



Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

2,4-Di-*tert*-butylphenol (2,4-DTBP) purified from *Streptomyces* sp. KCA1 from *Phyllanthus niruri*: Isolation, characterization, antibacterial and anticancer properties

Ayswarya Seenivasan^a, Radhakrishnan Manikkam^{a,*}, Manigundan Kaari^a, Amit Kumar Sahu^{b,d}, Madhukar Said^{c,d}, Syed G. Dastager^{b,d}^a Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai 600 119, Tamil Nadu, India^b NCIM Resource Center, CSIR-National Chemical Laboratory, Pune 411008, India^c Division of Organic Chemistry, CSIR-National Chemical Laboratory, Pune 411008, India^d Academy of Scientific and Innovative Research (AcSIR), CSIR-National Chemical Laboratory, Pune 411008, India

ARTICLE INFO

Article history:

Received 27 November 2021

Revised 28 April 2022

Accepted 10 May 2022

Available online 15 May 2022

Keywords:

Endophytic *Streptomyces*2,4-Di-*tert*-butylphenol

Antibacterial

Antiproliferative activity

ABSTRACT

Objectives: The present study reports the bioactivity of 2,4-Di-*tert*-butylphenol (2,4-DTBP) purified from an endophytic *Streptomyces* species KCA1 isolated from the leaves of *Phyllanthus niruri* and its antibacterial and anticancer properties.

Methods: The extracellular metabolites were produced from the strain KCA1 by submerged fermentation using ethyl acetate. The crude extract was further evaluated for antibacterial activity against set of pathogens. Active metabolite from the extract was purified using chromatography techniques and detected its antibacterial activity by disc diffusion assay. The molecular structure of the active molecule was identified through various spectral study. Moreover, the bioactive metabolite 2,4-DTBP was analyzed antibacterial and anti-proliferative activity.

Results: Strain KCA1 was identified as *Streptomyces* sp. In the preliminary screening, the crude extract exhibited broad spectrum activity against various bacterial pathogens. Based on the spectral properties, the active metabolite was identified as 2,4-Di-*tert*-butylphenol. The MIC of active compound 2,4-DTBP inhibited *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 at 50 µg/ml and 0.78 µg/ml, respectively. The IC50 value of 2,4-DTBP was found to be 11.0 µg/ml and 116.8 µg/ml, against breast cancer cell line (MCF7) and normal VERO cell line, respectively.

Conclusions: This study concluded that 2,4-DTBP, produced from the endophytic *Streptomyces* sp. KCA1, is the potential candidate to develop as promising antibacterial and anticancer agent.

© 2022 The Authors. Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Infectious diseases caused by the drug-resistant bacterial pathogens are the most significant health concerns (Serwecinska, 2020; Gasperini et al., 2021). Every year, an estimated 7 million people die due to the failed antimicrobial drugs, and the percentage is constantly increasing (Ukuhor, 2021). By 2050, a lack of

concerted efforts to address AMR is estimated to result in 10 million deaths annually worldwide, making AMR an even more prevalent reason of mortality than cancer disease (Mahoney et al., 2021). The loss of antibiotic treatment would threaten our capacity to combat infectious diseases. Once first- and second-line antibiotic drugs are reduced due to resistance or lack of availability, doctors are compelled to utilize toxic medicines, which are often more costly but also less efficient (Terreni et al., 2021; Serwecinska, 2020). As a result, finding new medicines to combat such dangerous infections is critical.

Most of the antibiotics in present clinical practice are derived mostly from natural products or inspired by them (Vuong, 2021; Atanasov et al., 2021). Among various diverse sources of natural antibiotics, microorganisms, especially members of the phylum actinobacteria remains in the top (De Simeis and Serra, 2021).

* Corresponding author.

E-mail address: mrkactinos@gmail.com (R. Manikkam).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

<https://doi.org/10.1016/j.jksus.2022.102088>

1018-3647/© 2022 The Authors. Published by Elsevier B.V. on behalf of King Saud University.

This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Actinobacteria, particularly the species *Streptomyces*, are estimated to have produced around two-thirds of all natural antibiotics (Maiti et al., 2020; Takahashi and Nakashima, 2018). Also, it has been reported that actinobacteria from rare sources like plants, the so called endophytic actinobacteria, are the promising source for new metabolites to develop as clinically relevant antibiotics (Singh and Dubey, 2018).

With this view, the present study was under taken for the purification of bioactive metabolites from actinobacterial strain KCA1, isolated from the leaves of *Phyllanthus niruri* and their evaluation for antibacterial and anticancer properties.

2. Methods and materials

2.1. *Streptomyces* strain KCA1

Using starch casein nitrate agar media, the endophytic strain KCA1 was isolated from the leaves of the medicinal plant *Phyllanthus niruri*. Viability of the strain KCA1 was maintained in ISP2 agar medium slants and 20% glycerol broth at 4 °C and –80 °C, respectively. Based on 16SrRNA gene sequencing, strain KCA1 was identified as species of the genus *Streptomyces* (data not shown).

