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Characterization of Secondary Metabolites Derived from Tomato Endophyte – *Streptomyces* sp. Shanivit

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Abstract

Endophytic actinomycetes are found in every plant. Actinomycetes are the largest source of antibiotics. The emergence of new diseases has created an urgency of finding new antibiotics that can be used for the treatment, which is the need of the hour. Therefore, we analysed the antimicrobial efficiency of 240 actinomycetes isolates from the Tomato plant *Lycopersicon esculin-dem*. The actinomycetes isolates were screened for *in vitro* ability to antagonize selective pathogens such as *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Bacillus subtilis*. Shanivit showed the highest inhibition compared to other isolates. 16S rRNA gene sequences analysis revealed that *Strepto-myces* sp. shanivit was 95% similar with *Streptomyces mutabilis* and *Streptomyces rochei* species, respectively. Ethyl acetate extract of isolate *Streptomyces* sp. shanivit showed good antibacterial activity. The GC–MS data showed the presence of 3-Pyrrolidin-2-yl- propionic acid**;** Oxazolid-2-one, 3-acetyl-4-methyl-5-phenyl-; Fumaric acid, 2-hexyl tetradecyl ester; Bromo-criptine; Cyclohexan,1,3,5-trimethyl-2-octa-decyl-; Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy; Actinomycin C2 and Didemnin B, and few more compounds. The results of the present study reveal *Streptomyces* sp. shanivit is an excellent source for antibacterial compounds.

Keywords: Endophytes, Actinobacteria, Antimicrobial activity, Pyrrolidine, Cyclohexane

Introduction

The urgency of antimicrobial-resistant pathogens had become a significant healthcare problem worldwide. According to World Health Organization [1], Novel antibacterial compounds are essential to treat bacterial infections. During the last three decades, only two new classes of antibiotics have been brought to the clinic for the treatment of contagious diseases [2]. The antimicrobial-resistant rate is a speedy increase and very difficult to treat, the problem is in the reduction of the number of new antibiotics being discovered and developed in recent years. The urgent need is to find new antimicrobial compounds required to fight against resistant bacteria. Two out of three parts of antibiotics are naturally produced by Actinobacteria. More than 74% of currently available antibiotics are the secondary metabolite product of the genus *Streptomyces* sp. Among the actino-bacterial group, *Streptomyces* produces large number of secondary metabolites and antibiotic compounds. *Streptomyces* produces significant source secondary metabolites for biomedical applications [3].

The genus *Streptomyces*, there are 800 species and published names in https://lpsn.dsmz.de/genus/streptomyces [4]. The major

limitations of the *Streptomyces* drug discovery research in the last few decades, contribute to the rediscovery of already reported compounds. Therefore, this is crucial time to find for novel potential *Streptomyces* species from untapped environments to new bioactive compounds [5]898 m.

In recent years, more focus on the unexplored areas such as deep-sea, desert, cryo, endophytic, deep forest soil and volcanic environments etc., for the isolation of potential *Streptomyces* species. Endophytic *Streptomyces* are an excellent source of a novel class of antimicrobial compounds. Endophytic *Streptomyces* species have been showing attention as a potential source of commercially interesting compounds such as antibacterial, antifungal, antiviral, antituberculosis, antihelminthic, larvicidal, antimalarial, cytotoxic, anticancerous, antidiabetic, biocontrol and even as plant growth promoters [6]the emergence of resistance to antimicrobials requires the constant development of new antibiotics. Recent scientific efforts have been aimed at the bioprospecting of microorganisms' secondary metabolites, with special emphasis on the search for antimicrobial natural products derived from endophytes. Endophytes are microorganisms that inhabit the internal tissues of plants without causing apparent harm to the plant. The present review article compiles recent (2006–2016. The endophytic *Streptomyces* strains and valuable secondary metabolites might be applied in the translational research at pharmaceutical industries as well as clinical research products [7]. This study aims to isolate and characterize secondary metabolites produced by *Streptomyces* sp. shanivit.

