



# Diketopiperazine derivative from marine actinomycetes *Nocardioopsis* sp. SCA30 with antimicrobial activity against MRSA

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Received: 9 August 2021 / Revised: 14 September 2021 / Accepted: 15 September 2021 / Published online: 10 October 2021  
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## Abstract

Actinobacteria isolated from marine sources are a potential source of novel natural products. In this study, we report isolation, biological activity and characterization of secondary metabolites from strain *Nocardioopsis* sp. SCA30, isolated from marine sediments of Havelock Islands, Andaman and Nicobar, India. The ethyl acetate extracts of the isolate on screening for biological activity demonstrated antibacterial potency and antiproliferative activity. The extracts showed anticancer activity in a panel of cell lines, including HCT 15, HT 29, MCF 7 and MDA-MB 468, at concentrations ranging from 62.5 to 1000 µg/ml. A dose-dependent reduction in cell viability was observed in all the tested cell lines. The extract at 15 µg/ml and 30 µg/ml inhibited growth of methicillin-resistant *Staphylococcus aureus* ATCC NR-46071 and NR-46171 with MIC's of 15.62 and 7.81 µg/ml, respectively. LC–MS and NMR studies revealed that the antibacterial and anticancer compound isolated from *Nocardioopsis* sp. SCA30 is 1-acetyl-4-(4-hydroxyphenyl)piperazine.

**Keywords** *Nocardioopsis* · Methicillin-resistant *Staphylococcus aureus* · Diketopiperazine · Actinomycetes · PKS · Antimicrobial · Anti-cancer

## Introduction

In the natural product drug discovery, actinobacteria are increasingly bioprospected as they are one of the largest sources of diverse lead compounds. Among the actinobacteria, the marine microorganisms are one focus for microbial drug discovery. They have distinct metabolic pathways as a response to their adaptation to marine environment, resulting in their ability to synthesize various bioactive compounds (Yang et al. 2019). The rare actinomycetes from marine

sources are known for producing diverse and structurally unique metabolites with therapeutic potential (Dhakal et al. 2017). It has been reported that 70% of the bioactive natural products in clinical use are derived from actinobacteria (Manivasagan et al. 2014).

In the last few years, a high number of novel bioactive compounds have been discovered from rare actinobacteria belonging to genera, such as *Nocardioopsis*, *Micromonospora*, *Salinispora* and *Pseudonocardia* (Subramani and Sipkema 2019). The genus *Nocardioopsis* is found in various ecosystems, including terrestrial and marine environments. They are considered to be a biotechnologically important group of Actinobacteria that produce a wide variety of chemical classes of compounds with diverse biological activities (Bennur et al. 2015). Secondary metabolites reported from this genus range from polyketides, cyclic peptides, macrolides, diketopiperazines, pyrones, naphthoquinones to phenazines and phenoxazine derivatives. These compounds are having pharmacological relevance for their antimicrobial, anticancer and immunomodulatory activity (Ibrahim et al. 2018).

In the present global health scenario, the emerging infectious diseases and multidrug-resistant pathogens necessitate

Communicated by Erko Stackebrandt.

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the discovery of novel antimicrobials and drug targets. Although the synthetic combinatorial libraries of compounds can be screened, microbial metabolites are major source of lead compounds to develop novel drugs (Chalinar and Bode 2015). Polyketide structure-based and phenazine derivative antibiotics have been characterized from various species of *Nocardioopsis*. Apart from antimicrobials, *Nocardioopsis* species have been reported to produce anticancer, antitumour and immunomodulatory compounds (Bennur et al. 2015).

In the present study, marine actinomycetes *Nocardioopsis* sp. SCA30 was screened for biological activity. A piperazine derivative was purified and characterized from the organism. The bioactive compound significantly inhibited methicillin-resistant *Staphylococcus aureus* and exhibited antiproliferative against the breast cancer cell line MDA-MB 468.

## Experimental procedures

### Sample collection, pretreatment and isolation of actinomycetes

Marine sediment samples were collected from a depth of 1–10 cm from Havelock in Andaman and Nicobar Islands (11.96°N, 93.00°E), India. Samples were transferred to the laboratory in a sterile container. Pre-treatment included air-drying the samples for a week, followed by drying them in a hot air oven for 2 days at 55 °C. Serial dilution of the sample followed by plating aliquots (100 µl) of the diluents up to 10<sup>-4</sup> in starch casein agar (SCA) led to the isolation of discrete colonies. The media were supplemented with nalidixic acid (20 µg/ml) and nystatin (25 µg/ml) to prevent the growth of contaminants. The plates were incubated at 28 °C until growth was observed. Further, pure colonies were isolated and maintained in ISP-2 slants at 4 °C. The selected isolate was observed for colony morphology, aerial and substrate mycelium, pigments and spore chain morphology by light microscopy and SEM analysis.