2.2. Production of bioactive metabolites

The fresh culture of KCA1 was inoculated into 50 mL of ISP2 (International *Streptomyces* Project) broth medium used as mother culture aseptically and incubated at 28 °C in a rotary shaker for 72 h with 120 rpm. Then 5% of seed culture was transferred to a 1L conical flask containing 200 mL of ISP2 broth as the production medium. This production medium incubated for 7 days at 28 °C in a rotary shaker with 120 rpm. The cell-free supernatant (CFS) was separated after fermentation by centrifugation at 5000 rpm for 30 min at 4 °C (Eppendorf AG, Hamburg, Germany). The extracellular bioactive metabolites from the CFS were extracted using ethyl acetate (1:1 v/v) with occasional manual shaking for 24 h. The crude organic extract was obtained by collecting the top organic layer and drying it using a rotary evaporator.

2.3. Preliminary antibacterial activity

The crude extract of *Streptomyces* KCA1 was tested antibacterial activity against various bacterial pathogens such as *Staphylococcus aureus* ATCC 29213, *Bacillus cereus*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 13882 by adopting disc diffusion method. Sterile filter paper disc (Himedia) with 5 mm diameter was impregnated with 20 µl of ethyl acetate extract (100 µg/ml) and kept for drying under sterile condition. The panel of bacterial pathogens, were grown in nutrient broth and set equivalent to 0.5 McFarland standards. After incubation, the bacterial pathogens were inoculated into Muller Hinton Agar (MHA) plates. The crude extract impregnated disc was placed over the pathogens inoculated MHA plates and incubated at 37 °C. The zone of inhibition was measured and represented in millimeter after 24 h of incubation (Radhakrishnan et al., 2021).

2.4. Purification of active metabolites

The active metabolite was purified from the crude ethyl acetate extract of *Streptomyces* KCA1 using column chromatography (35 × 1.0 cm) packed with silica powder (100–200 mesh size, Himedia) and eluted by various concentrations of hexane: and ethyl acetate. Totally 44 fractions were collected, concentrated and checked for antibacterial activity against *E. coli* ATCC 25922

and *S. aureus* ATCC 29213 as described earlier. The active antibacterial fractions were pooled together and re-purified using the column (20 × 1.0 cm) with methanol as the high polar eluent. Again, antibacterial activity was evaluated for the 11 re-purified fractions. Based on the results of antibacterial assay, active fractions were pooled together and further purified using semi-preparative UHPLC (Thermo Scientific UltiMate 3000). The flow rate was maintained at 3 mL/min and the UV detection was absorbed at 440 nm. The purified fractions were concentrated and evaluated for antibacterial activity.

2.5. Characterization and structure elucidation

Physicochemical characteristics such as appearance, colour, and solubility of purified metabolite was determined by adopting standard procedures. The Infrared (IR) spectral of the purified sample combined with potassium bromide (KBr) was measured using a high-quality Perkin Elmer Spectrum One FT-IR spectrometer. NMR spectrum was recorded on 500 MHz AVANCE III Bruker spectrometer. LC-Mass spectrum in positive ion mode was taken to determine the molecular weight using Thermo/Finnigan Surveyor system. All the spectral analysis was carried out at CSIR-National Chemical Laboratory, Pune and India. Chemical structure of the purified metabolite was determined based on their spectral properties.

2.6. Minimum inhibitory concentration (MIC) of purified metabolite

The purified metabolite was evaluated for MIC against the pathogens *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 by the micro broth dilution technique. The two-fold diluted concentrations of the purified sample (100–0.78 µg/ml) were prepared in 10% DMSO. Pathogenic bacteria viz. *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were grown to logarithmic phase (0.1 OD at 600 nm) and mixed with 100 µl of different concentrations of purified sample. 100 µl of test sample at various concentrations was mixed with 100 µl of nutrient broth served as control. The positive control well had 100 µl of pathogenic culture and 100 µl of nutrient broth, whereas the negative control had 200 µl of nutrient broth alone and incubated at 37 °C for 24 hrs. The optical density (OD) was calculated at 595 nm using microplate reader (Epoch 2, BioTek Instruments, Agilent Technologies, CA, USA).

2.7. Antiproliferative activity of purified metabolite

2.7.1. Cell lines maintenance and growth condition

Antiproliferative activity of purified metabolite against MCF7 (breast cancer cell line) and VERO (normal cell line) was determined by adopting MTT assay.

2.7.2. MTT assay

In a sterile flat bottom 96-well plate, 5000 cells per well concentration was maintained and incubated for 48 h. Each well received 20 µl of purified metabolite. The final concentration range was between 100 and 0.78 µg/ml. For this experiment, sterile DMSO at 0.05 % (v/v) was administered as a negative control. The microtitre plate was further incubated for 72 h. Later, 50 µl of MTT (Sigma) solution (5 mg/ml) was added to each well. The plates were further incubated in CO₂ incubator for 4 h at 37 °C with 5% CO₂ and 95% air. After gently aspirating the medium, to dissolve the formazan crystals, DMSO (100 µl) was added. Using a microplate reader, the absorbance of dissolved formazan product was measured at 570 nm.

