4. RESULTS

4.1 Physico-chemical properties of soil

More precisely, as nutrient behavior of the soils is governed by soil properties and environmental conditions, though measurement of such properties is often required. These include pH, salinity, contents of organic matter, calcium carbonate and texture. In drier areas, the presence of gypsum (CaSO₄·2H₂O) is also an important soil factor. Whereas, the soil nutrients are not permanently available in a particular soil varies according to crop rotation, flood, wind, application of various fertilizers and moisture holding capacity (MHC) of the soil, etc. The present study revealed the physico-chemical properties of the cultivation field rhizosphere soils of Thanjavur district, Tamilnadu, India. The pH values of the soil samples vary from one location to another and the results showed that majority of the soil sites were slightly alkaline. The soil pH ranged from 7.59 to 8.12, the maximum pH was recorded at Pappanadu soil, whereas minimum pH was recorded in Punalvasal soil. The maximum EC of the soil was recorded as 0.52 dsm⁻¹ recorded at Punalvasal and minimum value of 0.25 dsm⁻¹ recorded at Cholankudikadu. All the rhizosphere soils collected from nine various sampling stations were blackish brown in their colour. Similarly, the textural classes of the soils were sandy clay loam in all the sampling stations except Kurichy (sandy clay) and Thuravikkadu (clay loam). The lime content of the soil was found only in Pappanadu and Cholankudikkadu (data not shown). The soil of all the nine sampling stations were devoid of heavy metals (data not shown) (Table 1; Fig. 2a-g).

The macronutrients (N, P and K) contents of the rhizosphere soil samples are shown in Table 1 and Fig. 2a-g. They ranged from 0.613 to 0.982%, 0.016 to 0.035% and 0.215 to 0.266% respectively. The maximum contents of the N (0.982%), P (0.035%) and K (0.266%) were recorded at Ambalapattu, Pappanadu and Cholankudikkadu stations respectively, while the minimum contents of the N (0.613%), P (0.016%) and K (0.215%) were recorded at Kurichy, Thuravikkadu and Pappanadu stations respectively. The maximum contents of the Ca (13.6 C. Mol.
Proton/kg) and Mg (11.2 C. Mol Proton +/kg) were recorded at Cholankudikkadu station, whereas the minimum Ca (10.6 C. Mol Proton/kg) and Mg (8.6 C. Mol Proton/kg) contents were recorded at Kurichy and Ettupulikkadu stations respectively. Similarly, maximum Na (2.36 C. Mol Proton/kg) was recorded at both Kurichy and Pappanadu stations but the minimum (1.56 C. Mol Proton/kg) was recorded at Kanniyakurichy station. In addition, content of the OC ranged from 0.29 to 0.48%. Maximum OC content (0.48%) was recorded at Ambalapattu soil, whereas the minimum content (0.29%) was recorded at Cholankudikkadu station.

The maximum cation exchange capacity of the soil was recorded maximum (28.3 C. Mol. Proton/kg) at Thuravikkadu but minimum (20.9 Mol. Proton/kg) was recorded at Ulur station. Maximum content (48.61%) of fine sand was recorded in Ettupulikkadu station, whereas minimum (40.25%) in Cholankudikkadu. Likewise, maximum content (28.65%) of coarse sand was recorded at Ulur, and minimum (21.48%) in Ambalapattu station. The silt content was higher (26.41%) in Cholankudikkadu, whereas lower (18.45%) in Ettupulikkadu, and clay content was maximum (11.51%) in Kurichy but minimum (8.08%) in Punalvasal station Table 1; Fig. 2a-g. The crop rhizosphere soil samples were also analyzed for its micronutrients such as available Zn, Cu, I and Mn. The maximum contents of available Zn (0.96 ppm), Cu (0.96 ppm), I (5.36 ppm) and Mn (2.65 ppm) were recorded in rhizosphere soils of the Punalvasal, Kanniyakurichy, Cholankudikkadu and Ambalapattu respectively (Table 1; Fig. 2a-g).

