Journal of Pharmaceutical Research International

33(50B): 210-234, 2021; Article no.JPRI.77118
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Antimicrobial and Antioxidant Activities of *Streptomyces* sps Isolated from Muthupettai Mangrove Soil

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i50B33445
Editor(s):
(1) Dr. Asmaa Fathi Moustafa Hamouda, Jazan University, Saudi Arabia.
Reviewers:
(1) Rene Flores Clavo, UNICAMP, Brazil.
(2) Zahir Hafida, Sultan Moulay Slimane University, Morocco.
Complete Peer review History: https://www.sdiarticle4.com/review-history/77118

Original Research Article

Received 10 September 2021
Accepted 16 November 2021
Published 19 November 2021

ABSTRACT

Muthupet mangrove forest soil sediment was the abundant resource of the actinomycetes with distinct nature of bioactive compounds. The soil sediment was collected at 1-3meter away from bank. The present study was focused on isolation, identification and antimicrobial activity of the actinomycetes from Muthupet mangrove soil samples. Totally 32 actinomycetes strains was isolated and screened for antimicrobial activity against bacterial and fungal pathogens. Among 32 isolates 16 have antibacterial activity and 10 have antifungal activity but MG-3 and MG-4 showed maximum activity against both all the test pathogens. These two strains are gram-positive, rod-shaped, MG-3 possessing an earthy characteristic odour and MG-4 produce purple color pigment. The isolates were confirmed as *Streptomyces* sp. based on morphological, cultural, biochemical and physiological observations, as well as identification using the 16S rRNA gene sequence, it showed 98% similarity with *Streptomyces parvus* for MG-3 and *Streptomyces californicus* for MG-4. Bioactive compounds were extracted from *Streptomyces* using different solvents such as ethyl acetate, methanol, chloroform, hexane and antibacterial activities were assayed against test pathogens, ethyl acetate extract showed maximum zone of inhibition when compared with other

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solvents. The Minimum inhibitory concentration of ethyl acetate extract was found ranged between 1.96-3.9 µg/ml**.** The *invitro* antioxidant capacity of the crude extract was estimated by DPPH, ferric reducing power assay, H_20_2 radical scavenging assay, phosphomolybdenum assay and total antioxidant activities. The characterization of crude extracts was analyzed by FTIR and GC-MS. From the results, it is clear that the ethyl acetate crude extract of *S.parvus* MG-3 and *S.californicus* MG-4 possesses high antimicrobial and antioxidant activity and suggested that the isolated strains could be a potential for the nature resource of pharmaceutical.

Keywords: Physio-chemical parameters; antimicrobial activity; bioactive compounds; antioxidant activity and 16S rRNA sequencing.

1. INTRODUCTION

Soil is the complex combination of minerals, organic substances, gases and liquids. There are numerous micro and macro organisms in soil that sustain a wide variety of plant life. Soil quality is essential to forest management because of its influence in ecosystem processes and structure [1,2,3]. The quality of the soil and water resources depends on various physico-chemical parameters. There are a number of parameters like pH, temperature, soil type, salinity, organic matter, cultivation, aeration and moisture content that must be monitored in order to determine the pollution load in an area [4]. The mangrove forest is an internationally significant habitat for wildlife and is rich in biological diversity. Because of different microbial enzymatic and metabolic activities, mangroves have a salty ecology and are known to be huge suppliers of organic matter. Muthupettai mangrove ecosystem is largely unexplored which provides a rich source of the microorganisms that create unique and potent antimicrobial compounds. There is evidence that actinomycetes are abundant in mangrove sediments [5]. In nature, Actinobacteria are Gram-positive, aerobic, spore-forming bacteria that contain high level of GC and widely distributed in nature. They form extensive branching aerial and substrate mycelia. Actinomycetes are slow growing organisms compared with other bacteria and fungi [6]. *Streptomyces* is the largest group of actinobacteria, 'Geosmin' is a compound which is responsible for the earthy odour in soil and it is a *Streptomyces* metabolite. However volatile products secreted by *Streptomyces* may also be responsible for the characteristic smell [7].

The mangrove ecosystem is a largely unexplored source of *Actinobacteria* with the potential to produce active secondary metabolites [8]. Actinomycetes is one of the most economically and biotechnologically valuable prokaryotes [9]. Microorganisms produce over 23,000 bioactive metabolites, according to reports. Most actinomycetes have clinical applications based on their antibacterial, antiparasite and antiviral characteristics [10]. Streptomycin is one of the antibiotics is produced by *Streptomyces* [11]. Industrially important enzymes are produced by actinomycetes, such as cellulase, amylase, xylanase, lipase, pectinase and protease [12]. They play a vital role in the recycling of organic matter and a main source for the synthesis of antibiotics, pharmaceuticals, cosmetics, enzymes, anticancer agents and vitamins.

Recent studies focus on the bacterial antioxidative system, a major term in biotechnology like the development of *Streptomyces* in diverse oxidative stresses [13]. Antioxidants can protect humans from numerous illnesses and degenerative diseases by inhabiting and scavenging free radicals [14]. Oxidative stress can be caused by increase free radicals or decreased antioxidant, depending on the situation. Natural antioxidant can protect human cells from oxidative stress induced cell damage. In modern scientific research natural antioxidants from plants and microorganisms are now directly used as safe therapeutics [15]. The purpose of this study is to explore the antimicrobial and antioxidant property of actinomycetes from Muthupettai mangrove soil sample.

2. MATERIALS AND METHODS

2.1 Soil Sample Collection

The mangrove sediment soil samples were collected from five different locations of Muthupettai forest, Thiruvarur, Tamilnadu, India (Latitude of 10° 25'N; Longitude 79° 39'E). Each sample was collected at the depth of 2 inches and packed in sterile polythene bags and were kept in ice boxes and transported to the laboratory. After 15 days of shadow drying, the soil samples were used for further investigation.

2.2 Physicochemical Analysis

The collected soils were analyzed for physical and chemical soil quality parameter like pH [16], electrical conductivity (EC) [17], organic carbon (OC) [18], available nitrogen (N) [19], phosphorus (P) [20] and potassium (K) [21].

2.3 Isolation of Actinomycetes

Starch Casein Nitrate Agar medium (Starch -10 g/L, Casien -0.3 g/L, K2HPO4 - 2 g/L, KNO3 - 2 g/L, Nacl - 2g/L, MgSo4.7H2O - 0.05 g/L, CaCO3 - 0.02 g/L, FeSo4.7H2O - 0.01 g/L, Agar -15 g/L) was used for the isolation of actinomycetes supplemented with Nalidixic acid (20µg/ml) and Nystatin (20µg/ml) to prevent bacterial and fungal contamination respectively. One gram of mangrove soil sample was serially diluted upto 10^{-6} dilution and 0.1ml of diluted sample was taken from 10^{-2}, 10^{-3} and 10^{-4} dilution and spread over on the SCN agar plates separately and the control plate was maintain without any inoculation. Then the plates were incubated at 28°C for 7-9 days. Suspected actinomycetes colonies were isolated and transferred to actinomycetes isolation agar media. The pure culture was stored in 20% glycerol at -20°C for long time preservation [22].

2.4 Screening of Antimicrobial Activity

Antimicrobial activity of actinomycetes isolates were screened by agar well diffusion method [23]. Sterile Muller Hinton agar and Potato Dextrose agar plates were prepared and made 6mm diameter wells were punched on the agar medium using sterile well cutter against the test bacterial culture *Escherichia coli* (MTCC 1678)*, Klebsiella pneumonia* (MTCC 3384)*, Micrococcus luteus* (MTCC 106)*, Vibrio cholera* (MTCC 3906)*, Salmonella typhi* (MTCC 3231), *Proteus vulgaris* (MTCC 1771) and fungal culture *Candida albicans* (MTCC 183)*, Aspergillus niger* (MTCC 281), *Aspergillus fumigates* (MTCC 343), *Aspergillus flavus* (MTCC 277) and *Alternaria solani* (MTCC 2101) were procured from Microbial Type Culture Collection, IMTECH, India. The test bacterial and fungal strains were swabbed and 100µl of cell-free supernatant was loaded on the well. The antimicrobial activity was performed according to CLSI, USA guidelines on Muller Hinton agar medium using well diffusion method.

2.5 Identification of Potential Actinomycetes Isolates

The potent actinomycetes isolates were further identified based on microscopic and morphological features by scanning electron microscopy, cultural, biochemical and physiological characteristics according to the standard protocol of International Streptomyces Project [24-29].

2.6 Molecular Characterization

2.6.1 Genomic DNA isolation and 16S rRNA sequencing

Genomic DNA isolation of the selected isolate was prepared by the following method [30]. The primers (27F) (5'-AGAGTTTGATCCTGGCTCAG-3') and (1492R) (5'-GGTTACCTTGTTACGACTT-3') were used to amplify the 16S ribosomal sequence of genomic DNA by thermal cycler (Prima-96 Thermal cycler, Himedia). PCR product loaded on a 0.8% agarose gel and the gel was observed on the gel doc imaging system (UVP) [31,32]. The amplified PCR products were sequenced at Rajiv Gandhi Centre for Biotechnology (RGCB), Kerala, India.

2.6.2 Construction of phylogenetic tree

The sequence was submitted to Genbank and compared to all other sequences in the database. The 16S rRNA gene sequence was aligned with the nucleotide sequences using BLAST. For molecular taxonomy analysis, sequences with more than 98% homology were examined. Multiple alignments of 16S rRNA nucleotide sequences were performed with CLASTAL W program. A phylogenetic tree was generated using the neighbor-joining method with bootstrap testing in Molecular Evolutionary Genetics Analysis (MEGA 6) [33,34].

