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Antioxidant activity of newly discovered lineage of marine actinobacteria

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ABSTRACT

Objective: To investigate the antioxidant activity of marine actinobacteria. **Methods:** The content of total phenolics, the level of antioxidant potential by DPPH radical scavenging activity, metal chelating activity, FRAP method, β carotene assay and NO scavenging activity in extract were determined. **Results:** In all the methods the extract exhibited good scavenging activity except NO scavenging activity. The IC_{50} values of marine actinobacteria extract on DPPH radical were found to be 41.09 μg/mL. The zone of color retention was 12 mm in β-carotene bleaching assay. DNA protective efficiency of the extracts was also studied using UV- photolysed H_2O_2-driven oxidative damage to pBR322. HPLC analysis identified some of the major phenolic compounds in extracts, which might be responsible for the antioxidant potential and cyto-protection. It showed a 100% cytotoxic effect in brine shrimp lethality assay within 10 mins. The novel actinobacteria was identified as *Streptomyces* LK-3 (JF710608) through 16S rDNA Sequencing. **Conclusions:** The results obtained suggest that the extracts bear anti-cancer metabolites and could be considered as a potential source for anti-cancer drug development.

1. Introduction

During normal cell metabolism, reactive oxygen species (ROS) production occurs in both animals and plants. The intemperance of ROS leads to oxidative stress, ensuing in oxidative DNA damage which is implicated in the pathogenesis of abundant disorders, e.g. cardiovascular, atherosclerosis, reperfusion injury, cataractogenesis, rheumatoid arthritis, inflammatory disorders and cancer[1]. Many synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) have been used to retard the oxidation process; however, the use of synthetic antioxidants must be under strict regulation due to potential health hazards[2]. The search for natural antioxidants as alternative is therefore, of great interest among researchers.

Due to the important role of free oxygen radicals in various diseases, it have prompted to investigate novel and potent antioxidant compounds from microorganisms which are ultimately of therapeutic use. Among the two antibacterial antibiotics carbazomycin B and carazostatin, carbazomycin B showed much stronger antioxidant activity[3]. The new metabolites, benthocyanins A, B and C, and benthophoenin were isolated from *Streptomyces prunicolor* and Carquinostatin A from *Streptomyces exfoliatus* was found to have high antioxidant activity which was comparable to that of vitamin E. It showed brain-protecting activity and hippocampal neurone system, it also suppressed the glutamate toxicity[4]. In *Streptomyces* sp. OH-1049, three antioxidant isoflavonoids were isolated, in these 4′, 7, 8-trihydroxyisoflavone has shown antitumor activity[5].

Marine actinobacteria constitutes an infinite pool of novel chemistry. Out of 23 000 known microbial secondary metabolites, 42 % of secondary metabolites isolated from actinobacteria. Among various genera, *Streptomyces*, *Saccharopolyspora*, *Amycolatopsis*, *Micromonospora* and *Actinoplanes* are the major producers of secondary metabolite[6]. The secondary metabolites produced by actinomycetes have a broad spectrum of biological activities such as antibacterial, antifungal, antiviral, antiparasitic, immunosuppressive, antitumor, insecticidal, anti inflammatory, antioxidant, enzyme inhibitory, diabetogenic and others. Numerous soil actinobacteria species have been isolated and screened for bioactive compounds in the past decades. Now a day's the chance of isolating a novel bioactive compounds has reduced. Asha

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et al reported marine actinobacteria isolated from coastal water of Dhanushkodi, India showed the antibacterial and anti fungal effect against selected human pathogens[7]. The *Streptomyces* chibaensis AUBN1/7, isolated from marine sediment samples of Bay of Bengal, India showed potent cytotoxic activity[8] and Chinikomycin A and B, were isolated from a marine *Streptomyces* sp. isolate M045 exhibited antitumor activity[9]. The *Streptomyces* sp isolated from nicobar marine sediment samples showed potent antifungal activity[10]. The actinobacteria have adapted them to survive marine environment. Bioactive compounds from marine actinobacteria possess distinct lead molecule that may form the basis for synthesis of new drugs. To find out the novel antioxidant, innovative enrichments and screening methods were important. The present study focuses on the novel enrichment technique for to isolate potential antioxidants.

