

Bioactive compounds from marine *Streptomyces* sp. VITPSA as therapeutics

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BACKGROUND: Marine actinomycetes are efficient producers of new secondary metabolites that show different biological activities, including antibacterial, antifungal, anticancer, insecticidal, and enzyme inhibition activities.

METHODS: The morphological, physiological, and biochemical properties of the strain *Streptomyces* sp. VITPSA were confirmed by conventional methods. Antibacterial, anti-oxidant, anti-inflammatory, anti-diabetic, and cytotoxic activities of *Streptomyces* sp. VITPSA extract were determined. The media were optimized for the production of secondary metabolites. Characterization and identification of secondary metabolites were conducted by high-performance liquid chromatography, gas chromatography-mass spectroscopy, and Fourier transform infrared spectroscopy analysis.

RESULTS: The strain showed significant antibacterial, anti-oxidant, and cytotoxic activities, moderate anti-inflammatory activity, and no satisfactory anti-diabetic activity. The ethyl acetate extract of *Streptomyces* sp. VITPSA showed maximum antibacterial activity against two gram-positive and gram-negative bacteria at 0.5 mg/mL. The antioxidant potential of the crude extract exhibited strong reducing power activity at 0.5 mg/mL with 95.1% inhibition. The cytotoxic effect was found to be an IC₅₀ of 50 µg/mL on MCF-7 cell lines. Experimental design of optimization by one-factor analysis revealed the most favorable sucrose, yeast extract, pH (7.25), and temperature (28°C) conditions for the effective production of secondary metabolites.

CONCLUSION: This study revealed that *Streptomyces* sp. VITPSA is an excellent source of secondary metabolites with various bioactivities.

Keywords Marine actinomycetes, bioactive compounds, pathogens, oxidants

Introduction

Microorganisms are diverse and are found in every possible environment on Earth, including terrestrial environments, hot springs, and marine environments. The sea covers more than 70% of Earth's surface and exhibits unique biological diversity accounting for more than 95% of the whole biosphere (Qasim, 1999). This environment has been largely unexplored and is an enormous source for the discovery of natural products; new therapeutic agents for treating human diseases have been derived from marine microorganisms. Among these, the most economical and priceless prokaryotes are actinomycetes, whose representative genera are *Streptomyces*, *Actinomyces*, *Arthrobacter*, *Corynebacterium*, *Frankia*, *Micrococcus*, *Micromonospora*, and several others;

secondary metabolites derived from these bacteria possess a broad range of biological activities (Solanki et al., 2008). Actinomycetes produce numerous secondary metabolites, antibiotics, and bioactive compounds and are gram-positive bacteria with high guanine + cytosine content in their DNA (Hotam et al., 2013). Recently, many bioactive compounds showing antifungal, antibacterial, antioxidant, anti-inflammatory, antitumor, anti-parasitic, insecticidal, anti-viral, anti-infective, herbicidal, and enzyme inhibition activities have been isolated from marine actinomycetes (Wang et al., 1999). *Streptomyces* grow in various environments, with a filamentous form similar to that of fungi. The morphological differentiation of *Streptomyces* involves the formation of a layer of hyphae that can differentiate into a chain of spores. Approximately 75%–80% of drugs available on the market are derived from *Streptomyces* (Deepika and Kannabiran, 2009). A total of 100000 new compounds with diverse activities may be identified by continuous screening of actinobacteria, particularly *Streptomyces* (Williams et al., 1983). The genus *Streptomyces* is thought to be a rich source of bioactive metabolites (Zothanpuia et al., 2017). This study

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mainly focuses on isolation and screening of marine *Streptomyces* and identification of bioactive compounds.

Materials and methods

Sample collection

Marine soil samples were collected from south-east coast of India, Ramanathapuram-Sethu Karai (Lat. 9°50' N and 78°10' E) at depths of 10–100 cm from the littoral zone. Samples were stored at 4°C.

Isolation, screening, and characterization of marine *Actinomycetes*

Marine actinomycetes were isolated by serial dilution and the pour plate technique. Primary screening of the isolated marine actinomycetes was conducted to select the most potent strain. Biochemical characterization was performed using a HiMedia assorted biochemical test kit (Weissmann, 2006).

Molecular characterization

The 16S rDNA of the selected strain was PCR-amplified using universal primers. The purified PCR products of approximately 1012 base pairs (bp) were sequenced by using 2 primers as described above. Sequencing was performed by using Big Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system. The sequences were subjected to homology searching using the BLAST program from the National Center for Biotechnology Information. The partial 16S rDNA sequences derived in this study were deposited in GenBank under the Submission ID KR233808.

Extraction of bioactive compounds

The selected strain was inoculated in soya-bean casein digest broth and was incubated at 30°C in a rotary shaker for 7 days. Equal amounts of ethyl acetate were added to the broth and incubated in a rotary shaker for 24 h. The following day, the upper layer of the broth was separated, collected in a beaker, and allowed to dry. After drying, 2 mL of ethyl acetate was added and the samples were stored at 4°C (Pandey et al., 2005).

