

## Original Research Article

***Streptomyces peucetius* M1 and *Streptomyces lavendulae* M3 soil isolates as a promising source for antimicrobials discovery**

### Abbreviations:

MRSA, Methicillin-resistant *Staphylococcus aureus*; ISP, International Streptomyces Project; DPPH, 2,2'-diphenyl-1-picrylhydrazyl; ~~GC-MS, Gas Chromatography Mass Spectrometry; EI, Electron Ionization~~; NIST, National Institute of Standard and Technology; MTCC, Microbial Type Culture Collection, and Gene Bank; MH Muller Hinton; SDB, Sabouraud Dextrose Broth; SDA, Sabouraud Dextrose Agar; MIC, Minimum Inhibitory Concentration; IC, Inhibitory Concentration; MDR, Multi-Drug Resistant; PDR, Pan-Drug Resistant; NCBI, National Center for Biotechnology Information; PCR, Polymerase Chain Reaction; RAPD Random Amplified Polymorphic DNA.

### Abstract:

The ever-increasing fatality due to the surge of drug resistance has demanded the development of therapeutic metabolites with novel modes of mechanism. Despite the discovery of new antimicrobials, antibiotic resistance is increasing at an alarming rate. The emergence of novel molecules is necessary for targeting the Multidrug (MDR) and Pan-Drug resistant (PDR) pathogens which are the principal cause of life-threatening infections. *Streptomyces* strains commonly found in the soil produce secondary metabolites which have outstanding potential for being an antifungal and antibacterial agent. This study aims to isolate, identify, and characterize *Streptomyces* strains isolated from soil and extract their crude metabolites to determine their hidden therapeutic property. Out of four isolates identified, *Streptomyces peucetius* and *Streptomyces lavendulae* have shown significant antimicrobial activity against test organisms. To further determine the activity of their extracts GC-MS analysis, and MIC, as well as radical scavenging activity, were determined. GC-MS analysis revealed the presence of compounds 2,3-butanediol, 1-methyl-2-pyrrolidone-4-carboxamide, and L-Lactic acid which are frequently associated with potent antimicrobial nature. The most vulnerable pathogen to M1 extract was *Candida albicans* with a MIC value of 7.8125 µg/mL while the susceptible bacteria to M3 extract included *Staphylococcus aureus* with a MIC value of 3.90 µg/mL. The data obtained for antioxidant activity in the case of metabolic extracts M1 and M3 had an IC<sub>50</sub> value of 43.17 µg/mL and 22.65 µg/mL respectively. The above findings hint at the unrealized potential of *Streptomyces* strains.

**Keywords:** Fatality, Drug Resistance, Antimicrobials, Multidrug, Pan-Drug, *Streptomyces*, Soil, GC-MS, MIC, Radical scavenging activity

### 1. Introduction:

Drug-resistant illnesses are a serious health hazard responsible for 17 million deaths annually[1]. Given the swiftly diminishing arsenal of commercial drugs against major pathogens and steadily expanding drug resistance, the discovery and development of novel therapeutics are imperative, ideally naturally occurring ones with novel modes of action. New therapeutic drugs have entered the clinical field in recent years; however, they are accompanied by unpleasant negative effects[2]. Thus, there is an exclusive demand for novel therapeutic medications to combat the Multi- and Pan-drug resistant pathogens that are the main cause of life-threatening illnesses. The need for alternative antibiotics grows, particularly to combat super-infections, secondary infections, and nosocomial infections[3]. Control of these drug-resistant pathogens can be achieved by implementing another group of microorganisms, as the majority of antibacterial drugs are either natural products of microbial origin or analogues[4]. There are numerous sources where microorganisms capable of pharmaceutical and industrial application exist. Soil, in particular, is a heavily exploited ecological niche whose occupants generate a wide range of beneficial biologically active natural metabolites, including therapeutically relevant antibiotics[5].

*Streptomyces* are among the most abundant microorganisms that can be found in soil and aquatic environments. They produce secondary metabolites that have a variety of potential bioactive properties, including antibiotic, antifungal, and antiviral activity against a variety of microorganisms[6]. More than 70% of economically significant antibiotics are produced by the genus *Streptomyces*, which also produces a large range of bioactive substances with pharmacological and agricultural applications[7]. Natural manufacturers of bioactive compounds, *Streptomyces* are abundant in nature and are commonly used in the pharmaceutical sector owing to their seemingly limitless ability to produce secondary metabolites with different chemical structures and biological activity[8]. They are a vital source of secondary metabolites like antibiotics, biopesticide agents, plant growth hormones, antitumor compounds, antiviral agents, pharmacological compounds, pigments, enzymes, enzyme inhibitors, and anti-inflammatory compounds[9,10]. *Streptomyces* sp. VITBRK2 produced secondary metabolites which can be used to control several multi-drug resistance bacteria according to Rajan et al.[11]. *Streptomyces anulatus* NEAE-94's ethyl acetate extract exhibited strong biological activity against *Staphylococcus aureus*, Multidrug-resistant *S. aureus*, and *Bacillus subtilis* [12]. Kerase is an enzyme synthesized from *Streptomyces fradiae* while *S. griseus* secretes pronase is utilized to make hydrolysate proteins from various protein sources for application in biotechnology products [13]. *Streptomyces* can break down complex polymers and play a significant part in recycling organic carbon [14]. Ivermectin is produced by *Streptomyces avermitilis* and is effective against *Wucheria bancroftii* thus demonstrating the ability of *Streptomyces* species to produce high nematocidal compounds[15]. A crucial step in the manufacture of a wide range of structurally different bioactive chemicals, such as antibiotics, anticancer drugs, antiviral medications, and immunosuppressants, is fermentation.

