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Multipotential Secondary Metabolites from *Nocardiopsis dassonovillei* of Marine Actinomycetes and their *In Silico* studies

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Actinomycetes are one of the important secondary metabolite producers. Researchers focused on the exclusive marine areas for isolation and identification of marine actinomycetes. The present study focused on the isolation and identification of Nocardiopsis dassonovillei (ON627850) from TS Pettai region. The potential strainTSP1 showed effective antibacterial activity against Haemophilus influenza. TSP1 isolates showed IC50 value of 75.22 μg/ml effective antioxidant activity determined by DPPH assay. Cytotoxicity assay results were noted for the ethyl acetate extract of TSP1 screened against oral cancer cell lines (KB). The spectral characterization studies of UV, FT-IR and GC-MS results identified the compound 2,4-di-tert-butylphenol. The multi-potential 2,4-di-tert-butylphenol compound finally docked with KB cell lines protein for drug discoveries.

Keywords: Actinomycetes; Cytotoxicity assay; DPPH, Drug; KB cell; Molecular Docking.

Marine microorganisms are presently attracting a lot of attention as a novel and prospective source of biologically active compounds[1]. They produce a wide range of metabolites, some of which can be exploited in the creation of drugs[2]. The 95% of actinomycetes are present in both environmental habitats, both aquatic and terrestrial[3,4]. They are saprophytic, free-living bacteria that are a significant contributor to the manufacturing of antibiotics. They are crucial in the recycling of organic materials[5] Actinomycetes were extensively screened in marine plants, medicinal plants, sediments, and soil environments by recent researchers[6-7]. Actinomycetes have long been known as major manufacturers of enzymes, antibiotics, amino acids, anti-cancer medications, anti-diabetic pharmaceuticals, anti-obesity treatments, and pharmaceutically and industrially essential compounds. Around 80% of all antibiotics produced by bacteria, including streptomyces, are produced by this organism. It is also capable of producing several active secondary metabolites[8]. Secondary metabolites produced by actinomycetes continue to provide a chemically varied source for the discovery and development of pharmacological drugs as well as biochemical probes to investigate the mechanisms involved in human diseases[9].

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MATERIALS AND METHODS

Isolation of Actinomycetes

Marine sediment samples were collected from different sites of TS Pettai (Latitude 11.4110ºN, Longitude 79.7954ºE) Ponnanthittu (Latitude 11.483318ºN, Longitude 79.760164ºE) and Parangipettai (Latitude 11.49045ºN, Longitude 7976594ºE) at a depth of 10 cm, samples were taken 0.5 km from the coast. The sediments' surface layers were removed, and the center part of each sediment, weighing approximately 0.5 kg, was aseptically transferred into polythene bag. These samples were allowed to air dry for one week after the pretreatment sediment samples were serially diluted 10^{-7} and aliquots (0.1 ml) plated on Starch Casein Agar (SCA) and Actinomycetes Isolation Agar (AIA) mixed with 50% sea water and 50% distilled water. After the sterilization add the (fluconazole 25ìg/ml) to inhibit the fungal control of the petriplate. Plates were incubated for 7-10 days at 21 ºC. Subcultures of the isolated actinomycetes strains were stored at 4°C.

Antagonistic Activity

The antagonistic activities of the isolated actinomycetes were tested against human pathogenic bacteria by cross streaking method and incubate the plates for 37ºC at 24 for hours. After the incubation period the actinomycetes strains inhibit the pathogenic bacteria. The best actinomycetes strains were chosen and further investigated.

Identification of Actinomycetes

The potential actinomycetes strains were tested morphological, cultural, physiological and biochemical characteristics[10]. The majority of marine actinomycetes produced unpigmented grey and white colonies. The spore chain and spore bearing hyphae morphology were observed and using high power optical microscope at x1000 magnification[11]. Under a light microscope, the colors of the spore mass were analysed[12].

Extraction of Bioactive Compounds

The antagonistic actinomycetes isolates were inoculated into starch casein broth and incubated at 28ºC in incubator shaker 250 rpm for seven days. The broths were filtered on Whatman No. 1 filter paper after incubation period and were centrifuged at 4000 rpm for 15 minutes to extract the bioactive compound. The supernatant were aseptically transferred into a conical flask and kept at 4ºC for further analysis. To the supernatant and an equal volume of each solvent (*Viz*., Methanol, Ethanol, Ethyl acetate, Acetone, Hexane and Chloroform) on 1:1dilution. The activity of the compounds produced from each solvent was evaluated against the test pathogens[13].

Screening of Antibacterial Activity

The antibacterial activity of the actinomycetes isolates were evaluated against the bacterial Gram negative pathogens such as *Escherichia coli, Klebsiella pnemoniae, Serritia marcescens, Salmonella typhi, Proteus mirabilis,* and Gram positive pathogens such as *Streptococcus pyogenes, Staphylococcus aureus, Enterococcus faecalis, Listeria monocytogenes, Haemophilus influenza.* The different concentrations of 25 µl, 50 µl, 75 µl and 100 µl of crude extract were used. Chloramphenicol (10µg/ml) antibiotic used as a positive control and DMSO used as a negative control[14].

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MICs were calculated using test organisms that demonstrated sensitivity to crude extracts using the broth microdilution technique. To allow for a 50% dilution after the inoculum, solvents, or antibiotic were added, the medium used in the plates was prepared at twice the final strength. All 96 wells were filled with a 100 µl volume of Muller Hinton broth that was doubled in strength before different antibiotic concentrations were added in decreasing order along the wells. Finally, the test organism suspension was added to each well in a 50 µl volume. After that, the plates were incubated at 37°C for 18-24 hours. Even as growth controls, the wells in columns 8 and 9 are kept at the positive (crude extracts) and negative (Test organisms) controls[15].

Antifouling Activity

Biofilm producing bacteria (1 mL of seawater) were incubated with conical flask of nutrient broth medium at 37 ºC for 24 hours. As an antifouling agent, about 0.1 g of the crude extract was added to the flask. After dying for 10 minutes with 0.4% crystal violet solution, the cover glass was cleaned with water, air dried, and observed

under the microscope (OPTIKA microscopes, model: B-182, Italy). To compare, a control flask (without crude extract) was used[16].