Material and Methods

Sample collection: Fresh and healthy tomato (*Lycopersicon esculantum*) plant samples were collected from the agrofield of Madurai (9.9420° N, 77.9724°), Tamil Nadu (9.9420° N, 77.9724°), India during August and September 2017. Plants were collected in sterile polythene bags from the field at the fruiting stage and were brought to the laboratory for microbiology processing.

Isolation of endophytic actinobacteria: Endophytic actinobacteria were isolated using the ISP2 agar. The ISP2 medium was added with nalidixic acid (50 mg/l) and nystatin (50 mg/l) to suppress the growth of ISP2 Gram-negative bacteria and other endophytic fungi. The inoculated ISP2 agar plates were incubated for 10 days at 37^0C. The well-grown colonies on the media were selected and sub-cultured on nutrient agar medium at 37^0C for 7 days [8]. Isolates were labelled serially as VITGV01 to VITGV240. After 10 days, the incubated plates were taken for subculture.

Screening of potential cultures: Primary screening for evaluating the antimicrobial potential of *Streptomyces* strain was performed by spot inoculation method [9] against two Gram-positive bacterial strains (*Staphylococcus aureus* – MTCC 737 and *Bacillus subtilis* – MTCC 2756) and two Gram-negative strains (*Escherichia coli* – MTCC 1687, *Klebsiella pneumonia* - MTCC 109). Microbial strains showing good antibacterial activity were selected for secondary screening by the well diffusion method [10]. Culture filtrate was used to (25 - 100µl) the above four organisms. The experiments were done in triplicate for analysis.

Morphological and biochemical characterization: The morphological characters of the isolates, their colony morphology were observed with a light microscope and SEM. Standard biochemical tests such as indole, MR-VP, citrate utilization, catalase, nitrate reduction, carbon source utilization were performed. And hydrolysis tests such as starch and gelatin were performed.

Phylogenetic analysis: 16S rRNA sequencing was done identification of nearest phylogenetic neighbours upto 0.02 was done using BLASTN. Analysis of the 16S rRNA gene sequences used in the construction phylogenetic tree which was

retrieved from NCBI GenBank.

Extraction and characterization of bioactive compounds: The selected isolate was grown on nutrient broth for production in the 4x500ml flask and was kept in an Erlenmeyer culture flask over a shaker at room temperature for 28 days. The cultured broth was transferred in a 50 ml white sterile plastic centrifuge tube and then were centrifuged at 10000 rpm for 20 min and the supernatant was separated for further filteration process using Whatman No 1 The bioactive compound from the culture filtrate was extracted with using 1:1 ratio of ethyl acetate solvent. The filtrate and ethyl acetate mixture was transferred to a conical flask was shaken for 2 days at 150 RPM and allowed to stand in a separating funnel for 2 h. The extracted ethyl acetate solvent was separated and condensed for further compound characterization. GC-MS (GC trace ultra-version) was used to find out the chemical compounds. The peaks were identified with the library.

Antibacterial activity: The crude metabolic extract obtained from the potential *Streptomyces* isolate were tested by the well diffusion method using Muller Hinton Agar for the four bacterial species. The bacterial inhibition zone around the well is measured using Himedia antibiotic zone scale (PW297) for further analysis.

Results

Screening of the potential strains

Out of 240 actinomycetes strains, 44 showed antibacterial effect against both Gram-positive (*S. aureus* - MTCC737 and *B. subtilis* - MTCC2756) and Gram-negative (*E. coli* – MTCC1687 and *K. pneumonia* MTCC109) bacteria. 18 isolates (8%) had antibacterial activity against Gram-negative and 15 actinomycetes strains (6%) inhibited Gram-positive bacteria. Thus 77 (32%) actinomycetes strains exhibited antibacterial activity to explore their potentials. Among all the isolates, *Streptomyces* sp. shanivit recorded the highest inhibition zone (36 mm).