2. Materials and methods

2.1. Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH) and rutin were obtained from Sigma Co. (St. Louis, MO, USA). 2,2-azino-bis-(3-ethylbenzothiazoline- 6-sulphonic acid) (ABTS), ferrozine, gallic acid, 2, 4, 6-tripyridyl-s-triazine (TPTZ) and ascorbic acid were obtained from Himedia, Mumbai. Potassium ferricyanide, ferric chloride, trichloroacetic acid, aluminium chloride, potassium persulphate, ammonium persulphate, ferrous suphate, sodium salicylate, ammonium molybdate, sodium carbonate, aluminium chloride, sodium nitrate, sodium hydroxide, Folin-Ciocalteu's phenol reagent, ferrous chloride, sodium hydroxide and solvents were obtained from Merck, Mumbai.

2.2. Actinobacteria isolates

Three actinobacteria strains were used for screening to detect antioxidant activity. All the 3 strains used in the study were *Streptomyces*, isolated from nicobar marine sediments and maintained on glycerol stock. The isolated strains were inoculated into 100 mL of SS broth and incubated in rotary shaker incubator at 28 ℃ for 7 days[10]. It is prepared in 50% sea water. The potential strain was identified through 16s rDNA sequencing.

2.3. Optimization using response surface methodology (RSM)

A five-variable and optimal design was performed in the present study to optimize the process parameters of antioxidant activity of marine actinobacteria. The five independent variables were pH (A), temperature (B), glucose (C), casein (D) and NaCl (E). A set of 31 experiments with five variables were required. The experimental design for screening of medium components is shown in Tables 1. Each variable was set at two levels (-1 and +1). Trace element solution was constantly maintained[11]. The design was developed using a Design Expert, version 8.0.7.1.

Table 1
Media components for RSM

Variables	Levels	
	Low (-1)	High (+1)
A pH	6	9
B Temperature (℃)	25	40
C Glucose (%)	0.5	2.0
D Casein (%)	0.05	0.2
E NaCl (%)	0.1	0.5

2.4. Estimation of total phenolic content

Total phenolic content of the crude extract of marine actinobacteria was determined using the Folin-Ciocalteau reagent. The crude extracts were diluted in water to obtain different concentrations (125, 250, 500 and 1000 μg/mL). 50 μL of crude extract was mixed with 2.5 mL of Folin-Ciocalteau reagent (1/10 dilution in purified water) and 2 mL of 7.5% Na_2CO_3 (w/v in purified water). The mixture was incubated at 45 ℃ for 15 min. The absorbance was measured at 765 nm. Na_2CO_3 solution (2 mL of 7.5% Na_2CO_3 in 2.55 mL of distilled water) was used as blank. The results were expressed as gallic acid equivalence in μg[12].

2.5. Antioxidant assays

2.5.1. Reducing power

The reducing power measurement was performed according to the method of Oyaizu[13] with few modifications. The crude extracts of marine actinobacteria were diluted in Milli Q water to make 10, 20, 40, 60, 80 and 100 μg/mL dilutions. One milliliters of each dilution was mixed with 0.1 mL of 1% potassium ferricyanide and mixed thoroughly. The mixture was incubated at 50 ℃ for 30 min. After incubation, it was supplemented with 0.1 mL of 1% trichloroacetic acid and 0.1% $FeCl_3$. Incubate for 20 mins. Absorbance was measured at 700 nm using UV-Vis spectrophotometer. The higher absorbance of the reaction mixture was directly proportional to higher reducing power.

2.5.2. DPPH radical scavenging activity

The DPPH radical scavenging activity was performed according to the method of Priya *et al*[14] with few modifications. The crude extracts of marine actinobacteria were diluted in Milli Q water to make 10, 20, 40, 60, 80 and 100 μg/mL dilutions. Two millilitres of each dilution was mixed with 1 mL of DPPH solution (0.2 mM/mL) and mixed thoroughly. The mixture was incubated in dark at 20 ℃ for 40 min. Absorbance was measured at 517 nm using UV-Vis spectrophotometer with methanol as blank. Gallic acid was used as positive control. The percentage scavenging of DPPH by the extracts was calculated according to the following formula:

% DPPH Radical scavenging=[(Ac − At) / Ac] × 100

Here

Ac is the absorbance of the control (DPPH)

At is the absorbance of test sample.