Antioxidant Activity

The DPPH test is widely used in natural product antioxidant research. This method's simplicity and sensibility are among the factors. The idea behind this test is that an antioxidant is a hydrogen donor. It assesses chemicals that act as radical scavengers. The process by which DPPH takes hydrogen from an antioxidant is illustrated below. One of the few stable and easily obtained organic nitrogen radicals is DPPH. The reduction of DPPH in test samples reflects the antioxidant action. Due to its ease and precision, monitoring DPPH with a UV spectrometer has become the most popular technique. At 517 nm, DPPH has a significant absorption maximum (purple). When hydrogen from an antioxidant is absorbed, the colour changes from purple to yellow and DPPH is then produced. In terms of the quantity of hydrogen atoms absorbed, this reaction is stoichiometric. As a result, the reduction in UV absorption at 517 nm makes it simple to assess the antioxidant impact.

The 0.1 mM DPPH solution in methanol should be prepared in a hurry, and 100 ìl of this solution should be added to 300 ìl of the crude at various concentrations (500, 250, 100, 50, and 10 ìg/mL). The mixes must be briskly mixed and let to stand for 30 minutes at room temperature. The absorbance at 517 nm must then be measured with a UV-VIS spectrophotometer. (Ascorbic acid can be used as the reference). Higher levels of free radical scavenging activity are shown by reaction mixtures with lower absorbance values. The following formula can be used to calculate the capability of scavenging the DPPH radical[17].

$$\text{DPPH Scavenged (\%)} = (\text{A con} - \text{A test} / \text{A con}) \times 100$$

A con - absorbance of the control reaction
A test - absorbance of the sample

16S rRNA gene sequencing and phylogenetic analysis

Sequencing of the 16S rRNA gene showed some results. The 16S rRNA gene was sequenced using a PCR thermal cycler (Gene Amp PCR System 9700, Applied Biosystems). In order to get the closest match sequence, sequences were compared with the GenBank database using Blast N. The Pairwise alignment tool was used to align the sequences [18]. A consensus sequence was created using the aligned sequence. The produced consensus sequence was utilised to perform BLAST in NCBI to find related sequences. MEGA software was used to create a phylogenetic tree using the top ten sequences from the hit table[19].

SEM analysis

Streptomyces sp isolate TSP 1 was grown on Starch Casein Agar (SCA) media (28°C ± 1 for 7 days) for analysis using SEM.

GC-MS Analysis

The 8890 GC Agilent gas chromatography with front detector FID was used for the GC-MS study. The apparatus features a non-polar DB 35 - MS Capillary Standard column with measurements of 30 mm 0.25 mm ID 0.25 m film. Helium with a flow rate of 1.0 ml/min is the carrier gas utilised. The oven temperature was configured as follows, with the injector running at 250 °C: 15 minutes at 60 °C, followed by a 3 minute climb to 280 °C. The ratio of 1:100 was used to inject one microliter of extract in split mode into the GC column's injection port. The GC peak regions determined the sample's composition as a percentage. Willey and NIST libraries, as well as a comparison of their retention indices, were used to identify the components. After comparison with those in the computer library (NIST and Willey) connected to the GC-MS instrument, the components were identified, and the findings were tallied.

UV- Vis and FT-IR Analysis

The UV-spectra of *Nocardiopsis dassonovillei* obtained from this study were subjected to comparison of general pattern maximum absorbance peaks and range of wave length. Using UV-visible spectrophotometer Shimadzu – UV 1800, the UV region (200-800 nm) of each active extract was measured. The FTIR spectra of each active extract was then identified using a Shimadzu IR-470 plus. Moreover, the spectra were scanned between 400 and 4000 cm-1, and the results were shown as Transmittance (%) vs wavelength (cm^{-1})[20, 21].

Cytotoxic Activity [2,4-Di-tert-butylphenol (MTT Assay)]

The human oral cancer cell line NF-kB used for the study. The cell line was cultured in DMEM (Dulbecco's Modified Eagle Media)

medium with 10% FBS (Fetal Bovine Serum) supplementation at 37°C in an incubator with 5% CO2. To avoid bacterial contamination, antibiotics penicillin (100µg/mL) and streptomycin (100µg/mL) were added to the medium[22].

Molecular Docking

The compounds from marine *Nocardiopsis dassonovillei* were selected as ligands based on anticancer activity reported previously. The compound 2, 4-Di-tert-butylphenol was isolated from marine-derived *Streptomyces* sp and has been shown to possess significant cytotoxicity against oral cancer cell lines (KB cell).

RESULTS AND DISCUSSION

Actinomycetes from Marine Sediment Samples

As other researchers have documented the presence of actinomycetes in coastal and marine sediment. Several quantities of actinomycetes were also identified in the current investigation. The 3 different isolates in all were found in the various samples of marine sediment (Table 1 & Figure 1). The coastal mangrove sediments yielded the largest amount of isolates. According to other reports that have documented the presence of actinomycetes in the sediment of marine and estuarine settings, a significant portion of actinomycetes were isolated in the present research[24, 25]. The sediment samples are taken during tidal waves that are between 4 and 6 meters in depth. A minimum of 1.1x103/g and a maximum of 1.5 x 106 CFU of actinomycetes were found in different sediments[26].

Screening of Antagonistic Activity

The potential strains inhibited the growth of Gram negative pathogens such as *Escherichia coli, Klebsiella pnemoniae, Serritia marcescens, Salmonella typhi, Proteus mirabilis,* and Gram positive pathogens such as *Streptococcus pyogenes, Staphylococcus aureus, Enterococcus faecalis, Listeria monocytogenes* and *Haemophilus influenza.* The growth inhibition zone was around 6 - 30 mm in diameter on average. The diameter of the zone of growth inhibition produced by it reached 30 mm for *H. influenza.*

Identification of actinomycetes

Identification of actinomycetes using molecular techniques was found to be faster and less difficult than standard biochemical procedures. All of the actinomycetes isolates are members of the genus Streptomyces, according to the results of the direct sequencing of purified 16S rRNA. All of the isolates have spore chains of three or more, and results obtained with a research microscope at 100X revealed that they are all non-motile and have such spore chains. Streptomyces is a good source of physiologically active secondary metabolites. The *Nocardiopsis dassonovillei* (Accession number ON627850) is a moderately marine actinomycetes strain isolated and identified from the TS Pettai, Ponnanthittu and Parangipettai marine sediment areas based on morphological, microscopical and 16S rRNA molecular profiling.

