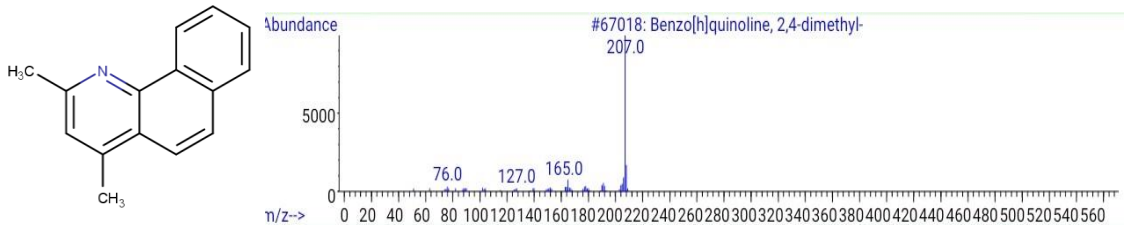
Fig. 4. FTIR spectrum of active fraction produced by *Streptomyces rochei* BF3A.

GC-MS analysis of active fraction. The identification of antibacterial metabolites (Table 3) in the active fractions of *Streptomyces rochei* BF3A was determined by GC-MS analysis. The seven compounds are identified in GCMS analysis such as Benzo [h] quinoline 2,4 dimethyl with RT 17.839, 1H-Indole,1-methyl-2-phenyl with RT 17.319, 1,2,4 -Triazol-3-amine,5 (1,3,5- trimethyl-4-pyrazolyl) amino with RT 17.745, 1H-Indole-2-carboxylic acid, 6-(4-ethoxy phenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester with RT 17.225, 2- methyl-7- phenylindole with RT 15.381, 5-methyl-2-phenylindolizine with RT 16.818, 2,4-Cyclohexadien-1-one, 3,5-bis with RT 16.100. This 7th active HPLC fraction contains several different groups of chemicals, they are highly bioactive and valuable compounds used for many types of infections (Table 3). The major area percentage containing the compound is Benzo [h] quinoline 2,4 dimethyl and 2,4- cyclohexadiene-1-one,3,5-bis. The IR spectrum shows peaks of CH3, three CH, CH2, CO, and OH stretching, 2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethyl ethyl)-4-hydroxy compound was identified in GCMS analysis of active fraction, this similar structural compound was identified in NMR.

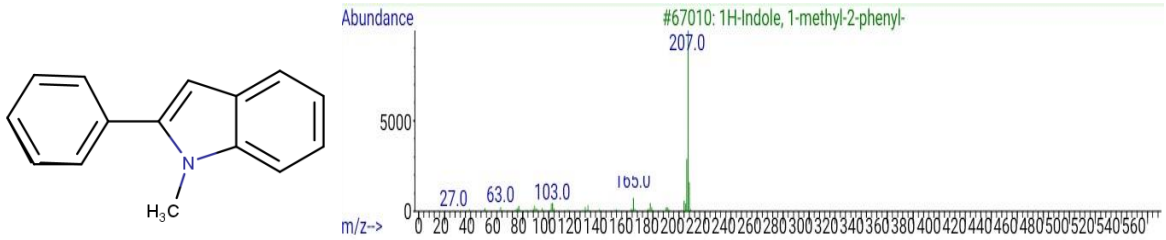
Table 3: Major compounds present in the active fraction and their biological activity.

Sr. No.	Retention time	Area (%)	Compound name	Molecular formula	Molecular weight	Applications
1.	17.839	33.24	Benzo [h] quinoline 2,4 dimethyl	$C_{15}H_{13}N$	207.27	Antibacterial, anticancer
2	17.319	9.35	1H-Indole,1-methyl-2-phenyl	$C_{15}H_{13}N$	207.27	Antiviral, antibacterial, antioxidant, antiproliferative
3.	17.745	3.98	1,2,4 -Triazol-3-amine,5 (1,3,5-trimethyl-4-pyrazolyl) amino	$C_8H_{13}N_7$	207.24	Antibacterial
4.	17.225	13.23	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester.	$C_{23}H_{29}NO_5$	399.49	Antimicrobial and antioxidant
5.	15.381	12.63	2- methyl-7- phenylindole	$C_{15}H_{13}N$	207.27	Antibacterial and antioxidant
6.	16.818	13.12	5-methyl-2-phenylindolizine	$C_{15}H_{13}N$	207.27	Antifungal agent
7.	16.100	14.45	2,4-Cyclohexadien-1-one, 3,5-bis	$C_{14}H_{22}O_2$	222.33	Antimicrobial, wound healing properties