4.2 Diversity of actinobacteria from terrestrial soil

Totally, 300 actinobacterial colonies were isolated from various terrestrial soil samples of Thanjavur district, Tamilnadu. Among them, the maximum (55 CFU/g) was recorded at Punalvasal soil, followed by Kanniyakurichy (40 CFU/g), Thuravikkadu (35 CFU/g), 30 CFU/g each were isolated from Ulur, Pappanadu and Ambalapattu, and 28, 27 and 25 CFU/g from Cholankudikkadu, Ettupulikkadu and Kurichy (Table 2). From these 300 colonies of actinobacteria, morphologically varying colonies were purified, sub-cultured and stored at 4°C on SCA medium for further studies (Plate II).
Among the 300 actinobacterial colonies, only 50 isolates were morphologically different (Plate III). From these 50 isolates, Thuravikkadu soil samples contributed maximum of 10 isolates, followed by Punalvasal (n=7), Kurichy, Ettupulikkadu, Kanniyakurichy, Ulur, Pappanadu and Ambalapattu contributed (each n=5) and 3 isolates from Cholankudikkadu (Table 2). Colours of aerial spore mass of the isolates were categorized into 4 groups including white, grey, pink and brown series on SCA medium. Among these 4 groups, most of the isolates were produced pink and white colour series than grey and brown series (Table 3; Fig. 3).

Further, many of the isolates produced pink coloured substrate mycelium (reverse side colour) on SCA medium after seven days of incubation at 28°C. Based on the colony and microscopic (sporopore) morphology, most of the isolates (66%; n=33) belonged to the genus *Streptomyces*, followed by *Actinomadura* (12%; n=6), *Nocardiopsis* and *Nocardia* (each 6%; n=3), *Micromonospora* (4%; n=2) and *Nocardiodes, Actinoplanes and Actinopolyspora* (each 2%; n=1) (Table 3; Fig. 4).

### 4.3 Correlation coefficient analysis

The correlation co-efficient analysis between physico-chemical parameters of soils and actinomycetes populations was also made. The significant positive correlation was observed between sodium and EC (r=0.679; P<0.05%); potassium and silt (r=0.699; P<0.05%); calcium and silt (r=0.708; P<0.05%); magnesium and nitrogen (r=0.638; P<0.05%); magnesium and phosphorus (r=0.691; P<0.05%); magnesium and calcium (r=0.769; P<0.05%) and magnesium and silt (r=0.825; P<0.01%). In contrast to this, the significant negative correlation was also observed between magnesium and fine sand (r=-0.855; P< 0.01%); calcium and fine sand (r=-0.751; P<0.05%); silt and fine sand (r=-0.733; P< 0.05%); copper and nitrogen (r=-0.738; P<0.05%); calcium and copper (r=-0.668; P<0.05%) and manganese and copper (r=-0.706; P<0.05%) (Table 4).
4.4 Screening of antimicrobial compound producing actinobacteria

4.4.1 Preliminary screening

In the primary screening of antimicrobial activity, out of 50 isolates, 29 (58%) isolates exhibited antimicrobial activity (Table 5). Among 29 antimicrobial activity possessed isolates, all the 29 isolates had antibacterial activity, but 28 isolates had antifungal activity and 28 isolates had both antibacterial and antifungal activities. Among 29 antibacterial isolates, 22 (75.89%) isolates had activity against Gram positive bacteria, 26 (89.66%) against Gram negative bacteria, and 20 (68.97%) isolates had both Gram positive and Gram negative bacteria (Plate IV). Individually, 16 isolates (55.17%) possessed activity against both B. subtilis and S. typhi, followed by 15 (51.72%) against E. coli, 14 (48.28%) against S. pneumoniae and K. pneumoniae and 9 (31%) against S. aureus exhibited antibacterial activity. About 23 (82%) isolates found antifungal activity against A. flavus, followed by 21 (75%) against Fusarium sp., 20 (80%) against A. niger and 19 (67.8%) against A. fumigatus had antifungal activity (Table 5; Fig. 5).