Extraction of actinomycetes

The six different solvent extracts were used to assess the isolates antimicrobial activity ethyl acetate extract provided the largest inhibitory zone against all the pathogens tested, followed by ethanol, methanol, hexane, acetone and chloroform extracts. The strain TSP1's ethyl acetate extract had the highest level of effectiveness against *Haemophilus influenza* (30 mm) and was followed by *Salmonella typhi* (27mm).

Screening of Antibacterial Activity

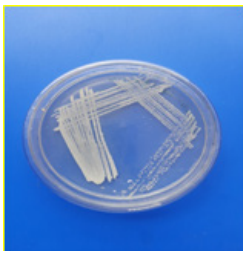
The crude extract shows the antibacterial activity against Gram positive bacteria such as *Haemophilus influenza* (30 mm) and gram negative bacteria such as *Salmonella typhi* (27mm) at 100 ìl of concentration mentioned above (Table 2) (Figure 2). The isolates TSP1 showed a broad spectrum of antibacterial activity against the variety of clinical bacterial pathogens concluded by antagonistic activity, well diffusion method, MIC and MBC. TSP1 ethyl acetate crude extract showed maximum zone of inhibition against *Haemophilus influenza* (30 mm) at 100 ìl concentration respectively.

Determination of MIC and MBC

As a result, the MIC wells could be distinguished visually with clarity. The extract's minimum inhibitory concentration (MIC), which is the concentration at which the growth of the test organisms is inhibited, were calculated (Figure 3). Following a procedure, the minimum bactericidal concentration (MBC) was measured from the MIC plate (Figure 4). It is described as the lowest concentration of an antibiotic that, when used in defined in vitro conditions, decreases the number of organisms in a medium containing a certain inoculum of bacteria by 99.9 percent in a specified amount of time. It was measured by inoculating

broths in the MIC range into a nutrient agar medium that was free of drugs. The antimicrobial concentration at which no growth was seen after a 48-hour incubation period was chosen as the MBC. The MIC results conclude and compare with other solvents finally the ethyl acetate will give the best minimum concentration are shown in (Table 3).

Table 1. Morphological characterization of actinomycetes

Actinomycetes isolates	TSP	PPT	PTU
Colony characteristics			
Size	Large	Medium	Medium
Shape	Regular	Regular	Irregular
Surface	Powdery	Powdery	Rough
Elevation	Convex	Convex	Raised
Pigmentation	Chalk white	Chalk white	Grey white
Gram staining	Large rod shaped branched filamentous gram positive bacteria	Large rod shaped branched filamentous gram positive bacteria	Large rod & Cocci shaped branched filamentous gram positive bacteria

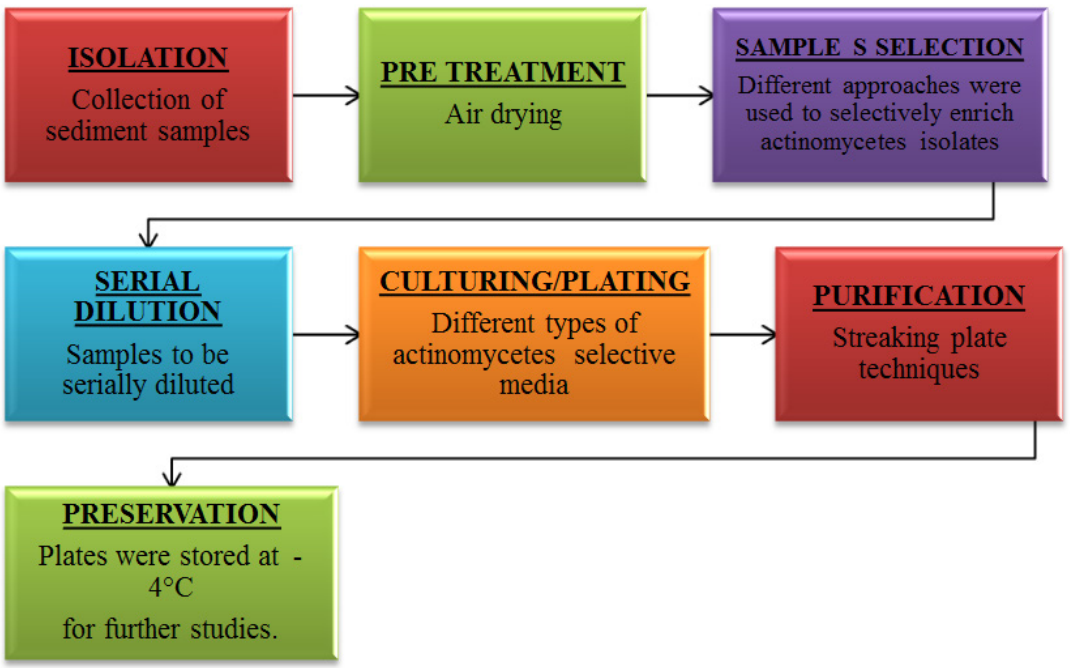


Fig. 1. Isolation of actinomycetes

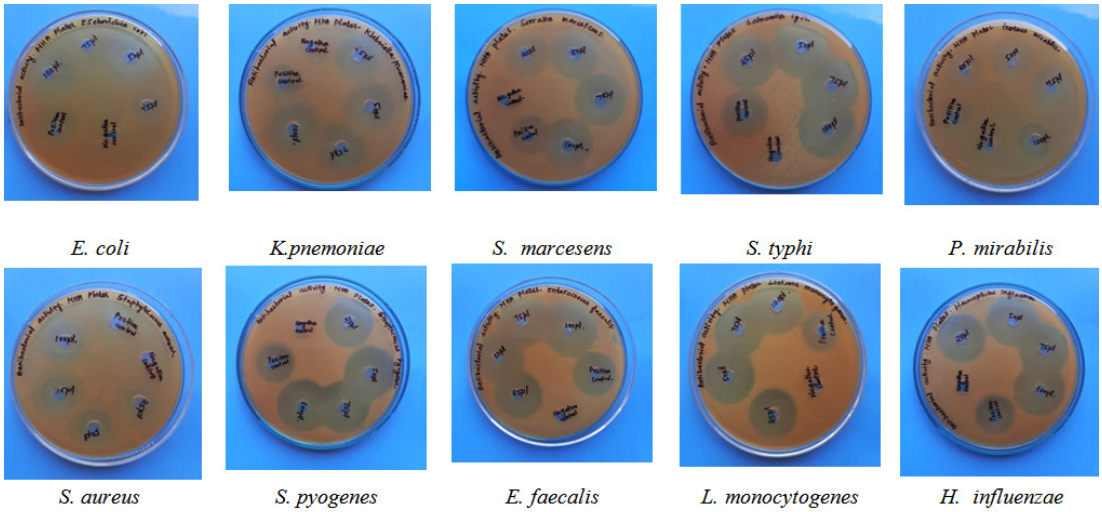


Fig. 2. Antibacterial activity of crude extracts

Table 2. The antibacterial activity for the crude extract of actinomycetes isolates against some bacterial pathogens

