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Original article

In vitro biological properties of *Streptomyces cangkringensis* isolated from the floral rhizosphere regions

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ABSTRACT

This context was investigated to determine *in vitro* antimicrobial, antioxidative, and anticancer traits of crude ethyl acetate extract of *Streptomyces cangkringensis* strain TSAS 04 isolated from soil sample of rhizosphere regions. The antimicrobial activity of ethyl acetate extract of strain TSAS 04 was determined against indicator pathogens using disc diffusion assay which exhibited maximum zones of inhibition of 20.6 ± 0.3 and 16.3 ± 0.6 mm against *Bacillus subtilis* and *Trichoderma viride*, respectively. *In vitro* antioxidant properties of the crude ethyl acetate extract were performed using standard methodologies. The extract revealed maximum $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ radical scavenging activities of 51.1 ± 0.39 and 81.25 ± 0.33%, respectively. Likewise, maximum phosphomolybdenum reduction and Fe^{3+} reduction of the crude ethyl acetate extract of strain TSAS 04 were estimated 76.18 ± 0.10 and 89.01 ± 0.44%, respectively. *In vitro* anticancer trait of the extract was determined against HeLa cell line using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay which showed anticancer activities in a dose dependent manner with an IC_{50} value of 410.5 μg/mL. Fourier transform infrared spectroscopy (FT-IR) and Gas chromatography–mass spectrometry (GC-MS) analyses indicated the presence of distinct functional groups and bioactive components in the extract, respectively. In conclusion, *S. cangkringensis* strain TSAS 04 showed its effectiveness as ideal bioactive agent by exhibiting substantial antimicrobial, antioxidant, and anticancer properties.

1. Introduction

Soil microflora is pivotal producers of bioactive metabolites, revealing colossal industrial and pharmaceutical applications (Arasu et al., 2013; Khusro and Sankari, 2015; Al-Dhabi et al., 2016; Aarti et al., 2018; Al-Dhabi et al., 2018a; Al-Dhabi et al., 2018b). *Streptomyces* spp. (Phylum - Actinobacteria) belong to complex gram positive bacteria with high Guanine and Cytosine content (Sivalingam et al., 2019). Currently, there are about 800 known species of *Streptomyces* and most of the streptomycetes live as saprophytes in the soil (Li et al., 2019). They have the unique potentialities to adapt in varied environmental conditions due to their complex developmental life cycle (Ser et al., 2017). The genus holds notorious position among soil bacteria and is known to secrete plethora of secondary metabolites as ideal therapeutic agents. In general, approximately 40% of all microbes' associated secondary metabolites correspond to *Streptomyces* spp. (Bérdy, 2012).

Over the past few decades, *Streptomyces* spp. have been identified as promising antimicrobial, anticancer, antiprotozoal, antidiabetic, anthelmintic, antiviral, and anticancer agents (Al-Dhabi et al., 2019a; Al-Dhabi et al., 2019b; Al-Dhabi et al., 2019c; Ser et al., 2017). Interestingly, approximately 60–70% of antibiotics

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discovered so far are associated with actinobacteria (Subramani and Aalbersberg, 2012; Arokiyaraj et al., 2015; Valsalam et al., 2019). Bioactive metabolites from *Streptomyces* spp. occupy the enviable pharmacokinetic traits which can be used for diversified medicinal applications (Abd-Elnaby et al., 2016). Additionally, actinobacteria have shown potentialities not only to improve biological properties of soils and contribute in nitrogen fixation but also degrade various hydrocarbons in order to maintain the cleaner ecosystem (Anandan et al., 2016).

Despite the discovery of diversiform bioactive constituents from actinobacteria from several years, the genus *Streptomyces* still remains a research hub for scientific communities worldwide. Hence, paramount efforts are being undertaken for harnessing the potency of streptomycetes, particularly as ideal therapeutic agents with maximum efficiency. In order to fill the gap of actinobacteria research, this investigation was initially focussed to isolate potential strain of *Streptomyces* sp. from soil samples of rhizosphere regions of plants and determine further the antimicrobial, antioxidant, and anticancer characteristics of its solvent extract. The presence of distinct bioactive components in the extract was further analyzed using varied analytical assays.

2. Materials and methods

2.1. Samples used

Soil samples were collected from the rhizosphere regions of *Ocimum tenuiflorum* and *Hibiscus rosasinensis* from inner depth regions of Chennai, Tamil Nadu, India in sterile plastic bags during March 2019. The collected soil samples were transferred to sterile petriplate and air-dried at 37^{0}C for three days with standard microbiological methods.

2.2. Isolation of terrestrial actinomycetes

Pre-treatment process was carried out for the air-dried soil in order to reduce bacterial population. Calcium carbonate (0.1% w/v) was mixed with the soil and agitated gently to ensure proper mixing. The pre-treated soil was kept undisturbed for three days and the soil was agitated at regular interval of time. Starch casein agar (SCA) medium (HiMedia) was preferred for isolating actinomycetes from the terrestrial soil. Serial dilution (10^{-1} to 10^{-10}), followed by spread plate method was performed for the isolation process. The medium was supplemented with nystatin to prevent bacterial and fungal contaminants. Inoculated plates were incubated at 37^{0}C for 7–12 days. The selected actinomycetial colonies were purified by streak plate (quadrant) method and stored at 4 °C on SCA slants for further investigation.

2.3. Preliminary characterization of selected isolates

Colony morphology of the purified actinomycetial isolates on SCA medium was recorded with respect to the colour of aerial spore mass, size and nature of the colonies, colour on the reverse side, and diffusible pigmentation. Starch casein agar medium was prepared and 3 to 4 sterile cover slips were inserted at an angle of 45^0. Purified actinomycete culture plates were carefully streaked on the surface of cover slip without any damage. Plates were incubated at 28 ± 2 °C for 4–7 days. Cover slips were removed at 2–3 days of interval and observed under the high power magnification. Structure and arrangement of conidiospores and arthospores on aerial and substrate mycelia of all the isolates were observed and classified according to the sub-types such as rectiflexibiles, straight to flexuous spore chain, and retinaculiaperti or spirals.

2.4. Production and extraction of secondary metabolites

Four actinomycetes isolates (TSAS 02, TSAS 04, TSAS 09, and TSAS 18) were selected based on the promising growth and then cultured freshly in yeast malt extract broth (or) International Streptomyces Project (ISP-2) medium aseptically. The inoculated broth of each of the isolate was subjected to fermentation process at 37 °C for 10 days at 150 rpm resulting in complete growth of the culture. The fermented broth was filtered and centrifuged at 8500 rpm for 20 min. Equal volume of mid polar solvent ethyl acetate was added along with the supernatant in 1:1 ratio. The supernatant-solvent combination was kept in rotary shaker undisturbed overnight. The organic solvent phase was separated and evaporated to dryness and the crude ethyl acetate extract of the selected isolates (selection was based on the substrate utilization, fast growers, and adaptation) were dissolved in methanol (Sambamurthy and Ellaiah, 1974) and further evaluated for pharmacological properties.