Secondary screening

The primary screening results exhibited the cell-free culture filtrate of *Streptomyces* sp. shanivit exhibits strong antibacterial potency against both Gram-positive and Gram-negative bacteria. This selected strain was taken for a detailed study on morphology, biochemical and taxonomy. Antibacterial efficiency of the culture filtrate by well diffusion method against test micro-organisms and their zone of inhibition in mm (diameter) is shown in Table 1.

Table 1: Showing the result of well diffusion method for shanivit culture filtrate

S. no	Pathogens	Zone of Inhibition (mm) / Culture Filtrate (µl)			
		25	50	75	100
1	*Bacillus subtilis* MTCC2756	8	10	12	16
2	*Staphylococcus aureus* MTCC737	9	12	15	18
3	*Escherichia coli* –MTCC1687	6	9	10	14
4	*Klebsiella pneumoniae* MTCC109	-	7	9	15

Characterization of the strains

The cultural, morphological and biochemical characteristics, of the strain *Streptomyces* shanivit are summarized in Table 2. This species records moderate to heavy growth with the production red colour diffusible pigment on ISP2, Luria Bertani agar, nutrient agar and starch agar medium. Excellent color change (from green to blue) of Simmons' Citrate Agar (SCA) was observed by the growth of *Streptomyces* sp. shanivit. The positive result in SCA as the evidence of citrate utilizing ability of shanivit. It utilizes all different carbon sources such as dextrose, maltose, lactose and starch.

Phylogenetic analysis

Genetic characterization of *Streptomyces* sp. shanivit strains was done by using 16S rRNA gene sequence analysis. Phylogenetic character analysis was carried out through blastn search (http://www.blast. ncbi.nlm.nih.gov/blast. cgi?) revealed that Streptomyces sp. shanivit is the members of the genus *Streptomyces.* The partial 16S rRNA sequence of *Streptomyces* sp. shanivit (859 bp) was deposited to NCBI GenBank with accession number MT792902.1. The 16S rRNA of *Streptomyces* sp. shanivit (859 bp) exhibited the highest sequence similarity (95%) to the 16S rRNA of their closest taxonomic match using BLASTN database. The construction of the phylogenetic tree (Figure 1) revealed 16S rRNA sequence similarity of 95% to *Streptomyces mutabilis* (95%)*, Streptomyces vinaceusdrappus* (95%)*, Streptomyces plicatus, Streptomyces rochei* (95%) and *Streptomyces olivoverticillatus* (95%). The phylogenetic tree (Figure 1) revealed that *Streptomyces* sp. shanivit affiliate to one cluster, very closest with other *Streptomyces* spp. strains.

Secondary metabolites Production

Streptomyces sp. shanivit produced metabolites in nutrient broth medium at 35^{0}C, pH 7.4 and grown for 21 days. shanivit metabolites were condensed to a total of 50 mg, which appeared dark brownish in colour.

Table 2: The cultural morphological and biochemical characteristics of the isolated *Streptomyces* spp. shanivit

S. no	*Streptomyces* spp. shanivit	Biochemical Activity
1	Colony morphology	Mucoid and Greyish mycelium
2	Pigment	Red colour diffusible
3	Aerial & substrate mycelium	Positive
4	ISP2 agar	Mycelia with red diffusible pigment
5	LB Agar	Mucoid with red diffusible pigment
6	Nutrient agar	Mycelia with red diffusible pigment
7	Starch agar	Powder with red diffusible pigment
8	Indole	Positive
9	Methyl Red	Positive
10	Voges–Proskauer	Negative
11	pH	7.4
12	Catalase	Positive
13	Gelatinase	Positive
14	Nitrate reduction	Positive
15	Citrate	Positive
16	Sucrose	Positive
17	Dextrose	Positive
18	Maltose	Positive
19	Starch	Positive
20	Glucose	Positive