The current research demonstrates the isolation, identification, and molecular characterization of the *Streptomyces* species from the soil along with the fermentation of their secondary metabolic extract. The results reveal the bioactive potential of the extracts along with the constituent compounds present in the extracts. The results impose the need for further investigation of the biologically significant extract.

## 2 Material & Methods:

### 2.1 Isolation of *Streptomyces* strain and Preliminary antimicrobial screening of isolated strains:

Soil samples from Kerala were acquired, sealed in sterile Ziploc bags, and transported to the laboratory. They were air-dried and suspended in sterile distilled water (1g/100mL), homogenized by vortexing, and 0.1mL of the serially diluted sample of  $10^{-5}$  dilution was pour plated on the International Streptomyces Project 4 (ISP-4) agar for isolation of *Streptomyces*[16]. Post incubation, the antimicrobial activity of the isolate was recorded by observing the inhibition zone around the inoculated spot with the isolated strains. The isolated *Streptomyces* colonies were carefully picked and sub-cultured on ISP-4 agar for the isolation of a single colony-[17].

### 2.2 Identification of *Streptomyces* strains:

Genomic DNA was isolated from 24-hour growing cultures using the Lysozyme-CTAB approach[18]. After qualitative examination of the isolated DNA using Agarose gel electrophoresis, the 16S rRNA gene was amplified using PCR. The isolates were then identified using 16S rRNA gene sequencing[19]. Using the BLAST Tool, the FASTA file obtained from the sequencing results was aligned with the nucleotide database. The sequences were then aligned using MUSCLE pairwise alignment, and the phylogenetic tree was built using MEGA 11 software's Neighbor-joining method, the Bootstrap Phylogeny test with 1000 bootstrap replications, and the Tamura-Nei model. The gene sequences were submitted to GenBank. The isolates' accession numbers are listed in Table 1.

SR No	Isolates	Organism name	Accession-ID
1	M1	<i>Streptomyces peucetius</i>	ON786999
2	M2	<i>Streptomyces fradiae</i>	ON787000
3	M3	<i>Streptomyces lavendulae</i>	ON787001
4	M4	<i>Streptomyces exfoliatus</i>	ON787002

Table 1: Isolate characterization and Sequence analysis

### 2.3 Fermentation and Metabolite Extraction:

Following the preliminary screening, the most potent strains M1 and M3 were taken for fermentation using a synthetic medium. Isolate M1 was grown in media containing Dextrose (60gm/L), Yeast extract (8gm/L), Malt extract (20gm/L), MOPS sodium salt (15gm/L), Sodium chloride (2gm/L), Magnesium chloride (0.1gm/L), Zinc sulphate (0.01gm/L), Ferrous sulphate (0.01gm/L). While Isolate M3 was fermented using media containing Dextrose (20 gm/L), Fructose (20 gm/L), Starch (10 gm/L), Soybean meal (40 gm/L), Sodium chloride (5 gm/L), Calcium carbonate (3 gm/L). The pH for the fermentation medium of both the isolate was maintained at  $7.0 \pm 0.2$ . 1 % inoculum was added to flasks comprising 300 mL of production media made specifically for each strain, respectively[20]. For 5 days, the flasks were incubated at 180 rpm at 37°C in an orbital shaker. For extraction of metabolite, the culture supernatant was subjected to liquid-liquid extraction using ethyl acetate. The solvent was added to the supernatant in a 1:1 (v/v) ratio and vigorously agitated for 10 minutes[21]. The metabolite comprising the ethyl acetate phase was separated from the aqueous phase using a separating funnel. The ethyl acetate layer was concentrated by evaporating the extract at room temperature, and the residue was reconstituted using methanol to obtain a

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yellowish-green extract[22]. The bioactive compounds were investigated for their antimicrobial property against pathogenic organisms.

## **2.4 Characterisation of isolated metabolic extracts:**

### **2.4.1. Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis and Chemical Components Investigations:**

The volatile components from the metabolic extracts of isolate M1 and M3 were analyzed in the GC-MS system (Shimadzu GCMS-QP2010)[23, 24]. The analysis was carried out on the Rtx-5MS column (30 m long, 0.25 mm in diameter, and with a 0.25 µm film thickness) having 5% diphenyl and 95% dimethylpolysiloxane chemistry. 1 µL of samples in a split ratio of 1:10 was analyzed using a helium carrier gas with a flow rate of 14 mL/min. To analyze the components of both the extracts, they were subjected to experimental conditions, which were regulated, including injector temperature, ion-source temperatures of 230 °C and 220 °C respectively, and oven temperature, which was programmed to vary in a gradient fashion from 60 °C with the isothermal environment for 2 min, with a 10 °C/min increase to 280 °C with 8.00 min isothermal condition. The program spanned 30 min in total to analyze the components present in the sample. The mass spectra were obtained in the region of 35 to 500 m/z. The components of the extracts were recognized by comparing their mass spectra to those present in the Wiley and NIST libraries[24].