2.5.3. Ferric reducing antioxidant power assay (FRAP)

The FRAP assay was performed according to Benzie and Strain[15] with some modifications. The stock solutions included 300 mM acetate buffer, pH 3.6, 10mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3$. $6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3.6H_2O$ solution and then warmed at 37 ℃ before using. Crude extract of marine actionbacteria (150 μL) were allowed to react with 2850 μL of the FRAP solution for 30 min in the dark condition. Readings of the colored product [ferrous tripyridyltriazine complex] were then taken at 593 nm. The standard curve was linear between 10 and 100 μg. Results are expressed in mM TE/ g fresh mass. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

2.5.4. Metal chelating activity

The chelating activity of the extract for ferrous ions was measured according to Dinis *et al*[16]. The reaction mixture containing 0.5 mL of extract, 1.6 mL of deionized water, 0.05 mL of $FeCl_2$ (2 mM) and 0.1 mL of ferrozine (5 mM) was incubated at 40 ℃ for 10 min and the absorbance measured at 562 nm. The chelating activity was calculated as

% Chelating Activity $=[(A_1 - A_2) / A_0] \times 100$

Where A_0 represents the absorbance of the control (without extract) A_1 represents the absorbance of reaction mixture, and A_2 represents the absorbance without $FeCl_2$.

2.5.5. β -Carotene bleaching assay

The β -Carotene bleaching assay was performed according to the method of Graven *et al*[17] with few modifications. Linoleic acid solution (10 mL of 2 mg/mL solution in EA) and β -carotene solution (10 mL, 2 mg/mL solution in acetone) were added to the molten agar (10 mL, 4% solution in boiling water). The mixture was then shaken to give an orange color. The agar was then poured into Petri dishes (25 mL per dish, diameter 9 cm) and was kept in dark and left standing to allow the agar to set. Holes (4-mm diameter) were then punched into the agar, extract (1 mg) each in DMSO were transferred into the holes, and the Petri dishes were then incubated at 45 ℃ for 4 h. A zone of color retention around the hole after incubation indicated sample with antioxidant activities. The zone diameter was also measured.

2.5.6. Anti-lipid peroxidation assay

The anti-lipid peroxidation of the goat liver homogenate was prepared according to the method used by Mandal and Chaterjee[18] after slight modification. Different concentrations of marine actinobacteria extracts were mixed with 2.8 mL of 10% goat liver homogenate and 0.1 mL of 50 mM $FeSO_4$. It is incubated for 30 min at 37 ℃. After incubation, 1mL of this extracts was taken with 2 mL of 10% TCA-0.67% TBA in acetic acid (50%) followed by boiling for 1 h at 100 ℃ and subsequent centrifugation at 10 000 rpm for 5 min. Absorbance of the test was recorded at 535 nm against the reagent blank. The control was without the extracts and $FeSO_4$. and induced without the extracts. ALP percentage was calculated using the following formula

%ALP = absorbance of Fe^{2+} induced peroxidation - absorbance of sample/ absorbance of Fe^{2+} induced peroxidation - absorbance of control X 100

2.5.7. Nitric oxide (NO) scavenging activity

Nitric oxide production from sodium nitroprusside was measured according to Kang *et al*.[19]. An equal amount (6 mL) of sodium nitroprusside (5 mM) solution was mixed with 6 mL of extract and incubated at 25 ℃ for 180 min. After every 30 min, 0.5 mL of the reaction mixture was mixed with an equal amount of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% napthylethylene diamine dihydrochloride), and absorbance was taken at 546 nm and compared with absorbance of 1 mg/mL of standard solution (sodium nitrite) treated in the same way with Griess reagent.

2.6. DNA damage inhibition efficiency

Potential DNA damage inhibition by marine actinobacteria extracts was tested by photolysing H_2O_2 by UV radiation in presence of pBR322 plasmid DNA and performing agarose gel electrophoresis with the irradiated DNA[20]. 1 μL aliquots of pBR322 (200 μg/mL) were taken in three polyethylene microcentrifuge tubes. 50 μg of each extract was separately added to two tubes. The remaining tube was left untreated as the irradiated control (CR). 4 μL of 3% H_2O_2 was added to all the tubes which were then placed directly on the surface of a UV transilluminator (300 nm). The samples were irradiated for 10 min at room temperature. After irradiation, 4 μL of tracking dye (0.25% bromophenol blue, 0.25% xylene cyanol FF and 30% glycerol) was added. The samples in all tubes were then analyzed by gel electrophoresis on a 1% agarose gel in TBE buffer (pH 8). Untreated non-irradiated pBR322 plasmid (C) was run along with the extract-treated UV-irradiated samples (methanolic extract treated = SM and aqueous extract treated = SA) and untreated UV-irradiated (CR) plasmid DNA. The gel was stained in ethidium bromide (1 μg/mL; 30 min) and photographed on Lourmat Gel Imaging System (Vilbar, France).