Pathogens	Zone of inhibition (mm)					
	25 µl	50 µl	75 µl	100 µl	+ ve control	-ve control
E. coli	17±1.53	19±1.53	22±0.58	23±1.15	16±1.00	-
K. pnemoniae	18±0.58	22±0.58	24±1.53	26±0.58	17±1.53	-
S. marcescens	17±1.53	21±1.00	22±0.58	24±1.53	26±0.58	-
S. typhi	21±1.00	23±1.15	25±1.00	27±1.00	19±1.53	-
P. mirabilis	14±1.00	16±1.00	19±1.53	21±1.00	15±0.58	-
S. aureus	16±1.00	18±0.58	21±1.00	22±0.58	15±0.58	-
S. pyogenes	19±1.53	21±1.00	23±1.15	24±1.53	16±1.00	-
E. faecalis	21±1.00	23±1.15	25±1.00	26±0.58	17±1.53	-
L. monocytogenes	22±0.58	24±1.53	26±0.58	29±1.00	18±0.58	-
H. influenza	23±1.15	25±1.00	27±1.00	30±1.00	19±1.53	-

Table 3. Minimum inhibitory concentration and minimum bactericidal concentration of crude extracts of *Nocardiopsis dassonoveilli*

Extract Code	Test organisms	Gram reaction	MIC (mg/ml)	MBC (mg/ml)
TSP1	*Echerichia coli*	-	10	20
	Klebsiella pnemoniae	-	15	30
	Serratia marcescens	-	20	40
	Salmonella typhi	-	10	20
	Proteus mirabilis	-	40	60
	Staphylococcus aureus	+	15	30
	Streptococcus pyogenes	+	20	40
	Enterococcus faecalis	+	40	60
	Listeria monocytogenes	+	05	10

Antifouling activity of *Nocardiopsis dassonovillei*

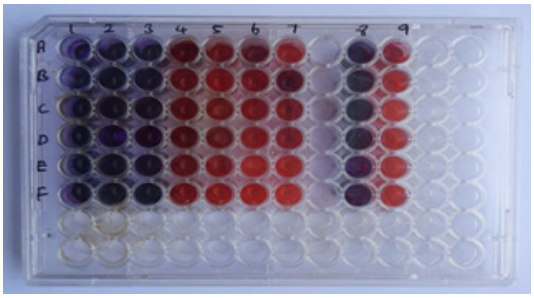
The *Nocadiopsis dassonovillei* crude extract inhibits the production of bacterial biofilms, as shown in Figure. The crude extract served as an anti-biofouling agent and decreased the density of bacterial cells. The results of the current study suggested that the marine *Nocardiopsis dassonovillei* crude extract may be a source for the synthesis of environmentally beneficial antifouling chemicals, which might be a better alternative to the pollution-causing synthetic antifoulants. This finding was consistent with research on the antifouling properties of *Nocadiopsis dassonovillei*, which decreased the growth of biofilm on glass.

Antioxidant activity

Free radicals are groupings that contain a single pair of electrons and have negative effects to living organisms by creating an oxidation mechanism that results in a deadly consequence. The antioxidants are the substance that inhibits this type of free radical from causing an oxidation process mentioned in (Tables 4, 5 and 6 & Figures 5, and 6). The TSP isolate has produced the dose IC_{50} Value of the tested sample: 75.22 ìg/ml (Figure 7). Antioxidant studies revealed the TSP1 ethyl acetate crude extract has antioxidant properties by inhibition of free radical scavenging[27].

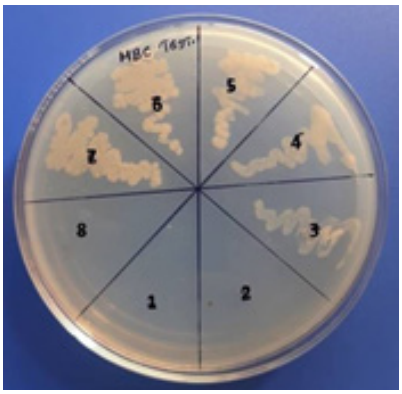
16s rRNA gene sequencing and phylogenetic analysis

It was amplified and sequenced to study the 16S rRNA gene of *Streptomyces* sp. A stretch of 1379 nucleotides made up of TSP1 complete 16S rRNA gene sequence, and the nucleotide sequence has been uploaded to GenBank (Accession number ON627850). The phylogenetic tree of *Streptomyces* sp. TSP 1 was built using the 16S rRNA gene sequence Figure 8. The strain's phylogenetic location was within a cluster that also contained *Nocardiopsis dassonovillei* (ON627850).



1. 500; 2. 250; 3. 125; 4. 62.5 5. 31.25; 6. 15.12; 7. 7.56 µg/mL 8. Control extract 9. Control broth
A. Methanol B. Ethanol C. Ethylacetate D.Chloropharm E. Hexane F. Acetone

Fig. 3. MIC of different extracts of *Nocardiopsis dassonvillei* against *Haemophilus influenza* ATCC 49247



1. 500; 2. 250; 3. 125; 4. 62.5 5. 31.25; 6. 15.12; 7. 7.56 µg/mL 8. Control broth

Fig. 4. MBC of different extracts of *Nocardiopsis dassonvillei* against *Haemophilus influenza* ATCC 49247

Table 4. OD Value at 517 nm

S. No	Tested sample concentration (µg/ml)	OD Value at 517 nm (in triplicates)		
1.	Control	0.724	0.756	0.971
2.	500 µg/ml	0.134	0.137	0.138
3.	250 µg/ml	0.138	0.152	0.160
4.	100 µg/ml	0.163	0.173	0.165
5.	50 µg/ml	0.180	0.185	0.189
6.	10 µg/ml	0.199	0.213	0.228
7.	Ascorbic acid	0.08	0.11	0.12

