Research Article

Bioactive compound isolated from marine *Bacillus safensis* MB8 and their cytotoxicity potential

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Abstract

Background: Marine bacteria represent a potential source for the production of medically useful compounds against cancer, diabetes, inflammation, infection, etc. **Objectives:** In the present study, isolation, characterization and identification of bioactive compound from marine *Bacillus safensis* MB8. **Methods:** Breast cancer (MCF-7) cell lines to determine the cytotoxicity activity of bioactive compound produced from marine *Bacillus safensis* MB8. **Results**: Marine *Bacillus safensis* MB8 (Genbank accession no. KJ531643) isolated from deep sea sediments of the Bay of Bengal, India, produced a bioactive compound, Bis (2-ethylhexyl) benzene-1,2-dicarboxylate (BEHBD) with a molecular formula of $C_{24}H_{38}O_4$. The molecular ion peak at m/z 391 (M^+). The MTT assay based cytotoxicity assessment of BEHBD in MCF-7 cancer cells revealed the IC_{50} to be 49.8µg/ml. The acridine-orange and ethidium bromide (AO/EB) staining of the BEHBD treated cancer cells showed typical characteristic of apoptosis such as nuclear condensation, cell shrinkage and formation of apoptotic bodies. **Conclusion:** Potent cytotoxicity compound of Bis (2-ethylhexyl) benzene-1, 2-dicarboxylate (BEHBD) produced from marine *Bacillus safensis* MB8. It was undoubtedly confirmed to BEHBD is a proved cytotoxicity compound. This is the first reported to BEHBD produced by marine *Bacillus safensis* MB8.

***Keywords*:** Marine bacteria, *Bacillus safensis,* Bis (2-ethylhexyl) benzene-1,2-dicarboxylate, MCF-7, Cytotoxicity activity, AO/EB staining

Introduction

Marine resources including vertebrate, invertebrate, microorganisms and plant are esteemed sources of bioactive compounds. A large number of drugs in medicinal practice have been developed from natural products (Amador et al., 2003).

To date, many anti-cancer drugs have been developed and applied in clinical trials. However, resistances to anti-cancer drugs and side-effects have been reported. Fighting against tumors through induction of apoptosis, without resistance and side-effects, has now been recognized as an essential strategy of cancer drugs (Lowe et al., 2000). Induction of apoptosis is a useful approach in cancer therapies. Apoptosis, a major process of programmed cell death that plays an important role in regulation of tissue development and homeostasis (Hengartner, 2000; Kaufmann et al., 2001). Since apoptosis was considered as potential anti-tumor character (Frankfurt et al., 2003), many efforts have been made to discover new drugs from natural products that hold apoptosis potential. Though the cells maintain the membrane integrity until late apoptosis, they display several morphological and biochemical alterations, including chromatin condensation, nuclear segmentation, internucleosomal DNA fragmentation, cytoplasmic vacuolization, cell shrinkage and membrane blebbing with shedding of apoptotic bodies at early stage (Wyllie et al., 1980; Häcker, 2000).

Based on World Health Organization data (WHO 2018), above 18.1 million new cancer cases and 9.6 million deaths were mentioned globally in the year 2018. Nearly, 80% of the world's population depend on traditional medicines and more than 60% of clinically approved anticancer drugs are derivatives of these medicinal plant (Iqbal et al., 2017; Cragg et al., 2016; Khan, 2014; Weaver, 2014). According to literature survey, there are many anticancer drugs clinically approved and are recommended for the cancer treatment (Singh et al., 2016; Branquinho et al., 2014). Among these different forms of cancer, lung cancer is reported the most in male followed by breast cancer in female.

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In recent years, revealed that matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI – TOF-MS) to evaluate relationship between isolates, two very distinct and consistent groups of *B. safensis* (Satomi et al., 2006; Dickinson et al., 2004). In previous report showed bioremediation of cadmium through *Bacillus safensis* (Accession No. JX126862). It's a bacterium remote from marine mangrove sediments. This bacterium, *B. safensis* exhibited potentially used in cadmium treatment (Rajesh et al., 2014). Earlier report showed that whole genome comparison analysis of the *B. safensis*, *B. pumilus* and other *Firmicutes* genome separate them into three distinct clusters. Two clusters are subgroup of *B. pumilus* while one houses all the *B. safensis* strains. The genome-genome distance analysis and a phylogenetic analysis of gyrA sequences corroborated these results (Tirumalai et al., 2018). Mahmoud et al., revealed that *B. sonorensis* KM374670 bacterium isolated from water and sediments of Suez bay, Timsah lake, Egypt. This bacterium was producing bioactive compounds and also tested with antagonistic activity against different bacterial pathogens (Mahmoud et al., 2019).