The following formula was used to compute the proportion of viable cells: cell viability (%) = (Treated cells absorbance/Untreated cells absorbance) × 100.

3. Results

3.1. Characterization and taxonomy of actinobacterial strain KCA1

Actinobacterial strain KCA1 produced powdery colonies when culturing on YEME agar plates with gray color aerial mycelium pale brown color reverse side pigment. The presence of aerial and a branched substrate mycelium was also noted under bright filed microscopic observation. The 16SrRNA gene sequencing of KCA1 showed 99.45% similarity with *Streptomyces* sp. and had a query length of 1452 bp. This partial sequence was submitted to GenBank with the accession number of MW470667.

3.2. Production and preliminary screening for antibacterial activity

In submerged fermentation, 47 mg of crude extract was obtained from 100 mL of ISP2 medium using ethyl acetate. In antibacterial assay, the crude extract was exhibited broad spectrum activity against bacterial pathogens. Maximum of 20.0 ± 0.3 mm zone of inhibition was observed against *S. aureus* ATCC 29213 followed by 19.83 ± 0.4 mm against *B. cereus* and 19.0 ± 0.3 mm against *K. pneumoniae*. Also, the crude extract showed least inhibition against *E. coli* ATCC 25922 (13.5 ± 0.8 mm).

3.3. Purification and characterization of active molecule

In the process of purification, totally 44 fractions were collected from the silica gel column chromatography in which 14 fractions (F13-F26) eluted with hexane: ethyl acetate was showed antibacterial activity against *E. coli* ATCC 25922 and *S. aureus* ATCC 29213. These 14 active fractions were concentrated after being pooled together based on their similar TLC patterns. Repurification through silica gel column chromatography, the F5 and F6 fraction that indicated antibacterial activity were pooled again before being subjected to semi-preparative UHPLC. The fraction collected at 18.817 min retention time was exhibited antibacterial activity. The active peak was subsequently chromatographed

using acetonitrile: water (90:10), yielding a single peak with a retention time of 16.242 min, indicating the metabolite purity.

The active metabolite (4.3 mg/L) was found to be yellow in color. It is soluble in water, chloroform, ethyl acetate and methanol. FT-IR spectra showed a peak at $3233\text{--}3302\text{ cm}^{-1}$, indicating the stretch of O–H phenol group. Furthermore, the C–C stretch of the alkyl group and C–O stretch of phenol group was confirmed by the peaks at $2849\text{--}2914\text{ cm}^{-1}$ and 1246 cm^{-1} , represented. The aromatic C–C stretch was confirmed by the peaks at $1465\text{--}1730\text{ cm}^{-1}$ (Fig. 1). Therefore, the above functional groups by FT-IR analysis confirmed the phenol group of molecules. LC–MS analysis was used to determine the molecular weight, with a mass of $[M+H]^+$ at m/z 209, matching to the primary peak, yielding an accurate mass of 206.17 in the positive ion (Fig. 2).

The presence of two singlets at 1.31 ppm and 1.33 ppm in the ^1H NMR data resembles di-substituted tertiary butyl groups (Fig. 3a). A singlet at 4.66 ppm indicates the presence of phenolic hydrogen. The remaining three protons were identified in the aromatic downfield area in the range of 6.60–7.31 ppm, indicating that the metabolite was tri-substituted aromatic. The ^{13}C NMR spectra indicated the existence of ten carbon signals, six of which were downfield carbon signals observed between 115.90 and 151.76 ppm in the aromatic region. In the up-field area, the remaining four carbon signals were discovered between 29.35 and 34.71 ppm. The aromatic substitution of six carbons was confirmed by the presence of three quaternary carbons at 135.17 ppm, 142.97 ppm, and 151.76 ppm. At 151.76 ppm, the presence of a phenolic OH group at the quaternary carbon was found in the furthest downfield position. Tertiary carbons were identified in two of the four carbon signals found in up-field locations (34.31 ppm and 34.26 ppm, respectively), whereas methyl signals were found in the other two (31.61 ppm and 29.65 ppm, respectively). Thus, the spectrum indicates that the molecule has two *tert*-butyl replacements on the aromatic ring's last quaternary carbons, at 135.17 ppm and 142.97 ppm, respectively (Fig. 3b). Based on the FT-IR (Fig. 1), LC–MS (Fig. 2), and NMR studies (Fig. 3a, 3b and 3c), the chemical structure of the molecule was observed to be 2,4-Di-*tert*-butylphenol ($\text{C}_{14}\text{H}_{22}\text{O}$) (Fig. 3d).