Bacterial pathogens

Bacterial strains *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were used for anti-bacterial studies. The *in vitro* antibacterial activity of the

ethyl acetate extract was determined by the agar well diffusion method. Log phase bacterial cultures were used to evaluate the antibacterial activity of the crude extract. The test was performed using different concentrations (0.0625, 0.125, 0.25, and 0.5 mg/mL) of crude extract with ethyl acetate as a solvent. Chloramphenicol (25 µg/mL) was used as a positive control. The plates were allowed to diffuse the crude extract for 15 min and the plates were incubated at 37°C for 24 h (Suthindhiran and Kannabiran, 2009).

DPPH scavenging assay

Antioxidant activity was determined by DPPH scavenging assay (Kekuda et al., 2010). Various concentrations (0.062, 0.12, 0.25, and 0.5 mg/mL) of ethyl acetate extract were added to separate tubes. Ascorbic acid was used as a reference compound.

Anti-inflammatory assay

The anti-inflammatory activity of the ethyl acetate extract of *Streptomyces* sp. VITPSA was assessed by an *in vitro* HRBC membrane stabilizing assay according to Yoganandam et al. (2010). The test was performed for both crude extract alone and with different concentrations (0.0625, 0.125, 0.25, and 0.5 mg/mL) of crude extract.

Anti-diabetic assay

Anti-diabetic activity was evaluated by an α -amylase inhibition assay. A total of 250 µL of 0.5 mg/mL extract was used to carry out the assay. A control was prepared using the same procedure by replacing the extract with distilled water. The α -amylase inhibitory activity was calculated as percentage inhibition. The concentration of extracts resulting in 50% inhibition of enzyme activity (IC₅₀) was determined graphically.

Cell viability assay

MCF-7 cells were obtained from NCCS Pune and cultured in RPMI-1640 medium on 10-cm tissue culture dishes (Greiner Bio-one™, Kremsmünster, Austria) supplemented with 10% heat-inactivated fetal bovine serum. Cells were incubated in a humidified incubator with 5% CO₂ at 37°C and subcultured when confluence reached 80%. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) MTT assay, was performed to assess cell viability (Mosmann, 1983). After starvation, the cells were treated with different concentrations of test compound (5–100 µg/mL) for 24 h. Spectrophotometric absorbance of purple blue formazan dye was measured in a microplate reader at 570 nm (680, BioRad, Hercules, CA, USA). Cytotoxicity was determined using GraphPad Prism5 software (GraphPad, Inc., Chicago, IL, USA).

Optimization of media components

Soya-bean casein digest broth was prepared and distributed into 100 mL in 6 × 50 mL conical flasks. The pH of the medium was adjusted to 6, 6.5, 7, 7.5, 7.25, and 8 with 10% seed inoculum of *Streptomyces* sp. VITPSA. Upon optimization, pH of the medium was adjusted to 7.25 and the medium was sterilized and inoculated with 10% seed inoculums of *Streptomyces* sp. VITPSA. The flasks were incubated at 25°C, 28°C, 32°C, 37°C, and 47°C for 7 days. Growth medium was prepared by varying the nitrogen source, such as peptone, tryptone, urea, yeast extract, and soya-bean meal. The inoculated medium was incubated at 28°C for 7 days. Growth medium was prepared by keeping one nitrogen source constant and varying the carbon source, such as fructose, sucrose, glycerol, maltose, and molasses. The pH of the medium was adjusted to 7.25. The medium was sterilized and inoculated with 10% seed inoculum of *Streptomyces* sp. VITPSA. The inoculated medium was incubated at 28°C for 7 days in a shaker at 120 rpm. The OD value was checked at 517 nm and DPPH scavenging activity was estimated (Kim et al., 2014).

Purification of secondary metabolites by TLC

The purified metabolite was analyzed by thin-layer chromatography with silica gel G-60 F25 (Merck, Kenilworth, NJ, USA) (Lynch et al., 1968). The chromatography chamber with the solvent was incubated for 20 min until equilibration. The sample was spotted on the silica gel sheet using a capillary tube and then air-dried. The thin-layer chromatography (TLC) sheet was then dipped into the solvent system. After 45 min, the TLC sheet was carefully removed and the retention factor value was calculated from the chromatogram.

Characterization and identification of secondary metabolites

HPLC analysis

The ethyl acetate extract was analyzed by high-performance liquid chromatography (HPLC). The metabolite was eluted with 70% (v/v) acetonitrile as the mobile phase at a flow rate of 1.0 mL/h and detected at 250 nm with a C18 column (3.0 × 300 mm).

GC-MS analysis

The extracted secondary metabolites were analyzed by gas chromatography-mass spectrometry. The following acquisition parameters used: oven: Initial temp 60°C for 2 min, ramp 10°C/min to 300°C, hold 6 min, InjAauto = 250°C, Volume = 0 µL, Split = 10:1, Carrier Gas = He, Solvent Delay = 2.00 min, Transfer Temp = 230°C, Source Temp = 230°C, Scan: 50–600 Da, Column 30.0 m × 250 µm. The chromatogram

obtained was subjected to Library Searching with TURBO-MASS software.

The ethyl acetate extract was subjected to FT-IR spectroscopic analysis (Nicolet, Avatar 370, Thermo Fisher Scientific, Waltham, MA, USA), equipped with KBr beam splitter and DTGS Detector over the 4000–400 cm⁻¹ range at a resolution of 4 cm⁻¹ and maximum source aperture. The infrared (IR) spectra of the crude extract were measured (as KBr discs). Important IR bands (C-N, OH, C-H, C = C, N-H, C-O, C-H) and symmetric, asymmetric, and stretching frequencies were studied to determine the presence of functional groups in the ethyl acetate extract.