## **2.5 Assessment of the Bioactive potential of the extracted metabolites:**

### **2.5.1 Determination of Anti-microbial property by Agar cup method:**

Petri plates with Mueller-Hinton and Sabouraud Dextrose (SDB) agar and a suspension of pathogenic bacteria with a final concentration of  $10^5$  CFU/ml were used to conduct the well diffusion procedure[25]. The crude metabolite extract of the isolates M1 and M3 were evaluated for antimicrobial activity against various pathogenic strains, such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Propionibacterium acne*, *Escherichia coli*, *Propionibacterium acne*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, Vancomycin-resistant *Enterococci* (VRE), Methicillin-resistant *Staphylococcus aureus* (MRSA), *Candida albicans* and *Aspergillus niger*. The above test organisms were obtained from MTCC Chandigarh and the few microbial cultures were obtained from existing laboratory test isolates. As a positive control, 20 µg/mL concentrations of chloramphenicol and fluconazole were administered. As a negative control, DMSO was employed. The plates were then incubated at 37°C for 24 hours.

### **2.5.2 Minimal Inhibitory Concentration:**

Minimum inhibitory concentration (MIC) determines the lowest concentration at which the crude metabolite extract inhibits complete growth[26]. The microdilution method was performed using 96 well microtiter plate, 100 µL of 1X Muller Hinton (MH), and Sabouraud Dextrose broth was dispensed in all the rows, except one row, to which 100 µL of 2X MH and Sabouraud Dextrose broth was added. Two-fold serial dilution was then performed from this row for five consecutive rows and 100 µL was discarded from the last row[27]. The extracted metabolites (10 mg) were suspended in 1 mL of DMSO (in the ratio of 1:10) and 100 µL was pipetted into each row[28]. The final optical density of the inoculum was adjusted according to the 0.5 McFarland standard and 10 µL was introduced into the

respective wells. Kanamycin (20 µg/mL) and DMSO were used as positive and negative controls. The plate was incubated for 18-24 hours at 37°C. After incubation, 5 µL resazurin was added to all the wells in the plate, incubated for 30 mins, and observed for colour change.

### 2.5.3 DPPH Free Radical Scavenging Activity:

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method was used to assess the free radical scavenging activities of the extracts[29]. *Streptomyces* extract concentrations ranging from 10µg/mL to 100µg/mL were prepared. The entire reaction volume was maintained at 4 mL, which included the sample, methanol as a blank, and 2 mL of DPPH reagent in each tube. The absorbance of each solution was measured at 517 nm using a spectrophotometer after 30 minutes of incubation in the dark.

For each metabolite, the % inhibition of the DPPH radical was computed using the following equation:

$$\% \text{ Free Radical Scavenging Activity} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100$$

Where  $A_{\text{blank}}$  is the absorbance of the control and  $A_{\text{sample}}$  is the absorbance of the test compound under study.  $IC_{50}$  was calculated from the graph plotted.

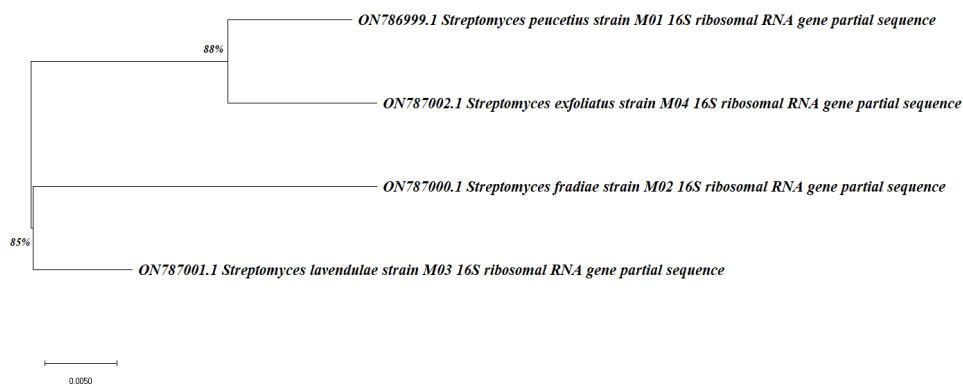
## 3 Result/Discussion:

### 3.1 Isolation and Preliminary screening of *Streptomyces* strain:

*Streptomyces* strains were successfully isolated from the soil samples by obtaining pure culture through growth on ISP-4 medium. The isolated strains exhibited a considerable zone of inhibition when pour-plated. Thus, it was selected further for experimental analysis.

