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ORIGINAL RESEARCH ARTICLE

Bioactivity of Marine *Streptomyces* sp. VITJS4: Interactions of Cytotoxic Phthalate Derivatives with Human Topoisomerase II α: An In Silico Molecular Docking Analysis

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Abstract Despite clinical advances in antimicrobial and anticancer therapy, there is an urge for the search of new bioactive compounds. In the present study, previously isolated *Streptomyces* sp. VITJS4 strain (NCIM No. 5574) (ACC No: JQ234978.1) crude extract tested for antibacterial activity showed a broad spectrum at the concentration of 20 mg/mL against pathogens. The antioxidant potential tested at 0.5 mg/mL concentration exhibited reducing power activity with a maximum of 90 % inhibition. The anticancer property by MTT assay on HeLa and HepG2 cells showed cytotoxic effect with IC_{50} of 50 µg/mL each. The DNA fragmentation pattern observed in both HeLa and HepG2 cell indicated laddering pattern at 40 µg/mL concentration. GC–MS analysis revealed that the significant peak corresponding at *m/z* 149 (M^+) was identified as phthalate derivatives. The extract was further separated by HPLC with their retention times (t_r) at 6.294 min. The above-obtained results were also supported by molecular docking studies which provide an insight into ligand binding to the active site of the receptor. The in silico docking studies revealed better binding affinity with a binding energy of −5.87 kJ mol^{-1} of the ligand toward topoisomerase II α.

Keywords Marine actinomycetes · Bioactive compounds · Cytotoxicity · *Streptomyces* sp. VITJS4

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1 Introduction

Natural ecosystems are rich sources of microbes that produce a broad range of compounds that exhibit diverse and versatile biological effects. Marine is a unique environment recognized widely for their most valuable resources with an immense diversity of bioactive metabolites from actinomycetes and remains to be attractive as it holds promising source for new entities. The South East Coast of India is indeed an ideal location for hunting new valuable species producing novel compounds. Although exclusive numbers of bioactive compounds are derived, still there is an urge for the new metabolites to treat emerging pathogens. Recently, marine actinomycetes have become a potential source for novel chemical structures with various pharmacological activities that could be utilized as drug leads. Gram-positive and G + C-rich actinomycetes possess impressive features and well known for its ability to produce biologically active molecules [1].

Cancer remains as one of the most serious human health problems [2] with chemotherapy as the standard treatment of choice [3]. Many adverse side effects of chemotherapeutics are mainly due to insufficient selectivity for tumor cells and represent significant limitation to the therapy [4]. Hence, developing new anticancer drugs from marine origin with high potency and specificity against cancer cells becomes a prime importance in today's biomedical research. *Streptomyces* strains isolated from the salt pans of Cuddalore, Tamil Nadu, India, were reported to possess excellent antimicrobial activity [5]. Most of the studies were focused and reported on the antimicrobial properties of the species that prevails in the marine environment. Few researchers have highlighted the significance of Indian marine environment especially South East coast of India for the novel class of compounds from *Streptomyces species*

Table 1 Antibacterial activity of *Streptomyces* sp. VITJS4 crude extract

Organisms	Reference drug Chloramphenicol 25 µg/mL	Zone of inhibition (mm) The crude extract of *Streptomyces* sp. VITJS4 20 mg/mL	MIC µg/mL Crude extract of *Streptomyces* VITJS4
Bacillus cereus	25 ± 0.5	22 ± 1	160
Staphylococcus aureus	21 ± 0.5	25 ± 0.6	320
Pseudomonas aeruginosa	20 ± 0.5	22 ± 06	640
Escherichia coli	27 ± 0.7	23 ± 0.2	640
Salmonella typhi	26 ± 1	21 ± 0.6	640
Clostridium perfringens	30 ± 0.5	21 ± 1.2	>1
Vibrio cholerae	27 ± 0.5	23 ± 1.5	320
Listeria monocytogenes	25 ± 0.5	24 ± 0.6	>1

Data presented are the mean ± SD of triplicate measurements from a representative experiment

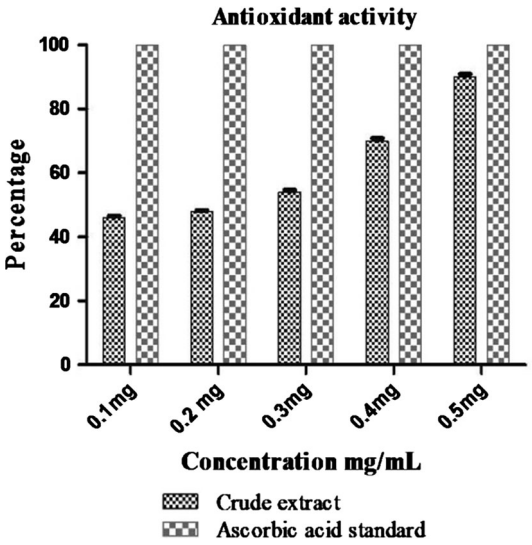

Fig. 1 Antioxidant activity of *Streptomyces* sp. VITJS4 crude extract with radical scavenging potential and its activity showing highly significance with the reference ascorbic acid

[6–8]. *Streptomyces* sp. VITJS4 strain crude extract isolated from the marine environment in South East coast of India, Puducherry, Thavalakuppam, showed better efficacy toward larvicidal and repellent activity against malarial and filarial vectors [8] (Fig. S1). In continuation, the present study is first of its kind in analyzing the antibacterial, antioxidant and cytotoxic activity using VITJS4 crude extract.