2.7. Determination of phenolic compounds: HPLC analysis

HPLC analysis was performed using a Waters 2487 HPLC system consisting of a dual λ detector and a Waters 1525 binary pump, and equipped with a Waters Symmetry® C18 column (5 μm, 4.6 mm × 150 mm) with Waters Sentry™ universal guard column (5 μm, 4.6 mm × 20 mm) (Waters Corporation, Milford, MA, USA). Phenolic compounds in the marine actinobacteria were analyzed using the phenolics reference standard for HPLC[21]. Gradient elution was performed at 35 ℃ with Solution A (50 mM sodium phosphate in 10% methanol; pH 3.3) and Solution B (70% methanol) in the following gradient elution program: 0-15 min - 100% of Solution A; 15-45 min - 70% of Solution A; 45-65 min - 65% of Solution A; 65-70 min - 60% of Solution A; 70-95 min - 50% of Solution A; 95-100 min - 0% of Solution A. Flow rate was 1 mL/min and injection volume was 20 μL (of 10 mg/mL extract solution). Detection was monitored

at diverse wavelengths (around λ_{max}) for various phenolic compounds, i.e., 250 nm for benzoic acids, isoflavones and most anthraquinones; 280 nm for some flavones, flavanones, catechins, theaflavins and some anthraquinones; 320 nm for cinnamic acids, most flavones and chalcones; 370 nm for flavonols; 510 nm for anthocyanins.

2.8. Brine shrimp bioassays

2.8.1. Brine shrimp hatchability test

The brine shrimp hatchability test is based on Migliore *et al.*[22] with some modification. The brine shrimp (Artemia salina) eggs or cysts were hatched in sterile seawater (1 g cyst per liter) at 28 ℃, under conditions of continuous lighting and strong aeration. The different concentration of marine actinobacteria extract (250, 500, 750, 1 000 μg/mL) was added along with eggs. The number of free nauplii in each treatment was calculated.

2.8.2. Cytotoxicity bioassay

The brine shrimp lethality assay was conducted according to Amat[23] with minor modifications to investigate the cytotoxicity of marine actinobacteria. The brine shrimp (Artemia salina) eggs or cysts were hatched in sterile seawater (1 g cyst per liter) at 28 ℃, under conditions of continuous lighting and strong aeration. Approximately 12 h after hatching the nauplii were collected with a pipette from the lighted side and concentrated in a test tube. Ten brine shrimp were transferred to each test tube containing 4.5 mL of sea water using adequate pipettes. Each test consisted of exposing groups of 10 Artemia aged 12 h to 0.5 mL of various concentrations (20, 40, 60, 80, 100 μg) of the marine actinobacterial extracts. The toxicity was determined after 12 h (mainly nauplii in instar I/II), 24 h (nauplii in instar II/III) and 48 h (mainly nauplii in instar III/IV) of exposure. The numbers of survivors were counted and percentage of deaths was calculated.

2.9. Statistical analysis

All analyses were performed in triplicate. Data are presented as mean±standard deviation (SD). One-way ANOVA were performed to determine the significance of differences between the groups at $P < 0.05$. Graphpad Prism v.5.00 (La Jolla, CA, USA) and Microsoft Excel 2007 (Roselle, IL, USA) were used for statistical and graphical evaluations.

3. Results

3.1. Optimization of media

Among the 3 *Streptomyces* sps tested for antioxidant activity, LK-3 strain showed very good antioxidant activity. ANOVA Analysis for response surface quadratic model gives the following equation. pH, temperature, glucose , casein and NaCl are indicated as A, B, C, D and E.

$$R1 = +85.95 - 0.69^{*}A + 2.17^{*}B + 1.49^{*}C - 0.85^{*}D - 4.712E-003^{*}E - 3.62^{*}A^{*}B - 0.40^{*}A^{*}C - 3.13^{*}A^{*}D + 1.48^{*}A^{*}E + 4.43^{*}B^{*}C - 4.18^{*}B^{*}D - 7.84^{*}B^{*}E + 1.09^{*}C^{*}D - 2.99^{*}C^{*}E + 8.77^{*}D^{*}E - 32.65^{*}A_2 + 6.62^{*}B_2 - 9.55^{*}C_2 - 6.35^{*}D_2 - 1.09^{*}E_2$$

ANOVA for the response surface Quadratic Model was shown in Table 1. The F value of 3.02 implies the model is significant. There is only a 3.78% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant. In this case DE, A2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The "Lack of Fit F-value" of 0.53 implies the Lack of Fit is not significant relative to the pure error. Non-significant lack of fit is good. In the present work, all the linear, interactive effects of AB, AC, AD, AE and DE were significant for antioxidant activity. The coefficient of determination (R2) for antioxidant activity was calculated as 0.8581. Figure 1 shows that the actual response values agree well with the predicted response values. The interaction effects of independent variables on antioxidant were studied by plotting 3D surface curves. The 3D curves of the calculated antioxidant activity for the interactions between the variables are shown in Figures 2(a)-2(e). The optimized values of the variables for antioxidant production were as follows: pH - 7.49, glucose - 2.0%, casein - 0.13%, temperature -30.25 ℃, and NaCl - 0.3%.