### 3.2 Identification of *Streptomyces* strains:

At taxonomic levels, PCR-based molecular techniques like 16S rRNA and RAPD, etc can be used[30]. Nevertheless, the most promising method for phylogenetic categorization of bacteria is the sequencing of genes encoding 16S rRNA. The 16S rRNA gene of *Streptomyces* strains was amplified in this study using the PCR method and universal primers. The PCR product was examined on agarose gel at a 1% concentration. The 16S rRNA sample that had been amplified underwent partial nucleotide sequencing. After performing a BLAST on the acquired nucleotide sequences for the *Streptomyces* strains, they were screened as *Streptomyces peucetius*, *Streptomyces fradiae*, *Streptomyces lavendulae*, and *Streptomyces erythraeus*. The evolutionary link between the microorganisms is determined by the phylogenetic analysis. As a result, a phylogenetic tree based on the 16S rRNA sequence of *Streptomyces* strains was built using the neighbor-joining method using MEGA 11. Isolates M1 and M4 showed 88% similarity while M2 and M3 exhibit 85% resemblance.



**Figure 1: Phylogenetic tree of isolated strains obtained by Neighbour-joining (NJ) method using MEGA 11 software. The branch node number shows percent bootstrap support. The accession numbers of the organisms are included in parentheses.**

### 3.3 Characterisation of isolated metabolic extract:

#### 3.3.1 GC-MS analysis to determine the components present in extracts:

To comprehend the potential pharmacological capability of the M<sub>1</sub> and M<sub>3</sub> extracts, GC-MS analysis was carried out in order to evaluate the chemical composition. The investigation of M<sub>1</sub> extract revealed 25 compounds, constituting the overall content of the extract, which is illustrated in Tables 3 and 4. The most abundant components of the M<sub>1</sub> extract were Benzene, 1,3-dimethyl- (34.80%), 2,3-bButanediol, [R-(R\*,R\*)]- (22.67%), pPyrrolo[1,2-a]pyrazine-1,4-dione, hHexahydro-3-(2-methylpropyl)- (22%) and L-lactic acid (11.77%). Furthermore, the analysis of the M<sub>3</sub> extract disclosed its own potential major constituents, namely o-xylene (28.87%) and (3-mMethyl-oxiran-2-yl)-methanol (9.35%), both of which had different retention periods of 3.330 and 2.019, respectively. Both the extracts possess potential antimicrobial action owing to the presence of constituents like 2,3-bButanediol[31], 1-mMethyl-2-pyrrolidone-4-carboxamide[32], [33], and L-lactic acid. Previous studies have indicated that bBis (2-ethylhexyl) phthalate could be a possible agent, which can inhibit microbial strains and showcase cytotoxicity[34]. 1-hHexadecanol[35], squalene[36] and Caryophyllene[37] present in the extracts have previously been shown to possess antioxidant activity against free radicals. Owing to the presence of above mentioned potential components in the microbial extracts, they could be developed as possible therapeutic agents.