UV analysis

Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. The ethyl acetate extract of *Streptomyces* sp. VITPSA was subjected to UV analysis at a wavelength 200–500 nm to determine the types of electronic transitions present in the secondary metabolites.

Determination of bioavailability and 3D structure

The compounds obtained by GC-MS were evaluated to determine their bioavailability based on Lipinski's rule of five, which states that no more than 5 hydrogen bond donors (total number of nitrogen-hydrogen and oxygen-hydrogen bonds), not more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms), molecular mass less than 500 Da, and octanol-water partition coefficient log P of not greater than 5. In addition, the 3D-structure was determined using Molinspiration software 2015 (Molinspiration Cheminformatics).

Results

Isolation and screening of marine actinomycetes

Isolation was performed and 5 different isolates were taken from the 10⁻¹ dilution and subjected to primary screening to identify the most potent strain. Gram staining was performed and the slide was observed under a light microscope to observe the spores and hyphae (Fig. 1). Genomic DNA was isolated and subjected to PCR analysis using universal primers and the DNA was found to be composed of 739 bp. The aligned sequence of the amplified 739-bp 16S rDNA fragment from the isolate was submitted to GenBank. The sequence of the isolate was similar to that of *Streptomyces achromogenes*. A phylogenetic relationship was established through alignment and cladistic analysis of homologous nucleotide sequences from bacterial species. The sequence of the *Streptomyces* isolate was submitted to GenBank with the

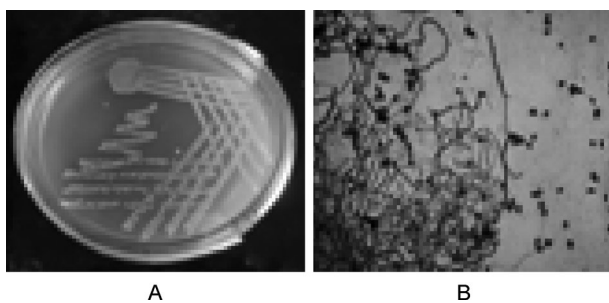


Figure 1 (A) Streak plate showing pure culture of *Streptomyces* sp. VITPSA on starch casein agar medium. (B) Microscopy 100 \times : showing morphology of *Streptomyces* sp. VITPSA

accession number KR233808 (Fig. 2). The ethyl acetate extract of *Streptomyces* sp. VITPSA in crude form was found to be active against pathogenic bacteria. The zones of inhibition against *P. aeruginosa* and *S. aureus* were 30 mm and 25 mm, which were higher than that of the standard chloramphenicol. The experiment with different concentrations of ethyl acetate extract did not show a zone of inhibition beyond 15 mm (Fig. 3). The result showed that the ethyl acetate extract of *Streptomyces* sp. VITPSA in crude form inhibited 95.11% of DPPH activity, which is good activity compared to the standard ascorbic acid (Fig. 4). In addition, radical scavenging activity evaluated using different concentrations of ethyl acetate extract showed good results. The percentage inhibition ranged from 93% to 86%. The ethyl

acetate crude extract of *Streptomyces* sp. VITPSA showed 70% hemolysis, indicating moderate anti-inflammatory activity (Fig. 5). Different concentrations of ethyl acetate extract showed moderate activity. The ethyl acetate extract of *Streptomyces* sp. VITPSA did not show significant anti-diabetic activity and was not sufficient to obtain an IC_{50} value (Fig. 6). In this present study, MCF-7 cell lines were incubated for 24 h with increasing doses of ethyl acetate extract, ranging from 5 to 100 $\mu\text{g/mL}$. MTT dye reduction assay to assess cytotoxic potential revealed that the proliferation of treated cells was decreased compared to the untreated controls. Ethyl acetate extract from *Streptomyces* sp. VITPSA induced apoptosis in MCF-7 cell lines in a dose-dependent manner with a mean IC_{50} of 50 $\mu\text{g/mL}$. Ethyl acetate extract was found to have a potent anticancer activity against MCF-7 cells. Cell viability analysis revealed 32% viable cells at 100 $\mu\text{g/mL}$ with 68% cytotoxicity (Fig. 7A, B). The maximum secondary metabolite levels were obtained at pH 7.25. The production of secondary metabolites slightly decreased at pH 6 and 6.5. Secondary metabolites activity was significantly low at pH 8. Maximum production of secondary metabolites was observed at 28 $^{\circ}\text{C}$ and production was slightly decreased at temperatures of 25 $^{\circ}\text{C}$, 32 $^{\circ}\text{C}$, and 37 $^{\circ}\text{C}$. Much lower production was observed at temperatures of 47 $^{\circ}\text{C}$. Maximum production of secondary metabolites was obtained using nitrogen soya bean meal; production was slightly decreased when yeast extract and tryptone were used. Much lower biomass and growth were obtained when the nitrogen sources were urea and peptone. The maximum