### Molecular characterization of isolate and detection of biosynthetic gene clusters

#### Genomic DNA extraction, amplification and sequence analysis of 16S rRNA gene

The genomic DNA from strain SCA30 was extracted by an optimized phenol–chloroform method according to Sidharth and Vittal (2018). The 16S rRNA gene was amplified using universal primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-ACG GCT ACC TTG TTA CGA CTT-3'). The PCR reaction conditions were as follows: Initial denaturation at 95 °C for 5 min, followed by

35 cycles at 95 °C for 1 min, primer annealing at 55 °C and extension at 72 °C for 1 min. The amplified PCR product was separated by electrophoresis in 1.5% agarose gel stained with ethidium bromide. The PCR product was subjected to sequencing using the same primers, compared with similar 16S rRNA sequences obtained from the BLAST search and submitted to NCBI database.

Mega 7.0 (Raynham, MA, USA) was used to determine the evolutionary phylogenetic relationship among different strains by the neighbour-joining tree algorithm method. 16S rRNA sequences of the isolate SCA30 were retrieved from the annotation results. Similarly, fifteen relevant *Nocardioopsis* species were selected and their sequences downloaded from the NCBI database to construct the phylogenetic tree. The consistency of the phylogenetic tree was maintained by using 1000 bootstrapped replicates (Glockner et al. 2000).

#### Detection of PKS-I and PKS-II gene clusters

Genomic DNA of the strain SCA 30 was used to amplify the PKS-I and PKS-II gene fragment with degenerate primer sets:

**PKS-I:** K1F (TSA AGT CSA ACA TCC GBC); M6R (CGC AGG TTS CSG TAC CAG TA) (Warwick et al. 2005).

**PKS-II:** KSF (TSG CST GC TTG GAY GCS ATC); KSR (TGG AAN CCG CCG AAB CCG CT) (Fischer et al. 2009).

The 50 µl PCR master mixture contained 2 µl (130 ng/µl) of template DNA, 25 µl of PCR master mix (dNTPs 10 mM; 10X reaction buffer; Taq DNA polymerase 3U/µL), each 2 µl of primer (10 pmol/µl), 2 µl of MgCl (25 mM) and 17 µl molecular grade water. The optimization of annealing temperature was performed in the Bio-Rad thermal cycler with gradient conditions as follows: Initial denaturation at 95 °C for 5 min, followed by 35 cycles at 95 °C for 1 min, primer annealing at 50 °C, 52.6 °C, 53 °C, 53.4 °C, 54.1 °C, 55 °C, 56.1 °C, 57 °C, 57.7 °C and 58 °C for 1 min, and extension at 72 °C for 5 min. The final extension was done at 72 °C for 10 min and was kept for cooling at 10 °C. A negative control without a DNA template was also included in PCR reactions. A 20 µl aliquot of these amplified products was electrophoresed in 1X TAE buffer at 100 V for 45 min with 100 bp ladder on 1.5% agarose gel electrophoresis stained with ethidium bromide. The amplicons were visualized in gel documentation system (Bio-Rad, USA) and product size of 1200–1400 bp (PKS-I) and 600–800 bp (PKS-II) was confirmed as keto synthase gene amplification (Webster and Taylor 2012). Obtained PKS gene nucleotide sequences were submitted to NCBI Gen Bank and accession numbers were obtained.

## Strain cultivation, fermentation and extraction of bioactive compounds

The isolate SCA30 was inoculated in a 1000 ml production medium (ISP-2 broth) and incubated under shaking condition for 21 days at  $28 \pm 2$  °C at 125 rev/min. After incubation, cell-free supernatant was extracted thrice with an equal volume of ethyl acetate. The organic phase was concentrated using a rotary vacuum concentrator at 55 °C (Hahn-Shin, Bucheon, South Korea). The crude extract thus obtained was dried using desiccators, dissolved in a solvent and stored at  $-20$  °C till further use.

## Biological activity

### Antibacterial activity

**Disc diffusion method** The antibacterial activity of the ethyl acetate extract (1 mg/ml) against methicillin-resistant *Staphylococcus aureus* (MRSA) strains was examined by disc diffusion method as previously reported by Siddharth and Vittal (2019). Chloramphenicol discs were used as a positive control. Discs impregnated with 0.5% DMSO were used as solvent control.

**Minimum inhibitory concentration (MIC)** The minimum inhibitory concentration (MIC) value against methicillin-resistant *Staphylococcus aureus* (MRSA) strains was determined by the microdilution method as previously reported by Siddharth and Vittal (2019). The serially diluted fraction of the extract was added to the well to get the required concentrations (1–3.8 µg/ml). The plate was incubated at 37 °C. The lowest concentration of the active compound that showed no visible bacterial growth (without turbidity) was considered as MIC.

### Anticancer activity

**Cell viability** Cells of cancer lines HCT 15, HT 29, MCF 7 and MDA-MB 468 were trypsinized and 10,000 cells in a 100 µL volume per well were plated in 96-well plates. The cells were allowed to grow in a CO<sub>2</sub> incubator till the cells reached 60–70% confluence. At this point, cells were treated with increasing concentration ranging from 62.5 to 1000 µg/ml of microbial extract along with positive control (diallyl disulfide-1 mM) for 48 h and cell viability was determined using the SRB assay.