2 Materials and Methods

2.1 Extract Preparation

The inoculum of *Streptomyces* sp. VITJS4 was prepared on starch casein broth at a seed concentration of 100 mL in 250-mL Erlenmeyer flask and incubated for seven days at room temperature. Various solvents, namely hexane, chloroform, benzene, dichloromethane, petroleum ether and ethyl acetate, were used for the extraction process [9]. All the crude extract powder was weighed and tested for antibacterial activity upon clinical pathogens.

2.2 Inoculum Preparation of Pathogens

Test MTCC strains, namely *Bacillus cereus* (MTCC No: 6840), *Staphylococcus aureus* (MTCC No: 7405), *Pseudomonas aeruginosa* (MTCC No: 4676), *Salmonella typhi* (MTCC No: 1167), *Escherichia coli* (MTCC No: 1588), *Vibrio cholerae* (MTCC No: 3906), *Clostridium perfringens* (MTCC No: 450), and *Listeria monocytogenes* (MTCC No: 657), were obtained from Microbial Culture Collection, IMTECH, Chandigarh, India, for further analysis. All the pathogenic microbial suspensions were maintained in nutrient broth and allowed to grow up to log phase to a final density of 10^8 CFU/mL at 37 °C for 18 h [10].

2.3 In Vitro Antibacterial Activity

In vitro antibacterial activity of all the VITJS4 solvent extracts was analyzed using agar well diffusion method [11]. A log-phase bacterial culture of 10^8 CFU/mL of 100 µL at various concentrations ranging 1 mg–20 mg/mL of crude extract was used, and zone of inhibition is measured regarding mm.

2.4 Minimum Inhibitory Concentration

The minimum inhibitory concentration was determined by microdilution method [12]. A stock solution of the extract