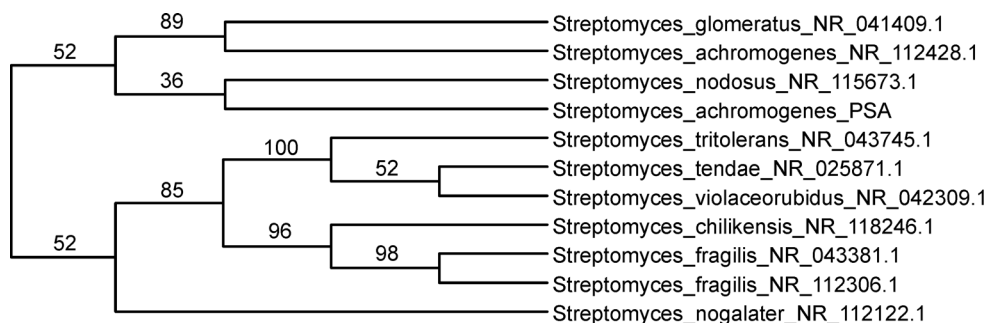


Figure 2 Phylogenetic tree showing relationship of *Streptomyces* sp. VITPSA to other species

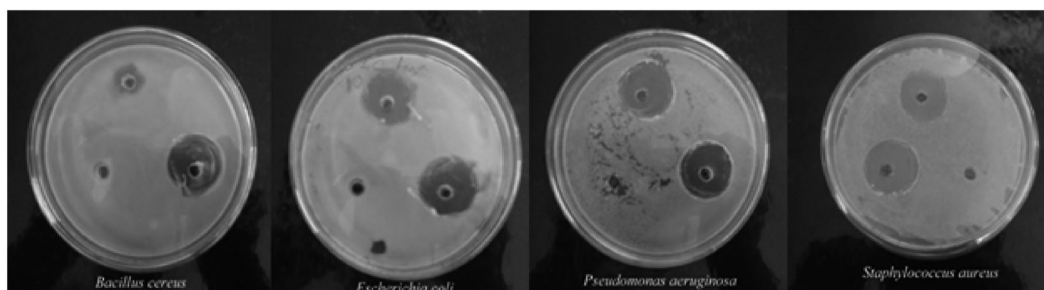


Figure 3 Antibacterial activity of crude extract compared with positive control

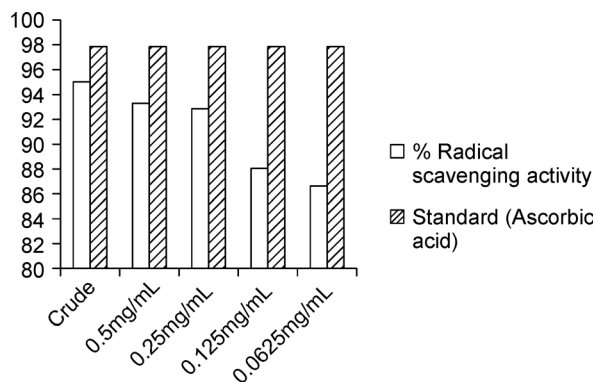


Figure 4 Radical scavenging activity of crude extract

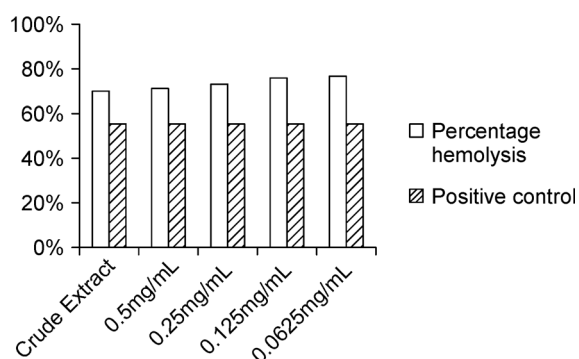


Figure 5 Anti-inflammatory activity of *Streptomyces* sp. VITPSA crude extract

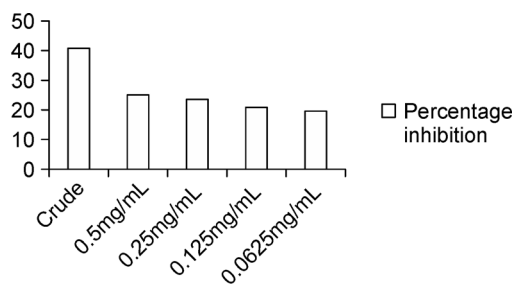


Figure 6 Anti-diabetic activity of ethyl acetate extract of *Streptomyces* sp. VITPSA

production of secondary metabolites was obtained when the carbon sources were sucrose and fructose along with yeast extract as the nitrogen source; production was slightly decreased when glycerol and sucrose were used as the carbon source along with tryptone. Comparatively lower secondary metabolites production was observed when carbon sources were maltose and the nitrogen source was soya bean meal.