Natural product derivatives like a plant is nontoxic to normal cells and also better tolerated hence they gain attention of new drug discovery. Appraised to plant kingdom comprises at least 250,000 species and only 10 percent have been investigated for pharmacological applications. Phytochemicals and their derived compounds present in flower, stem leaf, bark and root, perform numerous pharmacological functions in human systems viz., antitumor potential (e.g. ipomeanol, lycobetaine, tetrandrine, homoharringtonine, monocrotaline, curdione, vinblastine, vincristine, taxol, elliptinium, etoposide, colchicinamide, 10-hydroxycamptothecin, curcumol, gossypol, and indirubin) (Sukhdev et al., 2018; Singh et al., 2013). Previous report, 16 natural polyphenolic metabolite isolated from ethnomedicinal plant like *Helleborus purpurascens* (root and rhizomes). These metabolites were exhibited strong cytostatic and cytotoxicity activity against HeLa cancer cells. Horstmann et al., (2008), reported that MCS-18, a novel natural compound isolated from *Helleborus purpurascens* was showed strong immunosuppression potential (Vochita et al., 2011). In the present study, cytotoxicity potential of Bis-(2-ethylhexyl) benzene-1, 2-dicarboxylate **(**BEHBD) isolated from marine *Bacillus safensis* MB8.

Materials and Methods

Reagents

GTE (Glucose/Tris/EDTA) solution, lysozyme, SDS (sodium dodecyl sulfate), Phenol, Tris –EDTA (ethylene diamine tetraacetic acid), Rnase, Protease, Nuclear free water, 16s rRNA (ribosomal ribonucleic acid) Universal Primer, Taq DNA polymerase, Magnesium chloride (Mgcl2), deoxyribo nucleotide triphosphate (dNTPs), Buffer, were obtained from Sigma, Germany. Zobell marine agar (ZMA) and Zobell marine broth (ZMB), Silica gel 60- 120 mesh, 3,-(4,5 – dimetthylthiazol -2- yl-)-2,5- diphenyl tetrazolium bromide (MTT) were obtained from Himedia, India. Hexane, ethyl acetate, acetone, chloroform, methanol, thin layer chromatography (TLC) plate, were obtained from Merck, Germany.

Isolation of marine bacteria

Deep sea sediments (position 13'02.95'N 80'52.29'E, depth; 20 m) were collected from the Bay of Bengal (Moushumi Priya et al., 2012). In order to isolate marine bacteria 1 g sediment was added to 100 mL of saline water. Ten-fold dilution (10µL) was spread plated on Zobell marine agar (ZMA) medium with 1.5% (w/v) sodium chloride (NaCl) (Zobell, 1943). After incubation at 37ºC for 48 h, single colonies were isolated and subcultured to obtain pure cultures. Stock cultures were made in Zobell marine broth containing 50% (v/v) glycerol and stored at – 80°C.

Molecular characterization of marine bacteria by 16S rRNA gene analysis

Genomic deoxyribonucleic acid (DNA) was extracted by using standard sodium dodecyl sulfate (SDS) lysis methods. The 16S rRNA (ribosomal ribonucleic acid) genes were amplified by PCR (Polymerase chain reaction) using universal primers, fD1 (5'-AGTTTGATCCTGGCTCA-3') and rP2 (5'-ACGGCTACCTTGTTACGACTT-3'). The PCR cocktail (50 µL) contained 20 pM of primer, 50 ng of DNA, 1x *Taq* DNA polymerase buffer, 3 U of *Taq* DNA polymerase (Sigma, USA), 0.2 mM of each deoxynucleotide (dNTPs) , and 1.5 mM magnesium chloride ($MgCl_2$). PCR conditions consisted of an initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 59°C for 1 min and extension at 72°C for 2 min with a final extension at 72°C for 5 min. The amplification was examined by 0.8% agarose gel electrophoresis and purified using Quick PCR purification kit (Sigma-Aldrich, USA). The complete 16S rRNA gene was sequenced with automated DNA sequencer with specific primers using the facility at Macrogen Inc (Seoul, Korea) (Naik et al., 2008).

Construction of phylogenetic tree

The retrieved gene sequence was compared with other bacterial sequences by using (National center for biotechnology information) NCBI BLAST **(**Basic local alignment search tool) search for their pair wise identities. Multiple sequence alignment and the phylogenetic tree were constructed with molecular evolutionary genetics

analysis (MEGA 4.0) software by using the neighbor-joining (NJ) method with 1000 replicates using bootstrap. The 16S rRNA sequence was submitted to the GeneBank (http://www.ncbi.nlm.nih.gov) (Pathma et al., 2013; Naik et al., 2008; Tamura et al., 2007).

Extracellular extract of marine bacteria MB8

Potent marine bacteria was grown in Zobell marine broth (5 L) in Erlenmeyer flasks on a rotary shaker at 180 rpm for 72 h at 37 ºC. The cell-free culture supernatant was prepared by centrifuging the culture at 8000 rpm for 20 min at 4 ºC. To the cell free culture supernatant, equal volume of ethyl acetate was added and vigorously shaken for 5 min. Then, the organic (upper) layer was separated and evaporated to dryness in a rotary evaporator and the extract was dissolved in dimethyl sulfoxide (DMSO) (Lalitha et al., 2016).

