Original article

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

K. Saraswathi a, S. Mahalakshmi b, Ameer Khusro c, P. Arumugam d, Alarjani Khaloud Mohammed e, Roua M. Alkufei dy

a Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology, Madhuranthagam, Kancheepuram 603308, Tamil Nadu, India
b Department of Biotechnology, Pavendar Bharathidasan College of Engineering and Technology, Thanjai Natarajan Nagar, Madhuravadi Road, Tiruchirapalli 620024, Tamil Nadu, India
c Research Department of Plant Biology and Biotechnology, Loyola College, Chennai 600034, Tamil Nadu, India
d Industrial Fermentation Technology, ARMATS Biotek Training and Research Institute, Guindy, Chennai 600032, Tamil Nadu, India
e Department of Botany and Microbiology, College of Sciences, King Saud University, P.O. Box 22452, Riyadh 11495, Saudi Arabia

**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 **in vitro** antioxidant properties of the crude ethyl acetate extract were performed using standard methodologies. The extract revealed maximum DPPH and ABTS radical scavenging activities of 51.1 ± 0.39 and 89.01 ± 0.44%, respectively. Likewise, maximum phosphomolybdenum reduction and Fe³⁺ reduction of the crude ethyl acetate extract of strain TSAS 04 were estimated 76.18 ± 0.10 and 89.01 ± 0.44%, respectively. In **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 IC₅₀ 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.

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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 (Sivilingam 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, anti-diabetic, 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
discovered so far are associated with actinobacteria (Subramani and Aalbersberg, 2012; Arakiyaraj 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 petri-plates and air-dried at 37°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) 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°C for 7–12 days. The selected actinomycetrical 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 actinomycetrical 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 pigment. Starch casein agar medium was prepared and 3 to 4 sterile cover slips were inserted at an angle of 45°. 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 rectiflexibles, straight to flexuous spore chain, and retinaculaperti 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 medium to 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 Eliaiah, 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 Mycobacterial 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 antioxidative 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:

\[
\% \text{ DPPH scavenging} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100
\]

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⁺ radical cation degradation

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

\[
\% \text{ ABTS⁺ radical cation scavenging} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100
\]

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. Mo⁶⁺ reduction was estimated as:

\[
\% \text{ Phosphomolybdenum reduction} = \left(\frac{A_{\text{sample}} - A_{\text{control}}}{A_{\text{sample}}}\right) \times 100
\]

2.9.5. Ferric (Fe³⁺) 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. Fe³⁺ reduction was calculated as:

\[
\% \text{ Fe³⁺ reduction} = \left(\frac{A_{\text{sample}} - A_{\text{control}}}{A_{\text{sample}}}\right) \times 100
\]

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 × 10⁵ cells/well in 96-well plates for 24 h with 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 sulfoxide at room temperature for 1 h (Mossman, 1983). Absorbance was recorded at 570 nm on a scanning multi-well spectrophotometer and cancer cells inhibition (%) was determined for calculating the IC₅₀ 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 Rf values were calculated as mentioned below:

\[
R_f \text{ value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}
\]

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 IC50 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•+ 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 IC50 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 respect to the standard (21.19 ± 0.13 GAE/mg). Likewise, Fe3+ reduction ability of strain TSAS 04 increased in a dose dependent manner with maximum rate of 89.01 ± 0.44% (Table 5). RC50 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

| 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 | Rubberly |
| 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 |
37.45 ± 0.36 and 71.01 ± 0.24% of cells death at the lowest (100 mg/mL) and the highest (1000 mg/mL) ranges of the extract, respectively (Fig. 2). IC50 value of the crude ethyl acetate extract was calculated as 410.5 mg/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 Rf 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=S stretching of isothiocyanate. The absorption band at 2400 cm⁻¹ was mainly due to the presence of H=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-

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**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 | 19.0 ± 0.6 | 16.3 ± 0.3 | 21.3 ± 0.6 |

Values represent mean ± SD.

| 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.

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). IC50 value of the crude ethyl acetate extract was calculated as 410.5 µg/mL.

**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⁺ 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.
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

| S. No | Concentration (µg/mL) | Percentage of reduction | Mo⁶⁺ reduction | Ascorbic acid | Fe³⁺ 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.
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+ 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-methylspiro-3,3'- (2-(4-hydroxyphenyl)pyrrolidine)    |                                                                                   |
| 4     | Methyl 1-phenanthrenecarboxylate                   |                                                                                   |
| 5     | (1,1'-biphenyl)-2,2'-diol                          |                                                                                   |
| 6     | Quinoline, 2-chloro-4-methyl-6-nitro               |                                                                                   |
| 7     | 4H-1-benzyopyran-4-one, 5,7-dihydroxy-2-(4-hydroxyphenyl) |                                                                                   |
| 8     | But-2-endiamide, N,N'-bis(4-methoxyphenyl)         |                                                                                   |

**Fig. 3.** GC-MS chromatogram of the crude ethyl acetate extract of strain TSAS 04.
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; Ozakin 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 this strain revealed the dominance of versatile compounds. Further GC-MS chromatogram of 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 this strain revealed the dominance of versatile compounds. Further GC-MS chromatogram of 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 this strain revealed the dominance of versatile compounds. Further GC-MS chromatogram of 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 this strain revealed the dominance of versatile compounds. Further GC-MS chromatogram of 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.

5. Conclusions

The study described the potential of strain TSAS 04 as a magnific-ent agent with various pharmacological roles such as antimicrob-ial, antioxidant, 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.

Acknowledgements

Authors are thankful to IIT, SAIF, Madras and ARMTS Biotek Training and Research Institute for providing necessary facilities to carry out research work. The authors extend their appreciation to the Researchers supporting project number (RSP-2020/185) King Saud University, Riyadh, Saudi Arabia.

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