2.5. In vitro antimicrobial activities

2.5.1. Test microorganisms

Human pathogenic bacteria of representative groups such as Gram positive bacteria (*Staphylococcus aureus* MTCC 96, *Micrococcus luteus* MTCC 1538, and *Bacillus subtilis* MTCC 441), Gram negative bacteria (*Escherichia coli* MTCC 443, *Proteus vulgaris* MTCC 426, and *Shigella flexneri* MTCC 1457), and fungi (*Candida albicans* MTCC 227 and *Trichoderma viride* MTCC 800) were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh for the antimicrobial studies. Bacteria were sub-cultured on nutrient broth medium (g/L: peptone 10.0, beef extract 10.0, sodium chloride 5.0, and pH 7.2), while fungi were grown on potato dextrose broth medium (g/L; potatoes, infusion from 200.0, dextrose 20, and pH 5.2) for further experimental purposes.

2.5.2. Primary screening of terrestrial actinomycetes

Antagonistic activities of actinomycetes (TSAS 02, TSAS 04, TSAS 09, and TSAS 18) isolates were tested by perpendicular cross streak method. Single streak of 4–6 mm diameter of actinomycetes was streaked on the surface of the combined medium [equal volume of SCA and nutrient agar medium (g/L: peptone 10.0, beef extract 10.0, sodium chloride 5.0, agar 18.0, and pH 7.2) for bacteria and potato dextrose agar medium (g/L; potatoes, infusion from 200.0, dextrose 20, agar 18.0, and pH 5.2) for fungi] and incubated at 28 °C for 5 days. Fresh cultures of test microorganisms (bacteria and fungi) were streaked perpendicular (for bacteria) and fungal discs were placed to the actinomycetes streak. These perpendicularly cross streak plates were then incubated at 28 °C for 48 h and the zone of inhibition (mm) was measured (Panwar and Saini, 2012).

2.5.3. Secondary screening of terrestrial actinomycetes

The crude ethyl acetate extract of actinomycetes (TSAS 02, TSAS 04, TSAS 09, and TSAS 18) isolates were tested for antimicrobial potential by agar disc diffusion method. After 24 h growth of bacterial cultures and 48 h of fungal cultures, the microbial suspensions were swabbed on solidified Mueller Hinton Agar (HiMedia) plates using sterilized cotton swabs. The crude ethyl acetate extract (25 μL) of actinomycetes isolates were loaded on sterile discs (6 mm), transferred to the agar plates using sterile forceps, and plates were incubated at 37 °C for 24 h. Zone of inhibition (mm) was observed after 24 h. Azithromycin (for bacteria) and amphotericin (for fungi) were used as positive controls (Ravikumar et al., 2010).

2.6. Molecular identification of potent isolate

The potent isolate was cultured for 5–7 days on a sterile SCA slant, spore suspension was prepared, and then total genomic DNA was isolated. Polymerase chain reaction (PCR) amplification was carried out using primers (27F and 1492R) as per Macherey-Nagel PCR clean up kit. Finally, the 16S rDNA gene sequence of amplicon was sequenced by Sanger et al. (1977) and deposited in Genbank, National Centre for Biotechnology Information, and accession number was obtained.

2.7. Qualitative analysis of bioactive compounds

Qualitative analysis for the crude ethyl acetate extract of potent isolate was performed in order to find out the classes or nature of antioxidant compounds group such as phenols, alkaloids, terpenoids, steroids, and flavonoids (Harindran et al., 1999).

2.8. Phenols and flavonoids content determination

Total phenolic content of the crude ethyl acetate extract was determined following the methodology of Liu et al. (2007) and was estimated as gallic acid equivalent (GAE/mg of extract). Total flavonoid content of the crude ethyl acetate extract was assessed using aluminium chloride reagent method of Kefayati et al. (2017) and was estimated as quercetin equivalent (QE/mg of extract).

2.9. In vitro antioxidant activities

2.9.1. 1,1- diphenyl 2-picrylhydrazyl (DPPH·) degrading activity

The antioxidant activity of the crude ethyl acetate extract was measured based on the scavenging activity of the stable DPPH free radical (Khalaf et al., 2008). One millilitre of 0.1 mM DPPH solution in ethanol was added into crude ethyl acetate extract (50–300 μg/mL) and kept in dark for 30 min. Reduction in optical density was determined spectrophotometrically at 517 nm using ascorbic acid as standard. The DPPH· radical inhibition was estimated as:

$$\%\ \text{DPPH·scavenging} = [(\text{Acontrol} - \text{Asample})\text{Acontrol}] \times 100$$

2.9.2. Dot-blot assay

To the pre-coated thin layer chromatography (TLC) plate (silica gel 60 F254; Merck), drops of 0.1 mM DPPH solution dissolved in methanol were loaded onto each column accordingly. The TLC plate was allowed to dry for 3–5 min. DPPH solution was stained in the first row of plate (control). The ethyl acetate extract at varied doses was loaded on DPPH stained spot. In the next row, ascorbic acid was loaded on the DPPH stained spot and considered standard. Purple colour with yellow or white spots on TLC plates where scavenging occurred was exhibited on stained silica gel plates. The vanishing effect of purple colour depends upon the amount and nature of antioxidants (active compounds) present in the extracted secondary metabolites (Soler-Rivas, 2000).

2.9.3. $ABTS^{\cdot+}$ radical cation degradation

The antioxidative properties of extracts were determined by degrading 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) radical cation scavenging activity as per the methodology of Arnao et al. (2001) using ascorbic acid as standard. $ABTS^{\cdot+}$ radical cation degradation was calculated as:

$$\%\ ABTS^{\cdot+}\ \text{radical cation scavenging} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

2.9.4. Phosphomolybdenum reduction activity

The total antioxidative properties of different concentrations (20–120 μg/mL) of crude ethyl acetate extract were assessed according to the method of Prieto et al. (1999) using ascorbic acid as standard. Mo^{6+} reduction was estimated as:

$$\%\ \text{Phosphomolybdenum reduction} = [(A_{sample} - A_{control})/A_{sample}] \times 100$$

2.9.5. Ferric (Fe^{3+}) reducing power activity

The reducing power of the crude ethyl acetate extract was determined following the method of Oyaizu (1986) using ascorbic acid as standard. Fe^{3+} reduction was calculated as:

$$Fe^{3+}\ \text{reduction}\ (\%) = [(A_{sample} - A_{control})/A_{sample}] \times 100$$

2.10. In vitro anticancer activity

HeLa cell line was obtained from National Centre for Cell Science, Pune, India. Cell lines were cultured in Dulbecco Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum, antibiotics. The cell culture was maintained at 37 °C in a humidified atmosphere of 5% carbon dioxide and allowed to grow to confluence over 24 h. With slight modifications, HeLa cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 h in 200 μL of DMEM with 10% FBS. Then culture supernatant was removed, DMEM containing varied doses of crude ethyl acetate extract (100–1000 μg/mL) was mixed, and incubated for 48 h. Cells were incubated with (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) MTT solution (20 μL, 5 mg/mL in PBS, pH-7.2) at 37 °C for 4 h and then with dimethyl sulphoxide at room temperature for 1 h (Mosmann, 1983). Absorbance was recorded at 570 nm on a scanning multi-well spectrophotometer and cancer cells inhibition (%) was determined for calculating the IC_{50} value.