2.7 Mass Multiplication and Solvent Extraction

2.7.1 Fermentation and extraction of bioactive compounds

The bioactive metabolites were recovered from the yeast malt extract broth (Yeast extract- 4g/L, Malt extract-10g/L, Dextrose- 4g/L, Agar- 15g/L) by the solvent extraction method. Potential actinomycetes isolates were inoculated into a culture broth and incubated at 28˚C for 7-10 days

on a rotary shaker (220 rpm). The biomass was removed from culture broth followed by centrifugation at 10,000 rpm for 10 mins followed by filtration using Whatman no.1 filter. The supernatant was concentrated and extracted with equal volume of different solvents such as ethyl acetate, chloroform, hexane and methanol. The solvent and supernatant were transferred into separating funnel and shake vigorously for one hour. After shaking, the aqueous phase was separated from an organic phase. The separated aqueous phase was evaporated using a rotary evaporator and the obtained compound thus used as secondary metabolites [35]. The crude compounds were subjected for antimicrobial activity against bacterial and fungal pathogens by agar well diffusion method to confirm the active antibacterial metabolites.

2.7.2 Minimum inhibitory concentration (MIC)

MIC of the crude extract was determined by serial tube dilution technique or turbidimetric assay against Gram-positive (*S.aureus*) and Gram-negative (*E.coli*) bacterial strains [36]. 3ml of nutrient broth was taken in 9 test tubes and extracted compounds were add into first test tube (mg/ml), and the serial dilution was made at the final concentration 9th tube served as a control without extract. 20µl of bacterial pathogen was added into each tube and incubated in orbital shaker incubator (120rpm) at 37°C for overnight. The MIC was determined as the lowest concentration of crude extract that resulted in no observable bacterial growth (no turbidity) as compared to the control tubes [37]. After incubation the MIC was determined by Spectrophotometer at 570nm.

2.8 *In vitro* Antioxidant Activity

2.8.1 DPPH radical scavenging activity

The radical scavenging activity was determined by using DPPH (2.2-diphenyl-1-picrylhydrazyl) assay [38]. The ability of the extract to scavenge DPPH radicals was tested using a 0.1mM DPPH solution made by dissolving 4mg of DPPH in 100ml of methanol. 1ml aliquot of test extract at different concentrations (20-100µg/ml) in methanol of each fraction in separate tubes was mixed with 1ml DPPH (Himedia, Mumbai). The optical density was measured at 517nm using a UV-Vis spectrophotometer after 30 mins of incubation in the dark at room temperature. The colour change was observed in purple to yellow. Ascorbic acid was used as reference. In the control, 1ml methanol and 1ml of 0.1mM DPPH solution were used instead of extract. The formula was used to calculate the scavenging activity.

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

Where, A is absorbance of DPPH control and B is the absorbance of extract.

2.8.2 H_2O_2 radical scavenging assay

The ability of the extract to scavenge hydrogen peroxide was measured [39]. Hydrogen peroxide (40mmol/l) solution was prepared using phosphate buffer (pH 7.4). Extracts (20-100µg/ml) were added to hydrogen peroxide solution (0.6 ml). After 10 mins of incubation, the absorbance of hydrogen peroxide at 230nm was compared to that of ascorbic acid as a reference.

$$\text{Hydrogen peroxide activity (\%)} = [(\text{Abs control} - \text{Abs sample})/\text{Abs control}] \times 100$$

Where, Abs (control): Absorbance of the control and Abs (test): Absorbance of the extracts.

2.8.3 Total antioxidant activity

The green phosphomolybdenum complex production method was used to assess the antioxidant activity of samples [40]. Various concentrations (20µg-100µg/ml) from the prepared sample were mixed with 3.0 ml of reagents (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Reaction mixture was incubated at 95˚C for 90 mins in waterbath and the mixture absorbance was measured at 695nm following cooling of the samples at room temperature. Typical blank solutions which include 1 ml of reagent and an adeqate volume from the same solvent used for the sample and it were incubated under same conditions. Ascorbic acid was used as standard. From the study the potential reduction of phosphomolybdenum (PRP) in extracts has been shown as a percentage.

2.8.4 Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of the crude extracts was determined [41]. The reaction mixture contained various concentration of extracts (20-100 µg/ml), 1.0 ml of iron-EDTA solution (0.13% ferrous ammonium sulphate 0.26% EDTA), 0.5 ml of 0.018% EDTA, 1.0 ml of DMSO (0.85% in 0.1 mol/L phosphate buffer pH

7.4), 0.5 ml of 0.22% ascorbic acid and it was incubated in waterbath at 80–90˚C for 15 mins, the reaction was terminated by adding 1.0 ml of ice cold TCA (17.5%). 3.0 mL Nash reagent (75.0 g ammonium acetate, 3.0 mL glacial acetic acid, 2.0 mL acetyl acetone were mixed with distilled water and made up to 1L) was added to the reaction mixture specified above and colour development took place at room temperature for 15 minutes. Against a reagent blank, the intensity of yellow color generated was measured at 412 nm. As a control, ascorbic acid was employed. The % hydroxyl radical scavenging activity was estimated as follows,

HRSA (%) = Abs control – Abs sample / Abs control

Where, Abs control was the absorbance of solution without extract and Abs sample was the absorbance of extracts.

2.8.5 Total reducing power

The Reducing Power technique was used to measure the antioxidant activity of crude extract [42]. 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ were added to 1 ml of sample and the resulting mixture was incubated at 50˚C for 20 min, followed by the addition of 2.5 ml of Trichloro acetic acid (TCA) (Himedia, Mumbai) and the tubes were centrifuged at 3000 rpm for 10 min to collect the 2.5 ml upper layer of the solution, mixed with equal volume of distilled water and 0.5 ml of $FeCl_3$. The absorbance was then measured at 700 nm against blank and compared with ascorbic acid, the reference compound.

2.9 Spectroscopy of the Crude Extract

The infrared (IR) spectrum of the ethyl acetate extract was measured in the range of 400-4000 cm^{-1} by using Bruker Spectrum equipped with AT-XT Golden gate accessories [43]. GC–MS analysis of crude extract was performed using Shimadzu QP-2010 Plus with Thermal Desorption System TD 20.

3. RESULTS AND DISCUSSION

In the present study, actinomycetes were isolated from the soil sample of Muthupettai mangrove region and their antimicrobial and antioxidant properties were determined. It is well known that the mangrove environments are constantly exposed to environmental variations such as changes in tidal gradient and salinity [44,45]. In this context, the study of *Streptomyces* mangroves may offer a better chance of isolating novel *Streptomyces* sp. which may lead to the discovery of valuable bioactive molecules [46,47]. Researchers are still actively researching the diversity of the microbial community in the phylum *Actinobacteria* originating from various environments, often due to their ecological significance and biotechnological benefits [48,49,50].

Table 1. Physiochemical parameters of soil from different location of Muthupettai mangrove forest

S.No	Physiochemical parameters	Location 1	Location 2	Location 3	Location 4	Location 5
1	Soil Texture	Sandy clay	Sandy clay	Sandy clay	Sandy clay	Sandy clay
2	Soil Color	Black	Grey	Blackish Grey	Black	Grey
3	pH	7.98	8.08	8.29	7.98	8.10
4	$CaCo_3$ (mg/kg)	-	-	-	-	-
5	EC (dSm^{-1})	4.50	3.52	2.55	6.34	5.70
6	Nitrogen	60.2	37.8	49	63	77
7	Phosphorous	4.0	2.0	3.0	1.0	3.0
8	Potassium	228	251	241	245	228
9	Iron	5.16	5.07	5.00	5.10	4.91
10	Manganese	3.17	2.98	2.91	2.85	3.12
11	Zinc	0.86	0.97	0.84	0.95	0.88
12	Copper	0.94	0.97	0.91	1.00	0.94

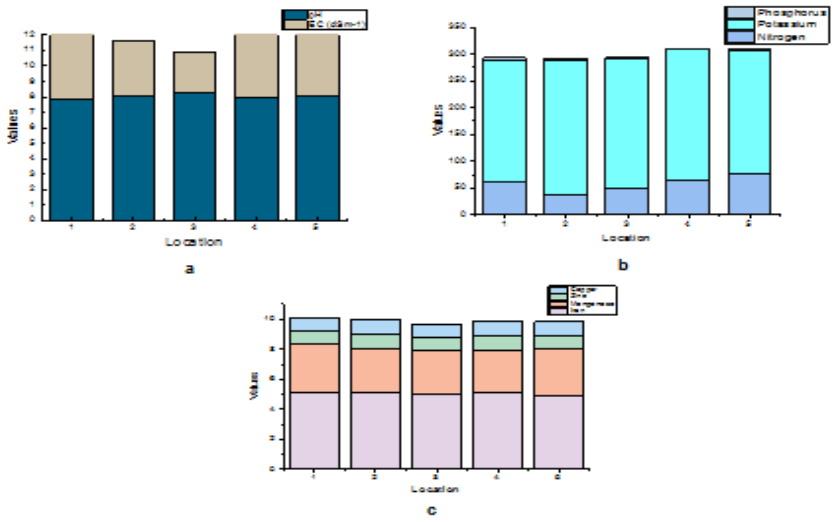


Fig. 1. Physiochemical parameters of soil collected from different location of Muthupettai mangrove forest. a) pH & Electric Conductivity, b) Available phosphorous, nitrogen and potassium, c) Available iron, manganese, zinc and copper

The physiochemical parameters and nutrient content of selected samples were estimated using different analytical methods and the results were representing in Table 1 and Fig. 1. Kiran and Chaudhari, [51] reported that the physiochemical study of soil is based on various parameters and these studies reveal the nutrient quality present in the soil. Adequate information on coastal wetlands such as estuaries, mudflats, coral reefs, mangroves etc., of Indian is available, and each has distinct physicochemical and biological characteristics [52].