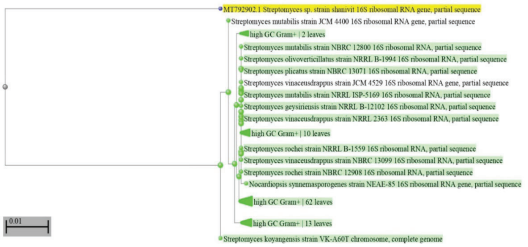

Figure 1: Phylogenetic tree of *Streptomyces* spp. shanivit isolated plant endophytes and the closest type strains based on the 16S rRNA sequence by maximum likelihood method

Antibacterial activity

The well diffusion method was used to analyse the antibacterial activity of the crude extract ranging from 25 – 100 µg/ml against the test microorganisms (Table 3 and Figure 2). The extract showed the lowest activity against *E. coli* (25µg/ml) whereas the highest antibacterial activity was recorded against *S. aureus* (100µg/ml).

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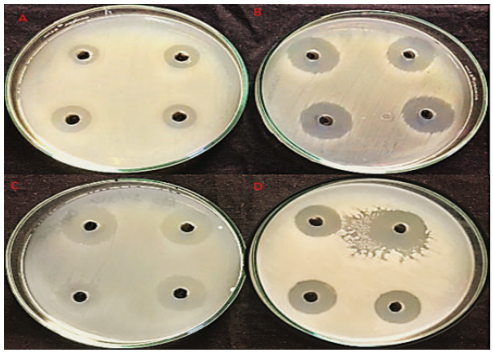
GCMS analysis

The chemical characterization of *Streptomyces* sp. shanivit composition of the extract was characterized with GCMS. Eight major chemical compounds were identified based on different retention time molecular weight and its molecular formula, all by comparing with mass spectra in the library as shown in Table 4 and Figure 3. The major identified compounds were 3-pyrrolidin-2-yl- propionic acid; Oxazolid-2-one, 3-acetyl-4-methyl-5-phenyl-; Fumaric acid, 2-hexyl Tetradecyl ester; Bromo-criptine; Cyclohexane, 1,3,5-Trimethyl-2-Octadecyl-; Ergotaman-3', 6', 18-Trione, 9, 10-Dihydro-12'-Hydroxy; Actinomycin C2 and Didemnin B are present in the sample. Their predicted chemical structures were reported in Figure 4.



A. E. coli - MTCC1687 B. *S. aureus* - MTCC737
C. B. subtilis- MTCC2756 D. *K. pneumoniae* -MTCC109

Figure 2: Antibacterial activity of the extract of *Streptomyces* spp. shanivit showing zone of inhibition in well diffusion method.

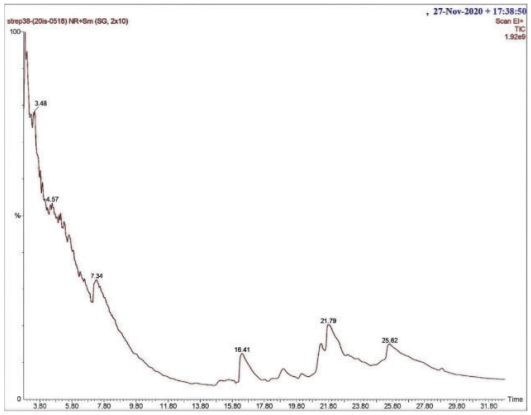


Figure 3: GC-MS profile of extract from *Streptomyces* spp. shanivit. The peaks in the chromatogram indicates the compounds identified from the library search.

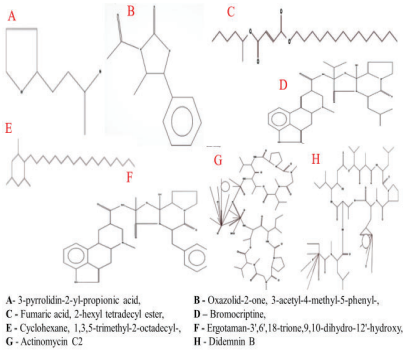


Figure 4: Chemical structures were predicted from the GCMS library search analysis of an extract of *Streptomyces* spp. shanivit.