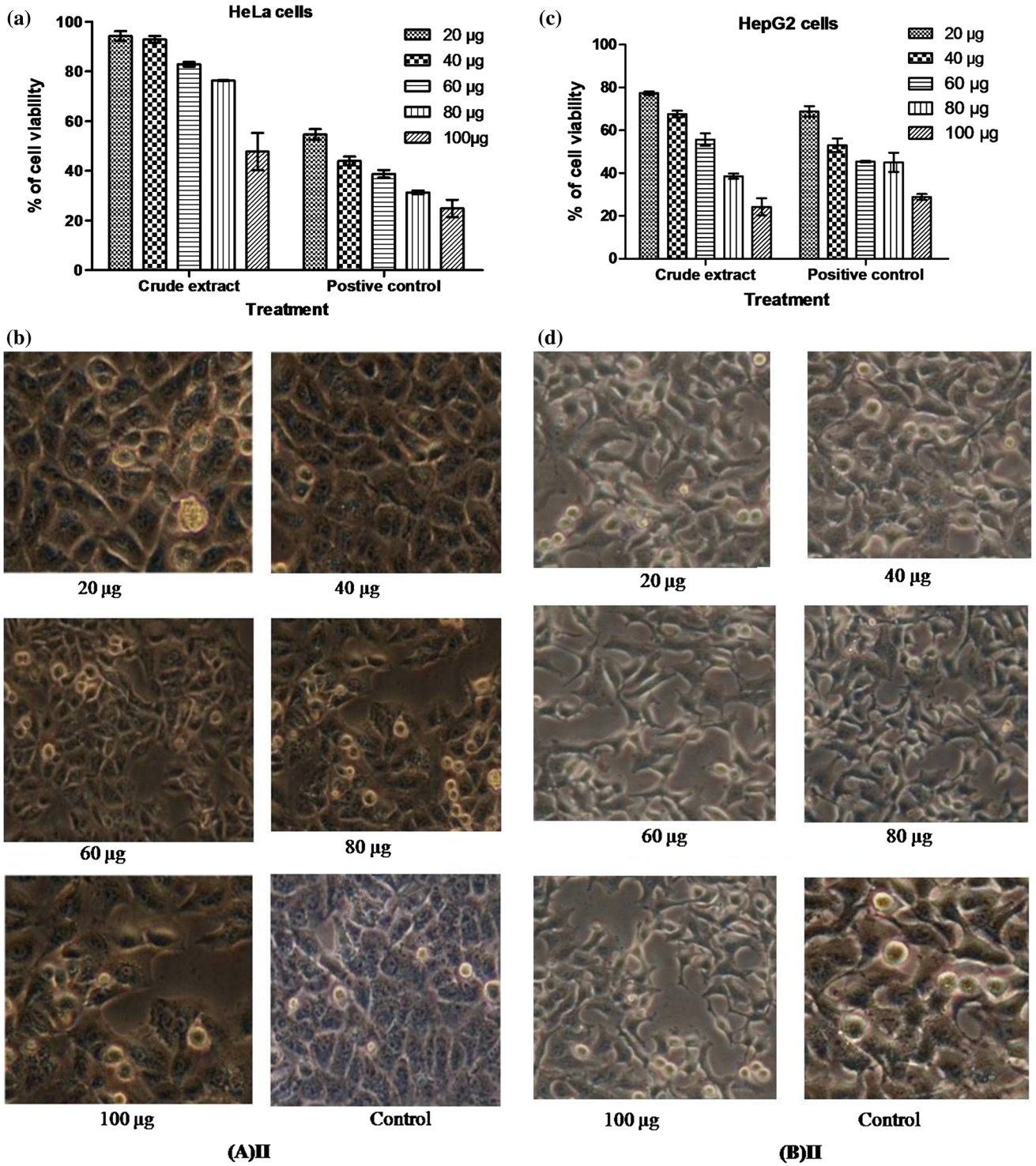

Fig. 2 **a** Percentage of cell viability, **b** *Streptomyces* sp. VITJS4 crude extract showing morphological changes in HeLa cell lines. **c** Percentage of cell viability. **d** *Streptomyces* sp. VITJS4 crude extract showing morphological changes in HepG2 cell lines

was prepared by dissolving in 20 % DMSO in different concentrations and stored at 4 °C. The extract was tested at various concentrations (10–1000 µg/mL). Chloramphenicol and DMSO were used as a standard drug and negative control, respectively. The optical density was recorded using a Bio-Rad model 680 microplate reader at 490 nm.

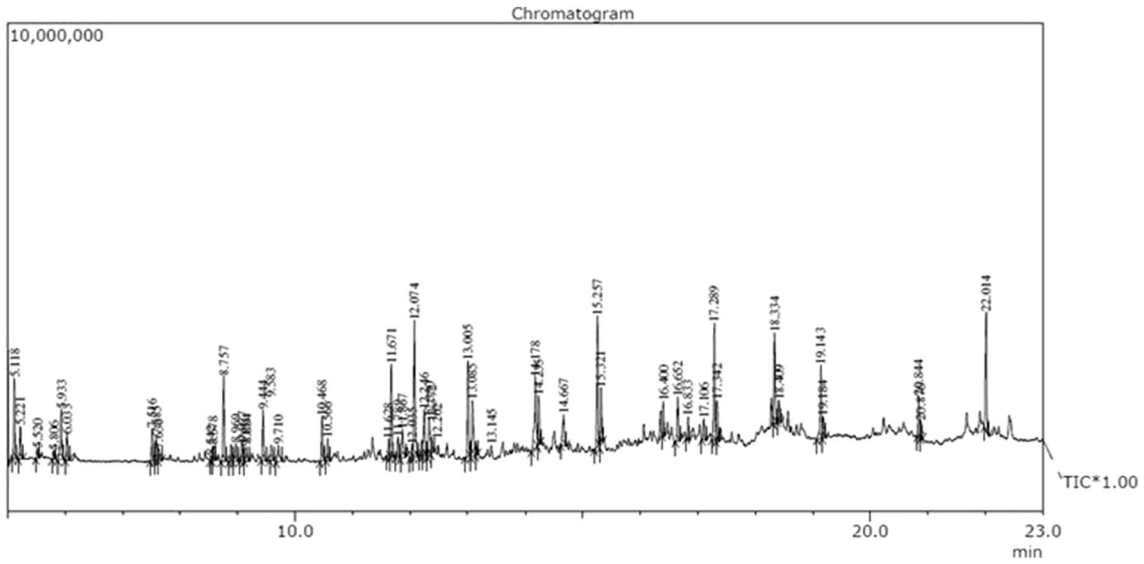

Fig. 3 **a** HepG2 cell lines and **b** HeLa cell lines showing fluorescence microscopic images of control and treated cells with crude extract. The control cells were found intact nucleus, whereas treated cells showed intense fragments of nucleus as signs of apoptosis by DAPI staining