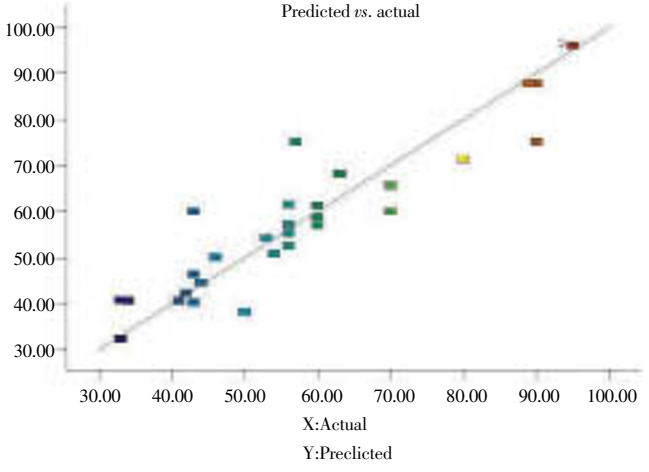

Figure 1. Predicted response versus actual value.

3.2. Total phenolic content and reducing power

Total phenolic content of the *Streptomyces* sp extract was expressed as gallic acid exultance (GAE) in μg (Figure 3a). The total phenolic compound found to be 22 μg GAE/g dry weights. Due to electron donating ability of the extract, it can serve as a significant indicator of potential antioxidant activity. In this assay, the green and blue colour represents the reducing power of test compound. Figure 3(b) represents the reductive capabilities of the *Streptomyces* sp LK3 extract and highest reducing power was found to be 1 000 μg.