**Table 2: Constituents present in crude metabolic extract M1 identified by GC-MS technique**

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Compound name	Molecular formula	Molecular weight	Retention Time
(3-Methyl-oxiran-2-yl)-methanol	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	88.11	2.019
<i>o</i> -Xylene	C <sub>8</sub> H <sub>10</sub>	106.16	3.33
1-Dodecene	C <sub>12</sub> H <sub>24</sub>	168.32	8.045
Dodecane	C <sub>12</sub> H <sub>26</sub>	170.33	8.169
Dodecane, 4,6-dimethyl-	C <sub>14</sub> H <sub>30</sub>	198.39	9.381
Decane, 3,7-dimethyl-	C <sub>12</sub> H <sub>26</sub>	170.33	9.69
Cyclohexasiloxane, dodecamethyl-	C <sub>12</sub> H <sub>36</sub> O <sub>6</sub> Si <sub>6</sub>	444.92	9.806
Dodecane, 4,6-dimethyl-	C <sub>14</sub> H <sub>30</sub>	198.39	10.059
<i>n</i> -Tridecan-1-ol	C <sub>13</sub> H <sub>28</sub> O	200.36	11.004
Tetradecane	C <sub>14</sub> H <sub>30</sub>	198.39	11.1
Caryophyllene	C <sub>15</sub> H <sub>24</sub>	204.35	11.515
Dodecane, 4,6-dimethyl-	C <sub>14</sub> H <sub>30</sub>	198.39	11.931
Tetradecane, 5-methyl-	C <sub>15</sub> H <sub>32</sub>	212.41	12.27
Dodecane, 4,6-dimethyl-	C <sub>14</sub> H <sub>30</sub>	198.39	12.306
Dodecane, 4,6-dimethyl-	C <sub>14</sub> H <sub>30</sub>	198.39	12.384
Decane, 5-propyl-	C <sub>13</sub> H <sub>28</sub>	184.36	12.44
Heptadecane	C <sub>17</sub> H <sub>36</sub>	240.5	12.5
Phenol, 2,4-bis(1,1-dimethylethyl)-	C <sub>14</sub> H <sub>22</sub> O	206.32	12.667
1-Decanol, 2-hexyl-	C <sub>16</sub> H <sub>34</sub> O	242.44	12.76
Eicosane	C <sub>20</sub> H <sub>42</sub>	282.5	12.96
1-Pentadecene	C <sub>15</sub> H <sub>30</sub>	210.4	13.611
Octadecane	C <sub>18</sub> H <sub>38</sub>	254.5	13.694
Heneicosane	C <sub>21</sub> H <sub>44</sub>	296.6	14.648
Dodecane, 4,6-dimethyl-	C <sub>14</sub> H <sub>30</sub>	198.39	14.73
Tetratriacontane	C <sub>34</sub> H <sub>70</sub>	478.9	14.855
Hexadecane	C <sub>16</sub> H <sub>34</sub>	226.4	14.894
Heptadecane, 2,6,10,15-tetramethyl-	C <sub>21</sub> H <sub>44</sub>	296.6	14.96
Eicosane	C <sub>20</sub> H <sub>42</sub>	282.5	15.014
Nonacosane	C <sub>29</sub> H <sub>60</sub>	408.8	15.12
Eicosane	C <sub>20</sub> H <sub>42</sub>	282.5	15.509
1-Nonadecene	C <sub>19</sub> H <sub>38</sub>	266.5	15.948
Nonadecane	C <sub>19</sub> H <sub>40</sub>	268.5	16.017
Cyclononasiloxane, octadecamethyl-	C <sub>18</sub> H <sub>54</sub> O <sub>9</sub> Si <sub>9</sub>	667.4	16.282
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl)	C <sub>18</sub> H <sub>26</sub> O <sub>4</sub>	306.4	16.86
Hexadecane, 2,6,10,14-tetramethyl-	C <sub>20</sub> H <sub>42</sub>	282.5	16.965
Hexadecane, 2,6,10,14-tetramethyl-	C <sub>20</sub> H <sub>42</sub>	282.5	17.01
Heneicosane	C <sub>21</sub> H <sub>44</sub>	296.6	17.094
Eicosane	C <sub>20</sub> H <sub>42</sub>	282.5	17.283
Eicosane	C <sub>20</sub> H <sub>42</sub>	282.5	17.363
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-	C <sub>17</sub> H <sub>24</sub> O <sub>3</sub>	276.4	17.436
2-Isopropyl-5-methyl-1-heptanol	C <sub>11</sub> H <sub>24</sub> O	172.31	17.681
Eicosane	C <sub>20</sub> H <sub>42</sub>	282.5	17.798
Dibutyl phthalate	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278.38	17.856
1-Nonadecene	C <sub>19</sub> H <sub>38</sub>	266.5	18.063
Heneicosane	C <sub>21</sub> H <sub>44</sub>	296.6	18.12
1-Chloro-1-(4-chlorophenoxy)-3-	C <sub>11</sub> H <sub>12</sub> Cl <sub>2</sub> O <sub>2</sub>	274.11	19.198

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methylbutan-2-one			
Cyclooctasiloxane, hexadecamethyl-	C <sub>16</sub> H <sub>48</sub> O <sub>8</sub> Si <sub>8</sub>	593.2	19.36
Tetracosane	C <sub>24</sub> H <sub>50</sub>	338.7	19.48
Bis(2-ethylhexyl) maleate	C <sub>20</sub> H <sub>36</sub> O <sub>4</sub>	340.5	19.652
Pentatriacontane	C <sub>35</sub> H <sub>72</sub>	492.5	19.782
Tetracosane	C <sub>24</sub> H <sub>50</sub>	338.7	19.868
n-Heptadecanol-1	C <sub>17</sub> H <sub>36</sub> O	256.5	19.995
Heneicosane	C <sub>21</sub> H <sub>44</sub>	296.6	20.04
Cyclononasiloxane, octadecamethyl-	C <sub>18</sub> H <sub>54</sub> O <sub>9</sub> Si <sub>9</sub>	667.4	20.697
<b>Component name</b>	<b>Molecular formula</b>	<b>Molecular weight</b>	<b>Retention Time</b>
Tetrachlorotricyclo[7.1.0.0(4,6)]decane			
Tetrapentacontane	C <sub>54</sub> H <sub>110</sub>	759.4	21.409
1-Heptacosanol	C <sub>17</sub> H <sub>36</sub> O	256.5	21.767
Heneicosane	C <sub>21</sub> H <sub>44</sub>	296.6	21.81
Cyclononasiloxane, octadecamethyl-	C <sub>18</sub> H <sub>54</sub> O <sub>9</sub> Si <sub>9</sub>	667.4	21.926
Phenol, 2-methoxy-4-(1-propenyl)-, (Z)-	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	164.2	22.953
Cyclononasiloxane, octadecamethyl-	C <sub>18</sub> H <sub>54</sub> O <sub>9</sub> Si <sub>9</sub>	667.4	23.075
Bis(2-ethylhexyl) phthalate	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390.6	23.124
Eicosane	C <sub>20</sub> H <sub>42</sub>	282.5	23.435
Cyclononasiloxane, octadecamethyl-	C <sub>18</sub> H <sub>54</sub> O <sub>9</sub> Si <sub>9</sub>	667.4	24.158
Cyclononasiloxane, octadecamethyl-	C <sub>18</sub> H <sub>54</sub> O <sub>9</sub> Si <sub>9</sub>	667.4	25.323
Squalene	C <sub>30</sub> H <sub>50</sub>	410.7	25.401
Cyclononasiloxane, octadecamethyl-	C <sub>18</sub> H <sub>54</sub> O <sub>9</sub> Si <sub>9</sub>	667.4	26.735