A single band with an R_f value of 0.8 was obtained after TLC with butanol: acetic acid: distilled water (3:1:1; v/v) (Fig. 8). The HPLC for secondary metabolites from *Streptomyces* sp. VITPSA was conducted. The retention time of the peak was 2.338 min. HPLC was also conducted

for ethyl acetate extract from *Streptomyces* sp. VITPSA (Fig. 9). The chromatogram showed three peaks. All peaks correspond to a compound has already been reported. For example, 17.45 corresponds to 3-methyldec-3-ene, 18.20 corresponds to pyrrolo[1,2-a]pyrazine-1,4-DIONE, HEXAHYDRO-3-(2-METHYLPROPYL) and 22.63 corresponds to PYRROLO[1,2-A]PYRAZINE-1,4-DIONE, HEXAHYDRO-3-(PHENYLMETHYL) (Fig. 10). Table 1 shows the 2D and 3D structures of the compounds obtained by GC-MS. It also depicts the molecular formula of all derived compounds. Table 2 shows that pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl), and pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl) follow Lipinski's rule, indicating higher drug likeliness. In contrast, 3-METHYLDEC-3-ENE violated one of Lipinski's rules and therefore would likely not function well as a drug. pyrrolo [1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl), and pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl) showed excellent protease inhibitor activity and good GPCR ligand and ion channel modulator activity. pyrrolo [1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) showed much lower enzyme inhibitor activity, while pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl) shows good enzyme inhibitor activity. FT-IR absorption in KBr for the ethyl acetate extract (Fig. 11) was dominated by sharp peaks at 1737.86, 1242.16, and 1045.42 cm^{-1} . The bond C–Br stretch corresponding to the functional group alkyl halide dominated the IR spectra. The bonds C–H stretch, C=O stretch, C–N stretch, and C–Cl stretch, which correspond to the functional groups alkanes, aldehydes, aliphatic amines, and alkyl halides, respectively were present in equal amounts in the IR spectra. The UV analysis for ethyl acetate extract of *Streptomyces* sp. VITPSA performed at 200–500 nm gave a peak at 275 nm (Fig. 12). This peak strongly suggests that the secondary metabolite extracts consist of pi-pi transitions corresponding to the presence of double bonds and aromatic rings. This also demonstrates that ethyl acetate is the best solvent for extracting the secondary metabolites of *Streptomyces* sp. VITPSA.

Discussion

Actinomycetes have a profound role in the marine environment apart from their antibiotic production (Das et al., 2006). The disc diffusion assay showed that the zones of inhibition formed by *Streptomyces* sp. VITPSA against *P. aeruginosa* and *S. aureus* were 30 mm and 25 mm, respectively, which are larger than those reported by Thirumalairaj et al. (2015). This indicates that *Streptomyces* sp. VITPSA has broad spectrum antibacterial activity. In 2010, Kannabiran et al. reported that the extracellular metabolite level of *Streptomyces* sp. VITTK3 DPPH scavenging activity was 96% at 5 mg/mL. *Streptomyces* sp. VITPSA crude extracellular extract