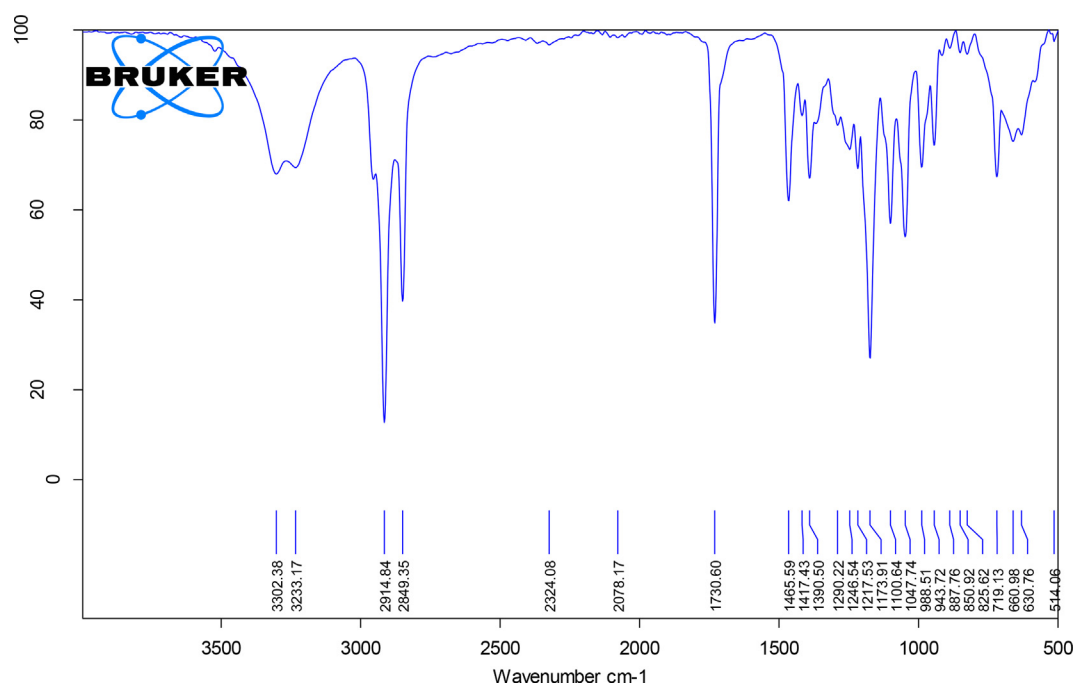


Fig. 1. FT-IR spectrum of 2,4-DTBP isolated from *Streptomyces* sp. KCA1.

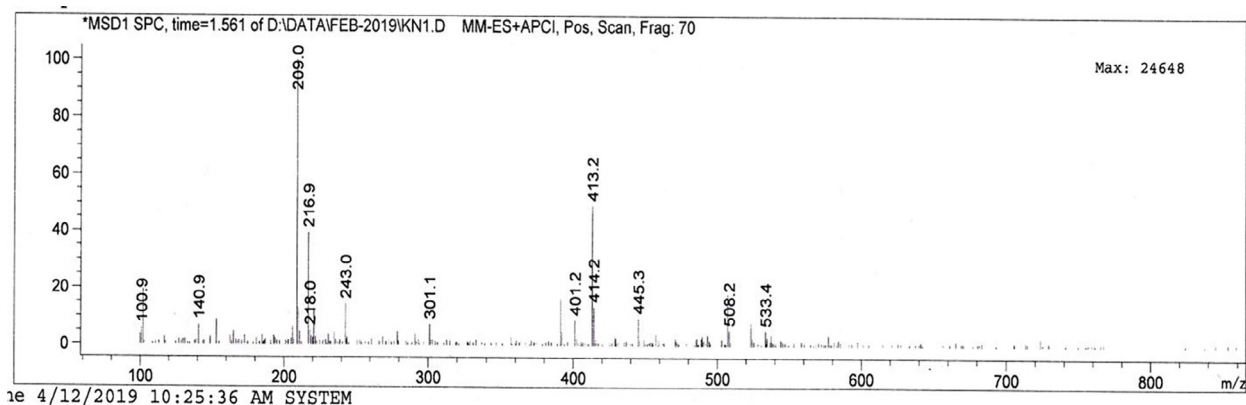


Fig. 2. LC-MS analysis of 2,4-DTBP isolated from *Streptomyces* sp. KCA1.