4.4.2 Secondary screening

In the secondary screening, 18 out of 29 actinobacteria with notable antimicrobial activities were further evaluated for their activity against test bacterial and fungal pathogens (Table 6). Individually, 17 isolates (94.5%) possessed activity against B. subtilis, 14 (77.77%) against S. aureus and E. coli, 13 (72.2%) against K. pneumonia, 11 (61.1%) against S. pneumonia and 10 (55.5%) against S. typhi. Of 18 isolates possessed antimicrobial activity, 13 (72.2%) isolates exhibited antifungal activity. Individually, 9 (69.2 %) isolates against Fusarium sp., 8 (61.5%) against A. flavus and A. fumigatus, and 7 (53.8%) isolates against A. niger had antifungal activity (Fig. 6).

Out of 18 antagonistic actinobacteria, the actinobacterial isolates, Streptomyces sp. (KV2) and Streptomyces sp. (PV1) were found strong antagonistic activity against all the 6 bacterial and 4 fungal pathogens tested than other actinobacterial isolates (Table 6). Hence, these 2 isolates were justifiably selected for further investigations.
4.4.3 Antimicrobial efficacies of selected streptomycetes

The antimicrobial efficacies of the two isolates *Streptomyces* sp. (KV2) and *Streptomyces* sp. (PV1) were evaluated with seven solvent extracts against the bacterial and fungal test organisms. The isolates possessed maximum antimicrobial activities when it was extracted with ethyl acetate and petroleum ether. The isolate namely *Streptomyces* sp. (KV2) had maximum antimicrobial activity in ethyl acetate against *E. coli* (14 mm), followed by *A. fumigatus* (13 mm), *S. pneumoniae* (12 mm), *Fusarium* sp. (10 mm), *A. niger* (9.5 mm), *B. subtilis* and *A. flavus* (9 mm), *S. typhi* (8.5 mm) and *S. aureus* (6 mm), no activity was found against *K. pneumoniae* (Table 7). The petroleum ether extract of *Streptomyces* sp. (PV1) exhibited maximum (12 mm) antibacterial activity against *K. pneumoniae* followed by *S. typhi* (11.5 mm), *S. aureus* (10.5 mm), *B. subtilis*, *S. pneumoniae* and *A. fumigatus* (9 mm), *E. coli* (8.5 mm) and *A. niger* (8 mm) (Plate V). In contrast, the ethyl acetate extract showed maximum (10 mm) activity against *Fusarium* sp. and *A. flavus* (8 mm) than petroleum ether extract (Table 8; Plate V).

4.4.4 Characterization and identification of selected *Streptomyces* spp.

The two isolates *Streptomyces* sp. (KV2) and *Streptomyces* sp. (PV1) found to have broad spectrum antimicrobial activities were selected for further characterization on the basis of morphological, physiological, biochemical and molecular characteristics (16S rRNA gene sequencing).

4.4.4.1 Microscopic observation of *Streptomyces* spp.

Based on the light and scanning electron microscopy, the antimicrobial producers *Streptomyces* sp. (KV2) formed straight to flexuous (rectiflexibles) spore chain on aerial mycelium with smooth surface, whereas *Streptomyces* sp. (PV1) formed retinoculiaperti spore chain on aerial mycelium with smooth spore surface. The microscopical studies of the two isolates undoubtedly placed these isolates under *Streptomyces* genera (Table 9; Plate VI).
4.4.4.2 Biochemical characterization

Among the various parameters studied, positive result were observed with both potential isolates [Streptomyces sp. (KV2) and Streptomyces sp. (PV1)] in production of catalase, urease and H$_2$S hydrolyses of casein, starch, esculin and lecithin, and negative results were observed in production of β-lactamase, melanin, DNase, RNase and xanthine, nitrate reduction test, hydrolyses of gelatin and lipid and heamolysis test (Table 10). The following biochemical tests such as oxidase showed positive result only for Streptomyces sp. (PV1).

4.4.4.3 Cultural characterization

Cultural characteristics of the two isolates were studied with seven different culture media. Both the isolates Streptomyces sp. (KV2) and Streptomyces sp. (PV1) produced grey, white and ash coloured spore mass and white, brown and yellowish reverse side in most of the media tested. None of the isolate produced diffusible pigments on any of the nine media tested (Table 11; Plate VII).