Table 3: Zone of inhibition (mm) produced by well diffusion method in the secondary screening of crude extracts of *Streptomyces* spp. shanivit.

Sreptomyces spp. Shanivit	Zone of inhibition (µg/ml)																
	Gram-Positive								Gram-Negative								
	B. subtilis				*S. aureus*				*K. pneumoniae*				*E. coli*				
	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100	
Crude Extract	10	11	12	22	16	18	19	25	15	18	21	22	9	10	13	14	
Positive control (Erythromycin)	10	11	13	18	14	15	17	20	10	14	16	24	10	11	12	14	
Negative control (Ethyl Acetate)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

Table 4: Chemical compounds detected in the Extract of *Streptomyces* sp. shanivit by GC-MS analysis.

S. no	Compounds name	RT (min)	MW (g/mol)	Formula	Nature of compound	Activity
1	3-pyrrolidin-2-yl-propionic acid,	16.41	143	$C_7H_{13}O_2N$	Pyrrolidine	Antibacterial (50)isolated from the freshwater fish, Zacco koreanus. Morphological, biochemical, and molecular characterization of 4I1 revealed it to be Pediococcus pentosaceus 4I1. The cell free supernatant (CFS
2	Oxazolid-2-one, 3-acetyl-4-methyl-5-phenyl-	18.96	219	$C_{12}H_{13}O_3N$	methyl	Antibacterial (35)
3	Fumaric acid, 2-hexyl tetradecyl ester	20.1	396	$C_{24}H_{44}O_4$	Ester	Antibacterial (51)
4	Bromocriptine	21.79	653	$C_{32}H_{40}O_5N_5B$	cyclol	Antitumor (52)
5	Cyclohexane, 1,3,5-trimethyl-2-octadecyl-	21. 80	378	$C_{27}H_{54}$	hexane	Anticancer (SheebaV2015)
6	Ergotaman-3',6',18-trione,9,10-dihydro-12'-hydroxy	25.62	583	$C_{33}H_{37}O_5N_5$	alkaloids	Antimicrobial (54)
7	Actinomycin C2	28.89	1268	$C_{63}H_{88}O_{16}N_{12}$	Antibiotic	Antimicrobial (48)
8	Didemnin B	28.91	1111	$C_{57}H_{89}O_{15}N_7$	Dispeptides	Antitumor (49)

RT – Retention time; MW – Molecular weight;

Discussion

The increasing resistance through poor medical practices and pathogenic bacteria to multiple antibiotics leads to an urgency for new antibacterial compounds [11]. Microbial communities of different ecological habitats are a proven natural source of effective novel biologically active compounds. The discovery of novel antimicrobial metabolites from endophytes is an effective untapped source to control the virulent antibiotic resistance pathogens Endophytic actinomycetes are believed to carry out a new mechanism to overcome the harmful pathogenic invasion by producing versatile secondary metabolites. Many antimicrobial compounds were isolated from endophytic

actinomycetes belonging to different structural classes like alkaloids, peptides, steroids, terpenoids, phenols, quinines, and flavonoids [12]. Secondary metabolites producing actinobacteria are still very interesting because of their enormous untapped number of metabolites producing gene clusters and their wide spectrum of antimicrobial activities. In this study, 240 actinobacterial strains were isolated from tomato plants of agrofield. Similarly, Goudjal et al. [13] and Romero et al. [14] have reported the isolation of actinobacteria from tomato plants. Similarly, Cao et al. [15] reported the maximum number of the endophytic actinobacteria, of genus Streptomyces, which is the most predominant actinobacteria in the soil and tomato plant. This media is supplemented with nystatin (50 mg/l) and nalidixic acid (50 mg/l) supplements to control the growth of fungal and Gram-negative bacterial contamination to promote the slow-growing actinobacteria. The same media was reported by Rashad et al. [16] the Streptomyces growth.