3.1 Isolation of Actinomycetes

Totally 32 actinobacteria isolates were isolated from the samples based on their color, odour and colony morphology. The isolates were purified for further study. As a result soil sediment continues to receive the most attention because it was shown to be good resource of mangrove actinobacteria [53]. Previous findings clearly reported the diversity and distribution of actinomycetes population were high in Muthupet mangroves [54]. Kumar and Kannabiran [55] used different types of media for isolating marine actinomycetes and among the three different media, the Starch Casein Agar was proved to be effective for isolation.

3.2 Antimicrobial Activity

All the 32 isolates were subjected to screening for antibacterial and antifungal activities where 16 isolates have antibacterial activity and 10 have antifungal activity. Among these 26 isolates four strains (MG-1, 3, 4 & 12) were inhibit the growth of all test bacteria and fungi. MG-3 and MG-4 were comparatively more active than the other isolates with a higher antimicrobial activity against both bacterial and fungal pathogens (Tables 2 and 3). The maximum zone of inhibition 20mm was observed in *K. pneumonia, V. cholera* in MG-3 and 22mm showed in *V.cholera,* 20mm was observed in *P.vulgaris, S.typhi* in MG-4 for bacteria. In fungi 20mm was observed in *A.solani*, 23mm was showed in *A.flavus,* 14mm was observed in *C.albicans*, 15mm was observed in *A.fumigatus* and *A.niger* for MG-4 and 16 mm was showed in *A.solani* for MG-3. These two strains were subculture in International Streptomyces Project Medium (ISP -2) for further identification and glycerol stocks stored at -20ºC for long time preservation (Fig. 2). The result revealed a broad spectrum of antimicrobial activity as it inhibited both Gram-positive and negative bacteria as well as fungus. In general, antifungal activity can be attributed to the degradation of cell wall hydrolytic enzymes and/or the production of antifungal metabolites. In consistent with a previous report by Sathiyaseelam and Stella [56] the present study suggests that the isolated actinomycetes strains from Muthupet mangroves were a promising candidate as an antibiotic source against bacterial pathogens. Similar findings were previously reported [57].

Table 2. Screening of antibacterial activity of actinomycetes isolates against bacterial test pathogens

S.No	Isolated strains	Bacterial Pathogens Zone of Inhibition (mm in diameter)					
		E.coli	***K. pneumonia***	***M.luteus***	***P.vulgaris***	***S.typhi***	***V.cholerae***
1	Positive control	20	20	24	20	25	24
2	MG-1	-	16	12	18	-	12
3	MG-3	16	20	10	18	10	20
4	MG-4	16	18	14	20	20	22
5	MG-12	-	14	16	14	-	17

Table 3. Screening of antifungal activity of actinomycetes isolates against fungal test pathogens

S.No	Isolated strains	Fungal pathogens Zone of Inhibition (mm in diameter)				
		C.albicans	***A.fumigatus***	***A.niger***	***A.flavus***	***A.solani***
1	Positive control	17	17	26	20	22
2	MG-1	11	10	10	9	11
3	MG-3	12	14	11	13	16
3	MG-4	14	15	23	15	20
4	MG-12	-	14	-	-	-

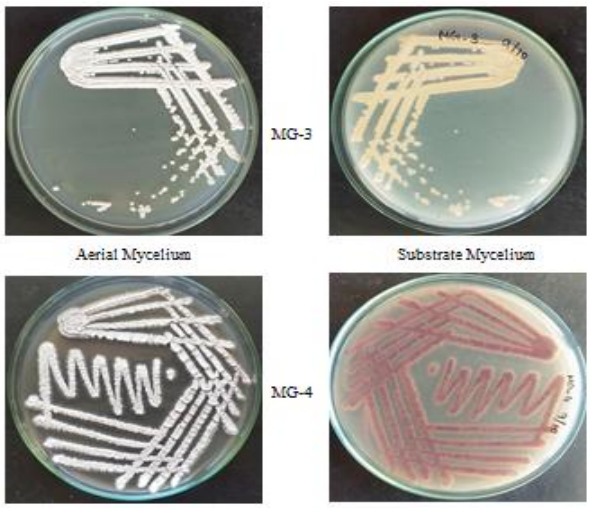


Fig. 2. Pure culture of actinomycetes

3.3 Identification of Actinomycetes Isolates

The distinct aerial hyphae arrangement, spore chain ornament and spore surface of the potential actinomycetes isolates were observed by light microscope. The variation among the sporophore size, ornamentation and spore surface were recorded in MG-3 and MG-4 actinomycetes isolates by scanning electron microscope the results were showed in (Figs. 3 and 4). In gram staining, the MG-3 and MG-4 were gram-positive, rod-shaped. Colony morphology of the MG-3 and MG-4 were analyzed using ISP-1 to ISP-7 culture medium and the color of the aerial and substrate mycelium were described according to the colors of the RAL code (Figs. 5 and 6). The smooth, powdery and earthy odour characteristics colonies were found in culture plate. On various ISP media, the strains produced different growth patterns and color morphologies. The results were given in Tables 4 and 5. The biochemical test evidenced that the both MG-3 and MG-4

isolates were positive in citrate, catalase, nitrate and urease test. Negative results were noted in indole, MR-VP, gelatin, starch hydrolysis and TSI test. The results were summarized in Table 6. The physiological properties of isolates were showed the highest growth in pH 7, moderate growth was noted in pH 5, 6 and 8. Temperature exhibited good growth at 25-30°C, with optimum growth occurring at 20°C, 35°C and no growth at 40-45°C. It could tolerate 5 and 7% NaCl concentration and has a moderate growth at 1%, 3%, 9% and no growth in 0%, 11% of NaCl. In carbon utilization all the sugars were utilized by active strains except sucrose and fructose. Nitrogen source such as asparagine, glutamine was found to be a best nitrogen source, moderate growth observed in tyrosine and no growth in alanine, urea (Fig. 7 and Table 7). From these analysis this active strains confirmed as *Streptomyces* sp.

Jeffrey, [58] reported that the methods have been used in the ISP (International Streptomyces Project) medium to characterize the *Streptomyces* sp. ISP-2 media were most frequently used for the biological characteristics of actinomycetes [59]. Based on the present and previous studies, it is concluded that the biochemical properties of actinobacteria varied depending on nutrients supplied in biochemical media and biochemistry of an organism; hence these aspects could potentially be used as a taxonomic criterion for genus level identification [60,61,62]. Eco-biological variables like pH, temperature, salinity, inhibitory compounds and the availability of nutrients influenced the proliferation of actinobacteria [63,64]. Recently several other investigators have been studied the spore surface morphology of *Streptomyces* sp [65].

Table 4. Cultural characterizations of MG-3 isolate on different ISP growth media

Cultural characterization	**International Streptomyces Project Medium (ISP)**						
	ISP-1	ISP-2	ISP-3	ISP-4	ISP-5	ISP-6	ISP-7
Growth	Good	Good	Good	Good	Good	Good	Good
Color of aerial mycelium	Grey	White	Grey	Pale Yellow	White	White	Grey
Substrate mycelium	Yellow	Pale Yellow	Pale Yellow	Yellow	Brownish Yellow	Yellow	Pale Yellow
Pigment production	No	No	No	No	No	No	No

Table 5. Cultural characterizations of MG-4 isolate on different ISP growth media

Cultural characterization	**International Streptomyces Project Medium (ISP)**						
	ISP-1	ISP-2	ISP-3	ISP-4	ISP-5	ISP-6	ISP-7
Growth	Good	Good	Good	Good	Good	Good	Good
Color of aerial mycelium	Grey	White	Grey	Grey	Purple	Purple	Grey
Substrate mycelium	Purple	Purple	Purple	Purple	Purple	Purple	Purple
Pigment production	No	Purple	No	No	No	No	No

Table 6. Biochemical characterization of actinomycetes

Biochemical Characteristics	**Actinomycetes isolates**	
	MG-3	**MG-4**
Indole	**-**	**-**
MR-VP	**-**	**-**
Citrate	+	+
Catalase	+	+
Gelatin	**-**	**-**
Nitrate	+	+
Starch Hydrolysis	**-**	**-**
TSI	**-**	**-**
Urease	+	+

(+: Positive; -: Negative)

Table 7. Physiological characterizations of actinomycetes

Physiological Characterization	Actinomycetes isolates	
	MG-3	MG-4
Ph		
4	-	-
5	++	++
6	++	++
7	+++	+++
8	++	++
9	-	-
Temperature		
20°C	++	++
25°C	+++	+++
30°C	+++	+++
35°C	++	++
40°C	-	-
45°C	-	-
NaCl Concentration		
0%	-	-
1%	++	++
3%	++	++
5%	+++	+++
7%	+++	+++
9%	++	++
11%	-	-
Carbon Source		
Dextrose	+++	+++
Fructose	-	-
Glucose	+++	+++
Lactose	++	+++
Maltose	++	+++
Sucrose	-	-
Nitrogen Source		
Alanine	-	-
Asparagine	+++	+++
Glutamine	+++	+++
Tyrosine	++	++
Urea	-	-

(+++: Excellent; ++: Moderate; - : No growth)