Purification and characterization of compound

The crude ethyl acetate extract of marine *Bacillus safensis* MB8 cell free extract was resolved in silica gel column chromatography (60-120 mesh) and eluted with methanol in chloroform (1:99). The active fractions of bioassay were pooled together, filtered (0.2µm, Millipore) and further purified by preparative HPLC (Shimadzu, Japan) using Axia packed C_{18} reverse phase column (Phenomenex, Torrance, CA, USA; 50 mm x 22 mm id., 10 µm particle size) and peak profiles were monitored at 254 nm using UV detector 10 AVP (Shimadzu, Japan). The purity was confirmed by analytical HPLC using Luna C_{18} reverse phage column (Phenomenex, USA; 250 mm x 10 mm id). Solvent condition included a flow rate 0.8 ml/min with 90% (v/v) acetonitrile in water for 10 min. Based on fourier transform infrared (FT-IR), nuclear magnetic resonance (NMR), liquid chromatography mass spectroscopy (LC-MS) and Gas chromatography–mass spectrometry (GC-MS) analysis the structure of bioactive compound was elucidated. NMR spectra for the compound dissolved in CDCl3 was obtained from NMR instrument operated at 400 MHZ for ^{1}H and 100 MHz for ^{13}C (Advance II, 400 MHZ, Bruker corporation, USA). The mass of the bioactive compound was analyzed in LC-MS 2010 (Shimadzu, Japan) (Lalitha et al., 2016, 2018; Kennedy et al., 2015).

Cell culture

Breast cancer (MCF-7) cell lines were obtained from the National Centre for Cell Science (NCCS), Pune. The cells were grown in T25 culture flasks containing dulbecco's modified eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and amphotericin B 3 m/L, streptomycin 75 mg/L, gentamycin 180 mg/L, penicillin 120 mg/L, cells were maintained at 37ºC in a humidified atmosphere containing 5% CO_2.

Cytotoxicity activity

Cell survival rate was determined by employing the 3-(4,5-Dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium Bromide (MTT) assay. Exponentially grown MCF-7 cells were seeded at a density of 0.2×10^5 cells in 96-well plate with a volume of 200µL per well. Cells were incubated with different concentrations (1, 10, 25, 50 and 100µg mL^{-1}) of compound and incubated at 37ºC for 24 h. At the end of the incubation periods, 10µL of MTT stock solution (5 mg mL^{-1}) was added to each well and the plates were further incubated for 24 h at 37ºC. The formazan crystals that formed due to the cleavage of tetrazolium salt were dissolved by the addition of 100µL of dimethyl sulfoxide (DMSO) per well. The soluble formazan produced was quantified spectrophotometrically using an enzyme linked immunosorbent assay (ELISA) reader at 570 nm using the following formula. Cell proliferation inhibition (%) = [1 (A value of the experimental samples/ A value of the 10 control)] ×100 (Lalitha et al., 2016, 2018). The IC_{50} was calculated using Graphpad prism software using nonlinear regression statistical method.

Morphological observations

Live cell imaging-cancer cells were seeded at 0.2×10^6 cells per well in 24-well tissue culture plate treated with the half maximal inhibitory concentration (IC_{50}) of compound for 24 h. After incubation, cells were observed under inverted phase contrast microscopy for morphological changes.

Acridine orange (AO) and ethidium bromide (EB) staining-cancer cells were seeded at 0.2×10^6 cells per well in 24-well tissue culture plate treated with the IC_{50} concentration of compound and incubated for 24 h. After incubation, the cells were washed thrice with phosphate buffered saline (PBS), stained with 10µl of dye mixture (10 mg mL^{-1} AO and 10 mg mL^{-1} EB) and cells were examined under fluorescence microscope. Digital images were obtained using the image acquiring software program (Eclipse TS, Nikon, USA) (Lalitha et al., 2016; Lalitha et al., 2018; Song et al., 2005).

Statistical analysis

The graph was plotted using Origin pro-8.0 software.

Results

Isolation and molecular characterization of marine *Bacillus safensis* MB8

In the present study, marine bacteria were isolated from deep sea sediment samples in Bay of Bengal, India and screened for antimicrobial and cytotoxicity activity (Lalitha et al., 2018). Among the isolates, the strain MB8 was noticed as potent bacterium based on screening of antimicrobial and cytotoxicity activity. Based on 16S rRNA gene sequence analysis the bacterium was identified as

Bacillus safensis MB8. Subsequent molecular phylogenetic tree analysis confirmed that the strain MB8 as *Bacillus safensis* (Figure 1). The 16s rRNA sequence of *Bacillus safensis* MB8 was deposited in the GenBank database under accession number KJ531643.