**SRB assay** After 48 h of incubation for fixation, 50 µL of 50% TCA was added to each well directly with the medium. The plates were incubated for 60 min at 4 °C. The plates were carefully washed with slow running tap water. Excess water was removed by tapping the plate on tissue paper.

After drying, 100 µL of SRB solution was added to each well and incubated for 30 min. The plates were washed with 1% acetic acid and kept for drying. Further, 100 µL of 10 mM Tris base solution was added to each well. The absorbance was measured at 510 nm for calculating percentage inhibition.

## Bioactivity-guided TLC assay

The detection and identification of active fractions separated on TLC were determined by agar overlay bioautography assay against MRSA strains (Choma and Grzelak 2011). The ethyl acetate extract of strain SCA30 was spotted on to developed TLC sheets in an optimized solvents chloroform:ethyl acetate:methanol in a ratio of 6:3:1. The sheets were kept at room temperature for drying and overlaid with Mueller–Hinton soft agar (0.5%) containing 1% microbial inocula. The plates were incubated for 24 h at  $37 \pm 2$  °C and sprayed with an aqueous solution of 2,3,5-triphenyl tetrazolium chloride (TTC, Sigma, India). The clear zone of inhibition was observed around the active fraction against the red background colour of the dye used.

## Purification and structural elucidation of bioactive compounds

The crude extract was purified using thin-layer chromatography and column chromatography and validated by preparative high-performance liquid chromatography (HPLC, Shimadzu, LC-20, Japan) as previously reported by Siddharth and Vittal (2019) with slight modifications. The crude extract-silica gel slurry was eluted with a gradient solvent system in the ratio 90:10 (hexane, chloroform, petroleum ether, ethyl acetate, acetone and methanol). The collected fractions were dried and further analysed by thin-layer chromatography (chloroform:ethyl acetate:methanol, 6:3:1). The purity analysis of fractions was determined by injecting them onto the C-18 column (5 µm; 230 × 4.50 mm) with a gradient run of acetonitrile and water for 20 min at a flow rate of 0.5 ml/min with detection at 210 nm. The structural elucidation and characterization of compounds were determined using spectroscopic methods, including mass spectroscopy (MS) and nuclear magnetic resonance (NMR). The mass spectrum data were recorded by the ion trap method on HR-ESI-MS (XEVO G2-XS QTOF, WATERS, UK). <sup>1</sup>H and <sup>13</sup>C NMR experiments were carried out on AM-500 FT-NMR spectrometer (Bruker, Rheinstetten, Germany) using tetramethylsilane (TMS) as the internal reference.

## Statistical analysis

The experiments on biological activity were carried out in triplicates. The data are reported as means  $\pm$  standard deviation. Student's t-test was used to analyse the differences between control and test samples. One-way analysis of variance (ANOVA) was used to study the differences among two assays. Differences with  $p$  values  $< 0.05$  were considered statistically significant. IBM SPSS Statistics 18 was used for data analysis.

## Results

### Characterization and identification of marine actinobacteria

Andaman and Nicobar islands, known for their rich biodiversity, were explored for isolation of actinobacteria for the production of bioactive metabolites. The actinobacterial isolate SCA30 was able to grow on starch casein agar and yeast extract malt extract agar (ISP-2 medium) and had developed branched aerial hyphae with elongated spores and smooth surface. The isolate was aerobic, Gram-positive and non-motile. Fragmented substrate mycelium was observed under the scanning electron microscope (Fig. 1).

Multiple sequence alignment and 16S rRNA gene sequence compared with the other sequences in NCBI confirmed that the strain SCA30 belonged to the genus *Nocardiopsis*. The sequence was submitted to the GenBank database under accession no: MT573349. Two

sets of degenerate primers were used to amplify specific domains of the PKS-I and PKS-II. The strain SCA30 possessed only PKS-II gene cluster (Fig. 2). The biosynthetic gene cluster sequence was submitted to NCBI under GenBank accession no: MT749355 (Table 1). Sequences were aligned with most closely related 16S rRNA and homology of amino acid sequences derived from PKS-II biosynthetic gene sequences from the GenBank. Phylogenetic tree was constructed using the neighbour-joining algorithm by MEGA 7.0 (Fig. S1).