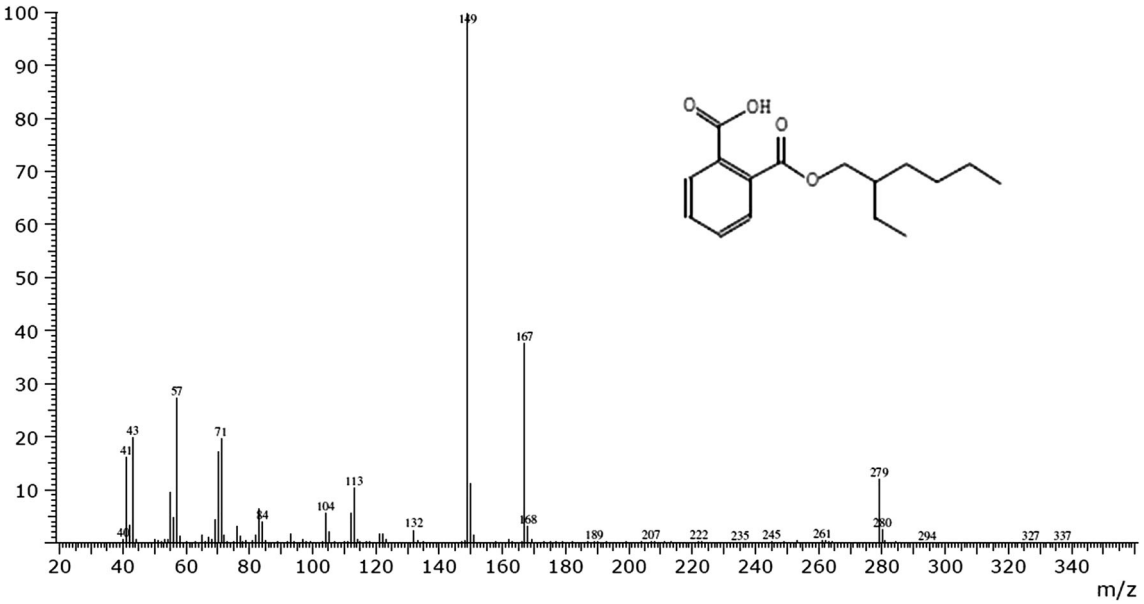

Fig. 4 DNA fragmentation **a** HepG2 cell lines and **b** HeLa cell lines. DNA laddering was visualized after treatment with the crude extracts for 24 h. L-1—1 Kb DNA ladder; L-2—control (untreated); L-3—40 µg/mL; L-4—60 µg/mL; L-5—80 µg/mL; L-6—100 µg/mL

2.5 Free Radical Scavenging Activity

The VITJS4 extract was tested for antioxidant activity by varying the concentrations ranging from 0.1 to 0.5 mg/mL and analyzed by DPPH scavenging assay [13].

2.6 Toxicity Studies on Cancer Cell Lines

The toxicity assay was performed using HeLa and HepG-2 cell lines obtained from NCSS Pune and cultured on Dulbecco's minimum Eagle's medium (DMEM) (HiMedia). About 25 µL cell suspensions, namely 5×10^3 cells/well, were seeded in each 96 wells and incubated for 48 h at 37 °C. Then, the cell lines were treated with various concentrations (20 µg–100 µg) of VITJS4 ethyl acetate extract. The MTT assay was performed to analyze the cell viability [14]. The percentage of recovery (% live cells) was plotted as Y-axis against the concentration of VITJS4 ethyl acetate extract as X-axis to interpolate the IC50 values.

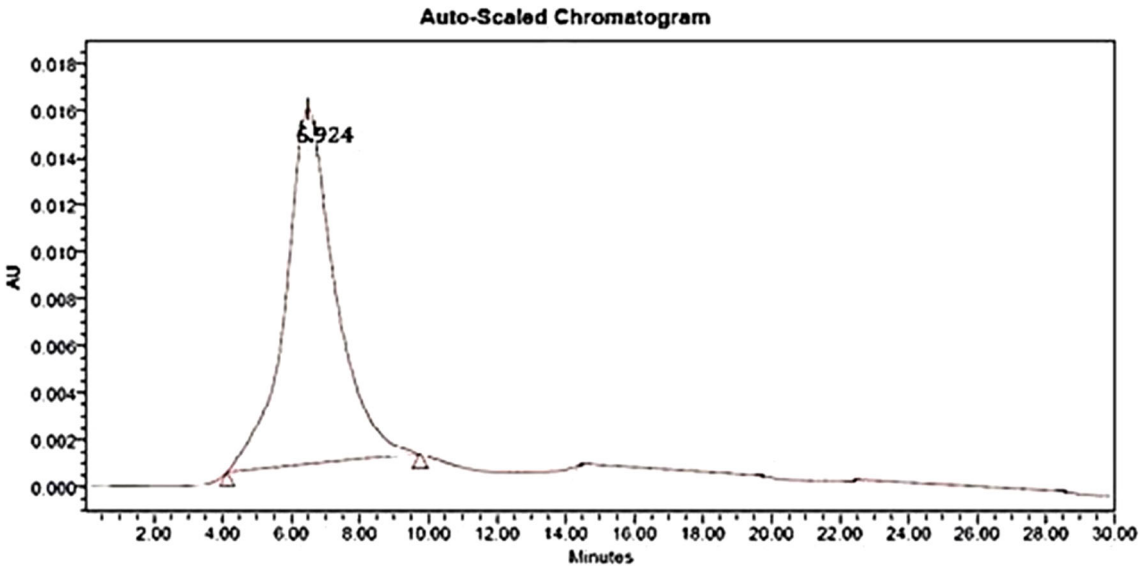

Fig. 5 Optimization of process parameters **a** effect of carbon sources, **b** effect of nitrogen sources, **c** effect of pH, **d** effect of temperature and **e** effect of RPM, on bioactive metabolite production by *Streptomyces* sp. VITJS4

Cyclophosphamide and DMSO were used as a standard and negative control.

2.7 Effect of Extracts on Cell Morphology and Apoptotic DNA Fragmentation

The HeLa and HepG-2 cells were incubated with extract at 60, 80 and 100 µg/mL concentrations for 16 h. The DAPI staining was done to observe the morphological changes of the negative control and treated cells under Zeiss fluorescence microscope. The cleavage pattern was analyzed using agarose gel electrophoresis [15].

2.8 Media Optimization

To evaluate the bioavailability, various carbon sources, namely starch, glucose, sucrose, maltose, lactose and mannitol, were supplemented at a concentration of 0.1 % into the starch casein medium. To evaluate metabolite production, the various nitrogen sources like beef extract, yeast extract, $(NH_4)_2HPO_4$, $(NH_4)_2SO_4$, NH_4NO_3 and corn steep liquor were supplemented at a concentration of 0.1 % nitrogen molar content. Dry cell weight estimation and zone of inhibition were determined by antibiogram. Process parameters like pH, temperature, and RPM were optimized to ensure maximum biomass and bioactive metabolite by varying temperature ranging from 25 to 40 °C. The effect of pH was measured ranging from 6.8 to 8. The range of different RPM was adopted from 50, 100, 150,200, 250 and 300 rpm for the enhanced cell mass yield. The maximum bioactive production was expressed regarding zone of inhibition.