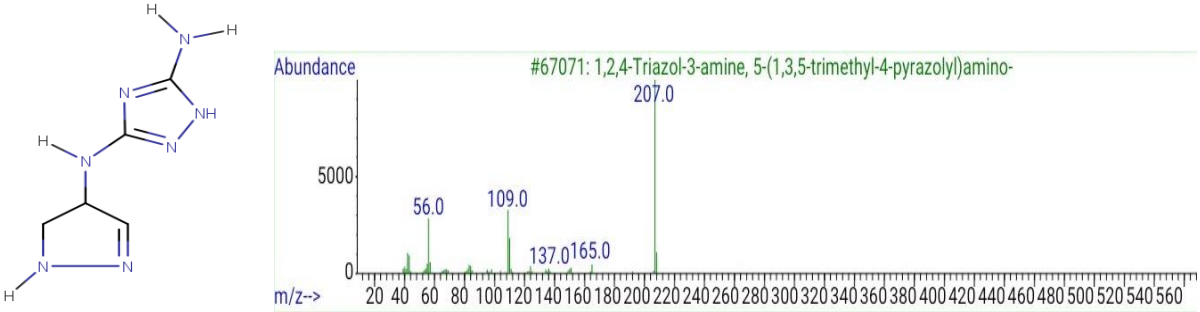
Benzo [h] quinoline 2,4 dimethyl



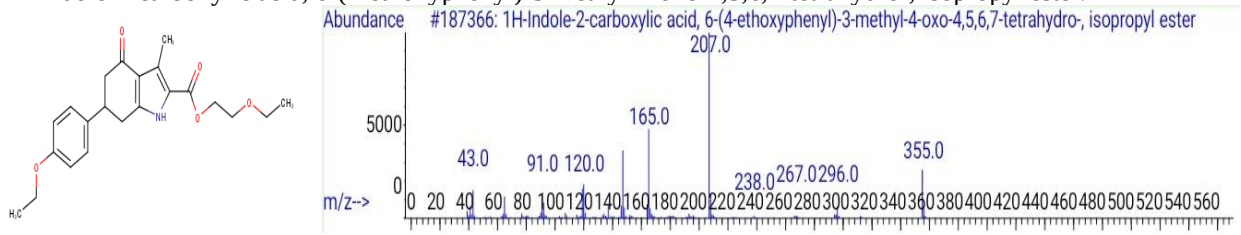
1H-Indole,1-methyl-2-phenyl



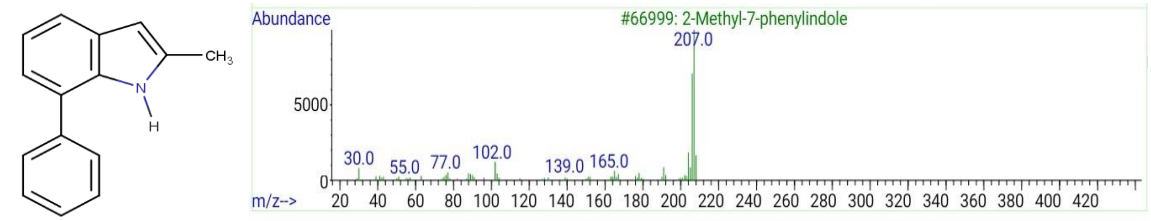
1,2,4 -Triazol-3-amine,5 (1,3,5- trimethyl-4-pyrazolyl) amino



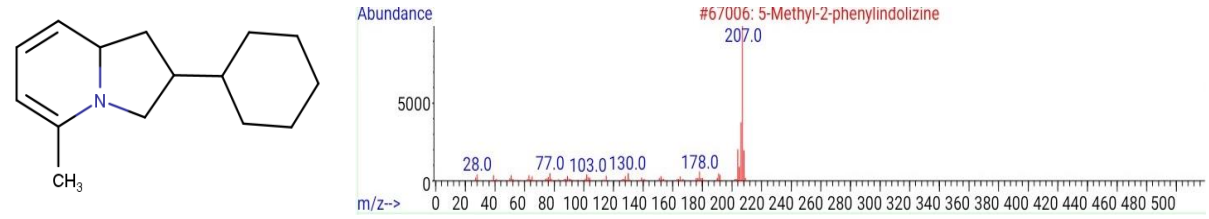
1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester.



2- methyl-7- phenylindole



5-methyl-2-phenylindolizine



2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy

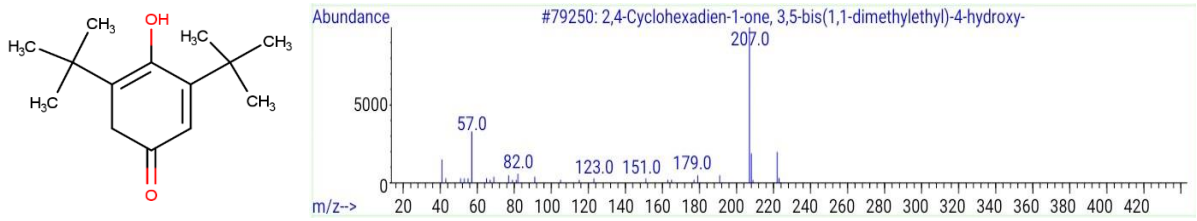


Fig. 5. Structure and chromatogram of the active fraction containing compounds produced by *Streptomyces rochei*.

