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

C. Nivetha a, T. Deepika a, A. Arjunan a, P. Sivalingam a, N. Revathi a and M. Muthuselvam a*#

a Department of Biotechnology, Bharathidasan University, Tiruchirappalli- 620024, Tamil Nadu, India.

Authors’ contributions

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

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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 1-3 meter 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
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, H₂O₂ 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 (UV) [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.

Scavenging activity (%) = [(A-B)/A] x 100

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

2.8.2 H$_2$O$_2$ 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.

Hydrogen peroxide activity (%) = [(Abs control-Abs sample)/Abs control] x 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 adequate 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 1 L) 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,

\[
\text{HRSA} (%) = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100
\]

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$_3$Fe(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 Muthupet 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 Muthupet 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 Muthupetai 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 Streptomycetes 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 Sathiayaseelam 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 | 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 | 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 strain 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  | White | White | Grey  |
| Substrate mycelium       | Yellow| Pale  | Pale  | Yellow| Brown  | Yellow| 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    | No    | 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
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.
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](image)

*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](image)

*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 | 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 | 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 1.95 μg/ml was found against E.coli and 3.9 μg/ml concentration was found against S.aureus in S.parvus MG-3 extract. In S.californicus MG-4 3.9 μg/ml was found against E.coli and 7.81 μg/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 100 μg/ml. The percentage of inhibition ranged from 40.26 at 20 μg/ml to 71.81% at 100 μg/ml for S.parvus MG-3 and 40.84 at 20 μg/ml to 72.45% at 100 μg/ml for S.californicus MG-4 extract. The IC50 value was found to be 16.24 μg/ml and 19.5 μg/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 100 μg/ml concentration with IC50 value of 10 μg/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 20 μg/ml to 100 μg/ml. The percentage of inhibition was existing from 31.16% at 20 μg/ml to 71.81% at 100 μg/ml and 41.72% at 20 μg/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 IC50 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 IC50 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 Fe3⁺ to Fe2⁺ by donating electron and the amount of Fe2⁺ 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 alkyl 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

| 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 |
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% | Name                                      |
|-------|--------|-------|-------------------------------------------|
| 1     | 5.950  | 2584312 | 3.05 Acetic acid, (acetylxy)-              |
| 2     | 6.178  | 174797  | 0.21 2-FURANCARBOXYALDEHYDE, 5-METHYL-     |
| 3     | 6.548  | 1769354 | 2.09 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one |
| 4     | 6.946  | 636325  | 0.75 2H-Pyran-2,6(3H)-dione                |
| 5     | 6.991  | 823625  | 0.97 2,5-FURANDIONE, DIHYDRO-              |
| 6     | 7.337  | 921553  | 1.09 Pentanoic acid, 4-oxo-               |
| 7     | 7.849  | 354526  | 0.42 Furfural, 4-oxo-                     |
| 8     | 8.519  | 1506085 | 1.78 Levoglucosenone                      |
| 9     | 9.378  | 647765  | 0.77 Furfural hydroxymethyl ketone         |
| 10    | 9.751  | 105833  | 0.13 Linalool                             |
| 11    | 9.992  | 819634  | 0.97 Caryophyllenyl alcohol               |
| 12    | 10.858 | 8472973 | 10.01 1,5-ANHYDRO-6-DEOXYHEXO-2,3-DIULOSE  |
| 13    | 11.756 | 162945  | 0.19 3-Tetradecene, (E)-                  |
| 14    | 11.913 | 274197  | 0.32 3-Octadecone, (E)-                   |
| 15    | 12.760 | 3312963 | 3.91 Dodecanoic acid, 1-methylethyl ester |
| 16    | 15.796 | 411814  | 0.49 1-Tridecane                          |
| 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-Octadecane, (E)-                   |
| 22    | 19.898 | 1613285 | 1.91 Dodecanoic acid, 1-methylethyl ester |
| 23    | 20.683 | 701797  | 0.83 Methyl decanoate                     |
| 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-OCTADECANOIC 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 Butyrolactone, methyl ester          |
| 38    | 24.693 | 1191675 | 1.41 Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methyl |
| 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-OCTADECANOIC ACID (Z)-, METHYL ESTER |
| 45    | 26.965 | 260653  | 0.31 METHYL DICYCLOHEXANE                     |
| 46    | 27.236 | 2235792 | 2.64 Methyl stearate                       |
| 47    | 27.783 | 2278001 | 2.69 OCTADECANOIC ACID                     |
| 48    | 28.119 | 2614735 | 3.09 1-Nonacosene                         |
| 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  | Octadecyl trifluoroacetate                                                                    |
| 53   | 30.567 | 388325 | 0.46  | Hexanoic acid, 2-ethyl-, hexadecyl ester                                                       |
| 54   | 31.578 | 687258 | 0.81  | Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl est                                       |
| 55   | 31.838 | 189100 | 0.33  | Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-me                                     |
| 56   | 31.999 | 275412 | 0.33  | Octadecyl trifluoroacetate                                                                    |
| 57   | 32.478 | 2294111| 2.71  | Octacosanol                                                                                   |
| 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-FURANCARBALDEHYDE, 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  | Levogluconone                                                                                   |
| 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  | 3-Hexadecene, (Z)-                                                                              |
| 12   | 15.798 | 380149 | 0.36  | 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-methyl              |
| 38   | 24.870 | 1124086| 1.06  | n-Hexadecanoic acid                                                |
| 39   | 25.165 | 1673336| 15.71 | 2-FURANCARBOXALDEHYDE, 5,5'-OXYBIS(METH                             |
| 40   | 25.383 | 1095392| 1.03  | 1-Octadecene                                                        |
| 41   | 25.472 | 1803338| 1.69  | Isopropyl palmitate                                               |
| 42   | 25.851 | 110898 | 0.10  | 9-Octadecenoic acid, methyl ester, (E)-                            |
| 43   | 26.891 | 291154 | 0.27  | Methyl stearate                                                    |
| 44   | 27.234 | 1204215| 1.13  | BENZENE, (2-DECYLDODECYL)-                                         |
| 45   | 27.383 | 183298 | 0.17  | 9-Hexadecanoic acid                                                |
| 46   | 27.475 | 995448 | 0.93  | 2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl est            |
| 47   | 27.708 | 167122 | 0.16  | OCTADECANOIC ACID                                                  |
| 48   | 27.777 | 2352776| 2.21  | Trifluoracetoxoy hexadecane                                        |
| 49   | 28.116 | 1333373| 1.25  | Benzaldehyde, 3-benzylxy-2-fluoro-4-methoxy-                        |
| 50   | 28.977 | 1125365| 1.06  | 2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl est            |
| 51   | 29.672 | 228098 | 0.21  | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmet            |
| 52   | 29.984 | 268418 | 0.25  | 9-Octadecanamide, (Z)-                                            |
| 53   | 30.175 | 419658 | 0.39  | Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-me          |
| 54   | 30.490 | 1722149| 1.62  | Hexanoic acid, 2-ethyl-, hexadecyl est                              |
| 55   | 31.573 | 1009334| 0.95  | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl est            |
| 56   | 31.830 | 192283 | 0.18  | Carbonic acid, bis(2-ethylhexyl) est                                |
| 57   | 31.991 | 238318 | 0.22  | Di-n-octyl phthalate                                               |
| 58   | 32.474 | 3855317| 3.62  |                                                                     |
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.](attachment:image)

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.

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**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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