2.9 Chromatography Analysis

The ethyl acetate extract of *Streptomyces* VITJS4 was subjected to gas chromatography–mass spectrum analysis (GC–MS) analysis using GC SHIMADZU QP2010 system [16]. The separated peaks were identified by NIST08 and WILEY8 database. The extract was also subjected to high-performance liquid chromatography (HPLC) analysis using LC-10 AT vp model, at 1 mL/min flow rate, and the peak was identified using the retention time of the compound.

2.10 In Silico Molecular Docking Studies

Three-dimensional structure (3D) of the receptor topoisomerase IV with PDB ID-1ZXM was retrieved from Protein Data Bank. The canonical SMILES of 1,2-benzenedicarboxylic acid, mono-(2-ethylhexyl) ester was converted to the 3D structure using online Corina (https://www.mn-am.com/online_demos/corina_demo). AutoDockTools-1.5.4 was used to study the interaction of the ligand with the receptor protein. Hydrogen atoms and Kollman charges were added to the protein, followed by torsion optimization of the ligand. AutoGrid was used to fix the grid around the active site of the protein. Ten best poses of interaction between protein and ligand were obtained using Lamarckian genetic algorithm. Best among the ten was visualized further using PyMol [17].

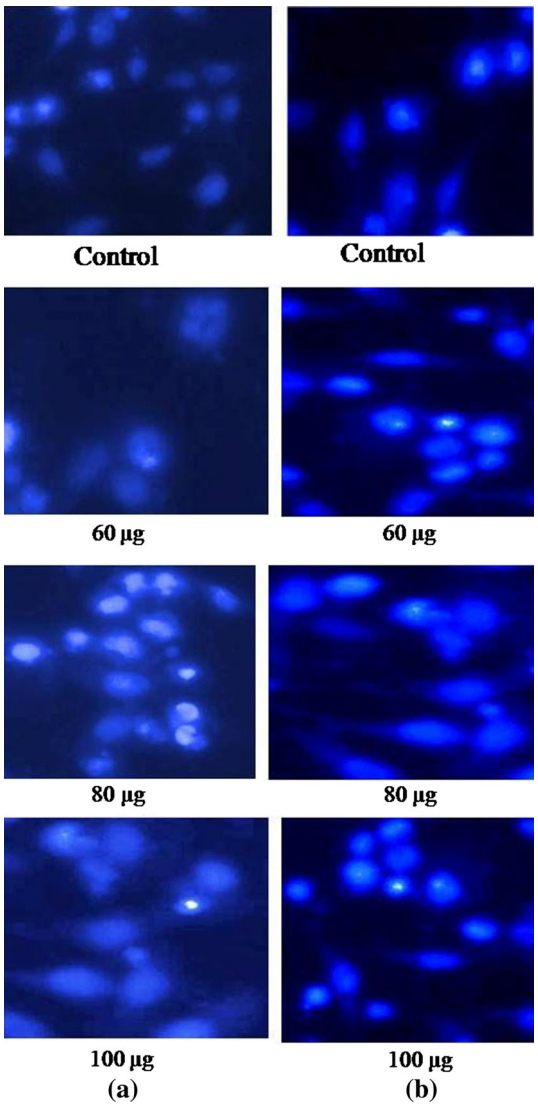

Fig. 6 GC–MS analysis of ethyl acetate crude extract of *Streptomyces* sp. VITJS4 indicates the presence of 52 compounds showing highest peak with retention time at 22.014

3 Results and Discussion

The current study was attempted to identify the antibacterial, antioxidant and cytotoxic activity of the VITJS4 ethyl acetate crude extract. The maximum antibacterial activity was noticed at 20 mg/mL against the pathogens, namely *B. cereus* MTCC No: 6840 (22 mm), *P. aeruginosa* MTCC No: 4676 (22 mm), *S. aureus* MTCC No: 7405 (27 mm), *L. monocytogenes* MTCC No: 657 (24 mm) and *V. cholerae* MTCC No: 3906 (23 mm). *E. coli* MTCC No: 1588 (23 mm) was highly susceptible, followed by *S. typhi* MTCC No: 1167 (21 mm) and *C. perfringens* MTCC No: 450 (21 mm) with moderate activity as compared to the standard drug chloramphenicol. In the current study, extract of *Streptomyces* sp. VITJS4 showed a better zone of inhibition against various tested pathogenic MTCC strains. Similarly, few studies have reported the effective antibacterial activity against bacterial pathogens from crude extracts of marine *Streptomyces* [18, 19]. The MIC values of extract tested against *B. cereus* MTCC No: 6840 were found to be 160 µg/mL (Table 1). The extract showing the lowest concentration of inhibition was considered as the MIC of the organism. The antioxidant activity of the VITJS4 extract showed maximum activity with 90 % percent inhibition at 0.5 mg/mL concentration and equivalent to the reference standard (Fig. 1). The cytotoxic activity of the extract varied from 20 to 100 µg/mL. The IC_{50} value was determined as 50 µg/mL for both HeLa and HepG2 cell lines and exhibited substantial growth inhibition. It was observed that VITJS4 extract revealed potent inhibitory effect against HepG2 cells with 27 % viability than HeLa cells with 45 %. The degree of morphological changes of HeLa and HepG2 cells was observed under the inverted microscope to depict apoptosis (Fig. 2a–d).

