RESEARCH PAPER

# Extraction and characterization of secondary metabolites derived from halophilic strain and its Pharmacological studies

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**Abstract**

The increasing prevalence of multidrug-resistant (MDR) bacterial pathogens has intensified the search for novel microbial sources of bioactive secondary metabolites. In the present study, a halophilic actinomycete strain, designated HA8, was investigated for its antimicrobial and antioxidant potential and chemically profiled using spectroscopic and chromatographic approaches. The secondary metabolites produced by HA8 were extracted using petroleum ether, hexane, chloroform, and ethyl acetate, and the resulting extracts were evaluated against clinically important bacterial pathogens by the agar well diffusion method. Among the tested extracts, the ethyl acetate extract exhibited the strongest antibacterial activity, with inhibition zones of 20 mm against *Acinetobacter baumannii*, 18 mm against *Pseudomonas aeruginosa*, 17 mm against *Enterococcus faecium*, and 16 mm against *Escherichia coli*. Concentration-dependent antibacterial activity was further observed for the ethyl acetate extract, with maximum inhibition zones of 21 mm against *A. baumannii* and 17 mm against *E. faecium* at the highest tested concentration. The active fraction also demonstrated concentration-dependent DPPH radical-scavenging activity, indicating the presence of antioxidant constituents. FT-IR analysis revealed characteristic absorption bands associated with diverse functional groups, while GC–MS analysis tentatively identified 30 compounds in the ethyl acetate extract. The major detected constituents included *n*-hexadecanoic acid, 9-octadecenamide (oleamide), undecanoic acid, 6-octadecenoic acid, and octadecanoic acid. Overall, the findings demonstrate that halophilic actinomycete HA8 produces chemically diverse metabolites with promising antibacterial activity against MDR-associated pathogens and antioxidant potential. Further purification, structural elucidation, MIC/MBC determination, antibiofilm evaluation, and mechanistic studies are warranted to establish the pharmaceutical potential of the bioactive metabolites.

**Keywords:** Multidrug Pathogens, Halophilic Strain, *Acinetobacter baumannii*, GCMS.

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## INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the greatest global public health threats, reducing the effectiveness of antimicrobial therapies and increasing the burden of infectious diseases worldwide. The ongoing development of antimicrobial resistance has made the search for new bioactive substances. Among microbial producers, actinomycetes have continuously shown remarkable ability for the creation of structurally diversified secondary metabolites with broad-spectrum biological activity. Actinomycetes are the source of more than two-thirds of clinically significant antibiotics, demonstrating their vital importance in contemporary drug discovery (Murray *et al.,* 2022).

The rapid emergence and spread of resistant pathogens have resulted in higher morbidity and mortality, particularly among vulnerable populations such as children, older adults, and immunocompromised individuals (Mancuso *et al.,* 2021; Naghavi *et al.,* 2024). Among the most concerning multidrug-resistant (MDR) pathogens are methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The increasing prevalence of these pathogens has severely limited available treatment options and poses a significant challenge to healthcare systems worldwide (Zowawi, 2016; Alotaibi, 2019; Thabit *et al.,* 2023; Kiran *et al.,* 2024).

Among them, halophilic actinomycetes, which inhabit saline and hypersaline environments such as salt pans, solar salterns, mangrove ecosystems, marine sediments, and salt lakes, have attracted considerable scientific interest owing to their remarkable physiological adaptations and biosynthetic potential. Adaptation to high salinity and osmotic stress has driven these microorganisms to evolve specialized metabolic pathways, allowing them to survive under extreme environmental conditions while producing a wide range of secondary metabolites with unique chemical structures (Barka *et al.,* 2016; Hamedi *et al.,* 2013; Rathinam *et al.,* 2023).

Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of

microorganisms but play important ecological roles, including defense against competing microbes and adaptation to environmental stress (Mohan & Rajamanickam, 2018). Marine and halophilic actinomycetes have received increasing attention because they produce unique secondary metabolites with potent biological activities, including the ability to overcome drug resistance in cancer cells and inhibit pathogenic microorganisms (Elmallah *et al.,* 2020). Consequently, extensive efforts have been devoted over the past several decades to isolating novel actinomycetes from diverse terrestrial and marine habitats as part of natural product discovery and drug screening programs (Dewi *et al.,* 2017).

These secondary metabolites include polyketides, non-ribosomal peptides, alkaloids, terpenoids, phenolic compounds, and several other biologically active molecules that exhibit diverse pharmacological properties, including antimicrobial, anticancer, antioxidant, and anti-inflammatory activities (Newman & Cragg, 2020).

Secondary metabolites produced by halophilic actinomycetes have demonstrated significant antimicrobial activity against several clinically important pathogens. The unique structural diversity of these metabolites provides opportunities for the development of new antimicrobial agents capable of overcoming existing resistance mechanisms (Thompson & Gilmore, 2024). Furthermore, advances in genome mining, metabolomics, and biosynthetic gene cluster analysis have revealed that many halophilic actinomycetes possess cryptic or silent biosynthetic pathways that remain unexpressed under conventional laboratory conditions, indicating enormous untapped potential for the discovery of novel therapeutic compounds (Chinnathambi *et al*., 2023).

Beyond their antimicrobial potential, secondary metabolites from halophilic actinomycetes have attracted growing interest because of their antioxidant properties. Oxidative stress, caused by excessive generation of reactive oxygen species (ROS), plays a critical role in the development of numerous chronic disorders, including cardiovascular diseases, diabetes, neurodegenerative disorders, cancer, and inflammatory diseases (Reuter *et al*., 2010; Liguori *et al*., 2018; Hamedi *et al*., 2013).