4.4.4.4 Physiological characterization

Both Streptomyces sp. (KV2) and Streptomyces sp. (PV1) grew well at pH 6 and 7, moderate growth of the isolates was observed at pH 8, and poor growth at pH 9. But they did not grow at pH 10. (Table 12). The growth pattern of Streptomyces isolates (KV2) and (PV1) were evaluated at eight different temperatures. Both the isolates showed their optimal growth at 30°C, whereas moderate growth was found at 25 and 35°C, and poor growth at 20 and 40°C. The isolates could not grow at temperatures 15, 45 and 50°C (Table 12).

*Streptomyces* sp. (KV2) was highly sensitive to streptomycin (35 mm), followed by gentamycin (26 mm), vancomycin (16 mm) and tetracycline (9 mm) and resistant to ampicillin, penicillin and co-trimazole, whereas, *Streptomyces* sp. (PV1) was more sensitive to tetracycline and gentamycin (26 mm) followed by, streptomycin (20 mm), co-trimazole (10 mm) and vancomycin (9 mm), and resistant to ampicillin and penicillin (Table 12). The tolerance capacity of the isolates against
some inhibitory compounds was studied. Both actinobacterial isolates were resistant to crystal violet (0.001%) and sodium azide (0.01%), and they were sensitive to phenol (0.1%) and potassium tellurate (0.001%). Similarly, they did not grow >4% of NaCl, moderate growth appeared in 3% NaCl, they could be able grew well at 1 and 2% of NaCl (Table 12). The organic acid utilization of *Streptomyces* sp. was evaluated against 4 different organic acids. Both the isolates were able to utilize sodium gluconate and sodium lactate, but they could not utilize sodium melonate and sodium citrate (Table 12).

The utilization of 18 different carbon sources by *Streptomyces* sp. was studied. Among the 18 carbon sources, both isolates were utilized dextrose, maltose, L-arabinose, D-arabinose, starch, fructose, mannose and D-sorbitol. Whereas, both the isolates did not utilize sucrose, lactose, D-galactose, L-rhamnose, inulin, xylose, D-rhamnose, salicin and meso-inositol *Streptomyces* sp. (KV2) alone utilized only lactose. *Streptomyces* sp. (PV1) alone utilized mannitol and galactose (Table 13). The utilization of nitrogen sources (amino acid) was also tested. Among 21 amino acids tested, 10 amino acids namely DL-2 amino-N-butric acid, L-tyrosine, DL-tryptophan, DL-ornithine, L-lysine, DL-leucine, L-arginine, DL-alanine, L-leucine and L-thrionine were utilized by both isolates, whereas, both the isolates did not utilize L-glutamic acid, L-cystine and DL-aspartic acid. *Streptomyces* sp. (KV2) alone utilized L-hydroxy proline, L-glycine and L-phenylalanine, whereas, *Streptomyces* sp. (PV1) alone utilized L-cysteine, L-isoleucine, L-histidine, L-serine and L-valine (Table 14).

### 4.4.4.5 Molecular characterization of *Streptomyces* spp.

The molecular characteristics of *Streptomyces* sp. (KV2) and *Streptomyces* sp. (PV1) were carried out by PCR amplification of 16S rDNA gene and their sequencing (Plate VIII). The sequences of both *Streptomyces* sp. (KV2) and *Streptomyces* sp. (PV1) 16S rRNA genes were deposited in genbank http://www.ncbi.nlm.nih.gov/genbank and received the accession numbers KF454869 (Fig. 7) KF728386 (Fig. 8) respectively. The sequences of the two isolates were compared with sequences of already existing species of streptomycetes from EMBL.
database to determine the phylogenetic relatedness using Neighbor joining tree method. It was revealed that, about 361 bp sequence of the isolate *Streptomyces* sp. (KV2) found 98% similarity with the existing species of *S. champavatii* (iafA) (Fig. 9). Correspondingly, 1321 bp sequences of *Streptomyces* sp. (PV1) found 100% similarity with *S. uncilais* JN177509 (Fig. 10).