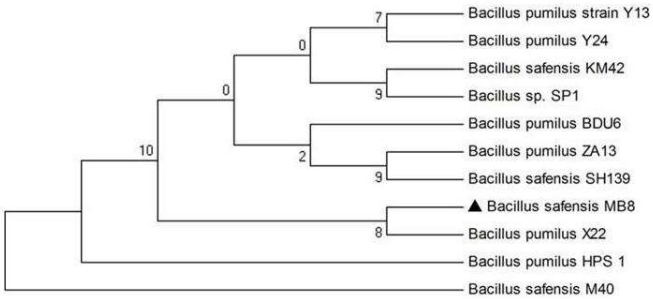

Figure 1. Phylogenetic tree analyses of *Bacillus safensis* MB8 isolated from the deep sea sediment of Bay of Bengal based on the nucleotide sequence of 16s rRNA. The multiple sequence alignment was done in CLUSTAL program. The pair-wise evolutionary distances were calculated using Kimura-2-parameter model. The phylogenetic tree was constructed by neighbor-joining (NJ) method with 1000 replicates using bootstrap.

Figure 2. Thin layer chromatographic analysis of crude and purified compound from marine *Bacillus safensis* MB8. C; Crude, P: Pure

Extraction and purification of compound from marine *Bacillus safensis* MB8

Extraction of supernatant from marine *Bacillus safensis* MB8 with ethyl acetate and subsequent dryness in rota evaporator, the crude extract ranged from 0.5 to 1g was obtained. The crude extract (0.1µl) dissolved in a solvent by thin-layer chromatography (TLC) revealed diverse compounds (Figure 2). Subsequent purification of the crude extract using silica gel column chromatography (60 - 120 mesh) yielded nine fractions and one major fraction was purified as greenish-yellow color compound. TLC of purified compound showed the Rf value of 0.87. The analytical high performance liquid chromatogram (HPLC) obtained using C_{18} reverse phase column and detection at 254 nm revealed the homogeneity of the compound at the retention time 7.56 min.

Spectroscopic analysis of purified compound from marine *Bacillus safensis* MB8

Ultraviolet – visible absorption spectrum of purified greenish-yellow color compound showed absorbance peak at 254 nm. Fourier transform infrared (FT-IR) spectrum of purified compound showed the functional groups such as a strong band at 1735.14 cm^{-1} that relates to C=O stretching of the ester carbonyl group. Strong bands in the region of 2927.58 to 2854.06cm^{-1} revealed aromatic and aliphatic C-H stretching. Weak bands in the region of 1655.00 cm^{-1} that corresponds to C=C stretching frequencies was also noticed. C-O stretching was observed by a characteristic band at 1120.44 cm^{-1} (Figure 3).

NMR analysis of purified compound

^{1}H NMR spectrum of purified compound displayed two multiplets at 7.70 and 7.52 ppm that correspond to H^{2} and H^{3} protons of the aryl ring. The multiplet at 4.22 ppm corresponds to H^{4} protons of the hexyl substituent of the ester. A multiplet at 1.41 ppm was due to H^{5} protons. The signals due to H^{6}, H^{7}, H^{8} and H^{10} were merged and appeared as a multiplet, centered at 1.32 ppm. The signals pertinent to methyl protons H^{9} and H^{11} appeared as a multiplet at 0.83 ppm (Figure 4).

^{13}C NMR spectrum of purified compound displayed a total of 12 signals that were appropriate for the twelve types of carbon atoms present in the molecule. The signals observed at 131.47, 130.12 and 129.04 were due to C^{1}, C^{2} and C^{3} carbons of the aromatic ring respectively. The ester carbonyl carbon (C^{4}) appeared at 165.03 ppm. The signals at 63.83, 59.35, 58.00, 45.88, 45.42, 40.75, 31.17, 29.17 and 21.93 ppm were due to C^{5}, C^{6}, C^{7}, C^{8}, C^{11}, C^{9}, C^{10} and C^{12} carbons of the alkyl residue respectively (Figure 5). The ESI-MS spectrum displayed the molecular ion peak at m/z