2.11. Analytical assays

2.11.1. Thin layer chromatography

Thin layer chromatography analysis was carried out for the crude ethyl acetate extract on silica gel aluminium sheet (Merck Silica gel 60 F254) as per the methodology of Esther Lydia et al. (2019) and R_f values were calculated as mentioned below:

$$R_f\ \text{value} = \text{Distance travelled by the solute} / \text{Distancetravelledbythesolvent}$$

2.11.2. Fourier transform infrared spectrophotometer (FT-IR)

The FT-IR spectrum of crude ethyl acetate extract was obtained following the methodology of Khusro et al. (2014).

2.11.3. Gas chromatography-mass spectrometry (GC-MS)

The GC-MS chromatogram of crude ethyl acetate extract was obtained as per the methodology of Esther Lydia et al. (2019). Mass spectra of unknown constituents were compared with the spectra of known constituents available in the NIST library.

2.12. Statistical analyses

Experiments were performed in triplicates. All data were reported as mean ± standard deviation (SD) of three replicates.

3. Results

3.1. Preliminary characterization of isolates

Isolates were diverse in nature with respect to growth pattern, aerial and substrate hyphae, colony margin, and pigments production. Isolates produced a range of aerial mycelium and substrate mycelium such as peach, pale sandal, ash white, purple, and yellow. Colonies were powdery, fluffy, rubbery, and chalky in nature and the colony margin appeared to be webbed and depressed on top (Table 1). Majority of the isolated strains produced the earthy odour of geosmin, which is an indication of predominant antibiotic produced by *Streptomyces* group. The selected isolates formed straight to flexuous spore chain, spirals on aerial mycelium with smooth spore surface from the cover slip culture technique.

3.2. Primary screening by cross streak method

Primary screening of potent actinomycetes isolates (TSAS 02, TSAS 04, TSAS 09, and TSAS 18) was carried out by perpendicular cross streak method which showed no growth of the test organisms after 48 h adjacent to the streaking of actinomycetes isolates, thereby indicating antimicrobial activity of the isolates. Among four different isolates, isolate TSAS 04 exhibited broad spectrum activity against all the tested pathogens (Figure not shown).

3.3. Secondary screening by agar disc diffusion method

Among four actinomycetes isolates, isolate TSAS 04 showed promising inhibitory effect against the bacterial and fungal pathogens (Table 2). Isolate TSAS 04 exhibited potent antimicrobial activities against *B. subtilis* (20.6 ± 0.3 mm), *S. aureus* (19.6 ± 0.6 mm), *P. vulgaris* (18.3 ± 0.3 mm), *M. luteus* (17.3 ± 0.6 mm), *E. coli* (17.6 ± 0.3 mm), *S. flexneri* (16.6 ± 0.6 mm), *C. albicans* (15.3 ± 0.3 mm), and *T. viride* (16.3 ± 0.6 mm).

3.4. Identification of potent isolate

Isolate TSAS 04 showed pronounced antimicrobial activity against the bacterial and fungal pathogens. This isolate was further identified as *Streptomyces cangkringensis* strain TSAS 04 (Accession number: MT476863) based on 16S rDNA sequencing.

3.5. Qualitative analysis of bioactive compounds

Qualitative screening was detected to study the nature of the compound resulting in various reactions. Results revealed the availability of flavonoids, terpenoids, steroids, phenols, and tannins in ethyl acetate extract of strain TSAS 04. On the other hand, alkaloids, glycosides, and saponins were absent in the extract.

3.6. Quantitative estimations of total phenols and flavonoids

Total phenolic content in the extract was estimated as 418.26 ± 0.37 GAE/mg and total flavonoid content was calculated 44.98 ± 0.24 QE/mg for the crude ethyl acetate extract (Figure not shown).

3.7. Antioxidant activities

Crude ethyl acetate extract of strain TSAS 04 reduced the stable DPPH radical. The DPPH· degrading trait of the extract increased (17.31 ± 0.13 to 51.10 ± 0.39%) with increase in concentrations (Table 3). The IC_{50} value for the crude ethyl acetate extract was estimated 268.1 μg/mL with respect to ascorbic acid (54.50 μg/mL).

Dot-blot assay exhibited coloured spot formation in the presence of crude ethyl acetate extract. Purple zone on the plate represents lack of antioxidative trait while yellow zone is an indication for antioxidant property. Fig. 1 illustrated that ethyl acetate extract of strain TSAS 04 possessed potent antioxidant activity.

The $ABTS^{\cdot+}$ radical cation scavenging trait of extract increased at varied doses with inhibition rate of 25.52 ± 0.35 to 81.25 ± 0.33% (Table 4). The IC_{50} value for the crude ethyl acetate extract of strain TSAS 04 was estimated 16.3 μg/mL as compared to ascorbic acid (5.69 μg/mL).

Maximum phosphomolybdenum reduction of ethyl acetate extract of strain TSAS 04 was estimated 76.18 ± 0.1% with RC_{50} of 51.38 μg/mL (Table 5). Likewise, Fe^{3+} reduction ability of strain TSAS 04 increased in a dose dependent manner with maximum rate of 89.01 ± 0.44% (Table 5). RC_{50} value for extract was found to be 41.8 μg/mL with respect to the standard (21.19 μg/mL).

3.8. Anticancer activity

The extract of strain TSAS 04 at varied doses (100–1000 μg/mL) inhibited HeLa cells proliferation. Viabilities of cells were reduced with increases in the extract concentrations. Results depicted

Table 1
Characteristic features of actinomycetes isolates.