The secondary structure of 16S rRNA gene of *Streptomyces* sp. (KV2) showed 22 stems in their structure (Fig. 11a), whereas *Streptomyces* sp. (PV1) showed 65 stems in their structure (Fig. 11b). However, both isolates are similar in energy threshold, cluster factor, conserved factor, compensated factor, conservativity, part of sequence, greedy parameters and treated sequence as indicated by RNA fold web server software (Fig. 12a; b).

The restriction sites found in both *Streptomyces* isolates are shown in Fig. 13; 14. Totally, 52 restriction enzyme sites were observed in *Streptomyces* sp. (KV2), whereas *Streptomyces* sp. (PV1) had 55 restriction enzyme sites. However, the cleavage sites and nature of restriction enzymes of *Streptomyces* sp. (KV2) were different from *Streptomyces* sp. (PV1). The GC contents of *Streptomyces* sp. (KV2) and *Streptomyces* sp. (PV1) were found to be 60% and 60% respectively (Fig. 13 and 14).

**4.4.4.6 Identification of potential Streptomyces sp.**

Based on the morphological, biochemical, physiological and quite significantly molecular features, antimicrobial compound producing *Streptomyces* spp. were identified as *S. champavatii* (KV2) and *S. uncilais* (PV1).

**4.5 Extraction of antimicrobial compounds**

The fermented broth containing antimicrobial compounds of selected two potential streptomycetes were extracted with eight different solvents. The compound of *S. champavatii* (KV2) dissolved completely in ethyl acetate followed by DMSO and weakly in methanol and chloroform, whereas it does not dissolved in acetone,
xylene, petroleum ether and water. In the same way, antimicrobial compound of *S. uncialis* (PV1) also strongly dissolved in ethyl acetate followed by, petroleum ether, chloroform, methanol and DMSO, but it does not dissolved in acetone, and water (Table 15). The extracted compounds were assessed for their antimicrobial ability.

4.6 Separation and purification of antimicrobial compounds

The ethyl acetate extracted compounds of the two isolates were purified and separated by thin layer chromatography. Single separated bands were observed and the Rf value was 0.49 cm and 0.58 cm for *S. champavatii* (KV2) and *S. uncialis* (PV1) respectively (Table 16). The Rf value of the separated compound was calculated by using the following standard formula.

\[
\text{Rf values} = \frac{\text{Distance traveled by the solute (Compound)}}{\text{Distance traveled by the solvent}}
\]

- *S. champavatii* (KV2) compound – Spot 1: Rf value = 3.7/7.5 = 0.49
- *S. uncialis* (PV1) compound – Spot 2: Rf value = 4.4/7.5 = 0.58

4.7 Characterization of antimicrobial compounds

The separated *S. champavatii* (KV2) compound was pale yellow in colour, viscous nature and melting point was 145°C. The compound was stable at pH from 4 to 7 and the temperature ranging from 30 to 45°C (Table 16). It showed positive reactions to silver mirror test for aldehyde and ketone, ninhydrin test for protein and peptide, Elson-Morgan test for amino acid. It showed negative reaction to Benedict’s test (absence of sugar moiety), Molish’s test (absence of reducing sugar) and Bial’s test (absence of pentose sugar) (Table 17).