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2,3-Epoxybutane	C <sub>4</sub> H <sub>8</sub> O	72.11	2.019
2,3-Butanediol, [R-(R*,R*)]-	C <sub>4</sub> H <sub>10</sub> O <sub>2</sub>	90.12	2.113
Silane, ethoxytriethyl-	C <sub>8</sub> H <sub>20</sub> OSi	160.33	2.494
Benzene, 1,3-dimethyl-	C <sub>8</sub> H <sub>10</sub>	106.16	3.325
L-Lactic acid	C <sub>3</sub> H <sub>6</sub> O <sub>3</sub>	90.08	3.441
1-Decene	C <sub>10</sub> H <sub>20</sub>	140.27	4.785
Decane	C <sub>10</sub> H <sub>22</sub>	142.28	4.921
3-Tetradecene, (Z)-	C <sub>14</sub> H <sub>28</sub>	196.37	8.045
Dodecane	C <sub>12</sub> H <sub>26</sub>	170.33	8.167
3-Hexadecene, (Z)-	C <sub>16</sub> H <sub>32</sub>	224.42	10.994
Tetradecane	C <sub>14</sub> H <sub>30</sub>	198.39	11.096
Phenol, 2,4-bis(1,1-dimethylethyl)-	C <sub>14</sub> H <sub>22</sub> O	206.32	12.666
1-Pentadecene	C <sub>15</sub> H <sub>30</sub>	210.4	13.609
Hexadecane	C <sub>16</sub> H <sub>34</sub>	226.44	13.693
1-Heptadecene	C <sub>17</sub> H <sub>34</sub>	238.5	15.948
Octadecane	C <sub>18</sub> H <sub>38</sub>	254.5	16.018
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C <sub>11</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub>	210	16.398
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C <sub>11</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub>	210	17.439
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C <sub>11</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub>	210	17.606
1-Nonadecene	C <sub>19</sub> H <sub>38</sub>	266.5	18.064
Octadecane	C <sub>18</sub> H <sub>38</sub>	254.5	18.12
1-Heneicosanol	C <sub>21</sub> H <sub>44</sub> O	312.6	19.995
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	C <sub>14</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub>	244	21.925
Phenol, 2,4-bis(1-phenylethyl)-	C <sub>22</sub> H <sub>22</sub> O	302.4	22.145
Bis(2-ethylhexyl) phthalate	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390.6	23.129

**Table 3: Constituents present in crude metabolic extract M3 identified by GC-MS technique**

Comment [PM10]: title before or after table; you must harmonize

### 3.4 Assessment of Bioactive potential of extracted metabolites:

#### 3.4.1 Determination of Anti-microbial property by Agar cup method:

Antimicrobial Resistance (AMR) of pathogenic bacteria emerges when the microorganisms acquire resistance to antibiotics, enabling them to no longer respond to antibiotics[38]. Crude extract from M1 and M3 isolates were screened for antibacterial activity against ten bacterial pathogens, and two fungus strains, using the agar well diffusion method[39]. Significant antimicrobial activity was observed in metabolite extracts obtained from the isolates *Streptomyces peucetius* (M1) and *Streptomyces lavendulae* (M3). Prominent activity against *Bacillus subtilis* (20 mm), and *Staphylococcus aureus* (19 mm), was shown by the extract of *Streptomyces peucetius*. Similarly, *Streptomyces lavendulae* extract, showed phenomenal activity against *Staphylococcus epidermidis* (19mm) and minimal activity against *Pseudomonas aeruginosa* (6mm). As previously known, fungal pathogens have been responsible for significant agricultural losses caused by the plant diseases they produce; they are also the origin of many medically related illnesses[40]. The highest antifungal activity was demonstrated by *Streptomyces peucetius* extract against *Aspergillus niger* (14 mm), and the extract from *Streptomyces lavendulae* displayed significant activity against *Candida albicans* (18mm). M1 extract demonstrated appreciable activity against both Gram-positive and Gram-negative bacteria. The presence of antimicrobial compounds in the extracts, which had previously been confirmed by GC-MS analysis, was most definitely linked to the activity that had been observed. Compounds such as bis(2-ethylhexyl) phthalate [41], 2,3-butanediol, [R- (R\*, R\*)]-[42], and L-lactic acid [43], have previously been reported to be potent antimicrobial and cytotoxic agent respectively. As aforementioned, the extracts work on the outer lipopolysaccharide cell membrane to transport them within and potentially change the inner cellular components and release intracellular components, asserting the extract's ability to kill microbial pathogens[44]. Thus, the extensive availability of antimicrobial compounds in the secondary metabolite produced by *Streptomyces sp.* revealed significant antimicrobial properties against the test pathogens[45].