Table 5. Percentage of inhibition

S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1.	Ascorbic acid	90.20	86.53	85.31	87.35
2.	500 µg/ml	83.59	83.23	83.10	83.31
3.	250 µg/ml	83.10	81.39	80.41	81.64
4.	100 µg/ml	80.04	78.82	79.80	79.55
5.	50 µg/ml	77.96	77.35	76.86	77.39
6.	10 µg/ml	75.64	73.92	72.09	73.88

Table 6. IC50 Value of tested sample: 75.22 µg/ml

log(inhibitor) vs. normalized response — Variable slope		
Best-fit values		
LogIC50	-	1.876
HillSlope	-	-1.535
IC50	-	75.22
Std. Error		
LogIC50	-	0.05021
HillSlope	-	0.2854
95% Confidence Intervals		
LogIC50	-	1.768 to 1.985
HillSlope	-	-2.152 to -0.9186
IC50	-	58.59 to 96.56
Goodness of Fit		
Degrees of Freedom		13
R square	-	0.9233
Absolute Sum of Squares	-	1510
Sy.x	-	10.78
Number of points		
Analyzed	3	15

TSP exhibited a 78% sequence similarity with *Nocardiopsis dassonovillei* (ON627850) and was presented as a single branch.

SEM analysis

In SEM analysis, the TSP1 potential strain showing 2 µm in size and rod shape observed under the Scanning electron microscope (Figure 9 & 10).

Gas Chromatography and Mass Spectrometry

The structure of compound TSP1 was further elucidated by GC-MS which exhibited molecular adduct ion peak at m/z 16.052 (Figure 11 and 12). The mass spectrum of compound TSP1 was found to be identical to 2,4 Di-tert-butylphenol and the structure of the compound was also confirmed in the GC-MS library.

UV Visible analysis

Studies on UV-visible absorption were carried out with a Shimadzu spectrophotometer (UV-1800) and matched quartz cuvettes with a 1 cm path length. Using the UV-Vis spectrum