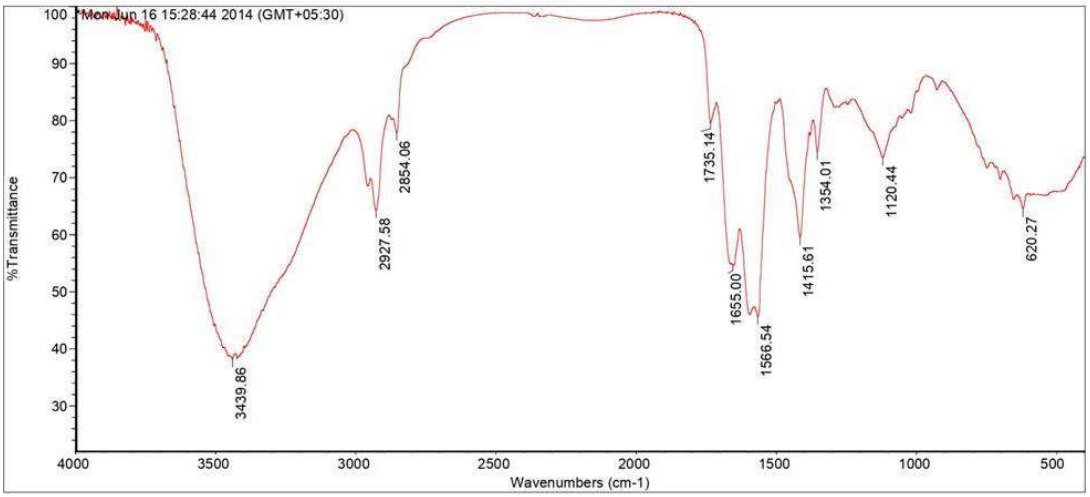

Figure 3. FTIR analysis of compound by marine *Bacillus safensis* MB8

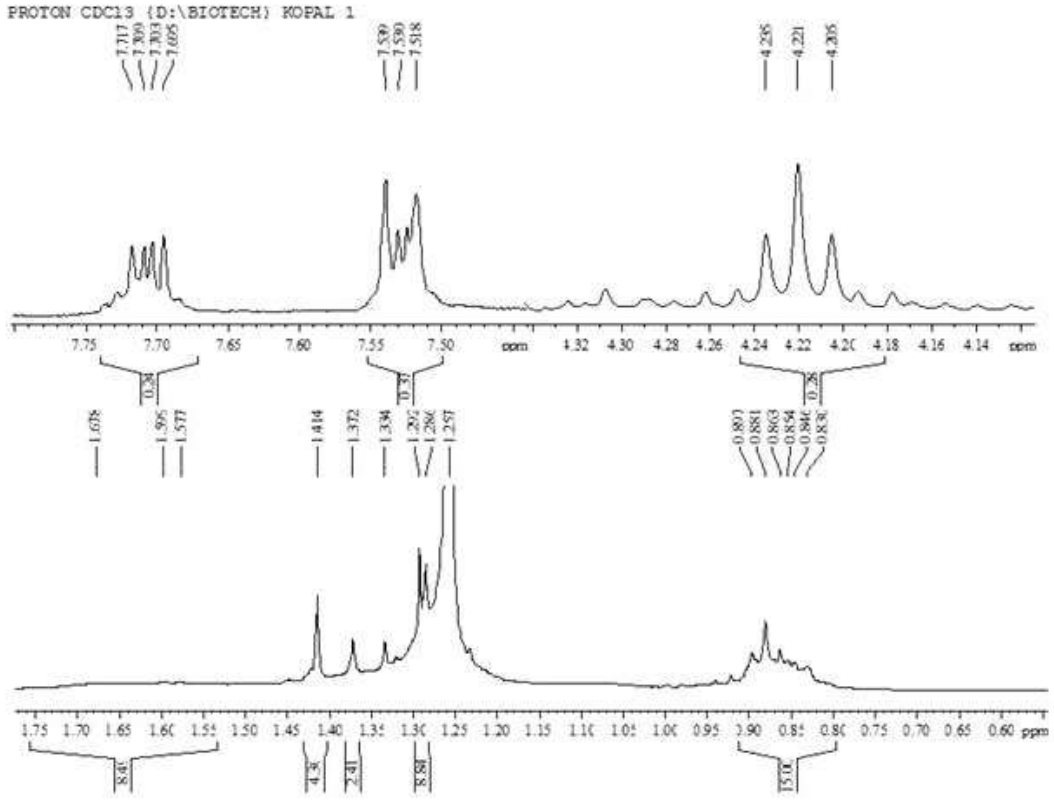

Figure 4. ^{1}H NMR of compound isolated from marine *Bacillus safensis* MB8

391.25 (M^+) that was matching with the molecular mass of the compound, thus confirming with the proposed structure of Bis (2-ethylhexyl) benzene-1,2-dicarboxylate (Figure 6). The GC-MS spectrum of the purified compound of marine *Bacillus safensis* MB8 showed the molecular ion peak at m/z 390 (Figure 7). The combined UV-spectroscopic, FT-IR, HPLC, NMR and LC-MS study showed that the compound was identified as Bis (2-ethylhexyl) benzene-1,2-dicarboxylate (BEHBD). This is the first reported to BEHBD produced by marine *Bacillus safensis* MB8. The molecular weight of the BEHBD was determined as m/z 391 (M^+) with empirical formula at $C_{24}H_{38}O_4$ (Figure 8).

Cytotoxicity activity of Bis (2-ethylhexyl) benzene-1,2dicarboxylate (BEHBD)

The MTT assay on BEHBD treated MCF-7 cell line revealed cytotoxic effect with IC50 concentration (49.8µg/mL). Whereas the BEHBD at IC50 concentration was not effective on PBMC cells.The BEHBD inhibited proliferation of MCF-7 cells in dose and time (24h and 48h) dependent manner (Figure 9).