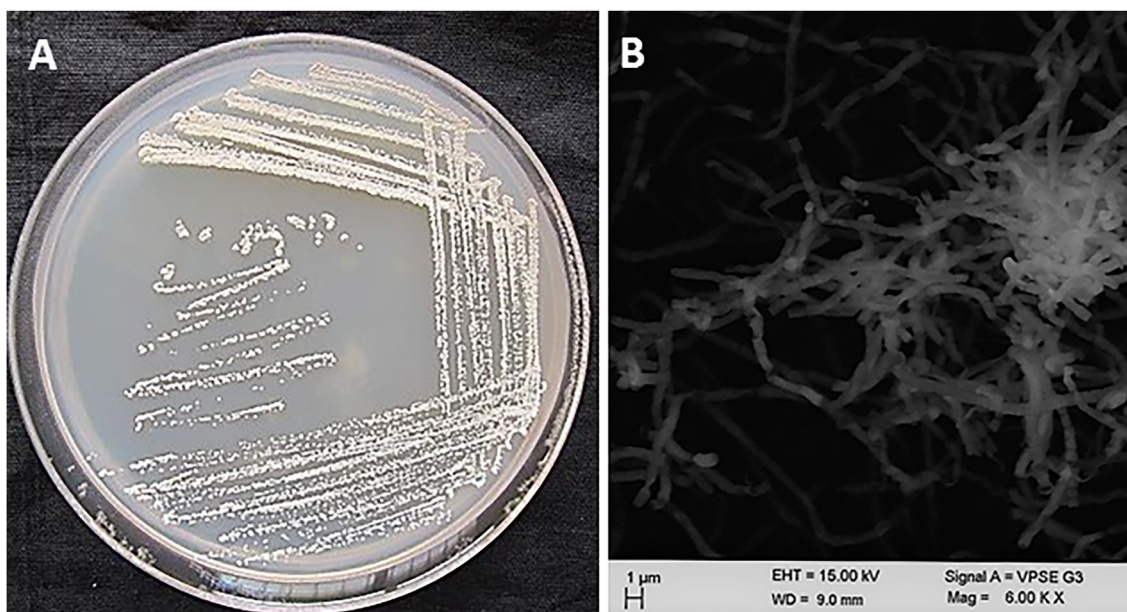
### Bioactive compound production and extraction

The selection of suitable production and seed medium is an important requirement for culturing actinobacteria to produce bioactive compounds. In this study, an optimized medium (ISP-2) was used as fermentation broth and extracted thrice with ethyl acetate to yield 1.09 g of extract. The dark gummy residue was tested for antibacterial activity against MRSA strains and anticancer activity.

### Biological activities

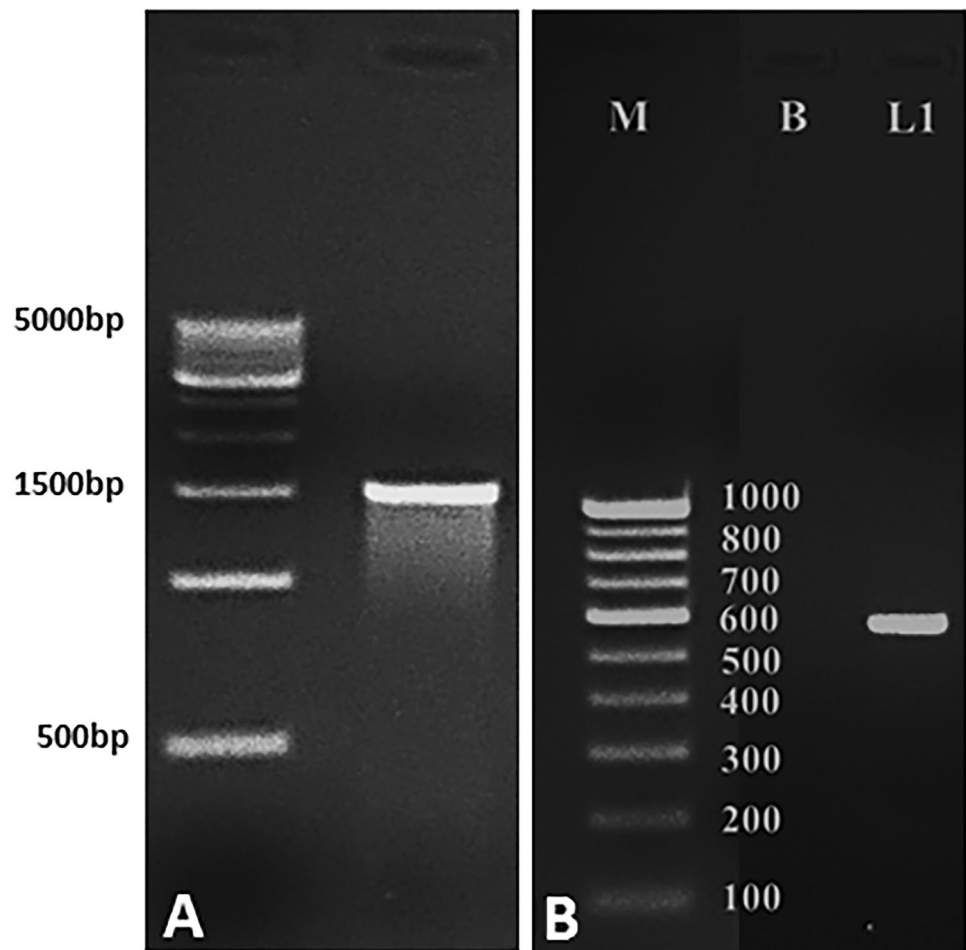
#### Antibacterial activity and minimum inhibitory concentration (MIC)

The extracted compound exhibited broad-spectrum antagonistic activity against MRSA ATCC-46071 and MRSA



**Fig. 1** **A** *Nocardiopsis* sp. SCA30 isolate on ISP 2 media. **B** Fragmented substrate mycelium observed under the scanning electron microscope under 6.00 KX magnification