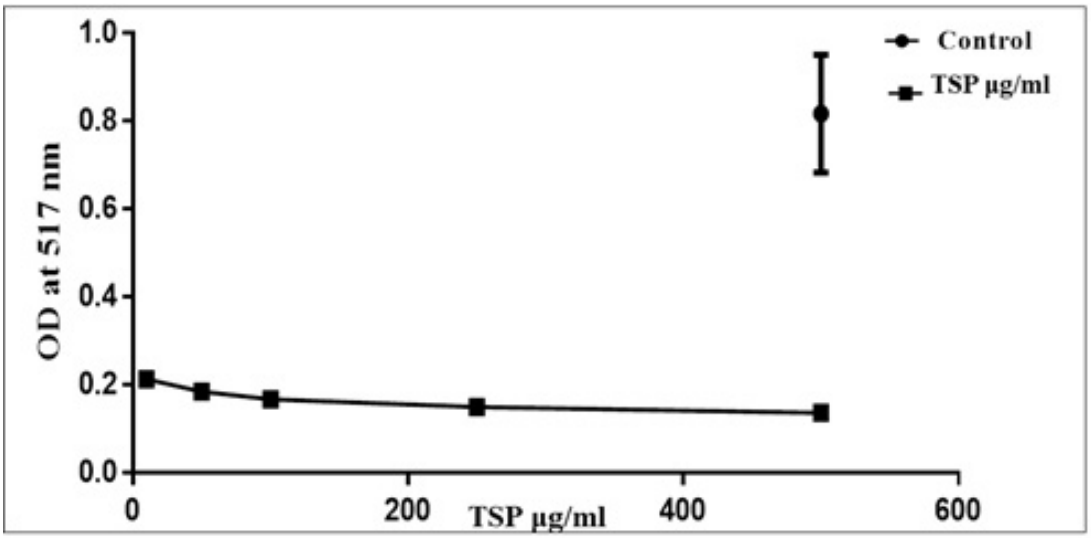


Fig. 5. 2,4 Di-tert-butylphenol µg/ml

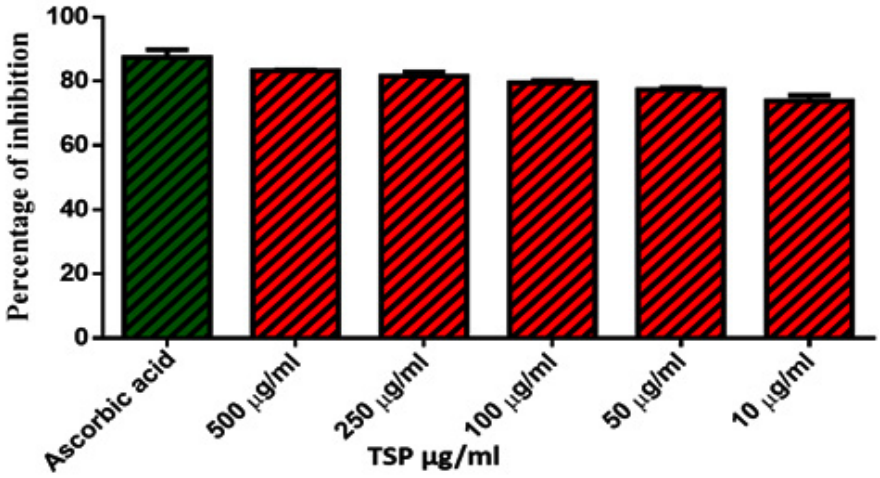


Fig. 6. 2,4 Di-tert-butylphenol µg/ml range

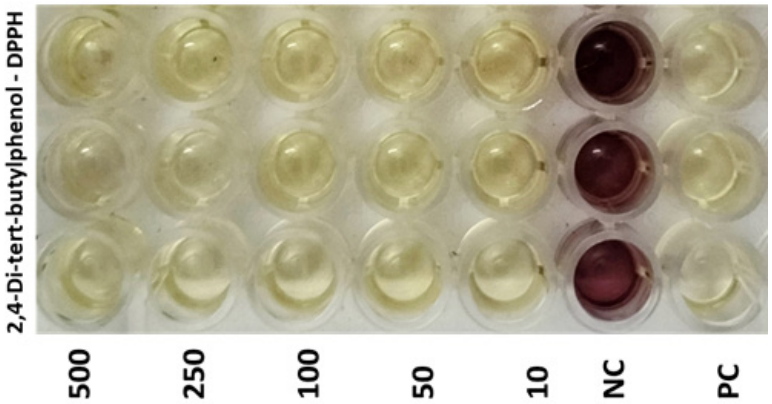


Fig. 7. IC50 Value of tested sample: 75.22 µg/ml

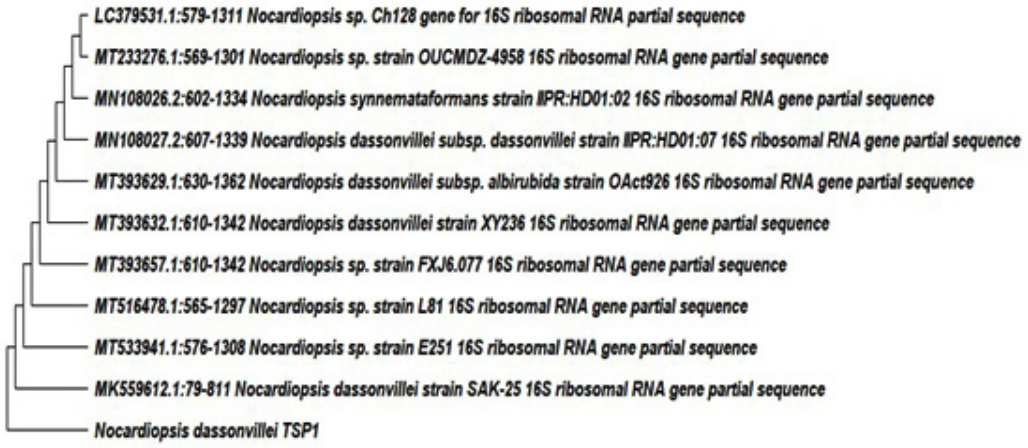


Fig. 8. Phylogenetic tree of *Nocardiopsis dassonoveilli*

between 200 and 800 nm, the TSP compound was observed. In (Figure 13) the pointed adsorption peak is displayed.

FT-IR analysis

The crude extracts of the TSP isolates were analyzed using GC-MS. The chemical compositions of the extracts were ascertained using GC-MS. The chemical compositions of the extracts were determined using elemental and functional group analysis data, as well as FT-IR investigations. The FT-IR spectrum (Figure 14) contained bands at 3446 cm^{-1} 2043 cm^{-1}, 1635 cm^{-1}, 1414 cm^{-1}, 1103 cm^{-1} and 562 cm^{-1}. A phenol belonging to the 2,4-di-tert-butylphenol class has two tert-butyl substituents at positions 2 and 4. All these indicated the presence of bioactive compounds which include n GC-MS. The multi-potential TSP1 ethyl acetate extracts spectral studies UV - Visible, FT-IR and GC-MS results identified as component 2,4-di-tert-butylphenol from *Nocardiopsis dassonovillei* is more effective against antimicrobial and anticancer activities

Microscopic analysis and MTT assay

Cancer cells were seeded in 96-well plates at a density of 5000 cells per well. The attached cells were incubated for 24 hours and treated with different concentrations of 2, 4-Di-tert-butylphenol (20 & 25 µM/ml) of DMEM media and were then incubated for 24 and 48 hours. The cells were

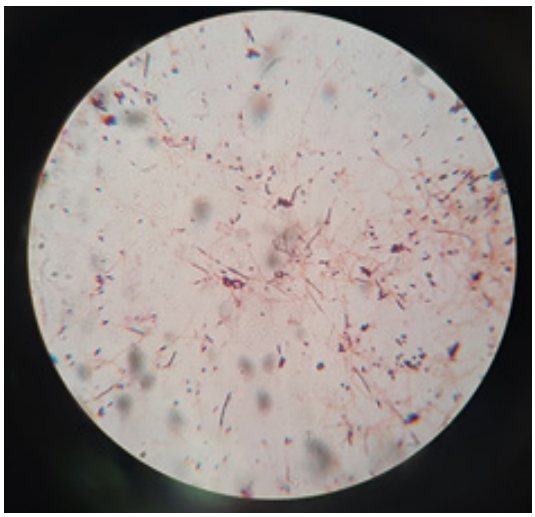


Fig. 9. A Microscope was used to examine the colony morphology

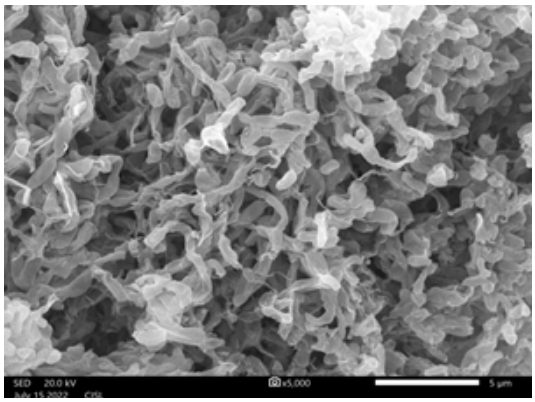


Fig. 10. A scanning electron microscope was used to examine the spore ornamentation and chain morphology

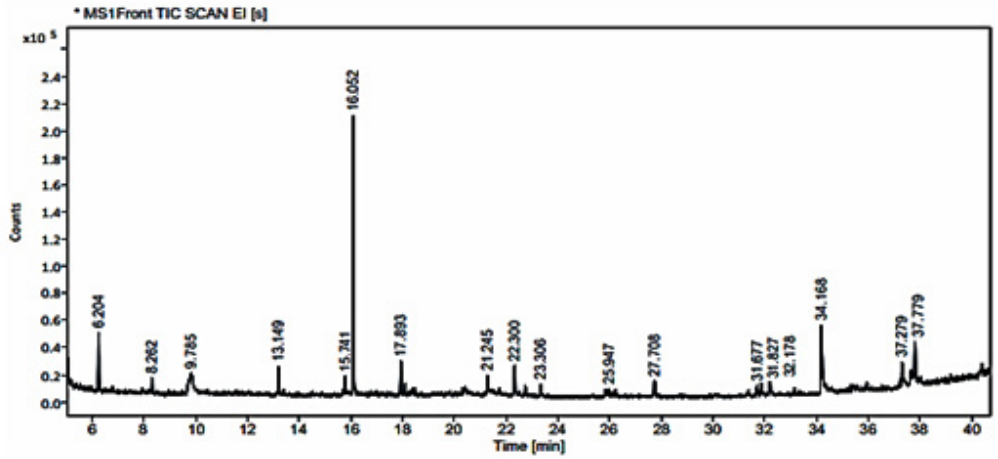


Fig. 11. GC-MS analysis peak at 16.052

examined under a microscope for morphological changes. By using 20 µl of MTT solution (5 mg/ml) was used to measure the cell viability. The plates were incubated for 4 hours at 37°C. The formazan crystals were dissolved in DMSO, and the soluble formazan product was measured spectrophotometrically at 575 nm using an ELISA reader are shown in (Figure 15).