Isolates	Mycelium		Colony margin	Texture
	Aerial	Substrate		
TSAS01	White	Pale yellow	Depression on top of colony	Chalky
TSAS02	Peach	Light sandal	Webbed colonies	Powdery
TSAS03	Pale purple	Purple	Depression on top of colony	Powdery
TSAS04	Pale sandal	Sandal	Dew drops on the colony surface	Fluffy
TSAS05	Peach	Light sandal	Webbed colonies	Leathery
TSAS06	White	Pale yellow	Webbed colonies	Powdery
TSAS07	White	Pale yellow	Dew drops on the colony surface	Chalky
TSAS08	Whitish gray	Sandal	Dew drops on the colony surface	Powdery
TSAS09	White	Light sandal	Dew drops on the colony surface	Powdery
TSAS10	Light sandal	Yellow	Webbed colonies	Rubbery
TSAS11	Light sandal	Yellow	Webbed colonies	Fluffy
TSAS12	Pale yellow	Light sandal	Depression on top of colony	Powdery
TSAS13	Ash white	Sandal	Webbed colonies	Powdery
TSAS14	Whitish Fanta	Yellow	Webbed colonies	Leathery
TSAS15	Yellow	Yellow	Dew drops on the colony surface	Fluffy
TSAS16	White	Pale yellow	Dew drops on the colony surface	Powdery
TSAS17	White	Light sandal	Webbed colonies	Chalky
TSAS18	Purplish white	Dark purple	Depression on top of colony	Powdery

Table 2
Secondary screening for antimicrobial activities of isolates by agar disc diffusion method.

Bacterial pathogens	Zone of inhibition (mm)				
	TSAS 02	TSAS 04	TSAS 09	TSAS 18	Standard
S. aureus	14.3 ± 0.3	19.6 ± 0.6	17.3 ± 0.3	15.6 ± 0.3	23.3 ± 0.3
M. luteus	14.6 ± 0.3	17.3 ± 0.6	19.3 ± 0.3	18.3 ± 0.3	25.6 ± 0.6
B. subtilis	16.6 ± 0.6	20.6 ± 0.3	17.6 ± 0.6	19.3 ± 0.3	23.3 ± 0.3
E. coli	13.3 ± 0.3	17.6 ± 0.3	14.3 ± 0.6	18.6 ± 0.3	25.3 ± 0.3
P. vulgaris	13.6 ± 0.3	18.3 ± 0.3	13.3 ± 0.3	19.6 ± 0.6	22.6 ± 0.3
S. flexneri	20.3 ± 0.6	16.6 ± 0.6	19.6 ± 0.6	16.3 ± 0.3	21.3 ± 0.6
Fungal pathogens	**Zone of inhibition (mm)**				
	TSAS 02	**TSAS 04**	**TSAS 09**	**TSAS 18**	**Standard**
C. albicans	11.6 ± 0.3	15.3 ± 0.3	10.3 ± 0.3	13.3 ± 0.6	19.6 ± 0.3
T. viride	12.3 ± 0.3	16.3 ± 0.6	12.6 ± 0.3	14.6 ± 0.6	22.6 ± 0.6

Values represent mean ± SD.

Table 3
DPPH radical scavenging activity of ethyl acetate extract of strain TSAS 04.

S. No	Concentration (μg/mL)	Percentage of inhibition	
		Strain TSAS 04	Ascorbic acid
1	50	17.31 ± 0.13	45.87 ± 0.25
2	100	26.40 ± 0.34	53.24 ± 0.31
3	150	38.76 ± 0.42	56.82 ± 0.19
4	200	44.49 ± 0.16	74.04 ± 0.37
5	250	47.13 ± 0.27	84.19 ± 0.45
6	300	51.10 ± 0.39	86.22 ± 0.12

Values represent mean ± SD.

Table 4
$ABTS^{\cdot+}$ radical cation scavenging activity of crude ethyl acetate extract of strain TSAS 04.

S. No	Concentration (μg/mL)	Percentage of inhibition	
		Strain TSAS 04	Ascorbic acid
1	5	25.52 ± 0.35	43.92 ± 0.14
2	10	33.33 ± 0.16	54.37 ± 0.45
3	15	46.35 ± 0.48	59.91 ± 0.39
4	20	60.42 ± 0.20	75.03 ± 0.18
5	25	69.27 ± 0.41	84.77 ± 0.22
6	30	81.25 ± 0.33	86.10 ± 0.10

Values represent mean ± SD.

37.45 ± 0.36 and 71.01 ± 0.24% of cells death at the lowest (100 μg/mL) and the highest (1000 μg/mL) ranges of the extract, respectively (Fig. 2). IC_{50} value of the crude ethyl acetate extract was calculated as 410.5 μg/mL.

3.9. Analytical assays

In TLC, methanol:ethyl acetate (1:1 ratio) was the choice of solvent system for ethyl acetate extract which demonstrated R_f values of 0.46, 0.41, and 0.37 under short UV light (254 nm) and iodine vapours (Figures not shown). Ethyl acetate extract of strain TSAS 04 showed the presence of hydroxyl, aldehydes, and aromatics groups in the frequency of 1000 cm^{-1} to 3200 cm^{-1}. Strong absorption peaks of 2024 cm^{-1} and 2055 cm^{-1} represented N═C═S stretching of isothiocyanate. The absorption band at 2400 cm^{-1} was mainly due to the presence of H—C═O stretching, representing aldehyde. Strong and broad peaks at 2276 cm^{-1} was due to N═C═O stretching representing isocyanate. Strong absorption band at 1700 cm^{-1} indicated conjugated aldehyde and C═O stretching. The absorption band at 1455 cm^{-1} was indication for C—H bend and alkanes. Strong peak at 1091 cm^{-1} was due to the secondary alcohol and C—O stretching (Figure not shown). On the other hand, GC-MS analysis exhibited the presence of certain bioactive components in the ethyl acetate extract as described in Fig. 3 and Table 6.

4. Discussion

Development of active potent drugs such as antibiotics from actinomycetes and their derived products by pharmaceutical companies is recommended for the treatment of several microbial dis-

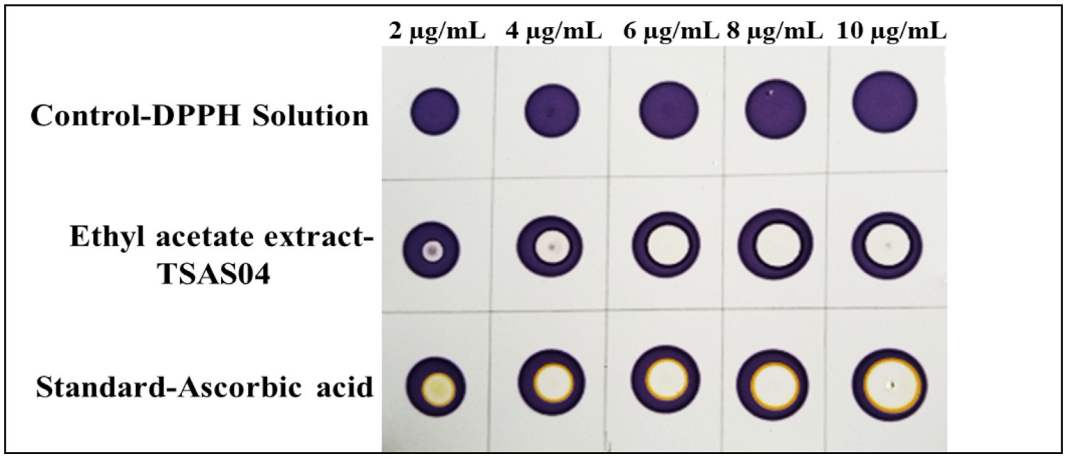


Fig. 1. Dot-blot assay for DPPH radical scavenging activity.