The detachment of cells with round morphology was considered to be the most significant characteristic features of apoptosis. Overall obtained data suggested that the VITJS4 extract induced apoptosis. Hence, induction of apoptosis is correlated inversely with decreased cell viability, confirming that apoptosis was mostly accountable for cell death. Furthermore, extract at the concentration used for cytotoxic activity showed no adverse effect on the viability of normal control cells signifying its particular activity toward cancer cells. Similarly, in few studies bioactive compounds obtained from marine sediment-derived actinomycete *Streptomyces avidinii* strain SU4, resistoflavine, from *Streptomyces. chibaensis, and* actinomycin D from *Streptomyces*. Streptazone from *Streptomyces* strains of HB117, HB122 and HB291 and fredericamycin A from HB116, HB118 and HB157 *Streptomyces* strains showed active cytotoxic activity [20–22]. Hence, the induction of apoptosis is known to be an efficient strategy for cancer therapy.

Data on the biological activity of the secondary metabolites from *Streptomyces* sp. VITJS4 were authenticated via GC–MS analysis (Fig. 3). The crude extract of *Streptomyces* sp. VITJS4 exhibited 52 bioactive compounds. The primary group of the compound was noticed with the presence of 1, 2-benzenedicarboxylic acid, mono-(2-ethylhexyl) ester, a phthalate derivative with the retention time of 22.014 from *Streptomyces* sp. VITJS4 ethyl acetate extract and confirmed based on the mass fingerprints showing the peak at *m/z* 149 (M) + with molecular formula $C_{16}H_{22}O$. The spectrum of the unknown metabolites was compared with known compounds, and the molecular structure of the corresponding peak was depicted (Fig. 4). HPLC chromatogram indicated the presence of

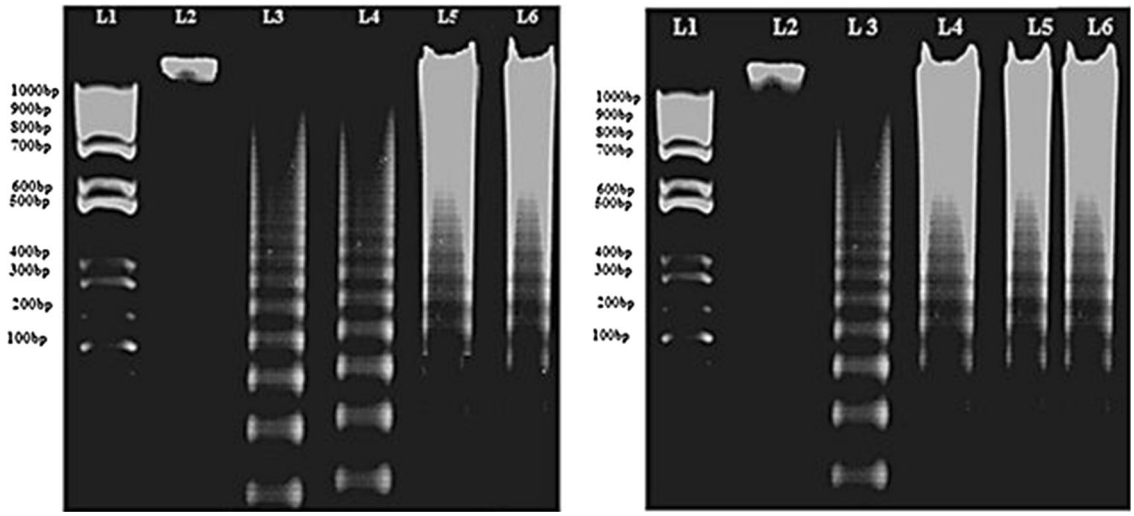

Fig. 7 Molecular structure of the major bioactive metabolite 1,2-benzenedicarboxylic acid, mono-(2-ethylhexyl) ester, a phthalate derivative from *Streptomyces* sp. VITJS4 crude extract

compound showing a single peak with the retention time at 6.924 min (Fig. 5).