**Fig. 2** **A** Amplification of genomic DNA (1500 bp) of *Nocardiopsis* sp. SCA30 **B** Amplification of PKS II genes (600 bp) in *Nocardiopsis* sp. SCA30



**Table 1** Detection of PKS-I and PKS-II gene clusters

Strains	Amplification of PKS genes		Accession No
	PKS-I	PKS-II	
SCA 30	–	+	MT749355

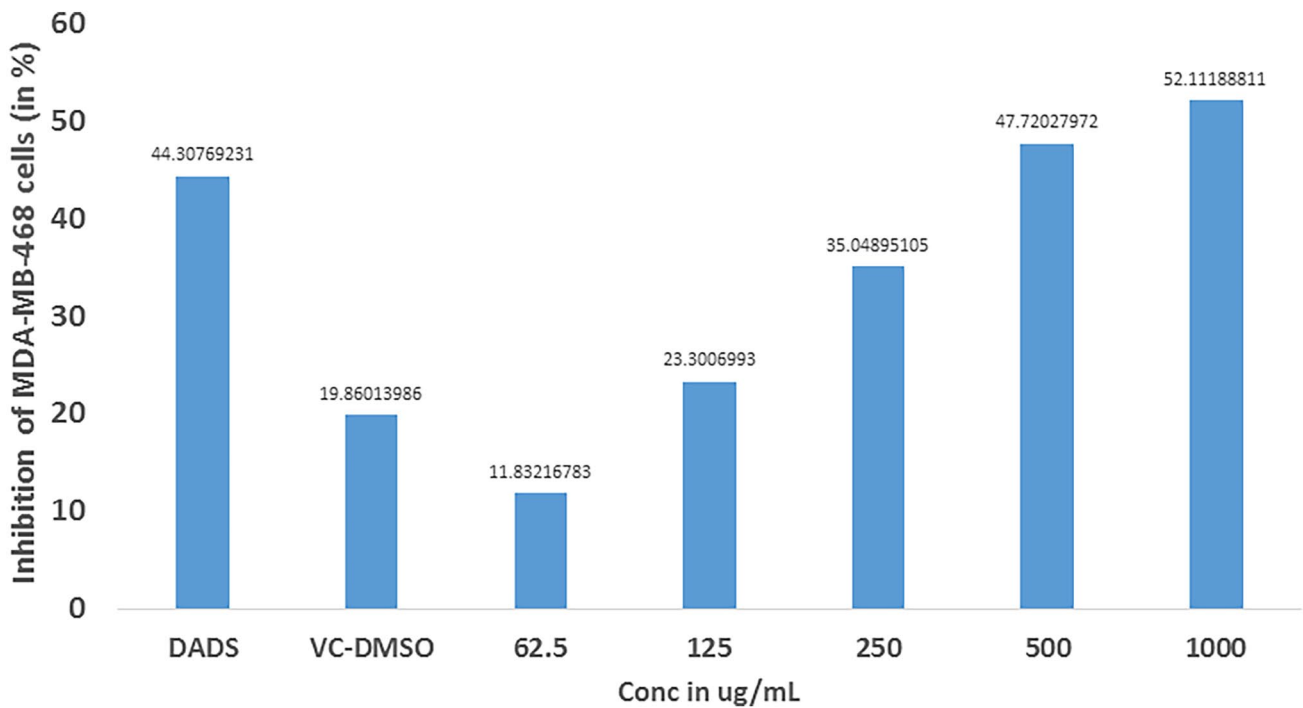
**Table 2** Antibacterial activity and minimum inhibitory concentration (MIC) of bioactive compound against MRSA strains

Test pathogens	Zone of inhibition (mm)		MIC ( $\mu\text{g/mL}$ )
	Compound	Gentamycin	Compound
MRSA ATCC NR-46071	22 $\pm$ 0.71	26 $\pm$ 0.11	15.62
MRSA ATCC NR-46171	18 $\pm$ 0.12	28 $\pm$ 0.07	7.81

ATCC-46171 on Mueller Hinton agar plates (MHA) (Fig. S2). The active compound showed significant inhibitory activity against MRSA ATCC-46071 (22  $\pm$  0.71) with MIC of 15.62  $\mu\text{g/mL}$  and against MRSA ATCC-46171 (18  $\pm$  0.12) with MIC of 7.81  $\mu\text{g/mL}$ , respectively (Table 2).