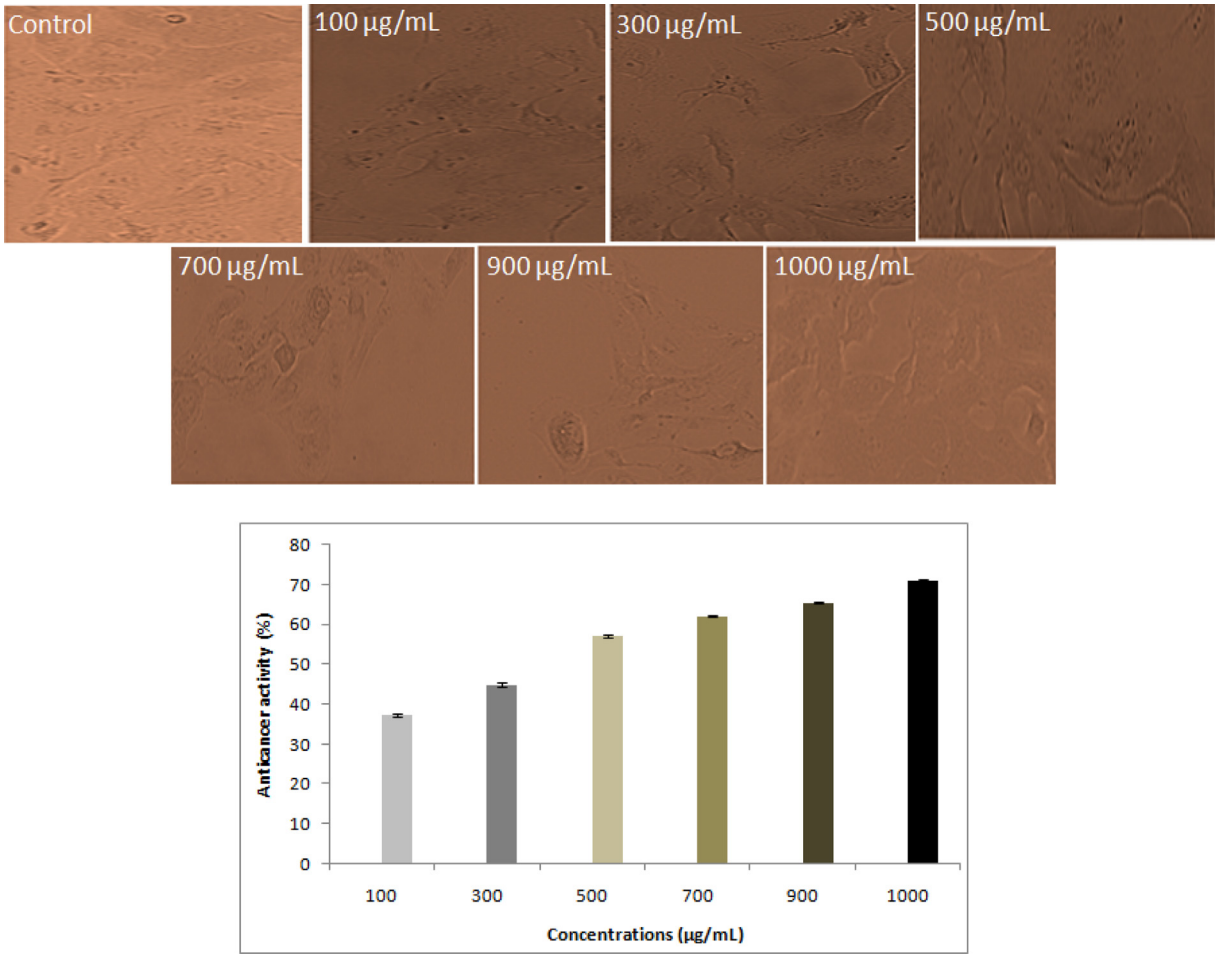


Fig. 2. Anticancer activity of ethyl acetate extract of strain TSAS 04 at various concentrations (100–1000 µg/mL) against HeLa cells.

eases (Kemung et al., 2020). In view of this, the present investigation entrenched the isolation of *S. cangkringensis* from floral rhizosphere regions and suggested its paramount role as biotherapeutic agents.

Cross streak method is the widely utilized primary screening method for determining the antagonistic role of bacteria (Palla et al., 2018). This method establishes foundation for secondary screening process. In this context, the preliminary screening of antibacterial activity of actinomycetes isolates by cross streak method revealed that strain TSAS 04 had potent antibacterial activity against tested pathogens. Furthermore, our findings were in complete agreement with the reports of Diekema et al. (2002) and Mayer et al. (2013) who demonstrated the antifungal activity of actinomycetes against *C. albicans*. The antifungal activities of strain TSAS 04 against selected fungi might be because of the availability of potential metabolites that were secreted during stationary phase of bacterial growth. Bioactive constituents such as flavonoids, steroids, and tannins are considered important secondary metabolites having significant role in their pharmacological properties (Sivakumar et al., 2011; Arasu et al., 2017; Arasu et al., 2019). In this context, the availability of bioconstituents in the crude ethyl acetate extract provided a preliminary effect for the active therapeutic applications.

The crude ethyl acetate extract of strain TSAS 04 demonstrated antioxidative trait by discolouring DPPH solution at varied concentrations. Findings exhibited promising antioxidant properties of extract, proving that active constituents are readily dissolved in the respective solvent. Also, the rapid radical scavenging effect by dot-blot using DPPH as the staining reagent for the crude ethyl acetate extract of strain TSAS 04 was highly acceptable to be the

Table 5
Phosphomolybdenum and Fe^{3+} reducing power activities of ethyl acetate extract of strain TSAS 04.