GC-MS analysis of the ethyl acetate solvent extract was performed and the compounds were identified based on their retention time. The following chemical compounds were reported from *Streptomyces* sp. (KV2) such as acetic acid, 1-
methylpropyl ester (CAS) 2-butyl acetate (3.250 min), butyl acetate (3.350 min), acetic acid, 2-methylpropyl ester (CAS) isobutyl acetate (3.450 min), benzene, ethyl-(CAS) EB – ethylbenzene (5.00 min), methyl laurate (5.100 min), benzene, 1,2-dimethyl- (CAS) o-xylene (5.00), benzene, 1,4-dimethyl- (CAS) p-xylene (5.140 min), benzene, 1,3-dimethyl- (CAS) m-xylene (5.14 min), pyrimidine, 2-methoxy-5-methyl (18.698 min), pyridine, 2-methoxy-5-nitro- (22.057 min), pyrimidine-2(1H)-thione, 4,4,6-trimethyl-1-(1-phenylethyl)- (29.023 min) and 1-phenylalanine, N-(2,6-difluorobenzoyl)-, methyl ester (29.291 min). Benzenepropanoic acid (16.391min), sulfuric acid, 2-ethylhexyl hexyl ester (17.063 min), 2- hexenoic acid, 5-hydroxy-3,4,4-trimethyl-, (E)- (21.408 min), 1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester (23.468 min), n-hexadecanoic acid (23.468 min), phosphonic acid, bis(1-methylethyl) ester (24.028 min), octadecanoic acid (25.327 min) and 1-phenanthrenecarboxylic acid, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodeca hydro-1,4a,7-trimethyl-, methyl ester, [1R-(1-alpha,4a (27.813 min), pyrrolo[1,2-a] pyrazine-1,4- dione, hexahydro- (21.587 min) and pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)- (26.940 min) and p-benzoquinone, 2-hydroxy-5-(methythio)- (25.932 min), propenylguaethol (17.175 min), phenol, 3-methoxy-(17.555 min) and phenol, 3, 5-dimethoxy- (22.416 min), tridecane, 1-iodo- (20.669 min), tridecane, 5-propyl- (20.736 min), 7-Acetyl-1,7-diazabicyclo[2.2.0]heptane (21.206 min), heneicosane (21.744 min), nonadecane (22.774 min), 1,4-dioxaspiro[4.5]decane, 6-methylene- (23.222 min), heptadecane (24.678 min), hexacosane (28.821 min), triacontane (29.560 min), nonacosane (31.240 min), tetracosane (33.592 min), caprolactam (15.248 min) and squalene (30.658 min) (Table 18; Fig.15).

S. champavatii (KV2) compound revealed that the absorption maximum was observed in 204, 212, 218 and 226 nm in ethyl acetate. The UV spectrum of compound is shown in Fig. 16. IR spectrum of compound showed two absorption peaks in the regions of 3440, 2926 and 2857 cm⁻¹ (Table 16). The IR spectrum of the compound indicated the presence of alcohols, phenols and alkenes group. The absence of Br stretch alkyl halides (C-Br), aliphatic amines, carboxylic acid (COOH),
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ester (COOR) and alkynes (C=C), was confirmed by the lack of bands in the region of 690 –515, 1250 –1020, 1250 –1020, 1700 – 1750 and 2000 – 2060 cm$^{-1}$ respectively (Fig. 17).

Large numbers of peaks throughout the $\delta$ (chemical shift) value of 0-10 were observed in the $^1$H NMR spectrum of purified S. champavatii (KV2) compound. The peaks (chemical shift) values 1-4, 2-5, 3.3, 4.2, 6-8 and 7-8 ppm indicated that the compound had $\text{N-H}$, $\text{O-H}$, $\text{C-O-CH}_3$, $\text{F-CH}_3$, $\text{N-H}$ and $\text{O}$ respectively (Fig. 18). Peak values of $^{13}$C NMR spectrum of the same purified compound indicated the presence of ether (55-90 ppm), alcohol (45-90 ppm), amines (10-70 ppm) and aromatic compound (90-160 ppm) (Fig. 19). The molecular formula of the compound has been documented as C$_{28}$H$_{26}$N$_4$O$_3$ on the basis of elemental analysis. On the basis of spectral and other supportive data, the antimicrobial compound of the S. champavatii (KV2) was identified as “Staurosporine” (Fig. 20).

The separated S. uncialis (PV1) compound was brownish in colour, powdery nature and melting point was 175°C. The compound was stable at pH from 5 to 7 and the temperature ranging from 25 to 45°C (Table 16). It showed positive reactions to silver mirror test for aldehyde and ketone, ninhydrin test for protein and peptide, Biuret test for peptide and Elson-Morgon test for amino acid. It showed negative reaction to Benetict’s test (absence of sugar moiety), Molish’s test (absence of reducing sugar) and Bial’s test (absence of pentose sugar) (Table 17).