### Antiproliferative activity

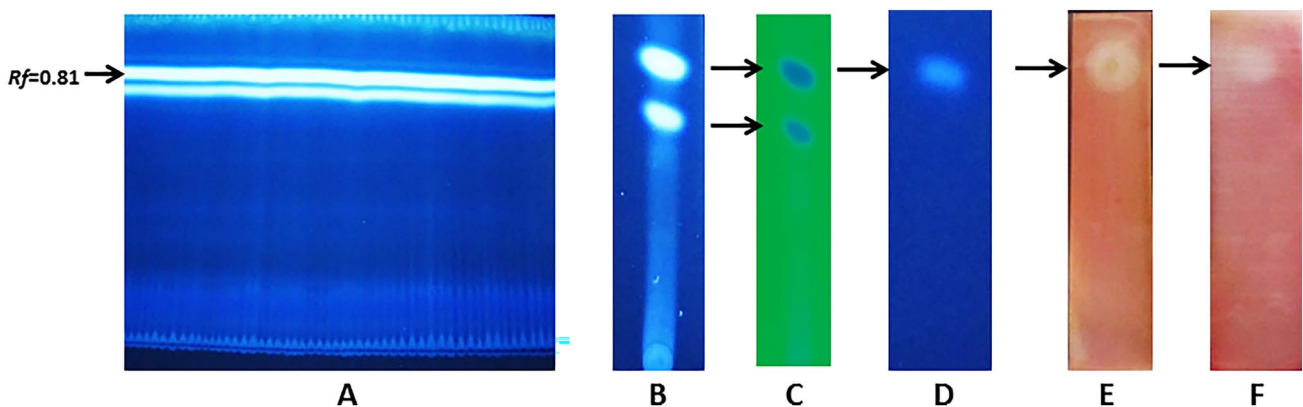
The antiproliferative effect of the active fraction on a broad-spectrum of cancer cell lines was evaluated by SRB assay. The active fraction was tested in cell lines HCT-15, HT-29, MCF-7 and MDA-MB-468 at concentrations ranging from 15.625 to 1000  $\mu\text{g/mL}$ . Diallyl sulfide (1 mM) was used as a positive control in this study. The active fraction had no observable activity at 15.625 and 31.25  $\mu\text{g/mL}$  in the four cell lines. However, a dose-dependent cell inhibition was observed in all the cell lines from 62.5 to 1000  $\mu\text{g/mL}$ . The fraction exhibited the highest cytotoxic effect in the MDA-MB-468 cell line at all tested concentrations with maximum cell inhibition of 52.11% at 1000  $\mu\text{g/mL}$ . In the colorectal adenocarcinoma (colon cancer cell line) HCT-15, the positive control DADS inhibited cell growth by 58.11% at 1 mM, and the fraction at 1000  $\mu\text{g/mL}$  inhibited the cell line by 48.78%. However, in colon cancer cell line HT-29, the activity of fraction (1000  $\mu\text{g/mL}$ ) was higher than that of DADS (1 mM), i.e., 44.34 and 38.75%, respectively. In the breast cancer cell line MCF-7, there was twofold increase in the activity from 500 to 1000  $\mu\text{g/mL}$ .



**Fig. 3** Antiproliferative activity of active fraction of extract tested at concentrations ranging from 62.5 to 1000 µg/mL in MDA-MB-468 breast cancer cell line by SRB assay

ml, i.e., the antiproliferation activity was 25% at 500 µg/ml and 52% at 1000 µg/ml. Again in the breast cancer cells MDA-MB-468, the fraction at 500 and 1000 µg/ml had higher inhibitory activity than DADS (1 mM) (Fig. 3). In previous studies, diketopiperazines, namely, nocazines F and G purified from the deep-sea sediment *Nocardiopsis* sp. YIM M13066 exhibited a broad-spectrum and excellent cytotoxicity against a panel of cancer cell lines PC3, H1299, HL7702, HeLa, MCF-7 and U251 (Sun et al.

2017). Similarly, many marine *Nocardiopsis* sp. have been reported for producing anticancer compounds belonging to polyketide (Kalafungin, Fijiolides, Nocapyrones, Apoptolidins and Griseusins) and peptides (Lucentamycins and Methoxyneihumicin) class (Bennur et al. 2015). Thus, the genus *Nocardiopsis* synthesises therapeutic leads with varied chemotypes and biological effects, of which the most frequently isolated class of compounds are α-pyrones and diketopiperazine derivatives (Ibrahim et al. 2018).



**Fig. 4** **A** Thin-layer chromatogram of crude extract of *Nocardiopsis* sp. SCA30 showing active bands at  $R_f=0.81$ , **B** TLC chromatogram at 364 nm and 254 nm, respectively. **C** Thin-layer chromatogram of

purified fraction at 364 nm. **D** TLC-bioautography assay of purified fraction showing zone of inhibition against MRSA strains **E** ATCC NR-46071 **F** ATCC NR-46171

Therefore, the discovery of novel species of *Nocardiopsis* could lead to the isolation of new and undiscovered bioactive natural products with a broad range of pharmacological activities.