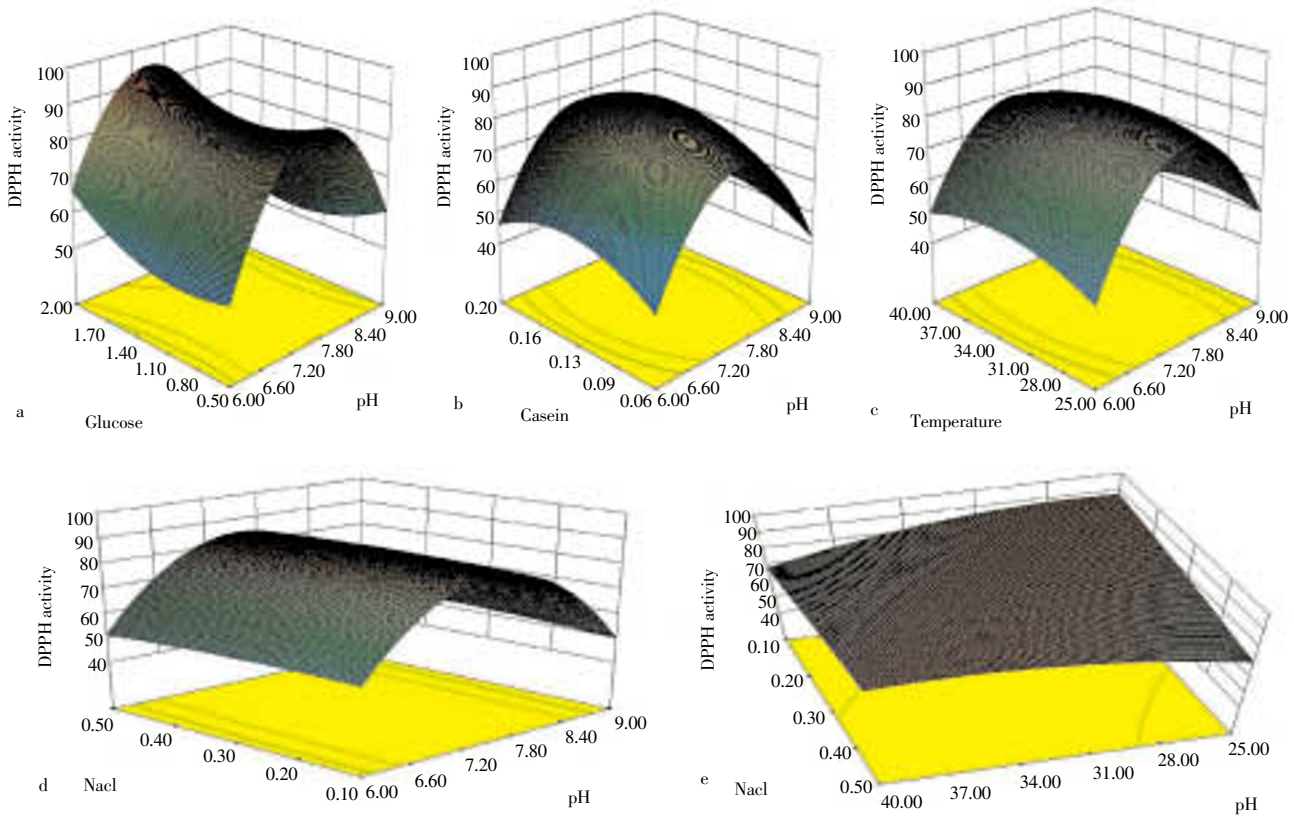

Figure 2. Effects of (a) pH and glucose, (b) pH and casein, (c) pH and temperature (d) pH and NaCl, (e) NaCl and temperature and their interactive effect on the DPPH activity.

3.3. DPPH Scavenging activity

DPPH is the reduction of purple colored DPPH in the presence of hydrogen donating antioxidants, by the formation of yellow colored non radical form of DPPH. The scavenging activity was increased when increasing concentrations of extract and the IC_{50} values was found to be 41.09 μg/mL. These results indicated that less concentration of marine actinobacteria extract exhibit the ability to reduce the DPPH radical, which indicated that extract was good antioxidant activity (Figure 3c).

3.4. NO scavenging activity

Dissolved oxygen and water was contact with NO, it is converting into nitric and nitrous acids and the end product reacts with Griess reagent, to form a purple azo dye. In presence of antioxidants, the colour intensity will reduce. The marine actinobacteria extract showed a concentration dependent elevation in NO scavenging activity and the best activity was seen at a high dose of 200 μg/mL where 46.33% scavenging was observed and the IC_{50} values was found to be 70.71 μg/mL (Figure 3d).

3.5. FRAP assay

The FRAP assay, TPTZ is used as an oxidant and it is working on the electron-transfer reactions. Results of the FRAP assay showed that the marine actinobacteria extract was a low Fe^{3+}-reductant activity at a high dose of 200 μg/mL where 11.60% scavenging was observed. The extracts showed considerably lesser antioxidant potential in FRAP assay in comparison to both DPPH and metal chelating activity.

3.6. Metal chelating activity and LPI assay

The antioxidant from marine actinobacteria was interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before it react with ferrozine. IC_{50} of the extract for chelating activity was 70.71 μg/mL (Figure 3e). which is similar to NO scavenging. During lipid peroxidation, malondialdehyde (MDA) will release and it is react with thiobarbituric acid at low pH and a pink chromogen (TBA-MDA adduct) was formed. The best activity was seen at a high dose of 200 μg/mL where 79.50% scavenging was observed and the IC_{50} values was found to be 88.15 μg/mL (Figure 3f).

3.7. β-Carotene assay

The antioxidant activity of the marine actinobacteria extracts were measured by the β-Carotene/linoleic acid model system. The zone of color retention was 16 mm, which was close to the value of positive control ascorbic acid (17 mm) and the order was 25 μg (8 mm)>50 μg (8 mm) >100 μg (14 mm) >200 μg (16 mm).