Based on the GC-MS analysis the following compounds were identified from Streptomyces sp. (PV1) such as benzene, ethyl- (CAS) EB –butyl acetate (4.960 min), methylaurate (4.960 min), benzene, 1,2-dimethyl- (CAS) o-xylene (5.100 min), xylene (5.100 min), 1,3-cyclopentadiene, 5-(1-methylethyridene)- (CAS) 6,6-dimethylfulvene (5.360 min), benzene, (1-methylethyl)- (CAS) isopropylbenzene cumene (6.220 min), benzene, propyl- (CAS) n-propylbenzene (6.810 min), benzene, 1-ethyl-2-methyl- (CAS) o-ethyltoluene (6.920 min), benzene, 1,2,3-trimethyl- 1,2,3-trimethylbenzene (7.100 min), benzene, 1,3,5-trimethyl- (CAS) 1,3,5-
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trimethylbenzene mesitylene (7.100 min), propanamide, 2-methyl- (9.694 min), N-methoxymethyl- N-methylformamide (11.015 min), L-prolinamide (21.072 min), acetamide, 2-chloro-N-(2-ethyl-6- methylphenyl)-N-(2-methoxy-1-methylethyl)-(23.737 min), Formamide, N-(2,4-diamino-1,6-dihydro-6-oxo-5- pyrimidinyl)-29.157 min), 2-ethylpiperidine (12.830 min) and pyrimidine-2(1H)-thione, 4, 4,6-trimethyl-1- (1-phenylethyl)- (29.381 min), acetic acid, 2-methylpropyl ester (5.416 min), 2-Propenoic acid, 2-methyl- (8.709 min), hexanoic acid, 2-methyl- (9.044 min), pentanoic acid (9.515 min), benzenacetic acid (15.450 min), benzenepropanoic acid (16.480 min), 5-oxohexanethioic acid, S-t-butyl ester (17.802 min), dodecanoic acid (19.146 min), N-hexadecanoic acid (23.535 min), octadecanoic acid (25.327 min), p-fluorophenoxyacetic acid (25.574 min), 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (28.508 min), octadecanoic acid, ethenyl ester (41.700 min), 1,2-ethanediol, monoacetate (7.678 min), p-dioxane-2,5-dimethanol (16.032 min), cyclohexanol, 4-methoxy- (18.272 min) and phenol, 3,5-dimethoxy- (22.528 min), 2-pyrrolidinone (12.449 min), 2, 5-piperazinedione, 3, 6-bis (2-methylpropyl) - (25.910 min) and 16-hentriacontanone (36.795 min), cyclohexane (13.681 min), tetradecane (17.0763), hexadecane (19.526 min), hexacosane (28.821 min), tetracosane (30.344 min), pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro- (21.766 min) and pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(phenylmethyl) (26.985 min), and caprolactam was also detected at (15.808 min) retention time (Table 19; Fig. 21).

S. uncialis (PV1) compound revealed that the absorption maximum was observed in 206, 210, 220, 224 and 228 nm in ethyl acetate. The UV spectrum of compound is shown in Fig. 22. IR spectrum of compound was shown five absorption peaks in the regions of 669, 863, 1265, 1463 and 2858 cm$^{-1}$ (Table 16). The IR spectrum of the compound was indicated the presence of alkynes (C≡C-H), aromatic amines (C-N), aromatic (C-C) and alkanes (C-H) group. The absence of Br stretch alkyl halides (C-Br), aliphatic amines, carboxylic acid (COOH), ester (COOR) and alkynes (C=C), was confirmed by the lack of bands in the region of 690 –515, 1250 –1020, 1250 –1020, 1700 – 1750 and 2000 – 2060 cm$^{-1}$ respectively (Fig. 23).
Large numbers of peaks throughout the $\delta$ (chemical shift) value of 0-7 were observed in the $^1$H NMR spectrum of purified $S.\ \text{uncialis}$ (PV1) compound. The peaks (chemical shift) values 1 -4, 1.6, 3.3, 4.1, 7 – 8 ppm indicated that the compound had $\text{N-H}$, -O-H, -C=CH$_3$, F-CH$_3$ and $\text{C}=\text{O}$ respectively (Fig. 24). Peak values of $^{13}$C NMR spectrum of the purified compound indicated the presence of ether (55-90 ppm), alcohol (45-90 ppm), amines (10-70 ppm), and aromatic compound (90-160 ppm) (Fig. 25). The molecular formula of the compound has been documented on the basis of elemental analysis as $\text{C}_{54}\text{H}_{90}\text{N}_6\text{O}_{18}$. On the basis of spectral and other supportive data, the antimicrobial compound of $S.\ \text{uncialis}$ (PV1) was identified as “Octa Valinomycin” (Fig. 26).