NMR structural prediction of active compound. From the 1HNMR spectrum data (Fig. 7), the signal at δ3.69 confirmed the methoxy group present in the chemical shift observed between δ 1.96- δ1.98 indicates the presence of aliphatic hydrogen methyl and methylene group present in the side chain of the compound. The 13C NMR spectrum (Fig. 6), and 13C DEPT (Fig. 7) of the antibacterial compound indicate that the single range of δC 21-28 ppm corresponds to methylene (CH_2). The peak range of δ39 corresponds methane CH group. The peak range of δ 207 ppm represents carbonyl carbon either amide or ester group. Bindu *et al*. (2018) reported *Streptomyces lavendulo color* VHB-9 strain active against tested bacteria to produce antibacterial compounds and was identified as (2R, 3R)-2, 3-butanediol, and nonadecanoic acid. The Compound cycloheptane carboxylic acid is commonly called hexahydrobenzoic acid or carboxycyclohexane. Cyclohexane is a derivative of shikimic acid and quinic acid. Sekiyama *et al*., 2003 reported Actinomycetes manufacture the polyketide-type antibiotics known as phospholactomycins (PLMs) A–F, which are formed from either a hydroxy cyclohexane carboxylic acid or a cyclohexane carboxylic acid starting unit. According to feeding studies using [2-13C]shikimic acid, the C-18 carbon in PLMs is derived from the C-5 of shikimate. Further feeding studies of cis and trans-3-hydroxy[7-13C] cyclohexane carboxylic acid, [7-13C]- and [2H11] cyclohexane carboxylic acid has suggested that the starter unit in the PLM biosynthesis is not cis-3-hydroxy cyclohexane carboxylate but cyclohexane carboxylate and that PLM-B is produced initially, and subsequently converted to other analogs by hydroxylation and acylation.

This is the first report to identify the antibacterial compound 4-formyl-2-hydroxy bicyclo [4.1.0] heptane-7-carboxylic acid produced by *Streptomyces rochei* BF3A. Identification based on the chemical shift value of C, H, and Dept 135 NMR, is closely matched with 4-formyl-2-hydroxybicyclo [4.1.0] heptane-7-carboxylic acid values. Based on the reference this compound has a nature of antimicrobial, anticancer, and anticandidal activity.

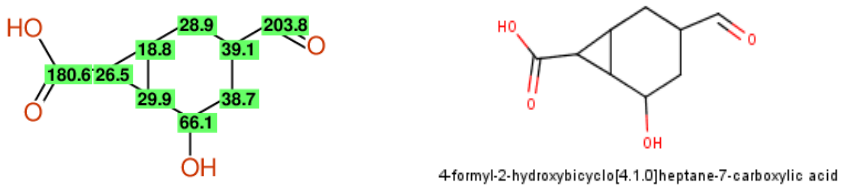


Fig. 6. The molecular structure of the 4-formyl-2-hydroxy bicyclo [4.1.0] heptane-7-carboxylic acid produced by *Streptomyces rochei* BF3A (OM746935).

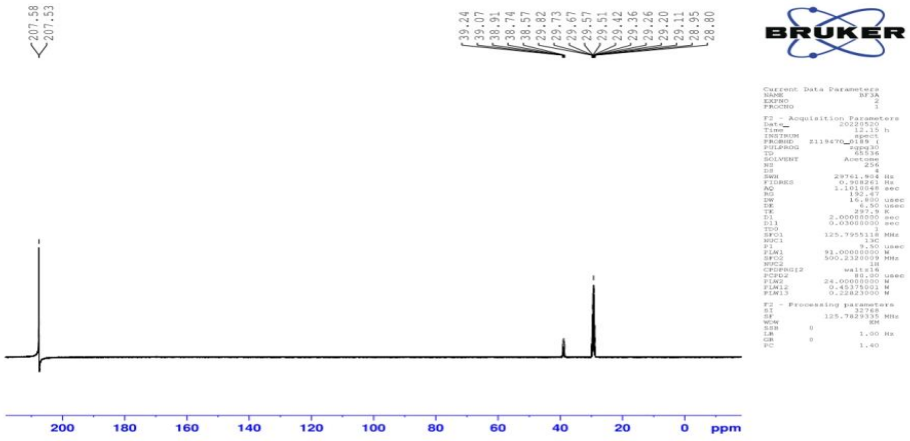


Fig. 7. 13C-NMR Spectrum of preparative HPLC active fraction from the *Streptomyces rochei* BF3A