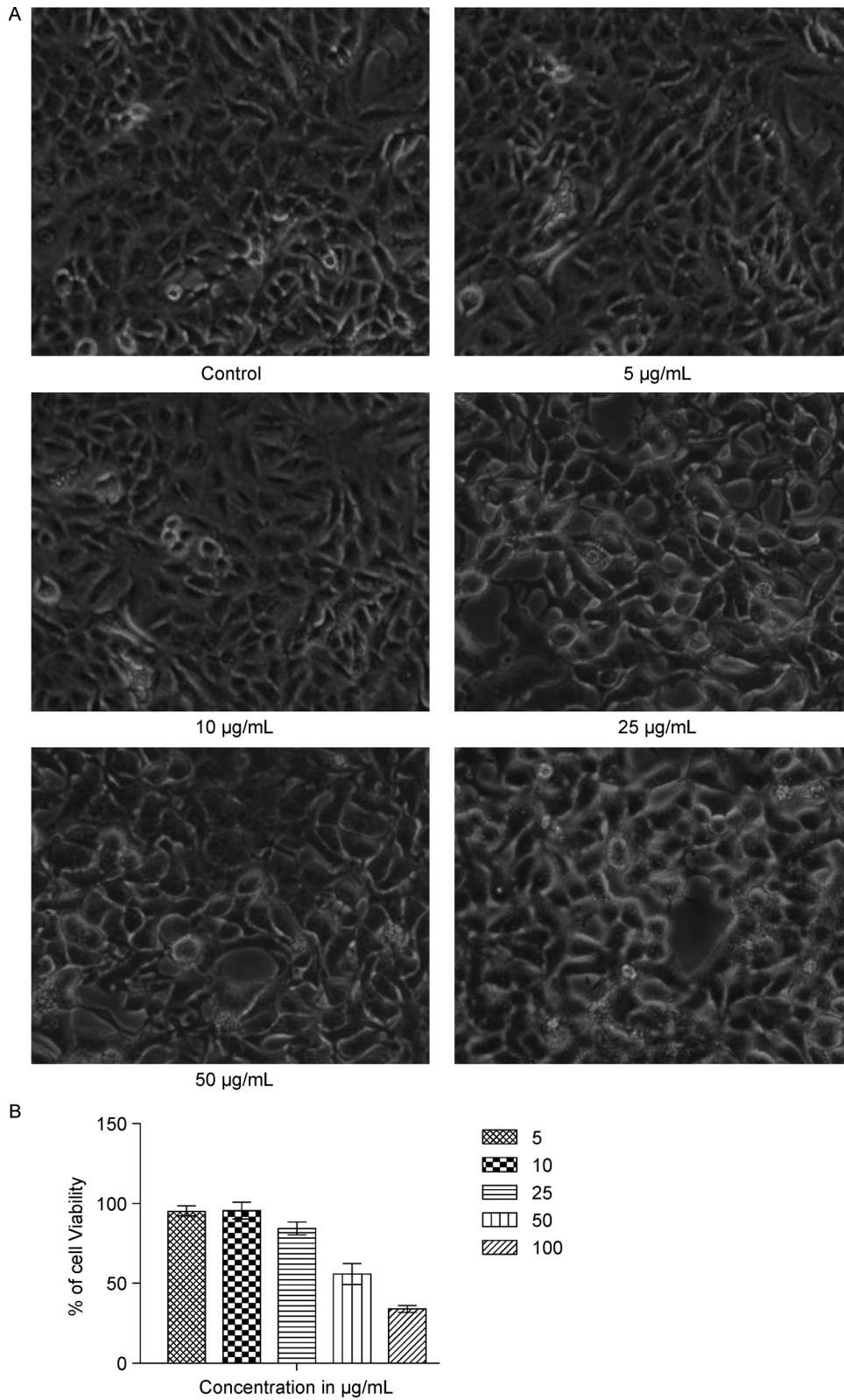


Figure 7 (A) Graphical representation of cytotoxic test activity; (B) Cytotoxic test results at different concentrations on MCF7 cell lines

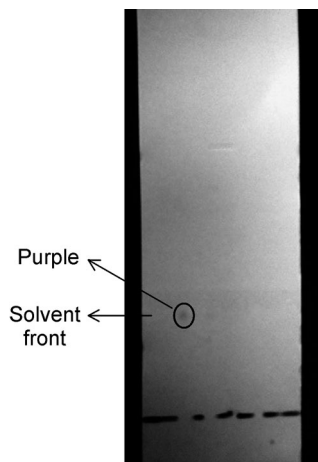


Figure 8 TLC plate showing spots

also showed 95.11% DPPH scavenging activity. This shows that *Streptomyces* sp. VITPSA secondary metabolites have an excellent antioxidant activity. The extracellular extract of *Streptomyces* sp. VITPSA showed 70% hemolysis. The anti-inflammatory activity reported by Yoganandam et al. (2010) from plant ethyl acetate extract showed 97.13% protection of RBC in 300 $\mu\text{g}/\text{mL}$. Therefore, *Streptomyces* sp. VITPSA has moderate anti-inflammatory activity.

The media containing soya bean casein digest broth components were optimized to maximize the production of secondary metabolites for antioxidant activity. At an optimized pH of 7.25 and temperature of 28°C, soya bean meal among all nitrogen sources, it showed the highest DPPH scavenging activity of 94.42%. The carbon source was optimized with different combinations of nitrogen sources, including soya bean meal, tryptone, and yeast extract. Media

containing soya bean meal and different carbon sources showed lower DPPH scavenging activity as compared to the other two nitrogen sources. Yeast extract and tryptone media at pH 7.25 and 28°C showed lower DPPH scavenging activity than the control media, but the highest antioxidant activity when used in combination with different carbon sources. Yeast extract with sucrose and fructose showed the highest DPPH scavenging activities of 92.45% and 92.14%, which are higher than the control value. Tryptone also showed good antioxidant activity in combination with glycerol and sucrose of 91.92% and 90.18%, which were also much higher than the control. This shows that soya bean meal along with dextrose as a standardized carbon source produced high levels of secondary metabolites with antioxidant activity; however, in combination with other carbon sources, DPPH scavenging activity was low. TLC was performed to isolate the purified compound. One spot was observed on the TLC plate and ethyl acetate extract when subjected to HPLC, which also showed one peak at a retention time of 2.338 min. The compound pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) showed a peak at 18.20 min and has been reported to exhibit antibacterial activity and quorum-sensing inhibition (Dash et al., 2009). pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl) showed a peak at 22.63 min and reported to have antibacterial activity by Melo et al. (2014). 3-methyldec-3-ene showed a peak at 17.45 min, but its bioactivity has not yet been studied previously. Each compound obtained by GC-MS should be isolated to study all other activities detected such as antioxidant, anti-inflammatory, and cytotoxic activities.

pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl), pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl) followed Lipinski's rule and thus may be