4.8 Antimicrobial activity of pure compounds of Staurosporine and Octa Valinomycin

The maximum antibacterial inhibitory effect of staurosporine compound produced by $S.\ \text{champavatii}$ (KV2) was exhibited on $E.\ \text{coli}$ (18 mm), followed by $B.\ \text{subtilis}$ (17 mm), $K.\ \text{pneumoniae}$ (16 mm), $S.\ \text{aureus}$ (14 mm), $S.\ \text{pneumoniae}$ (12 mm) and $S.\ \text{typhi}$ (12 mm), whereas octa valinomycin compound produced by $S.\ \text{uncialis}$ (PV1) had maximum antibacterial activity on $S.\ \text{pneumoniae}$ (18 mm), followed by $B.\ \text{subtilis}$ (16 mm), $S.\ \text{aureus}$ (15 mm), $K.\ \text{pneumoniae}$ (15 mm), $E.\ \text{coli}$ (14 mm) and $S.\ \text{typhi}$ (11 mm) (Table 20).

The maximum antifungal inhibitory effect of staurosporine was exhibited on $\text{Fusarium}$ sp. (17 mm), followed by $A.\ \text{fumigatus}$ (14 mm), $A.\ \text{niger}$ (12 mm) and $A.\ \text{flavus}$ (11 mm). But, octa valinomycin had maximum antifungal activity on $A.\ \text{fumigatus}$ (16 mm), followed by $\text{Fusarium}$ sp. (15 mm), $A.\ \text{niger}$ (13 mm) and $A.\ \text{flavus}$ (12 mm) (Table 20).
Actinobacteria are the dominant group of soil population together with bacteria and fungi and are originally considered as an intermediate group between bacteria and fungi. They are free living saprophytic bacteria, and a major source for production of antibiotics. They also play a major role in recycling of organic matter (Unaoguet et al., 1994), production of novel pharmaceuticals, nutritional materials, enzymes, antitumor agents, enzyme inhibitors, immune-modifiers and vitamins. Around 80% of the total antibiotic production has been obtained from Streptomycetes (Wellington et al., 1992). During the last few decades, actinobacteria have become the most creditable source for antibiotics. In the 20th century, 75-80% of the entire discovered antibiotics were derived from the order actinomycetales, mainly from Streptomyces species (Thakur et al., 2007). Considering the practically useful compounds, recent decades about 130-140 microbial products and a similar number of derivatives are derived from diverse group of actinobacteria and hence the members of this group are proved to be of commercial importance. Furthermore, some 10-20 compounds are used in agriculture mainly as pesticide, herbicides, plant protecting agents and food additives (Moncheva et al., 2002).

Actinobacteria play an important role in soil bio-geochemical processes which determine plant productivity, successful functioning of introduced microbial bioinoculants and their influence on soil health. Exhaustive efforts have been made to explore soil microbial diversity of indigenous community, their distribution and behavior in soil habitats (Hill, 2000). The wide distribution of Streptomyces in soil and their ability to produce novel antibiotics and non-antibiotic lead molecules had caused these bacteria to be targeted in drug screening programme. Discovery of novel antibiotics from actinobacteria are important in helping to cope with the growing proportion of antibiotic-resistant bacterial infections that become untreatable. Further, the frequency of novel bioactive compounds discovered from terrestrial actinobacteria decreases with recent time, because much attention has been focused on screening of actinobacteria from diverse environments for their ability to produce new secondary