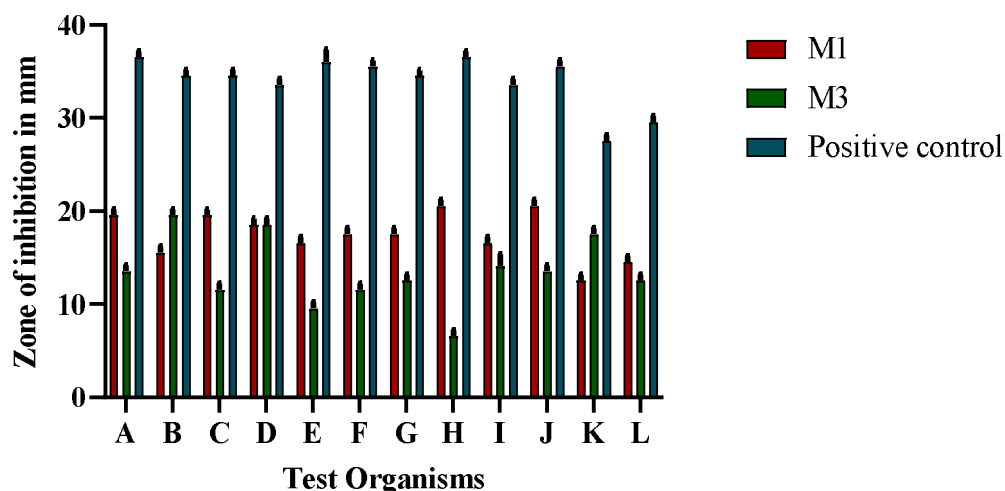


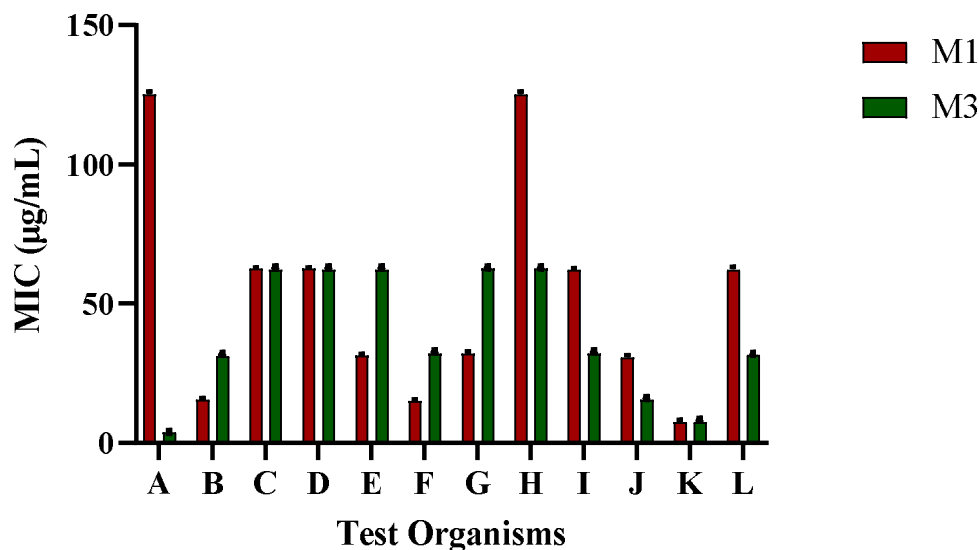
Figure 2: Zone of clearance in mm of M1 and M3 extract against Test organisms (A) *Staphylococcus aureus*, (B) *Staphylococcus epidermidis*, (C) *Bacillus subtilis*, (D) Methicillin-resistant *Staphylococcus aureus*, (E) *Propionibacterium acne*, (F) *Escherichia coli*, (G) *Salmonella typhimurium*, (H) *Pseudomonas aeruginosa*, (I) Vancomycin-resistant *Enterococci*, (J) *Vibrio cholerae*, (K) *Candida albicans*, (L) *Aspergillus niger*