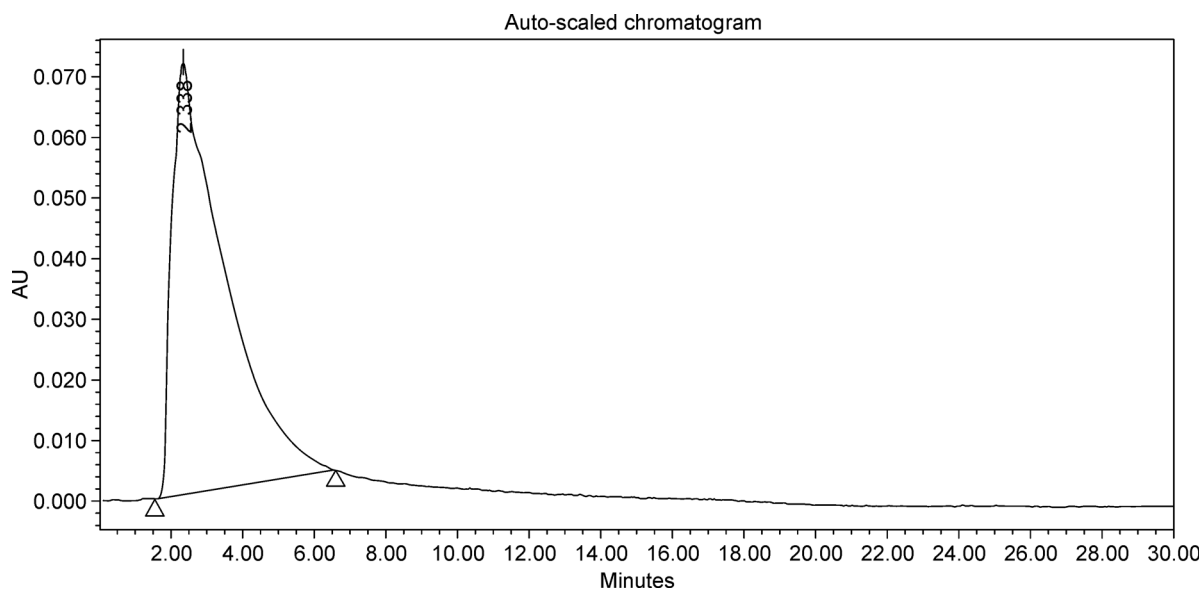


Figure 9 HPLC chromatogram for ethyl acetate extract of *Streptomyces* sp. VITPSA

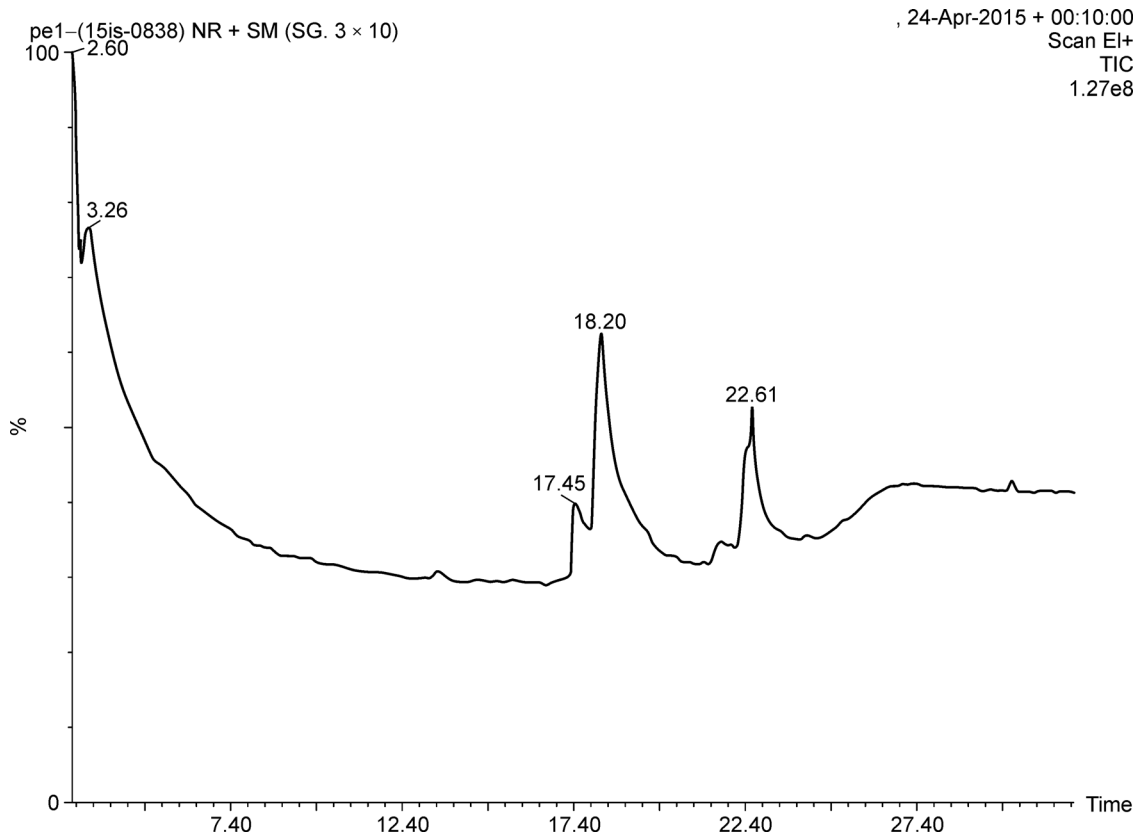


Figure 10 GC-MS chromatogram for ethyl acetate extract of *Streptomyces* sp. VITPSA

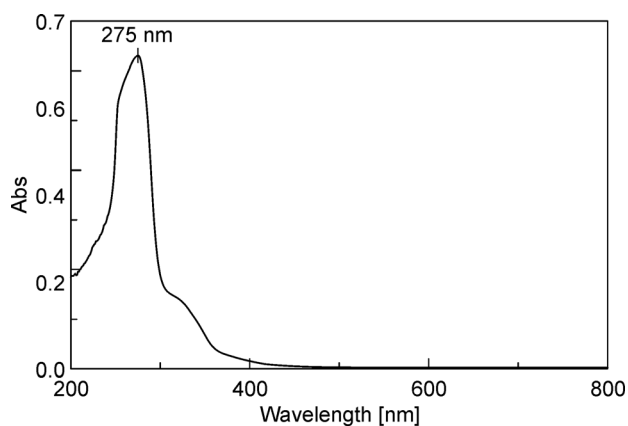


Figure 11 IR spectra for ethyl acetate extract of *Streptomyces* sp. VITPSA extract

orally active drugs in humans in the future. Moreover, these compounds show excellent protease inhibitor activity and can prevent viral replication. Thus, these compounds can be studied for their anti-viral activity. Saquinavir, an anti-viral drug used for HIV/AIDS, has protease inhibitor activity. The two compounds pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl), and pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl) showed protease inhibitor activities. Hence, these compounds show very good

antiretroviral activity and should be further examined for the potential clinical application.