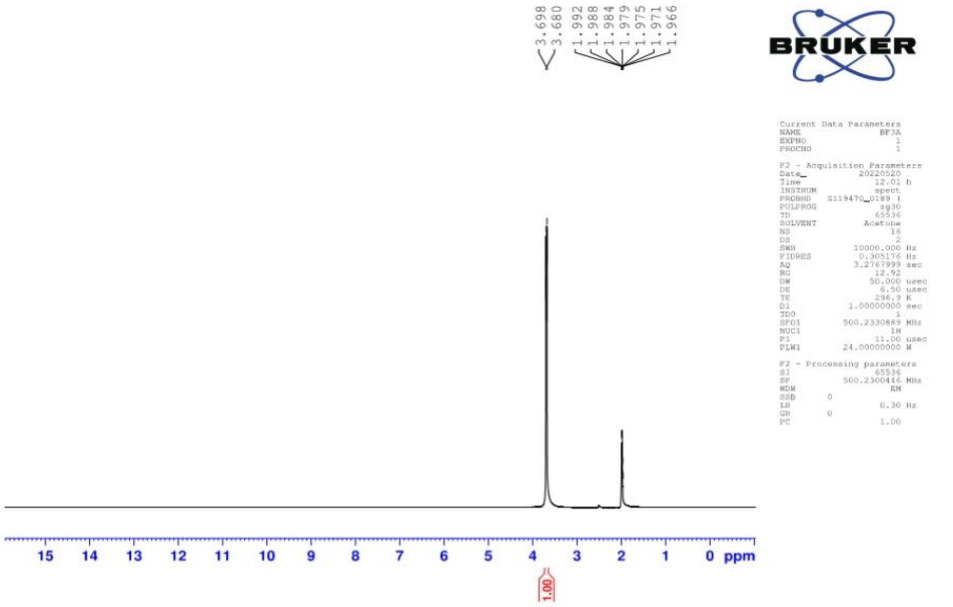


Fig. 8. 1H-NMR Spectrum of preparative HPLC active fraction from the *Streptomyces rochei* BF3A

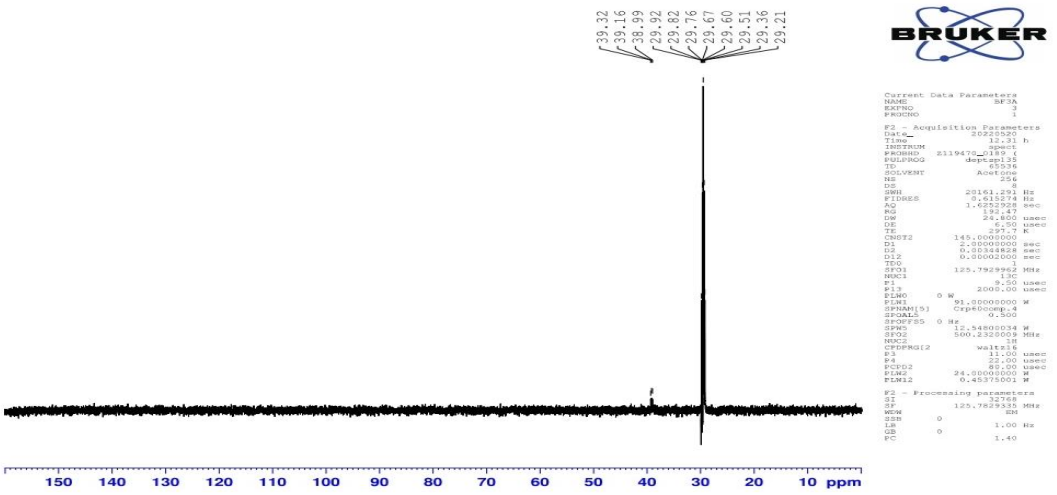


Fig. 9. 13C DEPT NMR spectrum of preparative HPLC active fraction form the *Streptomyces rochei* BF3A.

CONCLUSIONS

Strain *Streptomyces rochei* BF3A has the ability to produce antibacterial compounds. Purified antibacterial compound structurally identified as 4-formyl-2-hydroxy bicyclo [4.1.0] heptane-7-carboxylic acid. This compound is active against all the test organisms particularly the maximum zone of inhibition in *Staphylococcus aureus.* The Insilco docking approaches of a compound with the target sites of *Staphylococcus aureus* will be studied in the future.

FUTURE SCOPE

The findings are thought to have satisfied the study's goals and scope. Future goals include creating an in silico docking strategy and improving microbial target sites for the antibacterial substance.

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Conflict of Interest. None.

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