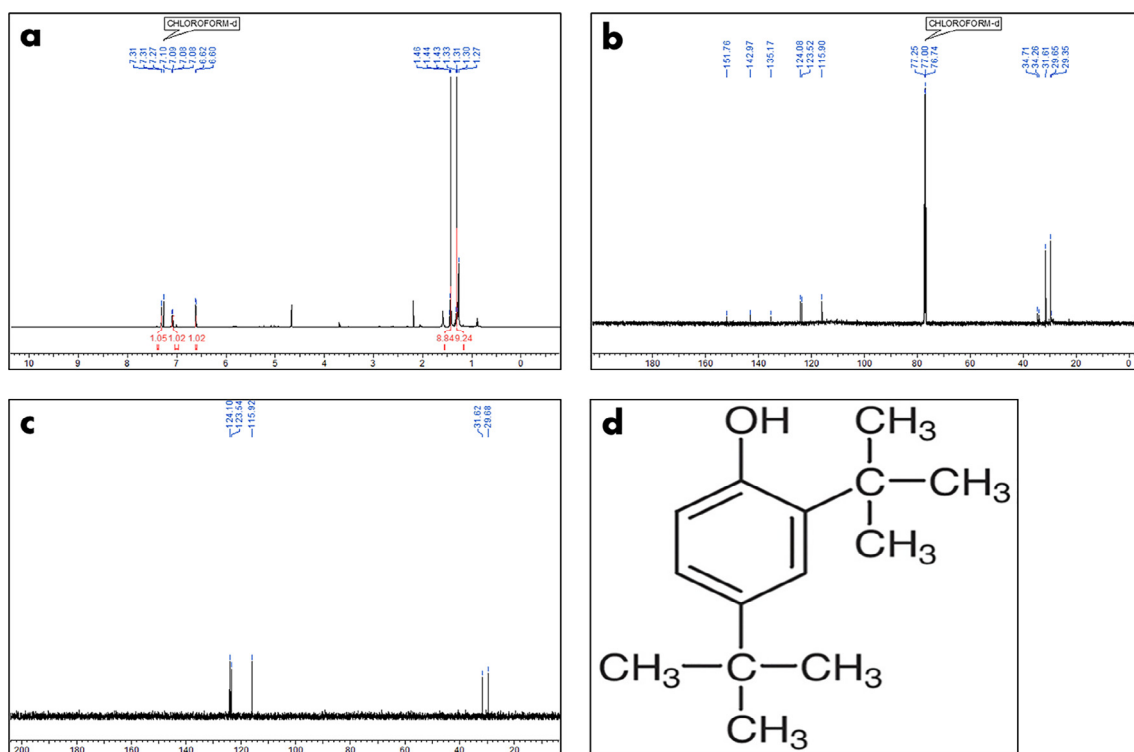


Fig. 3. NMR analysis and structural prediction of 2,4-DTBP (a) ^1H NMR, (b) ^{13}C NMR, (c) DEPT NMR and (d) chemical structure.

3.4. MIC of 2,4-DTBP against bacterial pathogens

The purified metabolite 2,4-DTBP was shown to be more efficient against *S. aureus* ATCC 29213, with MIC of 0.781 g/ml, compared to *E. coli* ATCC 25922, having a MIC of 50 g/ml.

3.5. Cytotoxicity assay of 2,4-DTBP

The MTT assay was used to investigate the cytotoxicity of 2,4-DTBP against MCF7 and VERO cell lines has been depicted in Fig. 4 and Fig. 5. The better effect of 2,4-DTBP on cell viability was observed, and the IC_{50} value was calculated. As shown in Table 1, 2,4-DTBP showed comparable cytotoxicity against MCF7 and VERO cell lines with IC_{50} values of 11.0 and 116.8 $\mu\text{g/ml}$, respectively.

4. Discussions

Streptomyces has been proven to be a dependable source of secondary metabolites having healthcare applications, therefore much research is being put into isolating novel *Streptomyces* strains for the isolation of clinically relevant bioactive metabolites (Parte, 2018; Anderson and Wellington, 2001). In the present study, an attempt has been made to identify the bioactive potential of the endophytic *Streptomyces* sp. KCA1 isolated from *Phyllanthus niruri*. According to literature, endophytic actinobacteria from medicinal plants are found to exhibit promising bioactivity and bioactive metabolite production. In this study also, during primary screening, the ethyl acetate extract of endophytic actinobacterial strain KCA1 was showed broad spectrum antibacterial activity. Findings of certain earlier studies also showed that the ethyl acetate extract of *Streptomyces* exhibited broad spectrum activity (Al Farraj et al.,