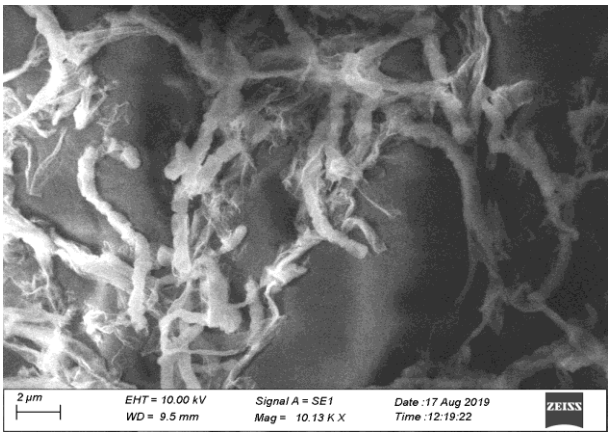


Fig. 3. Scanning Electron Microscopy of MG-3

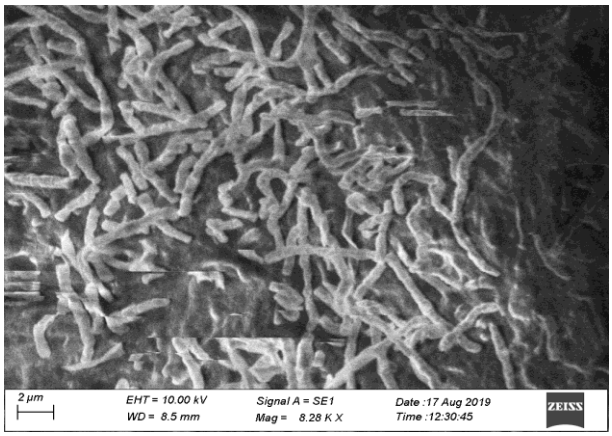


Fig. 4. Scanning Electron Microscopy of MG-4

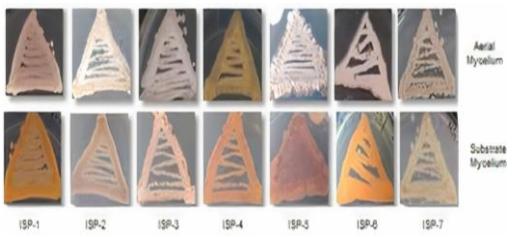


Fig. 5. Cultural characterizations of MG-3 isolate on different ISP growth media

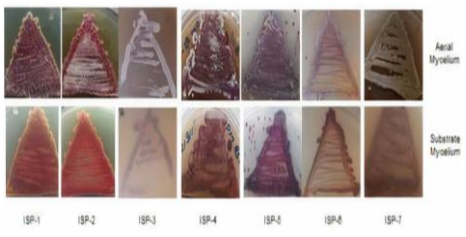


Fig. 6. Cultural characterizations of MG-4 isolate on different ISP growth media

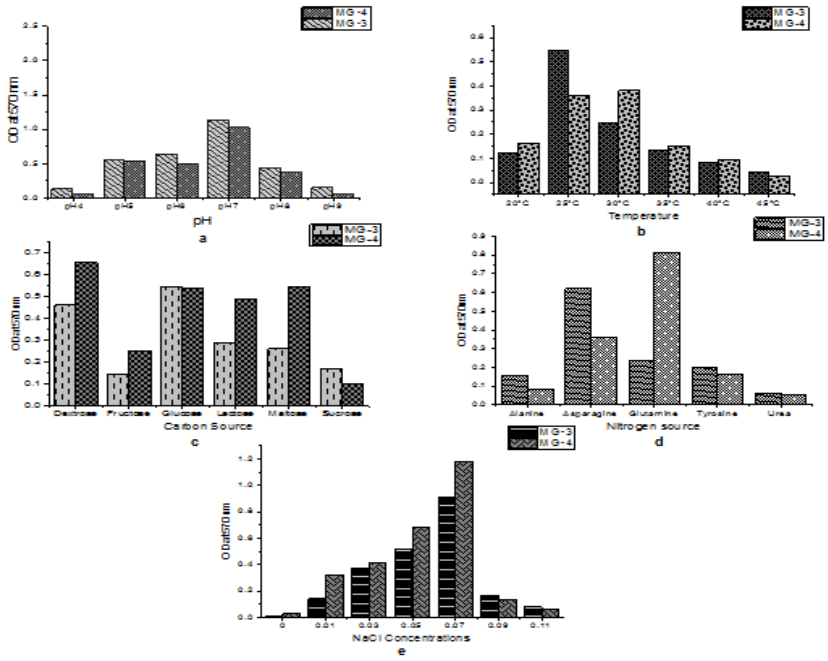


Fig. 7. Physiological characterization of actinomycetes. a) Effect of pH, b) Effect of temperature, c) Effect of carbon source, d) Effect of nitrogen source, e) Effect of NaCl concentration

3.4 Molecular Characterization

The genomic DNA of MG-3 and MG-4 were isolated using standard protocol. The isolated DNA was confirmed in 0.8% agarose stained with ethidium bromide. The PCR product of the both isolates were analyzed by agarose gel electrophoresis and the size MG-3 (1129 bp) and MG-4 (1134 bp) was confirmed. The PCR product was sequenced and confirmed by using NCBI BLAST tools. The Genbank accession number of MG-3 and MG-4 was MH595926 and MH595927 respectively (http://www.ncbi.nlm.nih.gov). Phylogenetic tree constructed using neighbor-joining method in MEGA 6 based on 16S rRNA gene sequence in comparison to MG-3 and MG-4 and showed its related member of the *Streptomyces* sp and confirmed MG-3 as *Streptomyces parvus* (GenBank Accession no. MH595926) and MG-4 as *Streptomyces californicus* (GenBank Accession no. MH595927) we propose the assignment of our strains as *S. parvus* MG-3 and *S. californicus* MG-4. (Fig. 8a and 8b).

Phylogenetic analysis of 16s rRNA have been reported by many workers [66,67,68]. Sottorff et al., [69] found that *Streptomyces* strains from different habitats with identical phylogenetic classification produced different secondary metabolites. As a result, these strains represent a diverse and putative source of novel secondary metabolites. In this present study cultural, morphological, biochemical, physiological and molecular characterizations of the isolated strains were found to be a member of *Streptomyces* genus.

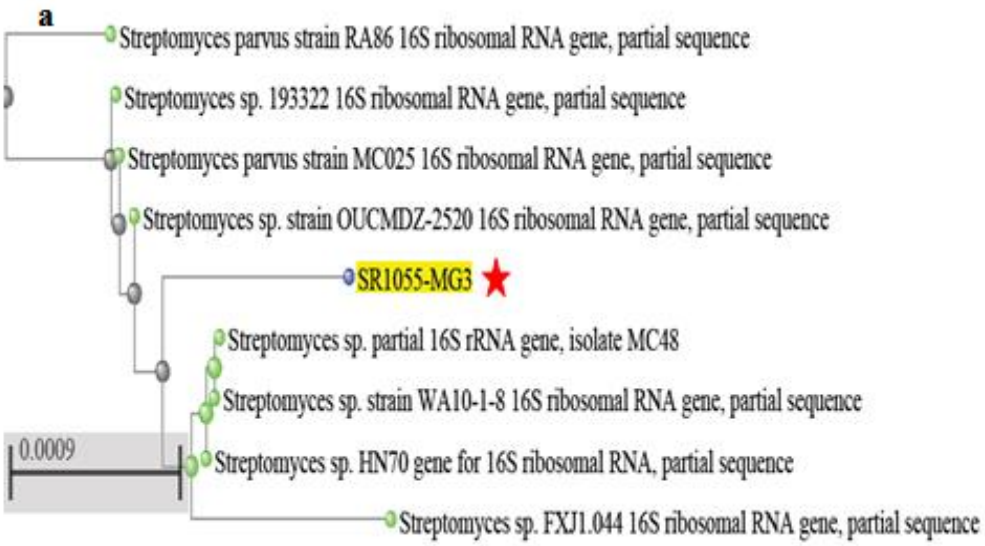


Fig. 8(a). Phylogenetic tree based on partial 16S rRNA sequences MG-3, showing the relationship with other species belongs to the genus *Streptomyces.*

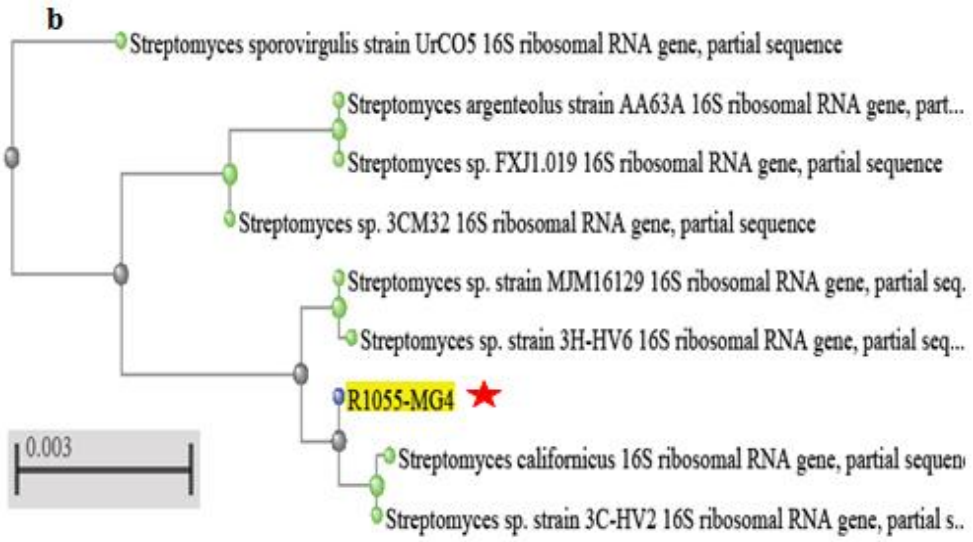


Fig. 8(b). Phylogenetic tree based on partial 16S rRNA sequences MG-4, showing the relationship with other species belongs to the genus *Streptomyces.*

3.5 Antimicrobial Activity of Secondary Metabolites

The different solvent extracted compound of *S.parvus* MG-3 and *S.californicus* MG-4 were screened for antimicrobial activity against *E.coli, K.pneumonia, M.leteus, P.vulgaris, S.dysentriae, V.cholerae* and fungal pathogens such as *A.niger*, *A.fumigatus, A.solani, A.flavus, C.albicans.* The results, revealed that all the extract of *S.californicus* MG-4 inhibit the growth of all the test bacterial pathogens except hexane. In *S.parvus* MG-3 ethyl acetate extract only inhibit the growth of the all the bacterial pathogens whereas chloroform extractions only inhibit the growth of *K.pneumonia* and other extracts does not inhibit the growth of test organisms (Fig. 9 and Table 8). *S.parvus* MG-3 and *S.californicus* MG-4 ethyl acetate extract inhibit all the fungal pathogens at maximum level. In *S.californicus* MG-4 the methanol and chloroform extract only inhibit the *A.fumigatus* (Fig. 10 and Table 9). From this study it was observed that, ethyl acetate solvent was suitable

for compound extraction. Ethyl acetate was chosen as it is a semi-polar solvent that can attract polar or non-polar compounds, has low toxicity and is easily evaporated. [70,71]. An extracellular bioactive molecule produced by *S.parvus* MG-3 and *S.californicus* MG-4 strains is responsible for its antimicrobial action [72,73]. Previously, Sudha and Masilamani, 2012 [74] reported that the marine mangrove region mediated actinomycetes is an excellent antibiotic producer due to the unpredictable environmental parameters.