The healthy cells can be differentiated from the cancer cells as there is a lack of equilibrium between cell division and apoptosis. The investigation of anticancer activity in the present findings has demonstrated that treatment of human HeLa and HepG2with phthalate derivatives results in cell death by apoptosis. This study was also supported by the previous finding, where 1, 2-benzenedicarboxylic acid, mono-(2-ethylhexyl) ester showed the onset of apoptosis [23]. Interestingly, previous literature on phthalate derivates was reported with antitumor, antidiabetic, antioxidant, antiscabies, anti-inflammatory, potent antimicrobial and antiviral activity [24–31]. The combination of the most effective synergy was observed through accelerated apoptosis triggered by phthalate derivatives, in particular, 1, 2-benzenedicarboxylic acid, mono-(2-ethylhexyl) ester extracted from marine *Streptomyces* sp VITJS4. Velmurugan et al. [32] reported the presence of phthalate derivatives as a major group of the compound with mass fingerprints showing the peak in GC–MS analysis of plant extract. These results confirmed that the phthalate derivatives were responsible for exerting such bioactivity. Therefore, any agents or compounds that can promote apoptosis are considered as a potential lead for developing anticancer drugs. The crude extracts evaluated for the apoptotic death of cancer cells revealed stained nuclei with round morphology in DAPI staining. Differently, stained DNA nuclei of treated and control cells were observed indicating chromatin condensation as a typical feature of apoptosis (Fig. 6). The evaluation of apoptosis was confirmed by DNA fragmentation patterns on HepG-2 and HeLa cells at 40 µg/mL concentration (Fig. 7). The optimized growth conditions of *Streptomyces* sp. VITJS4 in a different medium showed betterment of growth; starch casein broth influenced the higher yield of dry cell weight about 11 g/L. The best carbon source was found to be glucose with 15 g/L biomass. High yield was obtained from the medium supplemented with beef extract (11 g/L). Alteration of sources resulted in a change of biological activity caused by the variation in synthesis rate of bioactive compounds. The cultural conditions for the growth of bioactive metabolite production and biomass were more at pH 7.2 (11 g/L). Maximum metabolites and dry cell weight were attained at 27 °C (10 g/L). The influence of rpm on the bioactive metabolite production was found higher at 200 rpm with (11 g/L) biomass (Fig. 8a–e). The variability between the productions of bioactive secondary metabolites of all the parameters tested was evidenced by agar well diffusion assay.

Generally, in silico interaction studies elucidate the best docking ability between a receptor and ligand. Similarly, it can be observed from Table 2 that the complex (topoisomerase IV + 1,2-benzenedicarboxylic acid, mono-(2-ethylhexyl) ester) exhibited well-established bonds at the active pocket of protein with a binding energy of -5.87 kJ mol^{-1} (Fig. 9a, b). Based on the observed results, we conclude that the present study showed the