Molecular Docking analysis

The compound 2, 4-Di-tert-butylphenol was selected and docked with cancer target proteins and the least binding energy was calculated. Amino acids of NF-kB protein involved in the interaction with 2,4-Di-tert-butylphenol. The ligand 2,4-Di-tertbutylphenol interacted with the following amino acids ASP 534, CYS 533, GLY 409, ARG 408, GLY 407, LEU 406, VAL 414, LYS 429, ALA 427, MET 469, LEU 471, LEU 472, GLY 475, SER 476, GLN 479, LEU 522, ASN 520 and ASP 519. Among the ligands tested 2, 4-Di-tert-butylphenol was very effective in interacting with human oral cancer cell lines (KB Cell)[23]. Actinomycetes create

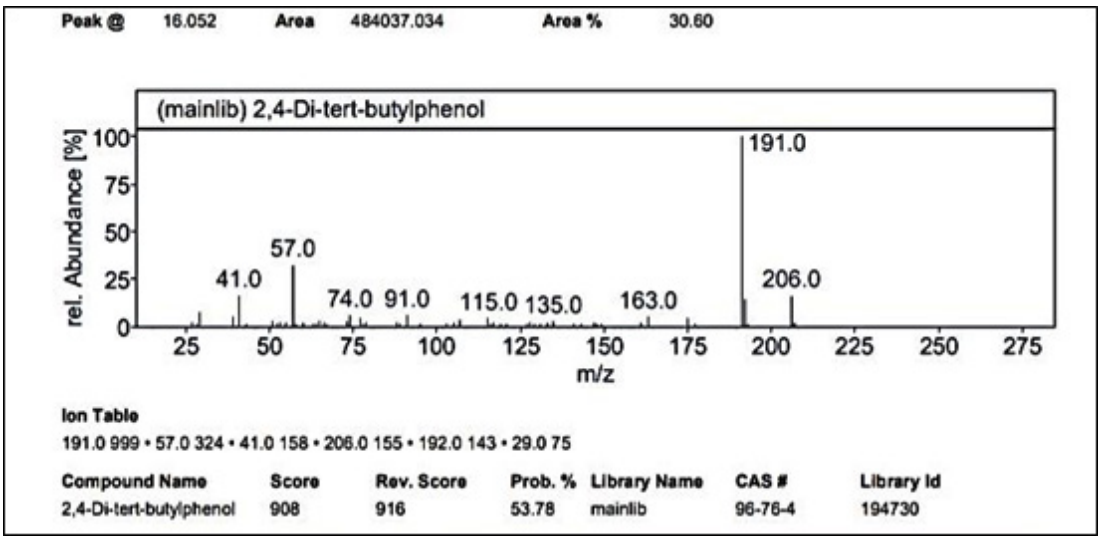


Fig. 12. GC-MS analysis (2, 4 Di-tert-butylphenol)

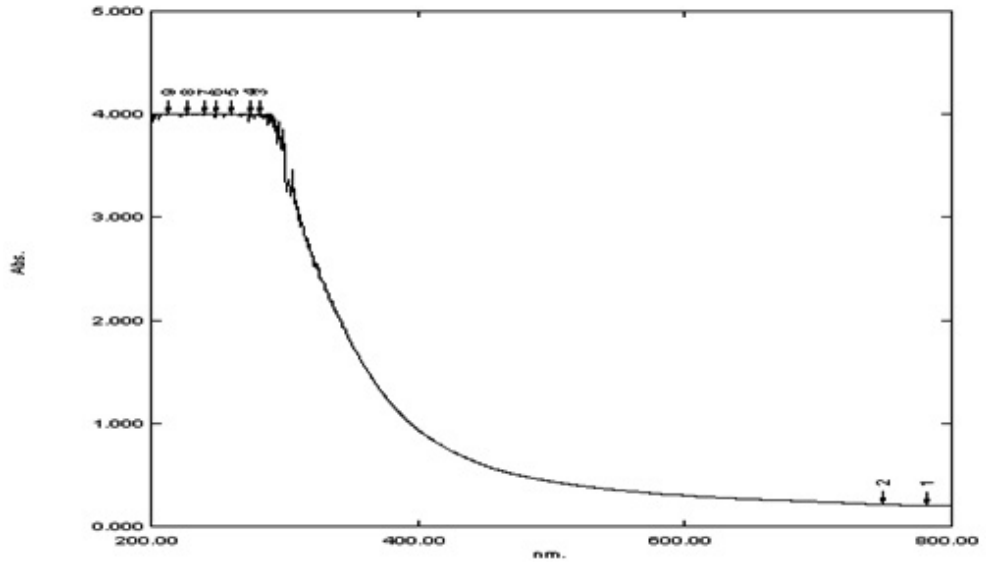


Fig. 13. UV-Visible analysis

a wide range of organic compounds with a variety of biological functions, including anti-tumor effects (Table 7 and 8 and Figure 16). The present *in silico* studies analysed the isolated compound 2,4- di-tert-butylphenol are docked with human oral cancer cell lines (KB cell) proteins[28].