Morphological observations

Live cell imaging of BEHBD-treated cancer cells showed dead

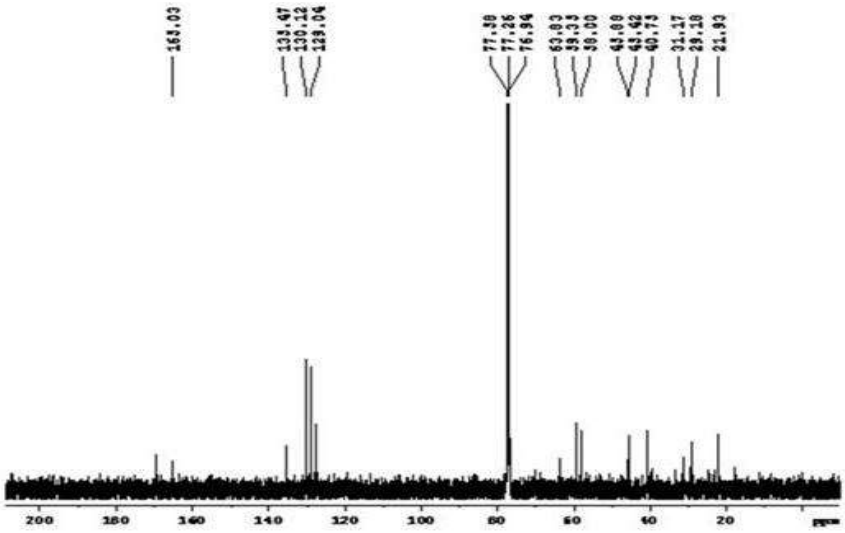

Figure 5. ^{13}C NMR of compound isolated from marine *Bacillus safensis* MB8

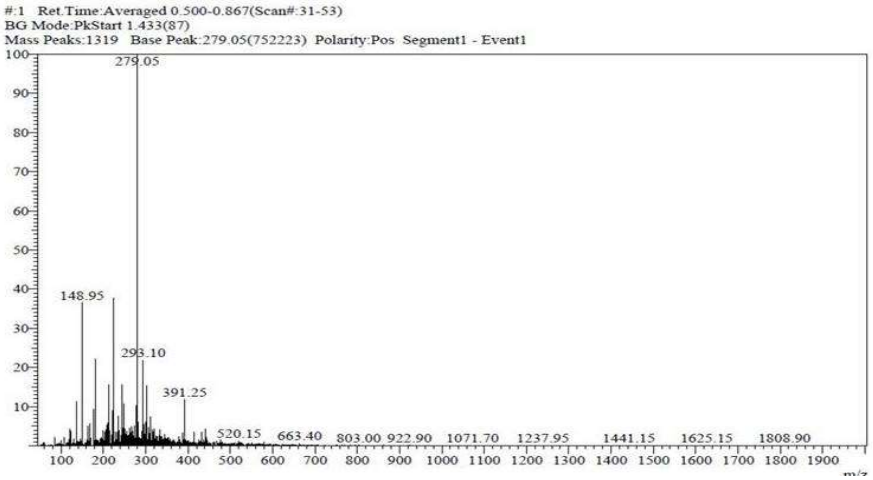

Figure 6. ESI-MS of compound isolated from marine *Bacillus safensis* MB8 with a molecular ion at m/z 391.25

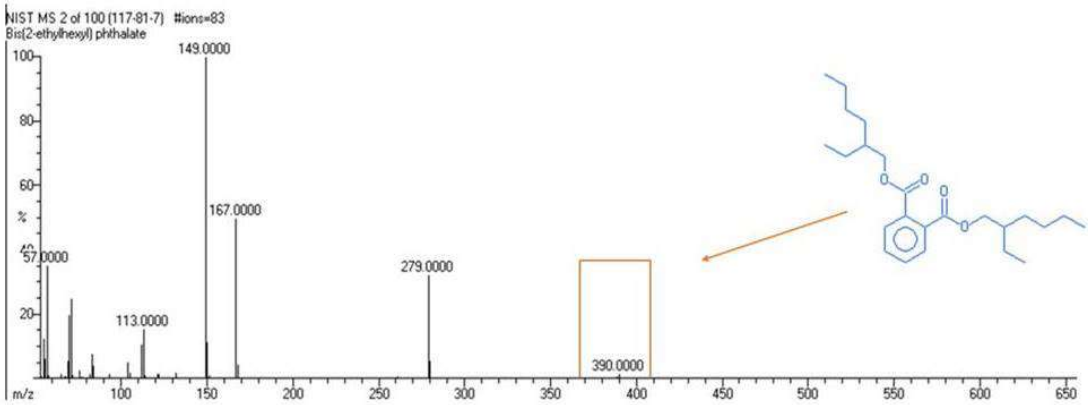

Figure 7. GC-MS of compound isolated from marine *Bacillus safensis* MB8 with a molecular ion at m/z 390

and floating cells (Figure 10). Fluorescent imaging of BEHBD-treated cancer cells showed different stages of apoptosis such as early (yellowish green) and late (orange-red) apoptotic cells with cell shrinkage compared to the uniformly stained green cells with normal morphology in control well (Figure 11). Greenish yellow color represents early apoptotic cells at 12 h (b) and reddish orange color represents the late apoptotic cells at 24 h (b). Arrows in b indicate nuclear condensation. Violet arrow in b indicates fragmented nuclei and blue arrow indicates apoptosis blabbing.