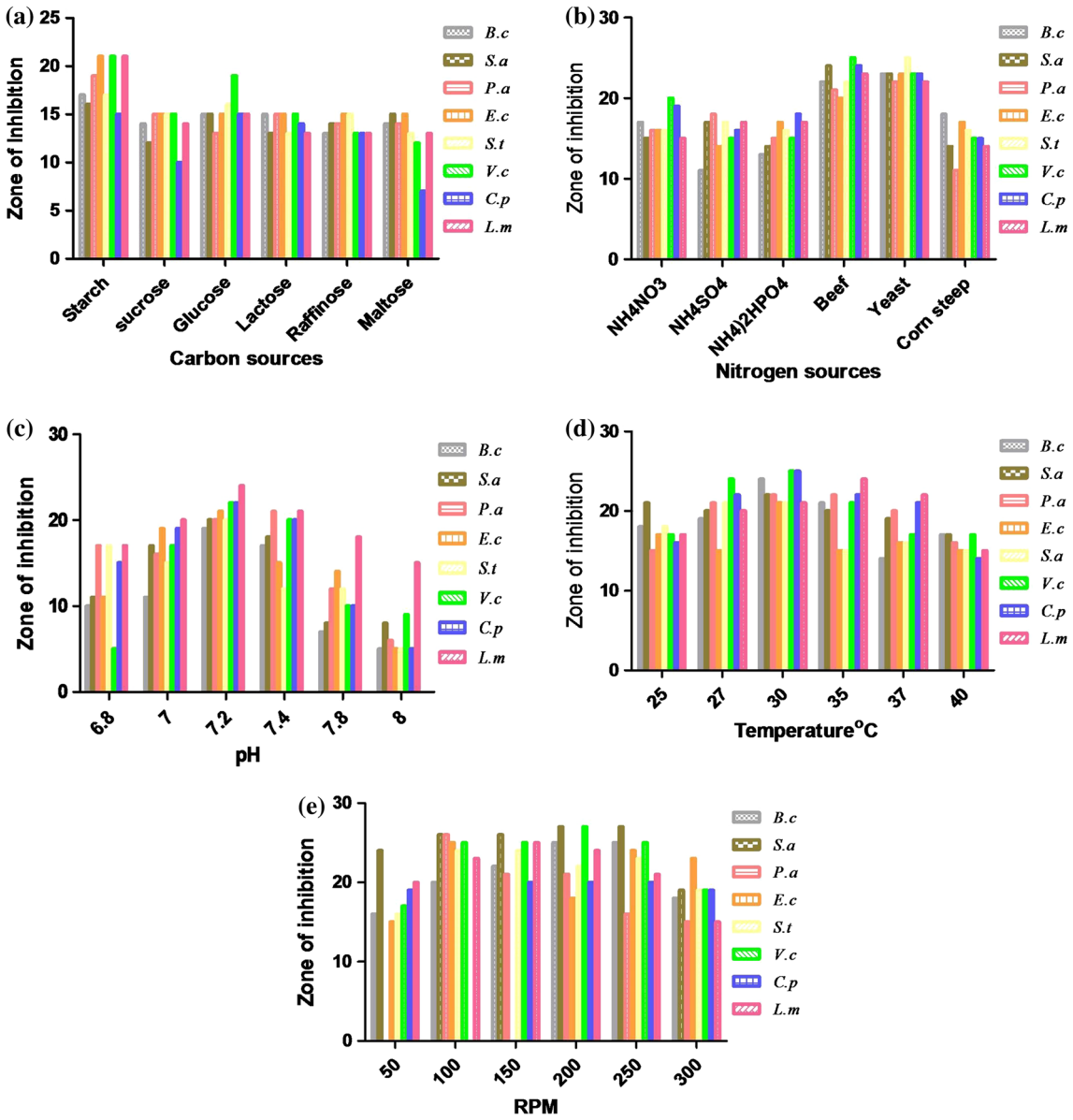

Fig. 8 HPLC analysis of *Streptomyces* sp. bioactive compound 1,2-benzenedicarboxylic acid, mono-(2-ethylhexyl) ester

Table 2 Docking results of protein–ligand binding interactions of DNA topoisomerase II α (PDB entry code: 1ZXM) with 1, 2-benzenedicarboxylic acid, mono-(2-ethylhexyl) ester in terms of energy in kcal/mol

Lead compound	Binding energy Kcal/mol	Ki (nm)	ΔG Inter-Molecular Kcal/mol	vdw + Hbond + desolv energy Kcal/mol	ΔG Electrostatic Kcal/mol	ΔG Internal Kcal/mol	Torsional Kcal/mol
	−5.87	49.55 um	−8.86	−8.21	−0.65	−0.7	2.98

highest degree of the novelty of the new strain *Streptomyces* sp. VITJS4 with bioactivity including antibacterial, antioxidant and cytotoxicity, which significantly expand such compounds in pharmaceutical therapeutics. Therefore, the obtained marine-derived *Streptomyces* sp. act as a prominent reservoir for novel drug molecules and solution to several diseases.

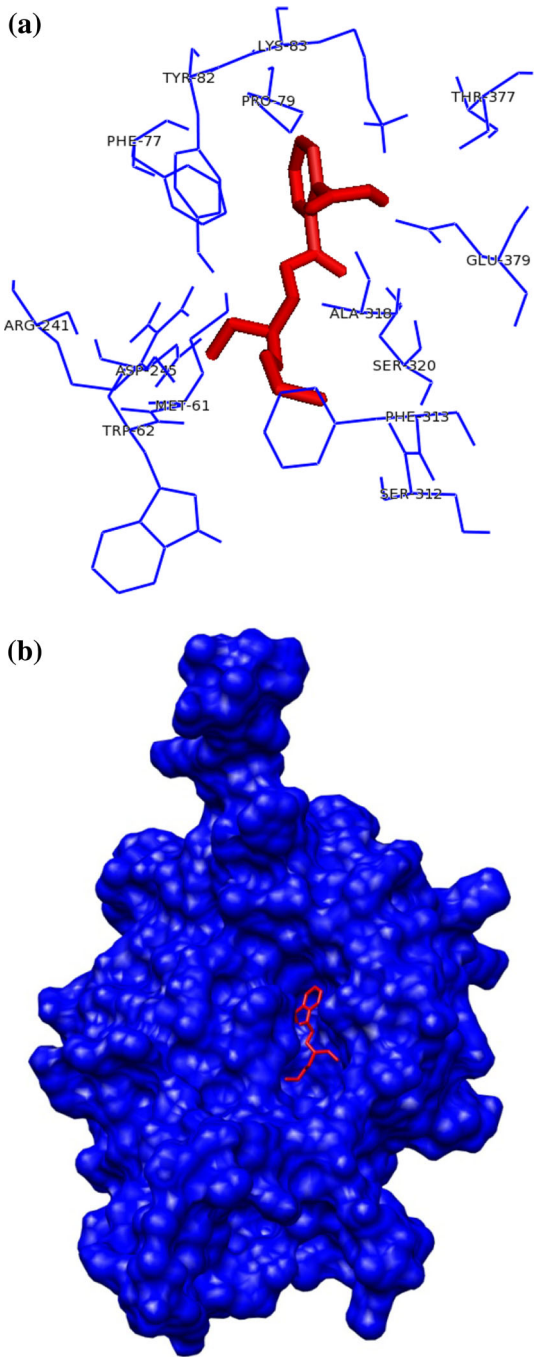

Fig. 9 **a** Aminoacids of topoisomerase IV interacting with 1,2-benzenedicarboxylic acid, mono-(2-ethylhexyl) ester with 4Å. **b** Schematic representation of docking revealing the compound interactions with the residues

4 Conclusion

Novel antibiotics and anticancer agents are the sources of marine-derived actinomycetes. Not only the presence of novel secondary metabolites makes *Streptomyces* interesting, but characterized by enormous diversity and also growing them under different conditions leading to an enrichment of bioactive compounds. In the present investigation, *Streptomyces* species showed a useful bioactive property for the search of new bioactive natural products. Based on the observed results, we conclude *Streptomyces* strain VITJS4 from marine environment South East coast of India as a potent source of novel antibiotics. It is foreseen that studies on *Streptomyces* can be useful in the discovery of novel isolates with therapeutic value and serve as alternatives drugs with fewer side effects, which will be economical and accessible to the greater section of the community.

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Compliance with Ethical Standards

Conflict of interest The authors have no conflict of interest to declare.

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