S. No	Concentration (µg/mL)	Percentage of reduction			
		Mo^{6+} reduction	Ascorbic acid	Fe^{3+} reduction	Ascorbic acid
1	20	39.64 ± 0.32	42.18 ± 0.18	35.32 ± 0.16	47.19 ± 0.28
2	40	49.13 ± 0.18	47.32 ± 0.23	54.52 ± 0.31	56.83 ± 0.34
3	60	52.88 ± 0.46	54.84 ± 0.40	59.01 ± 0.29	74.81 ± 0.13
4	80	54.43 ± 0.11	68.77 ± 0.13	68.17 ± 0.13	86.32 ± 0.25
5	100	65.32 ± 0.35	83.19 ± 0.34	77.04 ± 0.30	90.19 ± 0.43
6	120	76.18 ± 0.10	89.22 ± 0.11	89.01 ± 0.44	93.14 ± 0.16

Values represent mean ± SD.

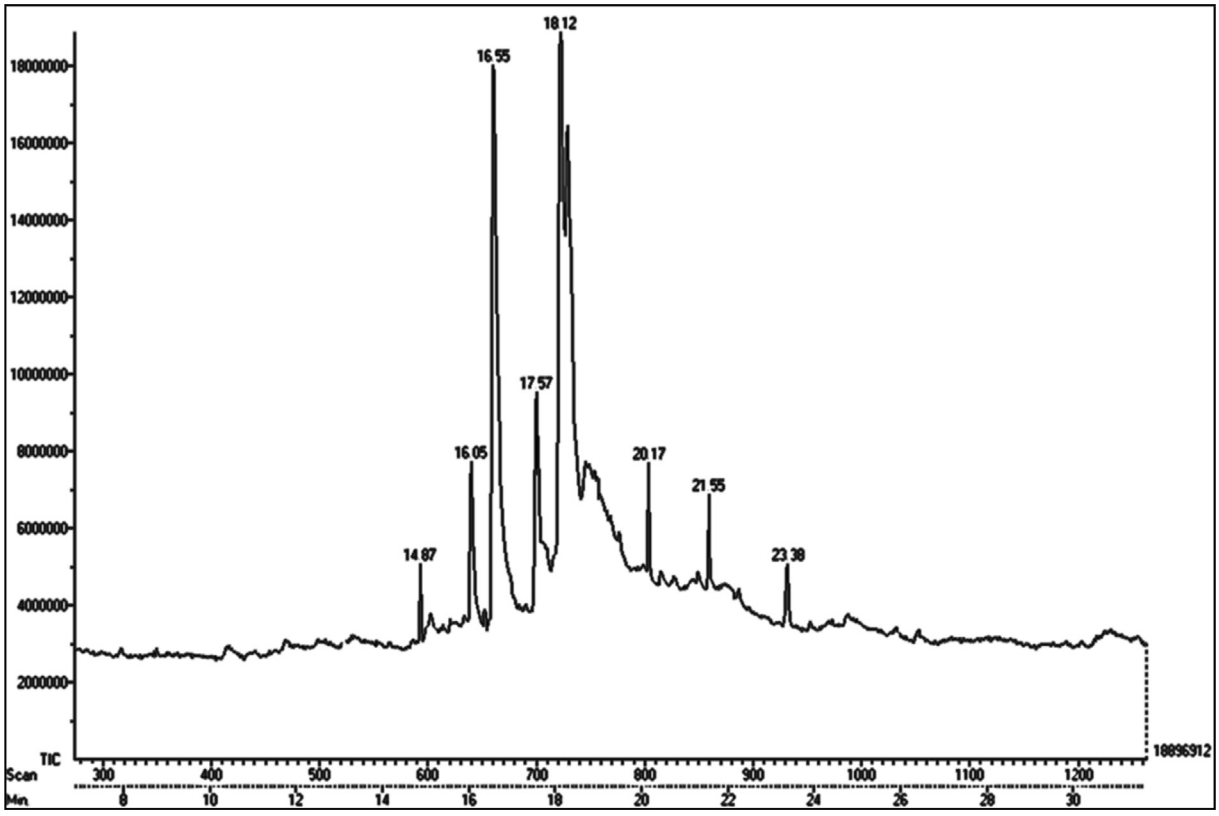


Fig. 3. GC-MS chromatogram of the crude ethyl acetate extract of strain TSAS 04.

potent antioxidant precursors for scavenging the free radicals. The present findings were more or less similar to Prashith Kekuda et al. (2015) who depicted promising antioxidant trait of actinomycetes extract. Findings of this context showed inhibition of various free radicals at varied concentrations, the crude ethyl acetate being the most potent. This study revealed a strong $ABTS^{\cdot+}$ radical cation

Table 6
Bioactive compounds and their structures as obtained from GC-MS spectrum of ethyl acetate extract.

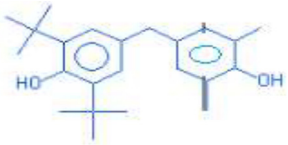
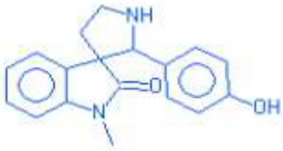
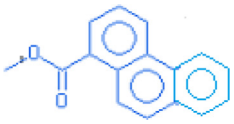
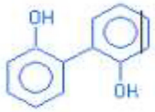
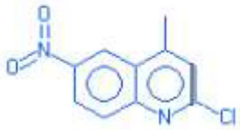
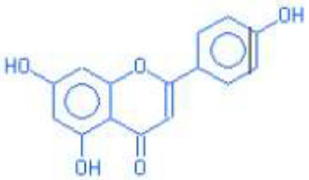
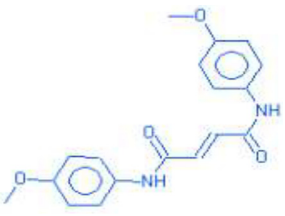
S. No	Compound name	Compound structure
1	(1-methylenebutyl)-benzene	
2	Phenol , 2,6-bis(1,1-dimethylethyl)-4-((4-hydroxy-3,5-dimethylphenyl) methyl)	
3	Indol-2(3H)-one,1-methyl-spiro-3,3′-(2-(4-hydroxyphenyl)pyrrolidine)	
4	Methyl 1-phenanthrenecarboxylate	

Table 6 (*continued*)

S. No	Compound name	Compound structure
5	(1,1′-biphenyl)-2,2′-diol	
6	Quinoline, 2-chloro-4-methyl-6-nitro	
7	4H-1-benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxyphenyl)	
8	But-2-endiamide, N,N′-bis(4-methoxyphenyl)	

scavenging effect too. Antioxidant activities of ethyl acetate extract were highly effective mainly because of the availability of phenols and flavonoids. On the other hand, roles of natural products as anticancer agents are of great demand nowadays (Latha et al., 2019). In this study, strain TSAS 04 extract at varied doses inhibited HeLa cells proliferation. Previous reports demonstrated pronounced role of actinobacteria as ideal anticancer agents (Lee et al., 2007; Özakin et al., 2016; Azman et al., 2017).