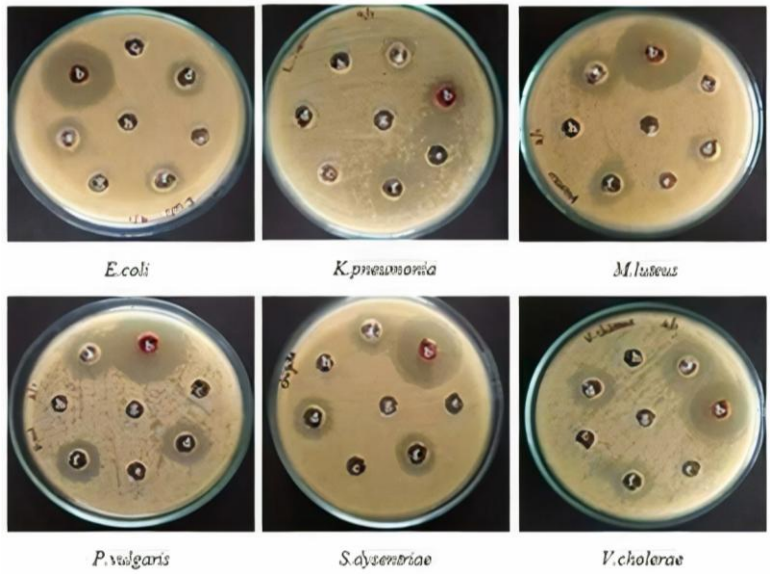


Fig. 9. Antibacterial activity of extracted bioactive compounds (a and b ethyl acetate extract of *S.parvus* and *S.californicus*, c and d methanol extract of *S.parvus* and *S.californicus*, e and f chloroform extract of *S.parvus* and *S.californicus*, g and h hexane extract of *S.parvus* and *S.californicus*)

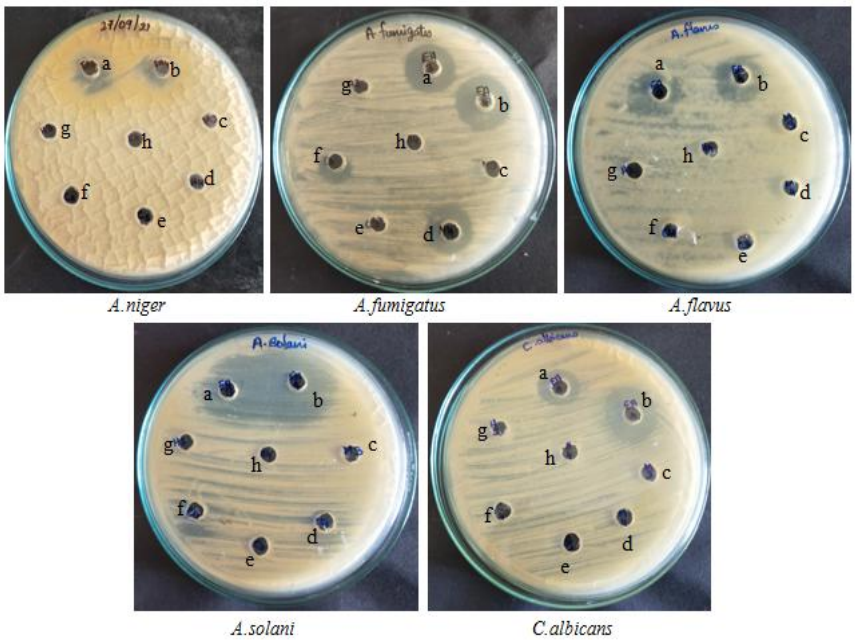


Fig. 10. Antifungal activity of extracted bioactive compounds (a and b ethyl acetate extract of *S.parvus* and *S.californicus*, c and d methanol extract of *S.parvus* and *S. californicus*, e and f chloroform extract of *S.parvus* and *S.californicus*, g and h hexane extract of *S.parvus* and *S. californicus*)

Table 8. Antibacterial activity of extracted bioactive compounds

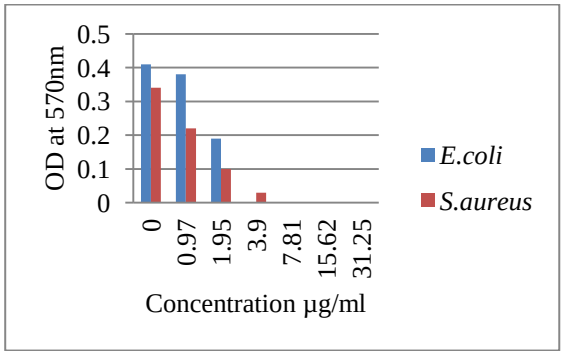
S.No	Test Organisms	Zone of Inhibition (mm in diameter)							
		***S.parvus* MG-3**				***S.californicus* MG-4**			
		Ethyl Acetate	**Chloroform**	**Hexane**	**Methanol**	**Ethyl Acetate**	**Chloroform**	**Hexane**	**Methanol**
1	*E.coli*	18	-	-	-	28	16	-	18
2	*K.pneumonia*	12	22	-	-	16	20	-	8
3	*M.luteus*	20	-	-	-	30	18	-	10
4	*P.vulgaris*	22	-	-	-	30	20	-	20
5	*S.dysentriae*	20	-	-	-	30	16	-	16
6	*V.cholera*	20	-	-	-	24	16	-	18

Table 9. Antifungal activity of extracted bioactive compounds

S.No	Test Organisms	Zone of Inhibition (mm in diameter)							
		***S.parvus* MG-3**				***S.californicus* MG-4**			
		Ethyl Acetate	**Chloroform**	**Hexane**	**Methanol**	**Ethyl Acetate**	**Chloroform**	**Hexane**	**Methanol**
1	*A.niger*	13	-	-	-	12	-	-	-
2	*A.fumigatus*	18	-	-	-	20	11	-	15
3	*A.flavus*	19	-	-	-	16	-	-	-
4	*A.solani*	20	-	-	-	24	-	-	-
5	*C.albicans*	14	-	-	-	19	-	-	-

Table 10. Minimum Inhibitory Concentration of crude extract of active strains

S.No	Actinomycetes isolates	Minimum Inhibitory Concentration (MIC) µg/ml	
		E. coli	***S. aureus***
1	*S.parvus* MG-3	1.95	3.9
2	*S.californicus* MG-4	3.9	7.81



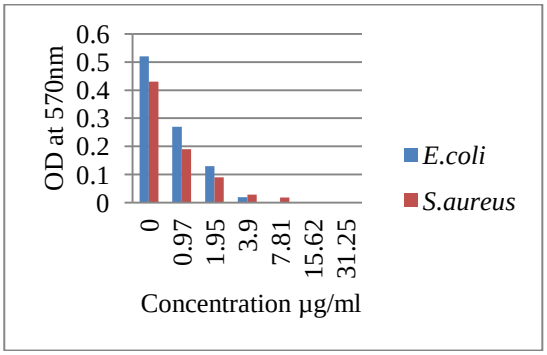


Fig. 11(a). Minimum Inhibitory Concentration of crude extract of active strain of *S.parvus* MG-3, (b). Minimum Inhibitory Concentration of crude extract of active strain of *S.californicus* MG-4

3.6 Minimum Inhibitory Concentration (MIC)

The MIC values of crude extract vary between 1.95-7.81*µg/ml.* The lowest MIC value 1.95µg/ml was found against *E.coli* and 3.9µg/ml concentration was found against *S.aureus in S.parvus* MG-3 extract. In *S.californicus* MG-4 3.9µg/ml was found against *E.coli* and 7.81µg/ml concentration was found against *S.aureus.* The results of MIC determination against bacteria are shown in the Figs. 11a, b and Table 9. The MIC of crude extracts is a potential source of antibiotics, which could lead to the development of new drugs for treating infectious diseases, according to Maleki and Mashinchian, [75]. Two actinomycetes isolates had different MICs and MBCs for both *S.aureus* and *E. coli*. These results were similar with the report [76].

3.7 DPPH Radical Scavenging Activity

The DPPH free radical scavenging assay is one of the most commonly used tests for assessing the antioxidant properties of drugs and other substances [77]. Many studies have shown that antioxidant substances can help reduce oxidative stress and slow or prevent the development of free radical-mediated diseases. Many synthetic antioxidants have been found to be toxic and/or carcinogenic. As a result, naturally occurring antioxidants have been considered [78]. In the current study, various in vitro assays were used to determine the antioxidant activity of *S.parvus* MG-3 and *S.californicus* MG-4 culture filtrate.

The percentage of scavenging effect on the DPPH radical were increased with the increase of concentration of extracts from 20 to 100µg/ml. The percentage of inhibition ranged from 40.26 at 20µg/ml to 65.60 at 100µg/ml for *S.parvus* MG-3 and 40.84 at 20µg/ml to 72.45 at 100µg/ml for *S.californicus* MG-4 extract. The IC50 value was found to be 16.24µg/ml and 19.5µg/ml for *S.parvus* MG-3 and *S.californicus* MG-4. Meanwhile, Ascorbic acid serving as the positive control exhibiting 76.92% of inhibition at 100µg/ml concentration with IC_{50} value of 10µg/ml. The *S.californicus* MG-4 extract has more scavenging activity than that of the *S.parvus* MG-3 strain (Fig.12a).