Out of 240 strains, 77 (32%) showed antagonistic activity minimum one test organism, but from these 77 strains, 44 (18%) showed a wide spectrum of antagonistic activity while 18 isolates (8%) inhibited Gram-negative and 15 isolates (6%) inhibited Gram-positive bacteria. Sharma and Thakur [17] also reported that out of 107 actinobacteria, 29 (37.6%) isolates showed potential inhibition on Gram-positive and Gram-negative bacteria. Among these isolated Streptomyces sp. shanivit was one strain that showed strong antagonistic activity in all four selected pathogen and this strain was used for further studies.

The isolate Streptomyces sp. shanivit is a slow-growing, aerobic, Gram-positive, pink coloured aerial, substrate mycelia. It produces brown to red pigments, on ISP2, LB, starch and Nutrient agar. The pigment-producing ability on ISP2 and nutrient agar may be due to enough amount of nutrient availability and the media's pH. This study coincides with reports Sapkota et al. [18] that slow-growing, aerobic and pigment-producing Streptomyces sp. Streptomyces sp. shanivit utilize citrate and all the sugars as a carbon source for growth and metabolism. This study is similar to the Tandale et al. [19] biochemical test and sugar sources for the growth of actinobacterial strains.

The analysis of 16S rRNA gene sequences was analysed and deposited in GenBank [accession number MT792902.1]. This analysis revealed that this strain shanivit belongs to the genus Streptomyces with 95% similar with closely related species such as Streptomyces mutabilis Streptomyces vinaceusdrappus, Streptomyces plicatus, Streptomyces rochei and Streptomyces olivoverticillatus. A similar type of results were reported by Law et al. [20] in 16S rRNA gene sequence similarity on S. monashensis MUSC 1JT strain showed the highest match (98.70%) with S. corchorusii NBRC 13032T strain.

The present study confirmed the antimicrobial compound produced in nutrient broth in the optimized conditions such as pH 7.4, incubation period was 21 days at 350C. The same scenario was reported by Onaka et al. [21] that production of pigmented antibacterial compound goadsporin from Streptomyces sp. TP-A0584. Ethyl acetate was used as 1:1 ratio to extract, a secondary metabolite from the culture filtrate. The same methodology was reported for the extraction of secondary metabolite by Sengupta et al. [22] and Ahsan et al. [23] in Streptomyces strain KX852461and actinomycetes strain, respectively.

Actinobacteria are an important producer of antimicrobial metabolites with different biological applications. In this study, antibacterial activity using crude extracts of Streptomyces sp. shanivit showed that the extract had a maximum zone of inhibition against test Gram-positive bacterial pathogen S. aureus (25 mm) at 100µg/ml and minimum zone of inhibition against test organism E. coli (9mm) at 25µg/ml (Figure 2 and Table 3). Duddu and Guntuku [24] have reported actinomycetes

strain (M3) from mangrove ecosystem which showed a maximum zone of inhibition against S. aureus (19.5 mm) and C. albicans (22.6 mm). Streptomyces parvus secondary metabolite compounds were active against pathogenic bacteria; the maximum inhibition was recorded on Pseudomonas aeruginosa (20 mm) and Escherichia coli (20 mm).

There are many previous reports on GC-MS-based chemical analysis of actinobacterial secondary metabolite [25–28]. In the current study, GC MS peaks show eight major secondary metabolite compounds with different retention times and molecular formulas were detected. The identified crude compounds were in the class of Pyrrolidine, methyl, esters, cyclol, hexane, alkaloids, antibiotics and peptide in nature. Pyrrolidine compounds are known to be effective antifungal and antitumor activity. Studies led by Guo et al. [29]; Bharose et al. [30]; Podoll et al., [31]; Kannan et al.[32] and Jalaluldeen et al. [33] were exhibited potential characteristics of pyrrolidine compounds in GC MS analysis. The antimycobacterial and antifungal activity of 3-pyrrolidin-2-Yl-propionic acid from Amphipterygium adstringens and Streptomyces indiaensis, respectively. Previous findings have coincided that this compound possesses promising antimicrobial activity. This 3-pyrrolidin-2-yi -propionic acid compound is present in the Streptomyces extract. This compound could be the contributor to the potent antimicrobial action of extract Streptomyces sp. shanivit. Few studies have reported the antagonistic potential of Oxazolidinone such as 1,3,4-Thiadiazole phenyl oxazolidinone, 2,5-Disubstituted 1,3,4-oxadiazole and 5-(4-methyl-1H-1,2,3-triazole) methyl oxazolidinones against an array of test pathogens [34–37] but Oxazolid-2-One, 3-Acetyl-4-Methyl-5-Phenyl- still has not been documented for any antimicrobial efficiency till date, which needs further research.