TLC profiling confirmed the presence of major active constituents in the crude ethyl acetate extract of strain TSAS 04 based on the solvent system and distinct Rf values. FT-IR spectrum of the extract confirmed the availability of distinct functional groups. GC-MS chromatogram of the crude ethyl acetate extract of the strain revealed the dominance of versatile compounds such as (1-methylenebutyl)-benzene; Phenol, 2,6-bis (1,1-dimethylethyl)-4-((4-hydroxy-3,5-dimethylphenyl)methyl); Indol-2(3H)-one,1-methyl-spiro-3,3′-(2-(4-hydroxyphenyl) pyrrolidine); Methyl-1-phenanthrenecarboxylate; (1,1′-biphenyl)-2,2′-diol; Quinoline, 2-chloro-4-methyl-6-nitro; 4H-1-benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxyphenyl); and But-2-endiamide, N,N'-bis(4-methoxyphenyl).

5. Conclusions

The study described the potential of strain TSAS 04 as a magnificent agent with various pharmacological roles such as antimicrobial, antioxidative, and anticancer properties. The crude ethyl acetate extract had the capability to inhibit bacterial and fungal pathogens. Presence of higher phenolic content correlated with stronger antioxidant and anticancer traits. FT-IR spectrum and GC-MS chromatogram of the crude ethyl acetate extract of this strain revealed the dominance of versatile compounds. Further studies are needed to purify bioactive components from this extract for understanding the mode of action of therapeutic agents against distinct pathogens and cancer cell lines.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Aarti, C., Khusro, A., Agastian, P., 2018. Carboxymethyl cellulase production optimization from *Glutamicibacter arilaitensis* strain ALA4 and its application in lignocellulosic waste biomass saccharification. Prep. Biochem. Biotechnol. 48, 853–866.

Abd-Elnaby, H., Abo-Elala, G., Abdel-Raouf, U., Abdelwahab, A., Hamed, M., 2016. Antibacterial and anticancer activity of marine *Streptomyces parvus*: optimization and application. Biotechnol. Biotechnol. Equip. 1, 180–191.

Al-Dhabi, N.A., Esmail, G.A., Duraipandiyan, V., Valan Arasu, M., Salem-Bekhit, M.M., 2016. Isolation, identification and screening of antimicrobial thermophilic *Streptomyces* sp. Al-Dhabi-1 isolated from Tharban hot spring, Saudi Arabia. Extremophiles 20, 79–90.

Al-Dhabi, N.A., Esmail, G.A., Duraipandiyan, V., Valan Arasu, M., 2019a. Chemical profiling of *Streptomyces* sp. Al-Dhabi-2 recovered from an extreme environment in Saudi Arabia as a novel drug source for medical and industrial applications. Saudi. J. Biol. Sci. 26, 758–766.

Al-Dhabi, N.A., Ghilan, A.K.M., Arasu, M.V., Duraipandiyan, V., Ponmurugan, K., 2018a. Environmental friendly synthesis of silver nanomaterials from the promising Streptomyces parvus strain Al-Dhabi-91 recovered from the Saudi Arabian marine regions for antimicrobial and antioxidant properties. J. Photochem. Photobiol. B: Biol. 189, 176–184.

Al-Dhabi, N.A., Ghilan, A.K.M., Arasu, M.V., Duraipandiyan, V., 2019b. Green biosynthesis of silver nanoparticles produced from marine *Streptomyces* sp. Al-Dhabi-89 and their potential applications against wound infection and drug resistant clinical pathogens. J. Photochem. Photobiol. B: Biol. 111529

Al-Dhabi, N.A., Ghilan, A.-K.M., Arasu, M.V., 2018b. Characterization of Silver Nanomaterials Derived from Marine Streptomyces sp. Al-Dhabi-87 and Its In Vitro Application against Multidrug Resistant and Extended-Spectrum Beta-Lactamase Clinical Pathogens. Nanomaterials 8 (5).

Al-Dhabi, N.A., Mohammed Ghilan, A.K., Esmail, G.A., Valan Arasu, M., Duraipandiyan, V., Ponmurugan, K., 2019c. Bioactivity assessment of the Saudi Arabian Marine *Streptomyces* sp. Al-Dhabi-90, metabolic profiling and its in vitro inhibitory property against multidrug resistant and extended-spectrum beta-lactamase clinical bacterial pathogens. J. Infect. Public Health 12, 549–556.

Anandan, R., Dharumadurai, D., Manogaran, G.P., 2016. An introduction to actinobacteria, in Actinobacteria: Basics and Biotechnological Applications, Eds D. Dhanasekaran and Y. Jiang (Rijeka: Intech), 3-37. DOI: 10.5772/62329.

Arasu, M.V., Thirumamagal, R., Srinivasan, M.P., Al-Dhabi, N.A., Ayeshamariam, A., Saravana Kumar, D., Punithavel, N., Jayachandran, M., 2017. Green chemical approach towards the synthesis of CeO2 doped with seashell and its bacterial applications intermediated with fruit extracts. J. Photochem. Photobiol. B: Biol. 172, 50–60.

Arasu, M.V., Arokiyaraj, S., Viayaraghavan, P., Kumar, T.S.J., Duraipandiyan, V., Al-Dhabi, N.A., Kaviyarasu, K., 2019. One step green synthesis of larvicidal, and azo dye degrading antibacterial nanoparticles by response surface methodology. J. Photochem. Photobiol. B: Biol. 190, 154–162.

Arasu, M.V., Duraipandiyan, V., Ignacimuthu, S., 2013. Antibacterial and antifungal activities of polyketide metabolite from marine Streptomyces sp. AP-123 and its cytotoxic effect. Chemosphere 90 (2), 479–487.

Arnao, M.B., Cano, A., Acosta, M., 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. 73, 239–244.

Arokiyaraj, S., Saravanan, M., Badathala, V., 2015. Green synthesis of Silver nanoparticles using aqueous extract of Taraxacum officinale and its antimicrobial activity. South Indian J. Biol. Sci. 2, 115–118.

Azman, A.S., Othman, I., Fang, C.M., Chan, K.G., Goh, B.H., Lee, L.H., 2017. Antibacterial, anticancer and neuroprotective activities of rare Actinobacteria from mangrove forest soils. Indian J. Microbiol. 57, 177–187.

Bérdy, J., 2012. Thoughts and facts about antibiotics: where we are now and where we are heading. J. Antibiot. 65, 385-395.