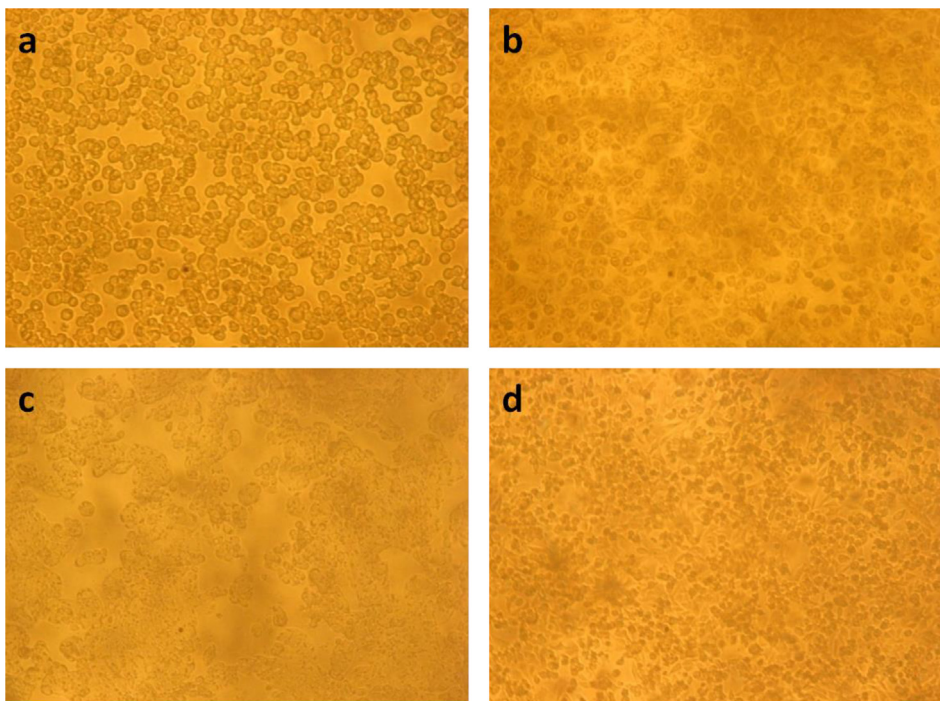


Fig. 4. Morphology of breast cancer cell line MCF7 after treatment with 2,4-DTBP at different concentrations. Comparison of the morphological features of MCF7 after the 72hrs with 2,4-DTBP at respective concentrations [(a) control, (b) 3 µg/ml, (c) 30 µg/ml, (d) 100 µg/ml].

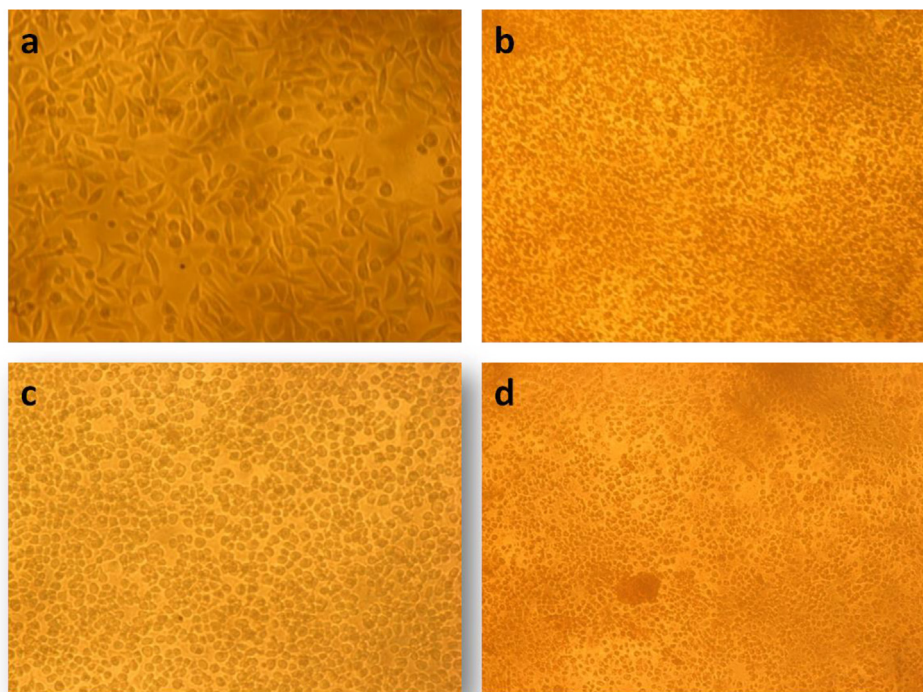


Fig. 5. Morphology of normal VERO cell line after treatment with 2,4-DTBP at different concentrations. Comparison of the morphological features of VERO after the 72hrs with 2,4-DTBP at respective concentrations [(a) control, (b) 3 µg/ml, (c) 30 µg/ml, (d) 100 µg/ml].

2020). The strain was found to exhibit antimicrobial activities against a set of pathogenic strains. An important finding of this present study was the purification of the active metabolite 2,4-DTBP from endophytic *Streptomyces* sp. KCA1. This metabolite is also produced by other bacterial strains such as *Sphingobium fuliginis*, *Flavobacterium johnsoniae* and *Pseudomonas putida*. (Toyama

et al., 2010; Sang and Kim, 2012; Dharni et al., 2014). In addition, some fungal species such as *Termitomyces heimi*, *Pleurotus sajorcaju* and *Hericium erinaceus* are also reported to produce 2,4-DTBP (Malek et al., 2009). Similar to this study 2,4-DTBP produced by *Streptomyces* sp. KB1 and *Streptomyces mutabilis* isolated from air and soil sample from different geographical habitat showed

Table 1
Antiproliferative activity of 2,4-DTBP isolated from *Streptomyces* sp. KCA1 against MCF7 and VERO cell lines.