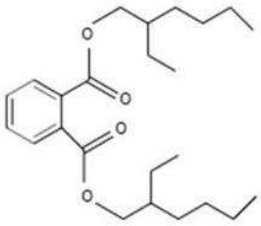

Figure 8. Structure of compound isolated from marine *Bacillus safensis* MB38 was identified as Bis (2-ethylhexyl) benzene-1,2-dicarboxylate

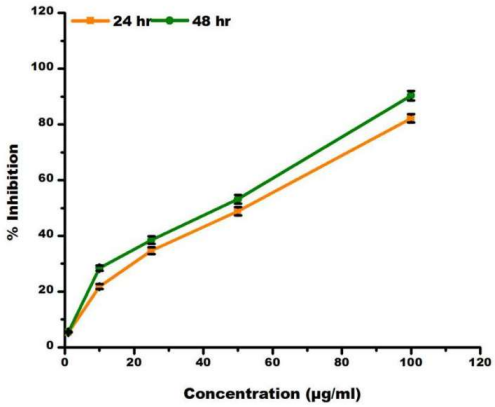

Figure 9. Dose- response analysis of BEHBD isolated from marine *Bacillus safensis* MB8 on inhibition of anti-proliferation of MCF-7 cells. 1×10^5 cells/well were seeded in 96 well tissue culture plate followed by treatment with different concentrations (1,10,25,50, and 100µg/ml) of BEHBD for 48h. Inhibition of cell proliferation was determined by MTT reduction assay. Results are mean values ±SD of three independent experiments.

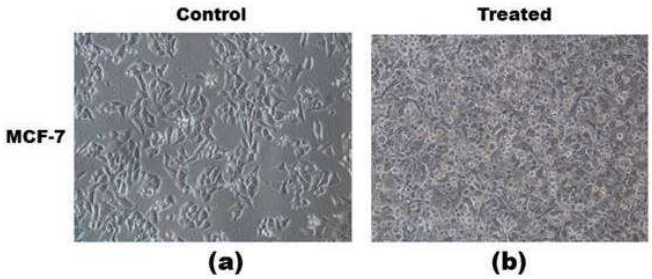

Figure 10. Live cell imaging of MCF-7 cancer cells treated with respective IC_{50} concentration of the BEHBD showing the induction of apoptosis.

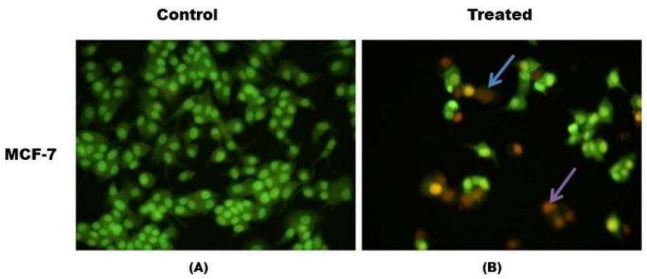

Figure 11. Fluorescent image of acridine-orange and ethidium bromide (AO/EB) double staining of MCF-7 cancer cells treated with BEHBD showing the induction of apoptosis