Diekema, D.J., Messer, S.A., Brueggemann, A.B., Coman, S.L., Doern, G.V., Herwaldt, L. A., et al., 2002. Epidemiology of candidemia: 3-year results from the emerging infections and the epidemiology of Iowa organisms study. J. Clin. Microbiol. 40, 1298–1302.

Esther Lydia, D., Gupta, C., Khusro, A., Salem, A.Z.M., 2019. Susceptibility of poultry associated bacterial pathogens to *Momordica charantia* fruits and evaluation of *in vitro* biological properties. Microb. Pathog. 132, 222–229.

Harindran, J., Gupte, T.E., Naik, S.R., 1999. HA-1-92, a new antifungal antibiotic produced by *Streptomyces* CDRIL-312: Fermentation, isolation, purification and biological activity. World J. Microbiol. Biotechnol. 15, 425–430.

Kefayati, Z., Motamed, S.M., Shojaii, A., Noori, M., Ghods, R., 2017. Antioxidant activity and phenolic and flavonoid contents of the extract and sub-fractions of *Euphorbia splendida* Mobayen. Pharmacog. Res. 9, 362–365.

Kemung, H.M., Tan, L.T., Chan, K.G., Ser, H.L., Law, J.W.F., Lee, L.H., et al., 2020. Antioxidant activities of *Streptomyces* sp. strain MUSC 14 from mangrove forest soil in Malaysia. Biomed Res. Int. 6402607.

Khalaf, N.A., Shakya, A.K., Al-othman, A., El-agbar, Z., Farah, H., 2008. Antioxidant activity of some common plant. Turk. J. Biol. 32, 51–55.

Khusro, A., Aarti, C., Preetamraj, J.P., Panicker, S.G., 2014. Comparative study on the effect of different solvent extracts of *Calotropis gigantea* and *Carica papaya* latex against new bacterial isolates – An *in vitro* study. Int. J. Pharm. Pharm. Sci. 6, 874–879.

Khusro, A., Sankari, D., 2015. Synthesis and estimation of total protein in *Bacillus subtilis* strain KPA under mild stress condition of certain antimicrobials. Asian J. Pharm. Clin. Res. 8, 86–90.

Latha, R., Rajanathan, T.M.C., Khusro, A., Chidambaranathan, N., Agastian, P., Nagarajan, S., 2019. Anticancer activity of *Mahonia leschenaultii* methanolic root extract and berberine on Dalton's ascitic lymphoma in mice. Asian Pac. J. Trop. Med. 12, 264–271.

Lee, C.H., Lim, H., Moon, S., Shin, C., Kim, S., Kim, B.J., Lim, Y., 2007. Novel anticancer agent, benzyldihydroxyoctenone, isolated from *Streptomyces* sp. causes G1 cell cycle arrest and induces apoptosis of HeLa cells. Cancer Sci. 98, 795–802.

Li, L.Y., Yang, Z.W., Asem, M.D., Fang, B.Z., Salam, N., Alkhalifah, D.H.M., et al., 2019. *Streptomyces desertarenae* sp. nov. a novel actinobacterium isolated from a desert sample. Antonie Van Leeuwenhoek 112, 367–374.

Liu, X., Dong, M., Chen, X., Jiang, M., Lv, X., Yan, G., 2007. Antioxidant activity and phenolics of endophytic *Xylaria* sp. from *Ginkgo biloba*. Food Chem. 105, 548–554.

Mayer, F.L., Wilson, D., Hube, B., 2013. *Candida albicans* pathogenicity mechanisms. Virulence 4, 119–128.
Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.
Oyaizu, M., 1986. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44, 307–315.
Özakin, S., Davis, R.W., Umile, T.P., Pirinccioglu, N., Kizil, M., Celik, G., et al., 2016. The isolation of tetrangomycin from terrestrial *Streptomyces* sp. CAH29: evaluation of antioxidant, anticancer, and anti-MRSA activity. Med. Chem. Res. 25, 2872–2881.
Palla, M.S., Guntuku, G.S., Muthyala, M.K.K., Pingali, S., Sahu, P.K., 2018. Isolation and molecular characterization of antifungal metabolite producing actinomycete from mangrove soil. Beni-Suef Univ. J. Basic Appl. Sci. 7, 250–256.
Panwar, D., Saini, V.K., 2012. Antagonistic activity of soil actinomycetes against common human pathogens. Int. J. Pharm. Biosci. 3, 500–506.
Prashith Kekuda, T.R., Onkarappa, R., Gautham, S.A., Mesta, S.C., Raghavendra, H.L., 2015. Antimicrobial, antioxidant and cytotoxic activity of *Streptomyces* species from Western Ghat Soils of Karnataka. India. Sci. Technol. Arts Res. J. 4, 164–180.
Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269, 337–341.
Ravikumar, S., Krishnakumar, S., Inbaneson, S.J., Gnanadesigan, M., 2010. Antagonistic activity of marine actinomycetes from Arabian Sea coast. Arch. Appl. Sci. Res. 2, 373–1280.
Sambamurthy, K., Ellaiah, P., 1974. A new streptomycete producing neomycin (B&C) complex - *S. marinensis* (Part I). Hindustan Antibiot Bull. 17, 24–28.
Sanger, F., Nicklen, S., Coulson, A.R., 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. 74, 5463–5467.
Ser, H.L., Tan, L.T., Law, J.W., Chan, K.G., Duangjai, A., Saokaew, S., 2017. Focused review: Cytotoxic and antioxidant potentials of mangrove-derived *Streptomyces*. Front. Microbiol. 8, 2065.
Sivakumar, J., Santhanam, P., Masilamani Selvam, M., 2011. Antimicrobial activity of actinomycetes isolated from the Western Ghats of Tamilnadu. Int. J. Pharm. Biosci. 2, 42–49.
Sivalingam, P., Hong, K., Pote, J., Prabakar, K., 2019. Extreme environment Streptomyces: potential sources for new antibacterial and anticancer drug leads? Int. J. Microbiol. 5283948.
Soler-Rivas, C., 2000. An easy and fast test to compare total free radical scavenger capacity of foodstuffs. Phytochem. Anal. 11, 1–9.
Subramani, R., Aalbersberg, W., 2012. Marine actinomycetes: an ongoing source of novel bioactive metabolites. Microbiol. Res. 167, 571–580.
Valsalam, S., Agastian, P., Arasu, M.V., Al-Dhabi, N.A., Ghilan, A.K.M., Kaviyarasu, K., Ravindran, B., Chang, S.W., Arokiyaraj, S., 2019. Rapid biosynthesis and characterization of silver nanoparticles from the leaf extract of Tropaeolum majus L. and its enhanced in-vitro antibacterial, antifungal, antioxidant and anticancer properties. J. Photochem. Photobiol. B: Biol. 191, 65–74.