Comparable reports with rich source of Streptomyces species were reported in marine environment, a compound 5-(2, 4-dimethylbenzyl) pyrrolidin-2-one extracted from marine Streptomyces VITSVK5 spp which exhibited potent antioxidant activity [79]. Similarly the *Streptomyces* VITTK3 species showed effective DPPH radical scavenging activity in both extracellular and intracellular metabolites [80].

3.8 Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging activity of crude extracts was determined form the concentration of 20µg/ml to 100µg/ml. The percentage of inhibition was existing from 31.16% at 20µg/ml to 71.81% at 100µg/ml and 41.72% at 20µg/ml to

72.44% at 10µg/ml for *S.parvus* MG-3 and *S.californicus* MG-4 extracts respectively. IC50 of the extracts was 57µg/ml for *S.parvus* MG-3 and 23µg/ml for *S.californicus* MG-4. Meanwhile, Ascorbic acid serving as the positive control exhibited 82.95% of inhibition at 100µg/ml concentration with IC_{50} value of 26.23µg/ml (Fig. 12b).

3.9 Total Antioxidant Activity

Total antioxidant capacity of crude extracts (*S.parvus* MG-3 and *S.californicus* MG-4) was evaluated by the phosphomolybdenum method with ascorbic acid as a standard. *S.californicus* MG-4 extract found to possess the highest total antioxidant capacity. At the concentration of 20 to 100µg/ml, *S.parvus* MG-3 and *S.californicus* MG-4 showed the inhibition at the percentage ranging from 63.18% at 20µg/ml to 86.18% at 100µg/ml and 44.63% at 20µg/ml to 76.90% at 100µg/ml respectively. IC50 of the extracts was 13.33µg/ml and 24.43µg/ml for *S.parvus* MG-3 and *S.californicus* MG-4 respectively. Meanwhile, Ascorbic acid serving as the positive control exhibited 88.11% of inhibition at 100µg/ml concentration with IC50 value of 10µg/ml. *S.californicus* MG-4 extract showed maximum total antioxidant capacity when compared with *S.parvus* MG-3 (Fig.12c).

3.10 Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging potential of extracts (*S.parvus* MG-3 and *S.californicus* MG-4) is shown in Fig.12d. Hydroxyl radical scavenging activity of each extract was increased with increasing concentration of sample extracts. The hydroxyl radical scavenging activity observed was in the range of 20 to 100µg/ml. The percentage of inhibition was existing from 40.57% at 20µg/ml to 59.03% at 100µg/ml and 35.33% at 20 µg/ml to 73.46% at 100µg/ml for *S.parvus* MG-3 and *S.californicus* MG-4 extracts respectively. IC50 of the extracts was 90µg/ml and 63µg/ml for *S.parvus* MG-3 and *S.californicus* MG-4 respectively. Meanwhile, Ascorbic acid serving as the positive control exhibited 73.65 % of inhibition at 100µg/ml concentration with IC_{50} value of 17.89µg/ml. *S.parvus* MG-3 extract was found to be higher hydroxyl radical scavenging activity. Hydroxyl radical is one of the reactive oxygen species generated in the body and removing hydroxyl radicals is important for antioxidant defence in living cell systems [81].

3.11 Reducing Power Activity

The reducing capacity of the actinobacterial extracts equivalent to the ascorbic acid was tested. Total reducing power assay of extracts were determined for the concentration from 20µg/ml to 100µg/ml. When compared with the *S.parvus* MG-3 (0.75 at 100µg/ml), *S.californicus* MG-4 extract showed higher absorbance (0.89 at 100µg/ml). Meanwhile, Ascorbic acid serving as the positive control exhibited 4.31 at 100µg/ml. Among the extract *S.californicus* MG-4 exhibited the most reducing power. This result indicates that the extracts show great reducing power (Fig.12e). Ferric reducing assay is performed to measure the reducing power of the compounds. In this assay, the reductants would cause the reduction of Fe^{+3} to Fe^{+2} by donating electron and the amount of Fe^{+2} complex formed can be monitored. Increasing absorbance at 700nm indicates an increase in reductive ability [82]. The result is in justification with the study of [83,84].

The results indicated that the ethyl acetate extract of *S.parvus* MG-3 and *S.californicus* MG-4 culture filtrate provides significant free radical potential under various in vitro assays. Hence, the present data suggest that the ethyl acetate extract of culture filtrate could be a potential source of natural antioxidant for the treatment of radical related diseases.

3.12 FTIR Analysis

FTIR spectrum of the crude extract *S.parvus* MG-3 showed that high absorption bands at 1017.04 cm^{-1} represent the P-F stretching and 3353.40 cm^{-1}indicate the N-H stretch in primary amide, 2926.58 cm^{-1} and 2851.91 cm^{-1} that indicate C-H stretch in alkaline and methylene (Tables 11 and 12). *S.californicus* MG-4 showed strong absorption bands 2833.51 cm^{-1} which indicates to methyl ether groups and 2358.70 cm^{-1} represent the amide. The band at 1405.53 cm^{-1} corresponds to Organic sulfates (Figs.13 and 14). FTIR spectra of crude extract showed some different vibrational peaks of these functional groups in the extract.

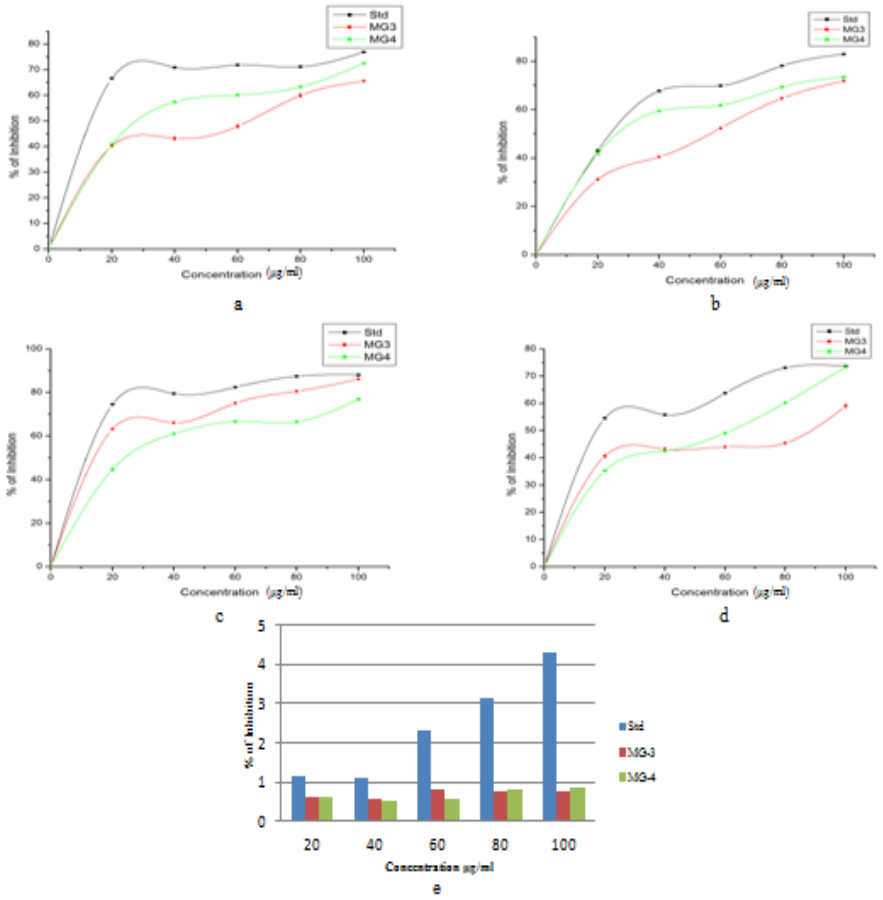


Fig. 12. Antioxidant results of the crude extract from *Streptomyces* sp. (a) DPPH scavenging assay, (b) Hydrogen peroxide scavenging activity, (c) Total antioxidant activity, (d) Hydroxyl radical scavenging activity, (e) Reducing power assay

Table 11. FTIR analysis of *S.parvus* MG-3

S. No	Group frequency, Wave number (cm-1)	Functional groups	Origin
1	3353.40	Primary amide NH_2 asymmetric Stretch	N-H
2	2926.58	Methylene C-H asym/sym. Stretch	C-H
3	2851.91	Alkane C-H Stretch	C-H
4	2358.24	C-H Stretching	C-H
5	1651.55	Alkenyl C=C Stretch	C=C
6	1403.13	N-H Stretching	N-H
7	1017.04	P-F Stretching	P-F

Table 12. FTIR analysis of *S.californicus* MG-4

S.No	Group frequency, Wave number (cm-1)	Functional groups	Origin
1	3339.64	Normal "polymeric" OH stretch	O-H
2	2833.51	Methoxy, methyl ether	C-H
3	2358.70	Amide	-
4	1655.24	Aromatic combination bands	-
5	1405.53	Organic sulfates	-
6	1019.94	Organic siloxane or silicone	Si-O-C