### TLC bioautography assay

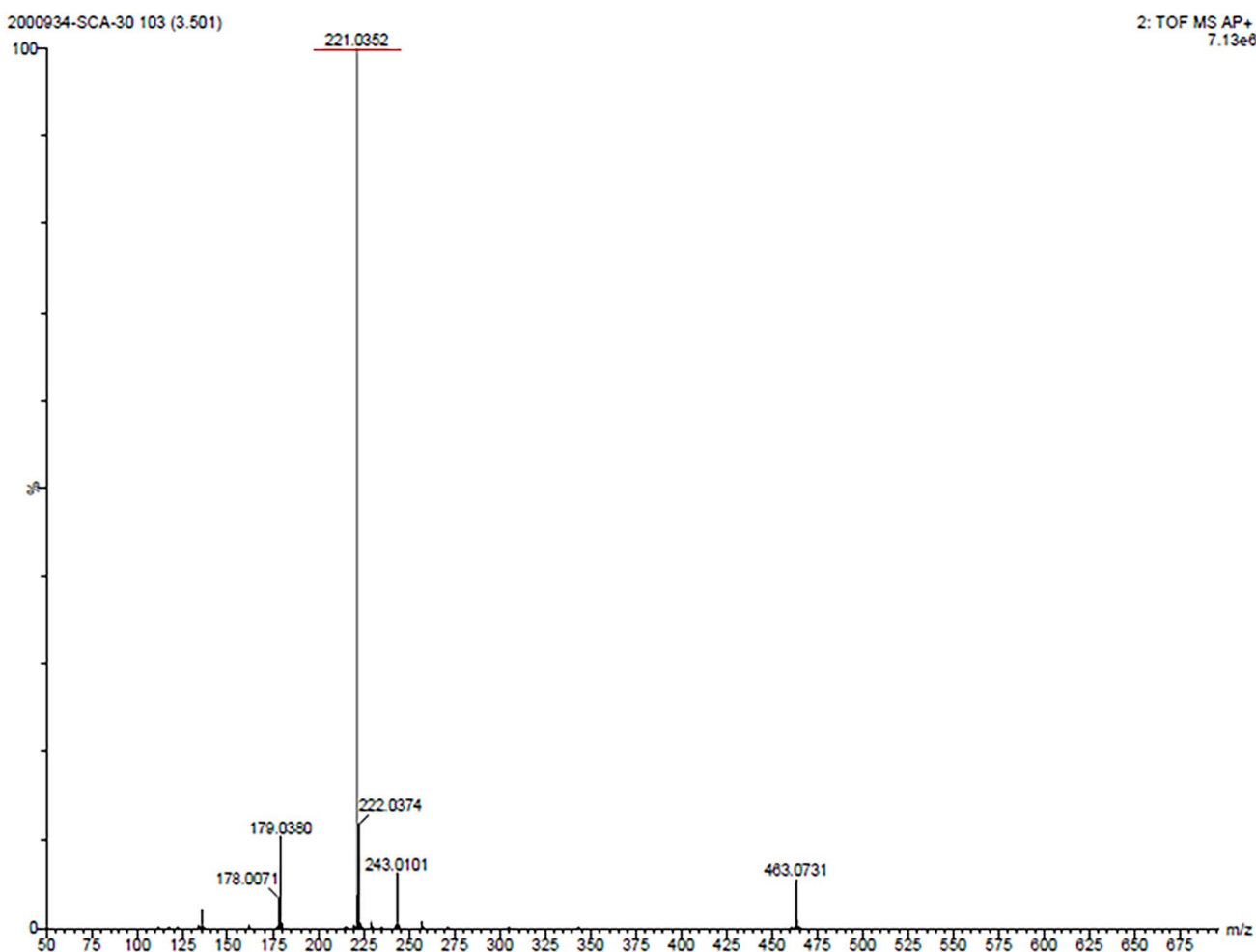
The ethyl acetate extract of strain SCA30 was screened for detection of antibacterial compounds by TLC bioautography assay. The extract showed two prominent bands with different  $R_f$  on TLC. Of them,  $R_f = 0.81$ , which appeared dark blue colour exhibited a clear zone of inhibition against ATCC MRSA-46071 and ATCC MRSA-46171 (Fig. 4). The fraction was further characterized using chromatographic and spectral studies.

### Purification and identification of compound

The ethyl acetate extract was subjected to fractionation using column chromatography

(hexane-chloroform-petroleum ether-ethyl acetate-acetone-methanol 10:90, v/v) yielding two fractions. Each fraction was evaluated for bioactivities. Of them, the active fraction at  $R_f = 0.81$  was further characterized by high-performance liquid chromatography (HPLC, Shimadzu, LC-20, Japan) to analyse the purity of the compound. The conditions were as follows: gradient run of water and acetonitrile (90:10 for 0–5 min, 30:70 for 5–15 min, 10:90 for 15–25 min and 5:95 for 20 min) at a flow rate of 0.5 ml/min and UV detection at 210 nm.

The structural identification of the active compound was determined by HR-ESI-MS (XEVO G2-XS QTOF, WATERS, USA),  $^1\text{H}$  and  $^{13}\text{C}$  NMR on AM-500 FT-NMR spectrometer (Bruker, Rheinstetten, Germany). The purified compound showed an ion peak at  $m/z$  221.03 ( $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_2$ )  $[\text{M}+\text{H}]^+$  (Fig. 5). The structure of the compound was further confirmed by following detailed NMR analysis:  $^1\text{H}$  NMR (DMSO, 400 MHz):  $\delta_{\text{H}}$  2.47–2.92 (m, 1H),  $\delta_{\text{H}}$  1.99 (m, 2H, Pr-H)  $\delta_{\text{H}}$  6.62–6.78 (d, Ar-H,  $J=9.2$  Hz),  $\delta_{\text{H}}$  8.845 (d, Ar-H,  $J=34.4$  Hz) (Fig. S3).  $^{13}\text{C}$