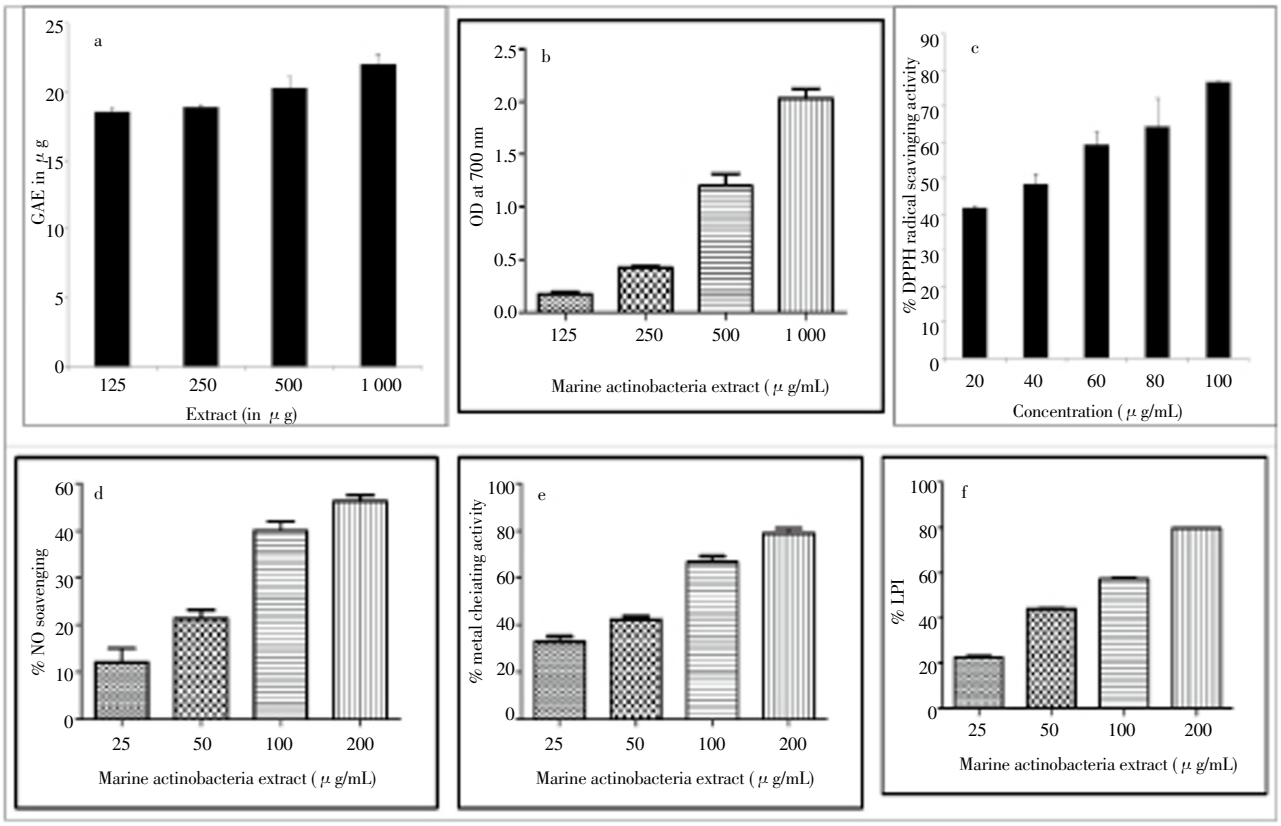

Figure 3. Antioxidant results (a) total phenolic content, (b) reducing power assay, (c) DPPH scavenging assay, (d) NO scavenging assay, (e) Metal chelating activity, (d) LPI activity

3.8. DNA damage inhibition efficiency

As represented in Figure 3, the electrophoretic pattern of pBR322 DNA with/without marine actinobacteria extracts following UV-photolysis of H_2O_2. The positive control pBR322 showed 2 bands in agarose gel electrophoresis and the negative control did not show any bands. The marine actinobacteria extract with pBR322 DNA exhibited same banding patterns of positive control, inferring that antioxidant from marine actinobacteria protect the DNA against oxidative stress (Figure 4).

3.9. Brine shrimp bioassays

The percentage of toxicity was detected at 12 h and 24 h of exposure to marine actinobacteria extract and no significant changes in hatchability of Artemia embryos up to 1 000 μg. It is highly sensitive to toxins at the early developmental stages. Hence, *Streptomyces* sp LK3 extract doesn't shows any toxicity. The brine shrimp lethality test was conducted on marine actinobacteria extracts at ten concentrations, 10 μg/mL-100 μg/mL. The marine actinobacteria was showed high lethality at 10 mins. The test compound was show shrimp death of 50% or more considered to be highly toxic. The 30 μg/mL of extract was showed 50% lethality and LC_{50} values was found to be 31.17 μg/mL; r^2=0.872 against brine shrimp. Results with $P<0.05$ were considered to be statistically significant.

Figure 4. Effect of marine actinobacteria extracts on the protection of plasmid DNA (pBR322) against oxidative damage caused by UV-photolysed H_2O_2.
Positive control=untreated non-irradiated DNA (PC), Negative control=untreated UV-irradiated DNA (NC) and *Streptomyces* LK3 extract (LK3)

3.10. HPLC analysis for phenolic compounds

Due to the diversity of natural compounds from marine actinobacteria, it is very difficult to identify them. Hence, HPLC analysis was performed to identify the different phenolic compound and it was compared with the reference retention times as reported earlier. The major components in the *Streptomyces* sp LK-3 extract was identified as daidzein-8-C-glucoside (puerarin), (-) gallocatechin gallate, sesamol, cyanidin-3-O-rutinoside, delphinidin (3,5,7,3′,4′,5′-OH) (Table 2).