UV Visible analysis

Studies on UV-visible absorption were carried out with a Shimadzu spectrophotometer (UV-1800) and matched quartz cuvettes with a 1 cm path length. Using the UV-Vis spectrum

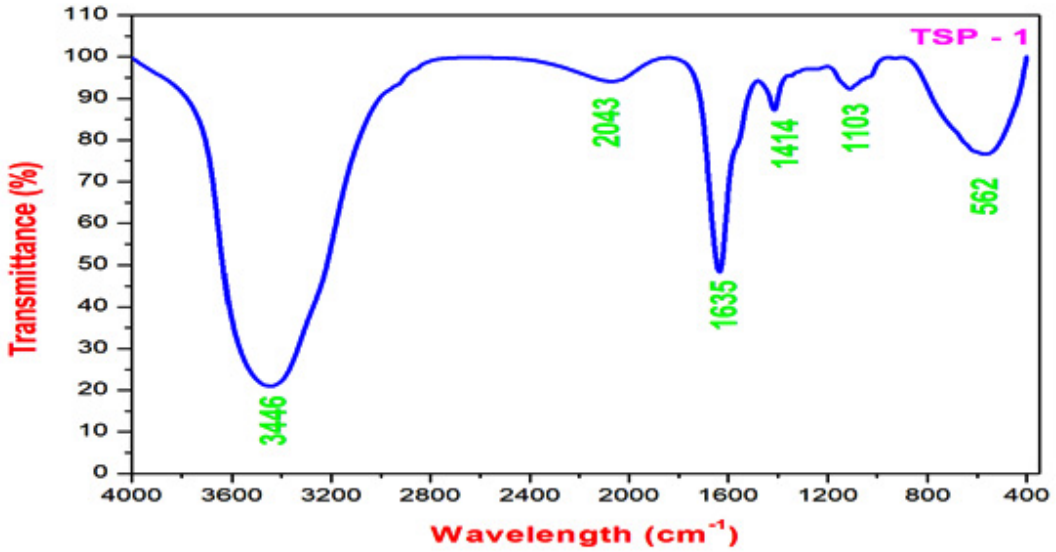


Fig. 14. FT-IR analysis

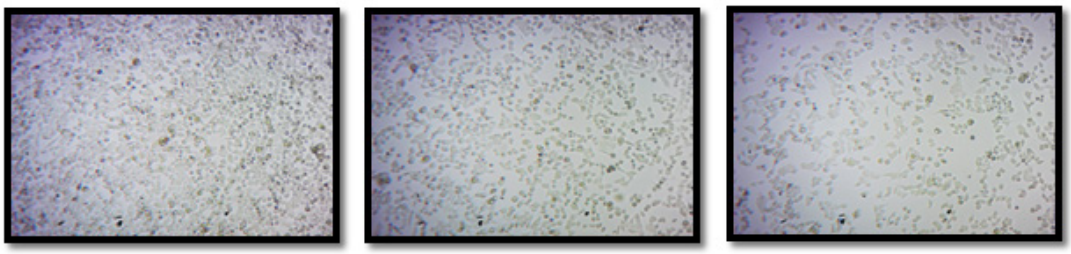


Fig. 15. Morphological changes of NF-kB cells after treatment by the 2,4-Di-tert-butylphenol

Table 7. Summary of molecular docking results of ligands with cancer drug target protein NF-kB

Ligand	Target Protein	Pdb ID	Binding Energy	Amino Acids
2,4-Di-tert-butylphenol	NF-Kb	Pdb4DN5	-6.8	ASP 534, CYS 533, GLY 409, ARG 408, GLY 407, LEU 406, VAL 414, LYS 429, ALA 427, MET 469, LEU 471, LEU 472, GLY 475, SER 476, GLN 479, LEU 522, ASN 520 and ASP 519

Table 8. Molecular Docking score value

Vina score	Cavity Size	Center			Size		
		X	Y	Z	X	Y	Z
-6.8	19984	-11	41	-13	35	35	35
-5.3	1317	-5	41	-12	18	18	18
-5.2	450	-6	32	9	18	24	18
-5	422	-3	51	-34	18	18	18
-4.8	226	-10	4	-8	18	18	18

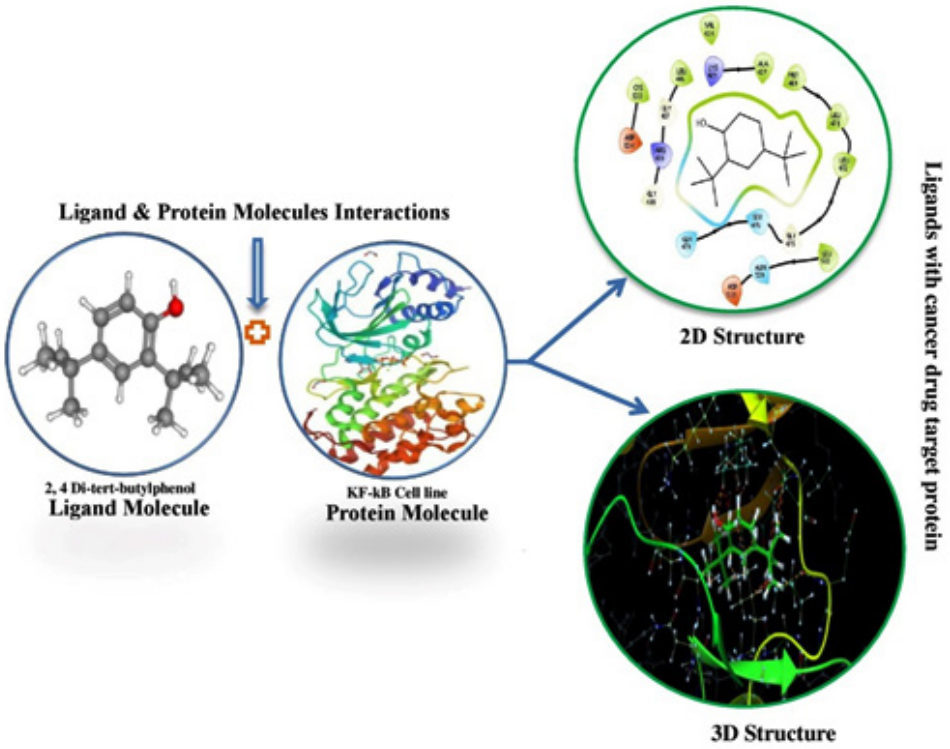


Fig. 16. Molecular Docking of 2,4-Di-tert-butylphenol interact with oral cancer cell line

between 200 and 800 nm, the TSP compound was observed. In (Figure 13) the pointed adsorption peak is displayed.

CONCLUSION

In recent years marine *Streptomyces* sp have been a rich source of novel and therapeutically active compounds. Actinomycetes have been extensively explored over the past 30 years, yet they have shown to be promising producers of new bioactive compounds. The secondary metabolite trends of an actinobacterial strain can change when heavy metals are added to the fermentation medium, as demonstrated by two chemical and pharmacological screening techniques. This research developed a rapid, accessible method for identifying secondary metabolites of the sort that are completely absent from regular culture when grown under good conditions. The current work is a small attempt to link in silico and in vitro data with potential screening of compounds from a library for drug development.

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Conflicts of Interest

All the authors to declare there are no conflicts of interest in this article to publish.

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