Conclusion

The *Streptomyces* sp. VITPSA secondary metabolites isolated from the south-east coast of India, Ramanathapuram-Sethu Karai (Lat.9°50'N and 78°10'E) at depths of 10–100 cm at the littoral zone showed significant antibacterial, antioxidant, anti-inflammatory, and cytotoxic activities. *Streptomyces* sp. VITPSA showed very good antioxidant activity; therefore, the production of antioxidant metabolites from this particular strain was chosen to optimize the medium components and growth conditions such as pH, temperature, nitrogen source, and carbon source. In addition, the compounds obtained by GC-MS previously showed antibacterial activity; additional bioactive assays of isolated compounds are needed in the future. We found that *Streptomyces* sp. VITPSA produced high levels of pigment in soya bean casein digest broth. Therefore, future studies should examine the pigment by extracting it and performing various bioactive assays. This would provide insight into the bioactive compounds present in the pigment. In addition, characterization of the pigment should be carried out to identify the compounds present.

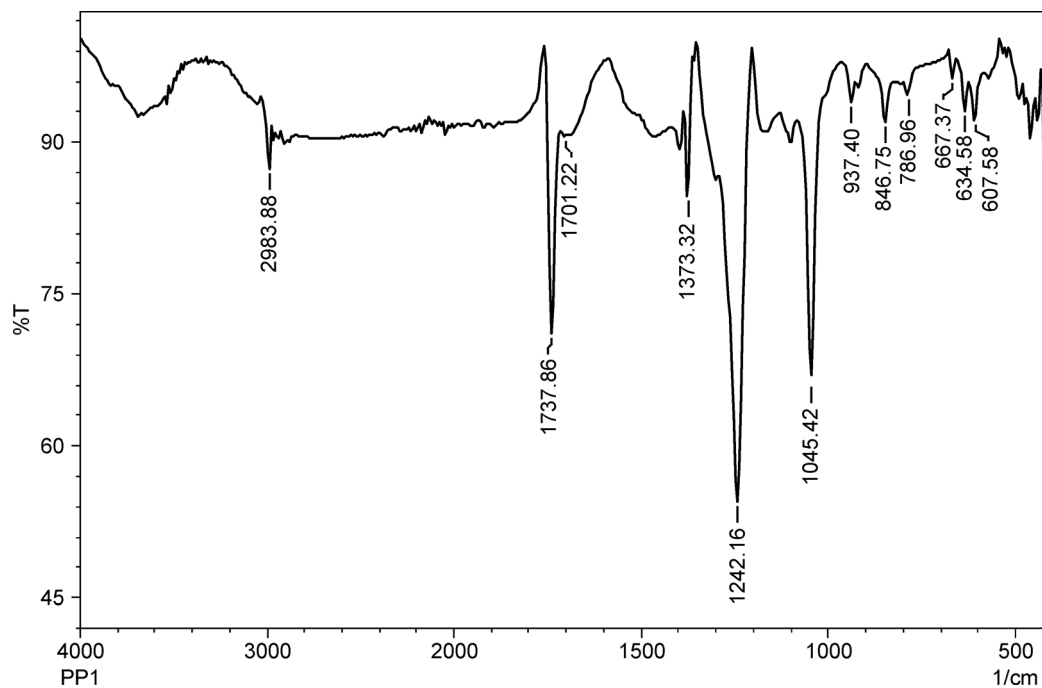


Figure 12 UV analysis spectrum of *Streptomyces* sp. VITPSA extract

Table 1 2D and 3D structure of the compounds

Compound	Peak	Molecular formula	2D structure	3D structure
3-METHYLDEC-3-ENE	17.45	C ₁₁ H ₂₂		
PYRROLO[1,2-A]PYRAZINE-1,4-DIONE, HEXAHYDRO-3-(2-METHYLPROPYL)	18.20	C ₁₁ H ₁₈ O ₂ N ₂		
PYRROLO[1,2-A]PYRAZINE-1,4-DIONE, HEXAHYDRO-3-(PHENYLMETHYL)	22.63	C ₁₄ H ₁₆ O ₂ N ₂		

Table 2 Drug likeliness based on Lipinski rule of five

Compound	logP	MW	No. of H bond acceptor	No. of H bond donor	Drug likeliness
3-METHYLDEC-3-ENE	5.45	154.30	0	0	++
PYRROLO[1,2-A]PYRAZINE-1,4-DIONE, HEXAHYDRO-3-(2-METHYLPROPYL)	0.66	210.28	4	1	+++
PYRROLO[1,2-A]PYRAZINE-1,4-DIONE, HEXAHYDRO-3-(PHENYLMETHYL)	0.81	244.29	4	1	+++

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Compliance with ethics guidelines

S. Pooja, T. Aditi, Jemimah Naine and C. Subathra Devi declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

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