Concentrations ($\mu\text{g/ml}$)	Breast cancer (MCF7)		Normal cells (VERO)	
	% of cell death	% Live cell	% of cell death	% Live cell
200	80.91 \pm 0.83	19.09	59.61 \pm 3.01	40.39
100	73.28 \pm 0.24	26.72	21.01 \pm 0.66	78.99
50	69.65 \pm 0.05	30.35	20.14 \pm 0.14	79.86
25	56.85 \pm 0.59	43.15	12.54 \pm 1.14	87.46
12.5	43.20 \pm 2.76	56.80	7.82 \pm 2.02	92.18
6.25	37.71 \pm 0.05	62.29	6.08 \pm 1.66	93.92
3.125	26.51 \pm 0.53	73.49	6.51 \pm 0.12	93.49
1.562	14.83 \pm 1.06	85.17	6.07 \pm 0.68	93.93
0.781	7.63 \pm 1.02	92.37	3.97 \pm 0.21	96.03

antimicrobial and anticancer activity (Chawawisit et al., 2015a, 2015b; Belghit et al., 2016).

In this present study the purified metabolite 2,4-DTBP possessed antibacterial activity against *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 with MICs of 0.78 $\mu\text{g/ml}$ and 50 $\mu\text{g/ml}$, respectively. Similarly, 2,4-DTBP isolated from *Streptomyces* sp. KB1 showed antibacterial activity against MRSA (methicillin-resistant *Staphylococcus aureus*) with the MIC of 31.25 $\mu\text{g/ml}$ (Chawawisit et al., 2015b). In addition, the molecule 2,4-DTBP isolated from *S. mutabilis* showed antimicrobial activity with higher MIC of 30 $\mu\text{g/ml}$ and >100 $\mu\text{g/ml}$, against *S. aureus* ATCC 29213 and *E. coli* ATCC 25922, respectively (Belghit et al., 2016).

Another important finding of this present study was the purified metabolite 2,4-DTBP showed moderate antiproliferative activity against the breast cancer cell line MCF-7 and less inhibition to the normal VERO cell line. This finding is also in agreement with the previous report by Chawawisit et al. (2015b). The cytotoxic property of 2,4-DTBP has been reported by different research groups against various cancer cell lines such as MCF7 (IC₅₀ 5.75 mg/ml), A549 (IC₅₀ 6 mg/ml), CasKi cells (IC₅₀ 4.5 mg/ml) and HeLa cell lines (IC₅₀ 10 mg/ml) (Malek et al., 2009; Varsha et al., 2015). This metabolite 2,4-DTBP is also well recognized for their antioxidant, anti-inflammatory, insecticidal, nematocidal, antiviral, antimalarial and anti-phytopathogenic activities (Zhao et al., 2020).

5. Conclusion

Based on the findings of this study, it can be concluded that the endophytic *Streptomyces* sp. KCA1 from *Phyllanthus niruri* produces 2,4-Di-tert-butylphenol, which possesses antibacterial and anticancer properties. To move this compound further, more research is needed on its mechanism of action, *in-vivo* cytotoxicity, and pharmacodynamics and pharmacokinetics.

Author contributions

Conceived and designed the experiments: RM. Performed the experiments: AS, MK, AKS. Analysis/interpretation of data: MS, SGD. Drafting of the manuscript: AS, MK. Supervising the work: RM, SGD. Critical revision of the manuscript: RM.

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 acknowledge the management of Sathyabama Institute of Science and Technology (SIST), Chennai Tamil Nadu, India and CSIR-National Chemical Laboratory, Pune, India for the research facilities provided.