### 3.4.2 Minimal Inhibitory Concentration:

Using the microdilution technique, the antibacterial activity of the extracts M1 and M3 was examined against several pathogenic Gram-positive and Gram-negative bacteria along with two predominant nosocomial fungal pathogens. The investigated strains showed growth inhibition at various MIC levels, as shown in the Figures. The most susceptible fungi to both extracts were *Candida albicans* and *Escherichia coli* was the most vulnerable bacteria to M1 extract with a MIC value of 15.625 µg/mL whereas M3 extract restricted the growth of *Staphylococcus aureus* with a MIC value of 3.90 µg/mL. The M1 extract showed significant potency against all the test organisms most notably against *Staphylococcus epidermidis*, *Propionibacterium acnes*, and *Vibrio cholerae* with respective MIC values of 15.62 µg/mL, 31.25 µg/mL, and 31.25 µg/mL. Additionally, M3 extract showed attributable antimicrobial nature against most test organisms notably against *Staphylococcus epidermidis*, Vancomycin-resistant *Enterococci*, and *Aspergillus niger* with respective MIC values of 31.25 µg/mL, 15.62 µg/mL, and 31.25 µg/mL. The data obtained through GC-MS revealed that the *Streptomyces* extract contains a range of compounds with known antimicrobial activity. Propionic acid is utilized as a preservative in several food products owing to its inhibitory effect on microorganisms especially fungus by inducing apoptotic cell death which can be further confirmed by our results [46]. 1-Methyl-2-pyrrolidone-4-carboxamide belonging to the family of carboxamides are efflux pump inhibitors which make them excellent antibacterial agents against several pathogenic Gram-positive and Gram-negative bacteria [47], [48]. All this data implies that the *Streptomyces* extract has the potential to

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inhibit several pathogenic microorganisms. Further research has to be dwelled upon to further purify and amplify its antimicrobial ability.



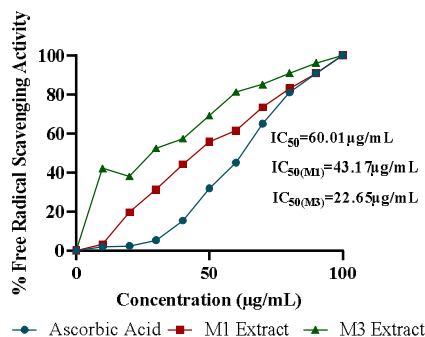
**Figure 3: MIC values of M1 and M3 extract against Test organisms (A) *Staphylococcus aureus*, (B) *Staphylococcus epidermidis*, (C) *Bacillus subtilis*, (D) Methicillin-resistant *Staphylococcus aureus*, (E) *Propionibacterium acne*, (F) *Escherichia coli*, (G) *Salmonella typhimurium*, (H) *Pseudomonas aeruginosa*, (I) Vancomycin-resistant *Enterococci*, (J) *Vibrio cholerae*, (K) *Candida albicans*, (L) *Aspergillus niger***

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### 3.4.3 DPPH Free Radical Scavenging Activity:

Antioxidant activity was determined using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay, which is one of the most widely accepted and efficient methods. DPPH has stable free radicals, which get reduced when a hydrogen donor is present i.e. free radical scavenging antioxidant [46]. When the reduction of the DPPH reagent takes place, the color changes from purple DPPH solution to yellow diphenylpicrylhydrazine [47]. Decolourization of DPPH by the metabolic extracts M1 and M3 indicated the scavenging ability of the extracts. The radical scavenging ability of M1 and M3 extracts, from concentrations ranging from 10µg/mL to 100 µg/mL, are illustrated in the figure below. Ascorbic acid was used as a standard for this assay and had an IC<sub>50</sub> value of 60.01 µg/mL. IC<sub>50</sub> values of metabolic extracts M1 and M3 were found to be 43.17 µg/mL and 22.65 µg/mL respectively. These values obtained were lower than the radical scavenging activity of standard ascorbic acid, but they are close to the standard IC<sub>50</sub> value. The free radical neutralizing capacity of the extracts could be attributed to components like 1-hexadecanol, squalene, and Caryophyllene, which were verified by our GC-MS analysis. Therefore, the metabolic extracts display a good level of antioxidant activity as per the results.



**Figure 4: Percent Free DPPH Radical Scavenging activity by *Streptomyces* crude extracts M1 and M3.**

### Conclusion:

Antimicrobial drugs are losing their curative effectiveness attributed to the prevalence of drug-resistant bacteria and a dramatic increase in infectious illnesses globally. There is a need to address these challenges by employing naturally accessible bioactive compounds. *Streptomyces* genus is responsible for their ability to produce active secondary metabolites, including antibiotic, antitumor, antimalarial and immunosuppressive agents. The objective of the current study was to isolate, characterize, and screen for the antimicrobial activity of different *Streptomyces* strains from the soil, ferment the secondary metabolic extracts and show the potential biological activity of the crude extract to further investigate the untapped potential of this significant therapeutic compound. During preliminary analysis, notable activity was shown by M1 and M3, later identified as *Streptomyces peuceetius* and *Streptomyces lavendulae* therefore their secondary metabolite were chosen for further analysis. Both extracts showed appreciable biological activities due to the presence of therapeutic components. GC-MS revealed the presence of various compounds like L-Lactic acid and bis-(2-ethylhexyl) phthalate. The metabolic extract was efficacious against both bacterial and fungal strains, emphasizing the extracts' potential antibacterial properties. Although the  $IC_{50}$  value was less than the standard ascorbic acid's ability to scavenge radicals, it was still relatively close to the amount. The aforementioned results are encouraging and unquestionably demonstrate the therapeutic ability of selected isolates. However, further analysis of secondary metabolites will be important to take into account for drug discovery.

Comment [PM12]: add perspectives

### Data Availability Statement:

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

**Ethical statement:**

No animals were harmed during this study.

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