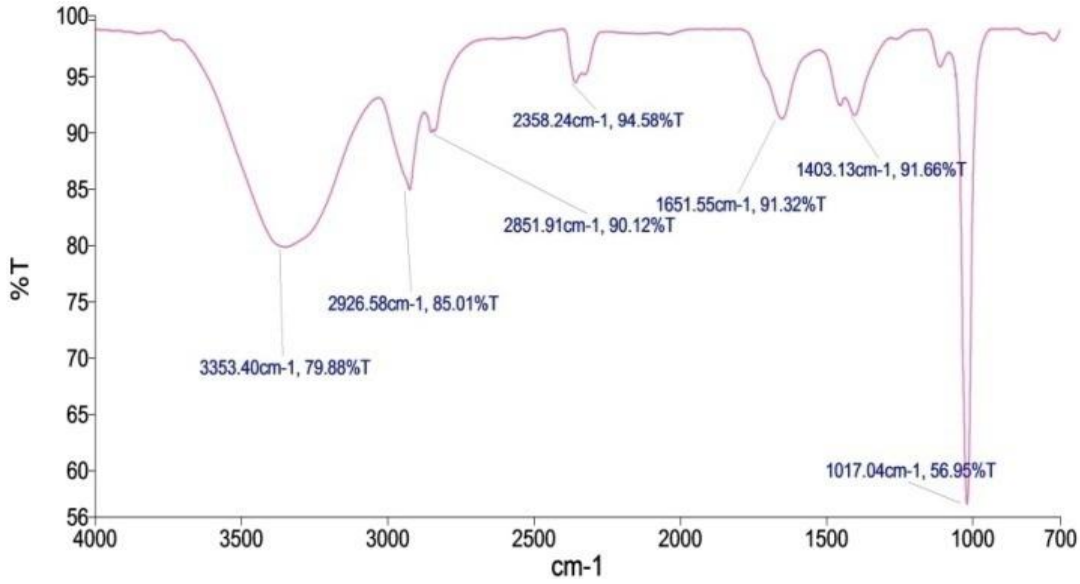


Fig. 13. FTIR spectral of functional groups present in crude ethyl acetate extracts of *S.parvus* MG-3

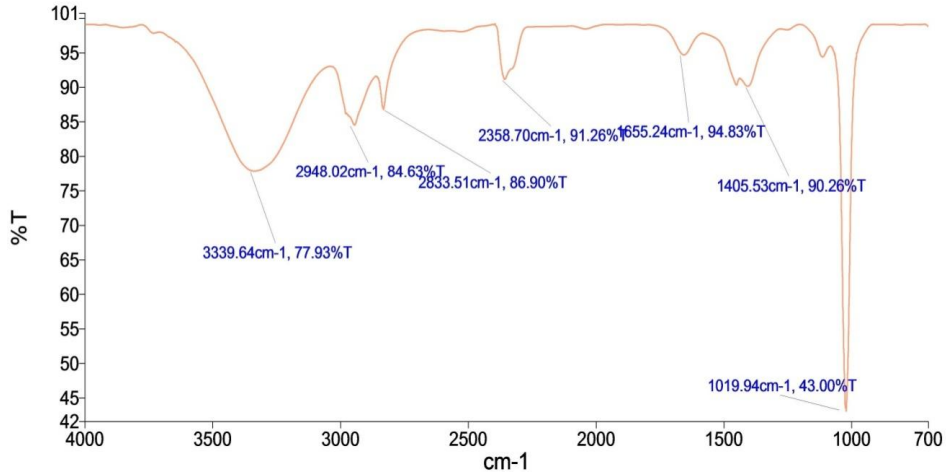


Fig. 14. FTIR spectral of functional groups present in crude ethyl acetate extracts of *S. californicus* MG-4

3.13 GCMS

Gas chromatography-mass spectrometry (GC-MS) analysis the crude ethyl acetate extract showed 63 compounds in *S.parvus* MG-3. These chemical compounds have antimicrobial, anticancer, anti-inflammatory and antioxidant properties. 3-Eicosene, (E)-, Hexadecanoic acid, n-Hexadecanoic acid, 1-NONADECENE, Phenol, 3,5-bis(1,1-dimethylethyl)-, these are the major compounds derived from *S.parvus* MG-3 (Fig. 15 & Table 13). 68 chemical compounds were found in *S.californicus* MG-4 which include TETRADECANOIC ACID, 3-Octadecene, (E)-, n-Hexadecanoic acid, 9-Hexadecenoic acid (Fig. 16 & Table 14). Similar results were reported by [85,86]. Jaina et al. [87] reported that the 1,2-benzenedicarboxylic acid has insecticidal, pesticide and antitumour effects; the hexadecanoic acid has antioxidant, nematicide, hypocholesterolemic and pesticide effects and the 9,12-octadecadienoic acid has anticarcinogenic, antiatherogenic, antioxidant and anti-inflammatory effects. In addition, Kumar et al. [88] concluded that compounds like hexadecanoic acid, methyl ester and 9,12-octadecadienoic acid (Z,Z)-, methyl ester have anticancer properties.

Table 13. GC-MS analysis of crude ethyl acetate extract of *S.parvus* MG-3

Peak#	R.Time	Area	Area%	Name
1	5.950	2584312	3.05	
2	6.178	174797	0.21	Acetic acid, (acetyloxy)-
3	6.548	1769354	2.09	2-FURANCARBOXALDEHYDE, 5-METHYL-
4	6.946	633625	0.75	
5	6.991	823625	0.97	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
6	7.337	921553	1.09	2H-Pyran-2,6(3H)-dione
7	7.849	354526	0.42	2,5-FURANDIONE, DIHYDRO-
8	8.519	1506085	1.78	Pentanoic acid, 4-oxo-
9	9.378	647765	0.77	Furyl hydroxymethyl ketone
10	9.751	105833	0.13	Linalool
11	9.992	819634	0.97	Levoglucosenone
12	10.858	8472973	10.01	1,5-ANHYDRO-6-DEOXYHEXO-2,3-DIULOSE
13	11.756	162945	0.19	3-Tetradecene, (Z)-
14	11.913	274197	0.32	
15	12.760	3312963	3.91	
16	15.796	411814	0.49	1-Tridecene
17	18.012	2891722	3.42	Phenol, 3,5-bis(1,1-dimethylethyl)-
18	18.168	545554	0.64	DODECANOIC ACID, METHYL ESTER
19	19.058	744044	0.88	DODECANOIC ACID
20	19.252	209625	0.25	Caryophyllenyl alcohol
21	19.367	1079038	1.27	3-Octadecene, (E)-
22	19.898	1613285	1.91	Dodecanoic acid, 1-methylethyl ester
23	20.683	701797	0.83	
24	21.492	623687	0.74	Methyl tetradecanoate
25	22.226	3764582	4.45	TETRADECANOIC ACID
26	22.472	476779	0.56	PENTADECANOIC ACID, METHYL ESTER
27	22.573	5888746	6.96	3-Eicosene, (E)-
28	22.676	582419	0.69	1-Decanol, 2-methyl-
29	23.016	926965	1.10	ISOPROPYL MYRISTATE
30	23.279	565356	0.67	9-OCTADECENOIC ACID (Z)-
31	23.565	201567	0.24	1,2-BENZENEDICARBOXYLIC ACID, BIS(2-METHYL
32	23.622	258308	0.31	1H-1,2,3,4-Tetrazol-5-amine, 1-ethyl-N-[(1-methyl-1H-py
33	23.964	616232	0.73	Pentadecanoic acid, 14-methyl-, methyl ester
34	24.181	912449	1.08	2H-Pyran, 3,6-dihydro-4-methyl-2-(2-methyl-1-propenyl)-
35	24.253	338266	0.40	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
36	24.498	4763731	5.63	Hexadecanoic acid, methyl ester
37	24.592	316165	0.37	
38	24.693	1191675	1.41	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylp
39	24.932	2226695	2.63	1,2-Benzenedicarboxylic acid, butyl octyl ester
40	25.142	8607707	10.17	n-Hexadecanoic acid
41	25.473	6187925	7.31	1-NONADECENE
42	26.256	175503	0.21	5-Methyl-1-phenylbicyclo[3.2.0]heptane
43	26.800	376711	0.45	9,12-Octadecadienoic acid, methyl ester
44	26.891	608001	0.72	9-OCTADECENOIC ACID (Z)-, METHYL ESTER
45	26.965	260653	0.31	METHYL DIHYDROMALVALATE
46	27.236	2235792	2.64	Methyl stearate
47	27.783	2278001	2.69	OCTADECANOIC ACID
48	28.119	2614735	3.09	1-Tricosene
49	28.610	158424	0.19	Cyclobutyl isopropylphosphonofluoridate

Peak#	R.Time	Area	Area%	Name
50	28.979	682902	0.81	Propanal, 3-(4-benzyloxyphenyl)-
51	30.017	542940	0.64	Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-me
52	30.525	275412	0.33	
53	30.567	388325	0.46	Octadecyl trifluoroacetate
54	31.578	687258	0.81	Hexanoic acid, 2-ethyl-, hexadecyl ester
55	31.838	189100	0.22	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl est
56	31.999	205017	0.24	Carbonic acid, bis(2-ethylhexyl) ester
57	32.478	2294111	2.71	Di-n-octyl phthalate
58	33.035	111830	0.13	Octanoic acid, tetradecyl ester
59	33.289	139617	0.16	Octacosanol
60	34.083	238284	0.28	Hexanoic acid, 2-ethyl-, hexadecyl ester
61	34.788	603141	0.71	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
62	35.558	195483	0.23	2,6,10,14,18,22,-TETRACOSAHEXAEN, 2,6,10,15,19,23
63	36.629	159560	0.19	Cholest-5-en-3-ol (3.beta.)-, tetradecanoate

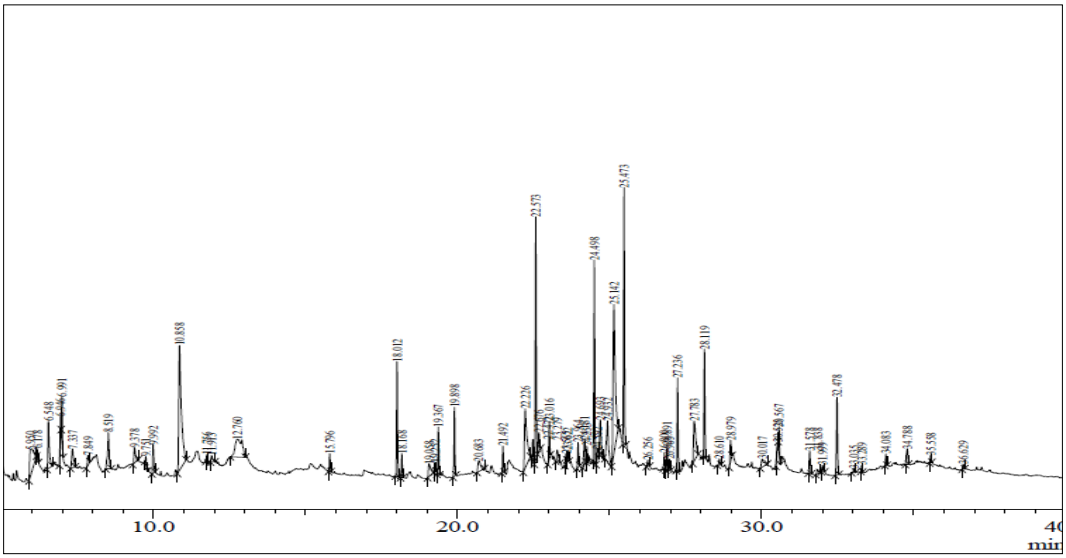


Fig. 15. GC-MS analysis of crude ethyl acetate extract of *S.parvus* MG-3.