Discussion

Now a day's great emphasis is given for the isolation, identification and characterization of bioactive compound produced by microorganisms of marine origin possessing a broad spectrum of activities such as anti-inflammatory compounds (e.g. seudopterosins, topsentins, seytonenin and manoalide), anticancer agents (e.g. bryostatins discodermolide, eleutherobin and sarcodictyin), antibiotics (e.g. marinone) and anti-parasitic compounds (e.g. valinomycin). So, nature abounds with a rich potential heritage of therapeutic resource that has been exploited for effective and beneficial use against many human cancers such as pancreatic, breast, bladder and lung cancer either in prevention strategy or therapeutic armamentaria to kill tumor cells (Bhatnagar, 2010). Potential bioactive compounds obtained from marine and terrestrial sources includes but not limited to auristatin, bryostatin, combretastation and dolastatin. The marine environments are enriched with both biological and chemical diversity and covers more than 70% of the biosphere (Jaiganesh et al., 2012). Earlier studies reported isolation, identification and characterization of many marine bacteria that are capable of producing bioactive molecules active against inflammation and cancer (Krishnaveni et al., 2009; Moushumi et al., 2012; Lalitha et al., 2016, 2018). In the present study, *Bacillus safensis* MB8 was isolated from deep sea sediment of Bay of Bengal, India. Ethyl acetate extraction of cell free supernatant from marine *Bacillus safensis* MB8 and was yielded 1g dry powder. Further bioassay guided fractions revealed the cytotoxicity activity of greenish-yellow color compound was showed TLC plates. Further structural elucidation of compound by using FTIR, NMR and LC-MS analysis were revealed Bis (2-ethylhexyl) benzene-1, 2-dicarboxylate (BEHBD). Moushumi et al., reported Bis(2-ethylhexyl) phthalate (BEHP) from *B. pumilus* MB40 inhibited proliferation of K562 cells in a dose and time dependent manner. Similarly Lee et al., (2000) isolated BEHBD exhibiting antileukemic and antimutagenic effects from *Aloe vera* plant. A structural analog of BEHBD, Dioctylphthalate obtained from marine brown alga *Sargassum wightii* was also reported to exhibit antimicrobial activity (Malaker et al., 2013). Antimicrobial activity of DEHP isolated from *Streptomyces avidinii* strain SB9 against various microorganisms is in accordance with previous reports proving that DEHP is a biologically active compound (Sastry et al., 1995; Lyutskanova et al., 2009; Habib et al., 2009; Al-Bari et al., 2005). A potent antimicrobial activity was also found for DEHP isolated from the microorganism *Streptomyces bangladeshiensis*

(Oie et al., 1997). The DEHP is considered as pro inflammatory agent in other studies (Gourlay et al., 2003; Bernan et al., 1997).

Cancer is the largest single cause of death in both men and women, claiming over 6 million lives each year in the world. The ability to induce tumor cell apoptosis is a desirable attribute of a candidate drug which prima facie discriminates between anticancer drugs and toxic compounds (Moushumi et al., 2012). Fluorescent imaging of BEHBD -treated cancer cells exhibited different stages of apoptosis such as early and late apoptotic cells with cell shrinkage as compared to the uniformly stained green in the control cells with normal morphology. Recently, Lalitha et al., (2016 and 2018) reported that Pyrrole (1, 2, a) pyrazine 1, 4, dione, hexahydro 3-(d-methyl prophy) 4, 6-Diamidino-2-phenylindole dihydrochloride (PPDHMP) produced from marine bacterium MB30 inhibition proliferation of A549 and HeLa cells in a dose dependent manner via apoptosis. In this direction great strides have been made in identifying compounds that influence apoptosis and their mechanism of action (Wang et al., 2007).

Conclusion

In the present study, a potent cytotoxicity compound of Bis (2-ethylhexyl) benzene-1,2-dicarboxylate (BEHBD) isolated from marine *Bacillus safensis* MB8. It was undoubtedly confirmed to BEHBD is a proved cytotoxicity compound. This is the first reported to BEHBD produced by marine *Bacillus safensis* MB8.

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Conflict of interest

The author declares that there are no conflicts of interest.

Source of funding

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Abbreviations

MCF-7 - Breast cancer
MTT - 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
BEHBD - Bis (2-ethylhexyl) benzene-1, 2dicarboxylate
AO/EB - Acridine-orange/Ethidium bromide
DNA - Deoxyribonucleic acid
MALDI-TOF-MS - Matrix-assisted laser desorption/ionization time of flight mass spectrometry
GTE - Glucose Tris EDTA
SDS - Sodium dodecyl sulfate
EDTA - Ethylene diamine tetraacetic acid
16 rRNA - Ribosomal ribonucleic aicd
dNTPs - Deoxyribo nucleotide triphosphate
ZMA - Zobell marine agar
ZMB - Zobell marine broth
TLC - Thin layer chromatography
NaCl - Sodium chloride
PCR - Polymerase chain reaction
$MgCl_2$ - Magnesium chloride
NCBI - National center for biotechnology information
BLAST - Basic local alignment search tool
MEGA - Molecular evolutionary genetics analysis
NJ - Neighbor-joining
DMSO - Dimethyl sulfoxide
HPLC - High performance liquid chromatography
FT-IR - Fourier transform infrared
NMR - Nuclear magnetic resonance
LC-MS - Liquid chromatography Mass spectroscopy
GC-MS - Gas chromatography -Mass spectrometry
$CDCl_3$ - Deuterated chloroform
NCCS - National Centre for Cell Science
DMEM - Dulbecco's modified eagle's medium
FBS - Fetal bovine serum
CO_2 - Carbon dioxide
ELISA - Enzyme linked immunosorbent assay
IC_{50} - Inhibitory concentration
PBS - Phosphate buffered saline
BEPH - Bis- (2-ethylhexyl) phthalate
ESI-MS - Electrospray ionization - Mass spectrometry
PPDHMP - Pyrrole (1, 2, a) pyrazine 1, 4, dione, hexahydro 3-(d-methyl prophy) 4, 6-Diamidino-2-phenylindole dihydrochloride