References

- Al Farraj, D.A., Varghese, R., Vágvölgyi, C., Soliman Elshikh, M., Alokda, A.M., Hossam Mahmoud, A., 2020. Antibiotics production in optimized culture condition using low cost substrates from *Streptomyces* sp. AS4 isolated from mangrove soil sediment. *J. King Saud Univ. Sci.* 32 (2), 1528–1535.
- Anderson, A.S., Wellington, E.M., 2001. The taxonomy of *Streptomyces* and related genera. *Int. J. Syst. Evol. Microbiol.* 51, 797–814.
- Atanasov, A.G., Zotchev, S.B., Dirsch, V.M., 2021. Natural products in drug discovery: advances and opportunities. *Nat. Rev. Drug Discov.* 20 (3), 200–216.
- Belghit, S., Driche, E.H., Bijani, C., Zitouni, A., Sabaou, N., Badji, B., Mathieu, F., 2016. Activity of 2,4-Di-tert-butylphenol produced by a strain of *Streptomyces mutabilis* isolated from a Saharan soil against *Candida albicans* and other pathogenic fungi. *J. Mycol. Med.* 26 (2), 160–169.
- Chawawisit K., Bhoopong P., Phupong W., Lertcanawanichakul M., 2015a. 2,4-Di-tert-butylphenol, the bioactive compound produced by *Streptomyces* sp. KB1. *J. Appl. Pharm. Sci.* 5,7–12.
- Chawawisit, K., Bhoopong, P., Phupong, W., Lertcanawanichakul, M., 2015b. Anti-MRSA activity, mode of action and cytotoxicity of 2, 4-Di-tert-butylphenol produced by *Streptomyces* sp. KB1. *Int. J. Pharm. Sci. Rev. Res.* 35, 114–119.
- De Simeis, D., Serra, S., 2021. *Actinomycetes*: A never-ending source of bioactive compounds—An overview on antibiotics production. *Antibiotics* 10 (5), 483.
- Dharni, S., Sanchita, Maurya, A., Samad, A., Srivastava, S.K., Sharma, A., Patra, D.D., 2014. Purification, characterization, and *in vitro* activity of 2,4-Di-tert-butylphenol from *Pseudomonas monteilii* psf84: Conformational and molecular docking studies. *J. Agric. Food Chem.* 62 (26), 6138–6146.
- Gasperini, B., Cherubini, A., Lucarelli, M., Espinosa, E., Prospero, E., 2021. Multidrug-resistant bacterial infections in geriatric hospitalized patients before and after the COVID-19 outbreak: Results from a retrospective observational study in two geriatric wards. *Antibiotics* 10, 95.
- Mahoney, A.R., Safaei, M.M., Wuest, W.M., Furst, A.L., 2021. The silent pandemic: Emergent antibiotic resistances following the global response to SARS-CoV-2. *IScience* 24 (4).
- Maiti, P.K., Das, S., Sahoo, P., Mandal, S., 2020. *Streptomyces* sp. SM01 isolated from Indian soil produces a novel antibiotic picolinamycin effective against multi drug resistant bacterial strains. *Sci. Rep.* 10, 10092.
- Malek, S.N.A., Shin, S.K., Wahab, N.A., Yaacob, H., 2009. Cytotoxic components of *Pereskia bleo* (Kunth) DC. (Cactaceae) leaves. *Molecules* 14, 1713–1724.
- Parte, A.C., 2018. LPSN—List of prokaryotic names with standing in nomenclature (bacterio.net), 20 years on. *Int. J. Syst. Evol. Microbiol.* 68, 1825–1829.
- Radhakrishnan, M., Vijayalakshmi, G., Gopikrishnan, V., Suresh, A., Manigundan, K., Joseph, J., 2021. Bioprospecting of actinobacteria from selected mangrove ecosystems, India effective against carbapenem resistant *Klebsiella pneumoniae* ATCC BAA-1705. *J. Appl. Pharm. Sci.* 11, 89–94.
- Sang, M.K., Kim, K.D., 2012. The volatile-producing *Flavobacterium johnsoniae* strain GSE09 shows biocontrol activity against *Phytophthora capsici* in pepper. *J. Appl. Microbiol.* 113 (2), 383–398.
- Serwecinska, L., 2020. Antimicrobials and antibiotic-resistant bacteria: MPDI. *Water* 12, 3313.
- Singh, R., Dubey, A.K., 2018. Diversity and applications of endophytic actinobacteria of plants in special and other ecological niches. *Front. Microbiol.* 9, 1767.
- Takahashi, Y., Nakashima, T., 2018. Actinomycetes, an inexhaustible source of naturally occurring antibiotics. *Antibiotics* 7, 45.
- Terreni, M., Taccani, M., Pregnolato, M., 2021. New antibiotics for multidrug-resistant bacterial strains: latest research developments and future perspectives. *Molecules* 26, 2671.

- Toyama, T., Momotani, N., Ogata, Y., Miyamori, Y., Inoue, D., Sei, K., Mori, K., Kikuchi, S., Ike, M., 2010. Isolation and characterization of 4-tert-butylphenol-utilizing sphingobium fuliginis strains from *Phragmites australis* rhizosphere sediment. *Appl. Environ. Microbiol.* 76 (20), 6733–6740.
- Ukuhor, H.O., 2021. The interrelationships between antimicrobial resistance, COVID-19, past, and future pandemics. *J. Infect. Public Health* 14 (1), 53–60.
- Varsha, K.K., Devendra, L., Shilpa, G., Priya, S., Pandey, A., Nampoothiri, K.M., 2015. 2,4-Di-tert-butyl phenol as the antifungal, antioxidant bioactive purified from a newly isolated *Lactococcus* sp. *Int. J. Food Microbiol.* 211, 44–50.
- Vuong, TV., 2021. Natural products and their derivatives with antibacterial, antioxidant and anticancer activities. *Antibiotics* 10 (1), 70.
- Zhao, F., Wang, P., Lucardi, R.D., Su, Z., Li, S., 2020. Natural sources and bioactivities of 2,4-di-tert-butylphenol and its analogs. *Toxins* 12, 35.