Table 2

Phenolic compounds identified in extract of *Streptomyces* sp LK-3 by HPLC.

Compounds	λ [a] (nm)	Et_R [b] (min)	Rt_R [c] (min)
Daidzein-8-C-glucoside (puerarin)	250	21.359	20.1
(-)-gallocatechin gallate	280	21.149	19.7
Sesamol	280	21.149	19.2
Cyanidin-3-O-rutinoside	510	17.201	17.4
Delphinidin (3,5,7,3′,4′,5′-OH)	510	21.672	28.2

[a] Wavelenghth for determination

[b] Experimental retention times

[c] Reference retention times

4. Discussion

The last few years have seen an increasing amount of knowledge about the important role of free oxygen radicals in various diseases. These pathological and clinical backgrounds have prompted to investigate novel and potent antioxidant compounds from marine actinobacteria which are ultimately of therapeutic use. Marine actinobacteria, *Streptomyces* sp LK-3 showed potential antioxidant activity against all the antioxidant studies. The 16s rDNA sequencing is a method of choice for tracing bacterial phylogeny and definite the taxonomy[24]. Hence the potential strain was identified as *Streptomyces* LK-3 (JF710608) through 16S rDNA Sequencing. It shows only 93% similarity with *Streptomyces* mutabilis.

The statistical experimental designs have wide application in biotechnology field. Many scientists have reported using RSM technique, satisfactory optimization of antioxidant production from microbial sources[25,26]. In the present study, RSM was shown to be efficient for the optimization of the antioxidant production.The regression coefficient value was larger with significant p-value indicates a more significant effect on the respective response variables[27]. In the vision of pharmaceutical industry utility of the antioxidant, a cost effective media formulation becomes a primary concern[28]. After Optimization, antioxidant activity of *Streptomyces* LK-3 was increased and the initial medium was very cheap cost.

There are only few reports are available on antioxidant activity of marine actinobacteria. Marine *Streptomyces* sp VITTK3 possess significant DPPH free radical scavenging at 5 mg/mL (96%)[29]. The compound 5-(2, 4-dimethylbenzyl) pyrrolidin-2-one (DMBPO) from marine *Streptomyces* VITSVK5 spp. showed 59.32% DPPH scavenging activity and it showed cytotoxic to cancer cells. It showed fewer chromosomal aberrations when compare to control[30]. Two phenolic compounds from *Streptomyces* sp, JBIR-94 and JBIR-125 showed DPPH scavenging activity with an IC50 value of 11.4 and 35.1 μM, respectively[31]. Likewise, *Streptomyces* sp LK-3 possesses significant DPPH free radical scavenging at 100 μg/mL (76%).

Understanding the roles of various antioxidants and their action to various diseases is challenging because it can act as a several mechanisms like donating hydrogen to radicals, reducing power, free radical scavenging activity, metal chelating ability, inhibition of β-carotene bleaching and quenching singlet oxygen[32]. To estimate multifaceted antioxidants, the use of one method is not enough. Hence we used several antioxidant tests to confirm the multifaceted antioxidants potential of *Streptomyces* LK-3 extract. Four radical-scavenging assays showed significant in Tukey's multiple comparison test at $P<0.05$. Coefficient of determination (R2) values for DPPH, LPI, Metal chelating activity and NO activity are 0.99, 0.99, 0.97 and 0.97 respectively.

Carballo *et al* suggested brine shrimp lethality bioassays can be used to test natural marine products for pharmacological activity[33,34]. Likewise, in the present study we suggest using brine shrimp lethality bioassays to test marine actinobacteria for cytotoxicity activity. This bioassay is easy, effective, and rapid way to study the cytotoxicity.

In conclusion, we have designed production media for antioxidant compound, produced by a novel *Streptomyces* sp LK-3 using RSM. It showed potential antioxidant activity in all antioxidant tests and short time showing cytotoxicity activity in brine shrimp assay. Brine shrimp lethality test can be used for rapid screening of cytotoxic compounds from marine actinobacteria. Results conclude that the isolate is a prominent producer of antioxidant and in future, this antioxidant compound can be used for anticancer treatment.

Conflict of interest statement

We declare that we have no conflict of interest.

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