**Fig. 5** HR-ESI-MS analysis. The purified compound showed an ion peak at  $m/z$  221.03 ( $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_2$ )  $[\text{M}+\text{H}]^+$

NMR (DMSO, 150 MHz):  $\delta_C$  21.95 (C5')  $\delta_C$  39.69–39.90 (C2')  $\delta_C$  116.269 (C4'),  $\delta_C$  119.226 (C2', C6'),  $\delta_C$  152.14 (C1')  $\delta_C$  169.94 (CO) (Fig. S4). Based on spectral data, the structure of the purified compound was confirmed as 1-acetyl-4-(4-hydroxyphenyl piperazine).

## Discussion

The marine ecosystems are one of the most productive habitats of a large number of organisms including actinobacteria, fungi, bacteria, invertebrates, birds and mammals (Saravana Kumar et al. 2014). Actinobacteria from unexplored environments, such as mangroves, marine sediments and estuaries, represent a functional source of bioactive metabolites and have the potential for therapeutic applications (Bennur et al. 2014). In the present study, from the Andaman and Nicobar Islands, we targeted marine-derived actinobacteria for the production of natural products. These actinobacterial isolates are, therefore, evaluated for antibacterial and cytotoxic activity and provide scope for the discovery of novel drugs. In this study, strain SCA 30 produced extracellular secondary metabolites, which were extracted using ethyl acetate and exhibited significant antibacterial activity against MRSA strains and cytotoxic activity against cell lines. Previously, many studies have reported that ethyl acetate is an appropriate solvent for the extraction of bioactive metabolites from actinobacterial strains (Vijayakumar et al. 2012).

The genus *Nocardiopsis* was described and reported by Meyer (1976). Members of the genus *Nocardiopsis* are characterized on their morphology and chemotaxonomic analysis (Grund and Kroppenstedt 1990). They are commonly found in extreme locations such as frozen soils, desert, saline and hypersaline (marine systems, salterns and soils) habitats (Chun et al. 2000). They are frequently isolated from the locations with moderate to high salt concentrations. Strains, including *Nocardiopsis alba*, *Nocardiopsis salina* and *Nocardiopsis lucentensis*, from the solar salterns of Thamaraiikulam, Kovalam and Puthalum were shown to have potential bioactivities (Al-Zarban et al. 2002). Members of the genus *Nocardiopsis* are known to produce a wide range of antimicrobial and cytotoxic agents (Hamedi et al. 2013). A new species *Nocardiopsis lucentensis* sp. nov. DSM 44,048 producing yellowish-brown substrate mycelium and a white aerial mycelium was reported from a salt marsh soil near Alicante, Spain (Yassin et al. 1993). Genus *Nocardiopsis*, well-known source for several antibiotics, reportedly produced pyranonaphthoquinone antibiotic, griseusin D, exhibiting antifungal activity against *Alternaria alternata* (Li et al. 2007). A protease-producing *Nocardiopsis*

sp. NCIM 5124 from marine sediment samples has been reported to degrade crude oil (Dixit and Pant, 2000). The sponge-associated marine *Nocardiopsis dassonvillei* MAD08 exhibited antimicrobial activity against multidrug-resistant pathogens. A total of 11 antimicrobial compounds, including anticandidal protein, were reported, making it the first strain to produce both water-soluble and organic solvent antimicrobial compounds (Selvin et al. 2009). *Nocardiopsis* sp. isolated from Puducherry coast produced metabolites with 18, 20 and 15 mm zone of inhibition against *P. aeruginosa*, *E. coli* and *K. pneumonia* (Vimal et al. 2009). Due to the presence of biosynthetic gene clusters namely PKS and NRPS, the *Nocardiopsis* species was highly determined to control an array of bacterial and fungal pathogens and possessed potent anticancer activity (Komaki et al. 2014). The discovery of many natural products from large multimodular enzymes was validated by genome sequencing to unveil the hidden production of natural products from microbes. Multiple gene clusters for secondary metabolism are a characteristic feature of several non-*Streptomyces* actinobacteria and myxobacteria (Li et al. 2004).

The extraction of secondary metabolites from ethyl acetate helped precipitate the potent antibacterial compounds. The extracts effectively inhibited the growth of MRSA strains. According to reports, the gene clusters in the *Nocardiopsis* strains had the ability to synthesizing antimicrobial compounds. The purification and characterization of active constituent led to the identification of 1-acetyl-4-(4-hydroxyphenyl piperazine), which has a molecular formula of  $(C_{12}H_{16}N_2O_2) [M+H]^+$

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00203-021-02582-2>.

**Author contributions** SS, JBA and MGK performed the experiments, analysed the results and wrote the manuscript. SRVM and RRV supervised the experiments, analysed the results and reviewed the manuscript.

**Funding** Not applicable.

**Availability of data and material** Not applicable.

**Code availability** Not applicable.

## Declarations

**Conflict of interest** The authors declare no conflict of interest.

**Ethical approval** Not applicable.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.



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