The GCMS analysis on compounds such as Fumaric acid, cyclohex-3-enylmeth l isobutyl ester, Fumaric acid, nonyl pentadecyl ester, Fumaric acid, 2-methylpent-3-yl nonadecyl ester, Fumaric acid, isohexyl undec-2-en-1-yl ester, Fumaric acid, 2- ethylbutyl undecyl ester and Fumaric acid, dodecyl 2-ethylbutyl ester (Karthikeyan and Dhanapal [40]; Manoon and Alnomani [39]; Al-Zubaidi et al. [38]. However, to date, there has been no report on the antibacterial potential of Fumaric acid, 2-Hexyl aetradecyl ester which is also one of the compounds in the extract of Streptomyces sp. shanivit.

Czyz et al. [41] reported a strong broad spectrum of intracellular pathogens activity by Bromocriptine compound. Recently, Wu et al. [42] reported computational biology data on SARS-CoV-2 for bromocriptine as potential RdRp inhibitors from the ZINC drug database. Based on this report, this study revealed bromocriptine could play a vital role in antiviral activity.

According to the reports by Madhaiyan and Annamalai [43] Cyclohexane, 1,3,5-Trimethyl-2-Octadecyl- exhibited excellent inhibitory activity against S. aureus, E. coli, Pseudomonas sp. and Klebsiella sp. Sangeetha et al. [44] also reported antiviral activity by Cyclohexane, 1,3,5-Trimethyl-2-Octadecyl- compound. Thus, it can be confirmed that Cyclohexane, 1,3,5-Trimethyl-2-Octadecyl- present in the extract of Streptomyces sp. shanivit might have a crucial role in this antibacterial activity against Gram-positive and Gram-negative bacteria, which is the first report. The following compound Ergotaman-3', 6', 18-Trione, 9, 10-Dihydro-12'- Hydroxy found in GC-MS analysis was demonstrated previously demonstrated from the extract of Ulothrix flacca (RT 26.202) and Pseudomonas aeruginosa (RT. 559) by Altaee et al., [45] and Vasudevarao and Sravanthi [46]. The same compound Ergotaman-3',6',18-trione,9,10-Dihydro-12'-Hydroxy compound was found in ethyl acetate extract of Streptomyces sp. shanivit at a retention time of 25.62 in the present study.

Actinomycin (C63H88O16N12) is

an antibiotic that inhibits the replication of DNA. Saravana Kumar et al. [47] identified actinomycin C2 from Streptomyces lavendulae strain SCA5 characterized by the GCMS analysis. This compound is a potential antimicrobial compound against five different Gram-positive, eight different Gram-negative bacterial pathogens and ten pathogenic fungi. In addition, Actinomycin C2 has been reported to inhibit the growth of S. aureus as well as M. tuberculosis by Shah et al. [48].

The didemnin is a natural product based on the structure which is classified cyclic depsipeptide. Chun et al. [49] reported that didemnin B has good antitumor, antiviral and potent immunosuppressive properties. A few compounds that had antimicrobial activity were proved from previous studies. The presence of these eight potential compounds might be the key contributor to the antimicrobial action of the extract from Streptomyces sp. shanivit. The endophytic actinobacteria were a diverse and largely underexplored resource for the isolation of Streptomyces producing effective antimicrobial compounds. Our findings can add to a novel source for the discovery of valuable and needful antibiotic compounds of pharma, and industrial values for human welfare.

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