Table 14. GC-MS analysis of crude ethyl acetate extract of *S.californicus* MG-4

Peak#	R.Time	Area	Area%	Name
1	5.962	5239179	4.92	2-HYDROXYPROPANOIC ACID
2	6.542	7435228	6.98	2-FURANCARBOXALDEHYDE, 5-METHYL-
3	6.947	432419	0.41	ETHANONE, 1-(3-HYDROXY-2-FURANYL)-
4	6.998	1149865	1.08	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
5	7.347	1440512	1.35	2H-Pyran-2,6(3H)-dione
6	8.459	654752	0.61	Benzeneacetaldehyde
7	8.523	534958	0.50	Pentanoic acid, 4-oxo-
8	9.996	547100	0.51	Levoglucosenone
9	10.886	24819453	23.30	1,5-ANHYDRO-6-DEOXYHEXO-2,3-DIULOSE
10	12.076	607411	0.57	4-METHOXY-2,5-DIMETHYL-3(2H)-FURANONE
11	12.912	1141393	1.07	
12	15.798	380149	0.36	3-Hexadecene, (Z)-
13	18.019	138561	0.13	Phenol, 3,5-bis(1,1-dimethylethyl)-

Peak#	R.Time	Area	Area%	Name
14	18.174	363716	0.34	Acetamide, N-(2-phenylethyl)-
15	18.591	505686	0.47	Cyclopentane, 1,1,3,4-tetramethyl-, trans-
16	19.063	1233775	1.16	DODECANOIC ACID
17	19.370	406078	0.38	3-Octadecene, (E)-
18	19.898	504837	0.47	Dodecanoic acid, 1-methylethyl ester
19	20.680	789547	0.74	[15N]-ANILINE
20	20.996	451706	0.42	2-PROPENOIC ACID, DODECYL ESTER
21	21.483	185726	0.17	Methyl tetradecanoate
22	21.652	1728849	1.62	
23	22.241	6013442	5.65	TETRADECANOIC ACID
24	22.475	720178	0.68	
25	22.573	1302923	1.22	3-Octadecene, (E)-
26	23.016	324366	0.30	Isopropyl myristate
27	23.076	229813	0.22	3-ISOBUTYLHEXAHYDROPYRROLO[1,2-A]PYRAZI
28	23.167	765709	0.72	Pentadecanoic acid
29	23.295	2763343	2.59	9-OCTADECENOIC ACID (Z)-
30	23.557	274490	0.26	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
31	23.704	387691	0.36	PENTADECANOIC ACID
32	23.965	270857	0.25	TETRADECANOIC ACID, 12-METHYL-, METHYL ES
33	24.190	337181	0.32	2,3-NONADIENE
34	24.256	265058	0.25	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
35	24.497	886203	0.83	Hexadecanoic acid, methyl ester
36	24.608	305203	0.29	n-Hexadecanoic acid
37	24.692	1494124	1.40	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylp
38	24.870	1124086	1.06	
39	25.165	16733336	15.71	n-Hexadecanoic acid
40	25.383	1095392	1.03	2-FURANCARBOXALDEHYDE, 5,5'-[OXYBIS(METH
41	25.472	1803338	1.69	1-Octadecene
42	25.851	110898	0.10	Isopropyl palmitate
43	26.891	291154	0.27	9-Octadecenoic acid, methyl ester, (E)-
44	27.234	1204215	1.13	Methyl stearate
45	27.383	183298	0.17	BENZENE, (2-DECYLDODECYL)-
46	27.475	995448	0.93	9-Hexadecenoic acid
47	27.708	167122	0.16	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl este
48	27.777	2352776	2.21	OCTADECANOIC ACID
49	28.116	1333373	1.25	Trifluoroacetoxy hexadecane
50	28.977	1125365	1.06	Benzaldehyde, 3-benzyloxy-2-fluoro-4-methoxy-
51	29.672	228098	0.21	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl este
52	29.984	268418	0.25	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmet
53	30.175	419658	0.39	9-Octadecenamide, (Z)-
54	30.490	1722149	1.62	Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-me
55	31.573	1009334	0.95	Hexanoic acid, 2-ethyl-, hexadecyl ester
56	31.830	192283	0.18	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl est
57	31.991	238318	0.22	Carbonic acid, bis(2-ethylhexyl) ester
58	32.474	3855317	3.62	Di-n-octyl phthalate

Peak#	R.Time	Area	Area%	Name
59	33.033	152142	0.14	Octanoic acid, tetradecyl ester
60	33.270	215136	0.20	Docosanoic acid, ethyl ester
61	34.078	363298	0.34	Hexanoic acid, 2-ethyl-, hexadecyl ester
62	34.784	607749	0.57	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
63	35.193	311268	0.29	9-Octadecenamide, (Z)-
64	35.553	363443	0.34	Squalene
65	36.627	226670	0.21	Cholesta-3,5-diene
66	37.224	193709	0.18	cis-9-Tetradecenoic acid, heptyl ester
67	40.128	299098	0.28	1-Hydroxy-3-(octanoyloxy)propan-2-yl decanoate
68	40.441	291284	0.27	1-Hexacosene

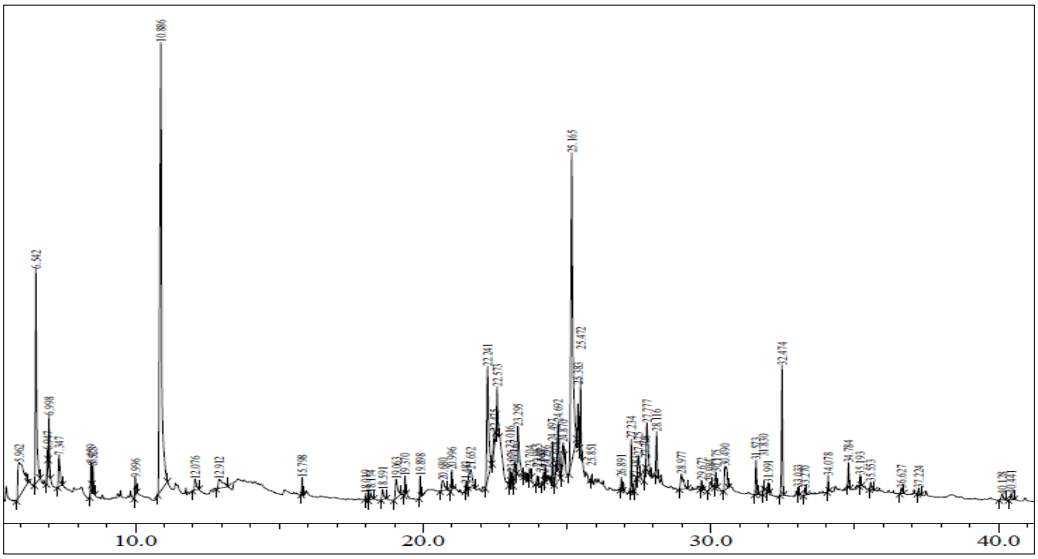


Fig. 16. GC-MS analysis of crude ethyl acetate extract of *S.californicus* MG-3.

Chromatogram D:\GCMS DATA\GC-MS Data\Tamil nadu\Nivetha\ *S.parvus*.qgd

Chromatogram D:\GCMS DATA\GC-MS Data\Tamil nadu\Nivetha\ *S.californicus*.qgd

5. CONCLUSIONS

Previously, a few strains of *S.parvus* and *S.californicus* were found in the soil. To the best of our knowledge, there have been no reports of *S.parvus* and *S.californicus* mangrove sediment soil isolates. This is the first report *S.parvus* MG-3 and *S.californicus* MG-4 were isolated from the mangrove region. From these present findings, it could be concluded that the working actinobacterial strains *S.parvus* MG-3 and *S.californicus* MG-4 isolated from Muthupettai mangrove sediment soil samples, has antagonistic properties against bacterial and fungal pathogens and its crude ethyl acetate extract showed high antibacterial, antifungal as well as antioxidant activities. The present investigation clearly reveals that the biodiversity and distribution of actinomycetes in mangrove soil is a major source to produce novel bioactive compounds against the pathogenic microbes and they are pharmacologically important for the development of natural drugs.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study does not involve experiments on animals or human subjects.

ACKNOWLEDGEMENTS

Authors are sincerely thanks to University Research Fellowship (URF), Bharathidasan University, Tiruchirappalli, Tamilnadu and DST-

SERB New Delhi, India for providing financial support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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