Therefore, the present study aimed to isolate halophilic actinomycetes from saline environments, evaluate their ability to produce biologically active secondary metabolites, and investigate the antimicrobial activity against multidrug-resistant pathogens together with their antioxidant and anti-inflammatory properties. The findings of this study may contribute to the identification of promising natural products for the development of next-generation antimicrobial and therapeutic agents.

## MATERIALS AND METHODS

### Sample Collection

Soil samples were collected from the different locations of costal saltpan in Nagapattinam District, Tamil Nadu, India. Samples were collected using sterile spatulas and transferred to sterilized, labelled polyethylene bags. Samples were collected from the top layer with a thickness ranging from 5-10 cm. Samples were brought to the lab for further processing. Some description of the sampling site was recorded when it was related to the environment, such as salinity and pH. Samples were processed for the isolation of halophilic actinomycetes within 24 hours post-collection.

### Isolation of Halophilic Actinomycetes

An appropriate selective culture methodology was adopted for the isolation and identification of halophilic actinomycetes. Using sterile techniques, about 1 g of each saline soil/sediment sample was immersed in 9 mL of sterile saline solution and appropriately diluted. A suitable dilution was spread on the surface of Starch Casein Agar (SCA) supplemented with the appropriate concentration of NaCl for the growth of halophilic/halotolerant actinomycetes. Plates were incubated at 28 ± 2°C and examined on a daily basis for the appearance of the characteristic actinomycete morphology including dry, powdery, chalky, leathery, and/or filamentous growth. Actinomycete colonies that exhibited morphological characteristics were isolated, and purified by repeated streaking on fresh SCA plates of the appropriate salt concentration and processed for the preparation of pure cultures. Purified isolates were held on SCA slants and preserved as working cultures for later examinations and secondary metabolite production. Isolates were identified with respective source and isolation order codes; the bioactive isolate was termed HA8.

### Extraction of Secondary metabolites

The most promising secondary metabolite producing strains were selected for scale-up extraction. Seed cultures were prepared by inoculating strains into 25 mL Starch Casein Agar (SCA) broth at optimum salt concentration and incubating at 28°C, 140 rpm for 7 days on a rotary shaker. This seed culture was subsequently scaled up to 1–2 L in the same medium and incubated for an additional 2 weeks under identical conditions. Following fermentation, the culture broth was centrifuged (10,000 rpm, 10 min) to separate the biomass, and the cell-free supernatant was collected. Metabolites were extracted using four organic solvents of increasing polarity such as petroleum ether, hexane, chloroform, and ethyl acetate at a 1:1 (v/v) ratio of supernatant to solvent. The organic phase was separated and concentrated to dryness

under reduced pressure using a rotary vacuum evaporator at 45°C. The resulting crude extracts were reconstituted in 1 mL of their respective solvents and subsequently evaluated for antimicrobial activity against test pathogens (Wang *et al*., 2009).

**Screening of Antibacterial activity**

The antibacterial activity of crude extracts and active fractions was tested against the bacterial pathogens by the well diffusion method. For this, bacterial pathogens were inoculated in nutrient broth and incubated for 24 h before the antibacterial assay. All the bacterial strains were individually spread on the Mueller-Hinton agar plates. Wells were made in the plates at 6 mm using a cork borer. The different concentrations 25µl, 50µl, 75µl and 100µl of fractions were added on the wells and incubated at 37°C for 24 hours (Saadoun and Muhana, 2008). The assay was carried out in triplicates. The zone of inhibition was measured in mm after the completion of the incubation period.

**Antioxidant assay**

The samples were allowed to react with the stable DPPH (α, α--Diphenyl- β-picryl-hydroxyl) radical in ethanol solution. The reaction mixture has 0.5 ml sample, 3 ml of absolute ethanol and 0.3 ml of 0.5 mM DPPH radical in ethanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read at 517 nm after 30 min of reaction using a UV spectrophotometer. The mixture of ethanol (3.3 ml) and sample (0.5 ml) serve as blank. The control solution was prepared by mixing ethanol (3.5 ml) and DPPH radical solution (0.3ml). The scavenging activity percentage was determined. Different concentrations of fractions and reference standard 20, 40, 60, 80 and 100 ug/ml Ascorbic Acid was used as reference standard (Silva *et al.,* 2005).

**FT-IR analysis**

The FT-IR spectrum of the purified fraction was recorded using an EXI-Spectrum One FT-IR spectrophotometer. Samples were analyzed by the KBr pellet technique, wherein 100 mg of vacuum-dried (AR grade) KBr was used to prepare the pellet. Spectra were recorded in the range of 4000–400 $cm^{-1}$ at a resolution of 1.0 $cm^{-1}$, and the resulting spectrum of intensity versus wavenumber was analyzed to identify characteristic functional groups present in the sample (Sahoo *et al*., 2015).

**GCMS Spectrum**

The crude extract was analyzed using a Shimadzu GCMS-QP2010 Plus system equipped with an AOC-20i auto sampler and an RTX-5MS capillary column (30 m × 0.32 mm, 0.50 µm film thickness). Helium of 99% purity was used as the carrier gas at a constant flow rate of 1.73 mL/min. The sample was injected at 0.5 µL with a split ratio of 10:1 and an injector temperature of 270°C. The ion-source temperature was maintained at 200°C, and mass spectra were acquired in electron ionization mode at 70 eV over an m/z range of 40–450, with a scan interval of 0.5 s. The oven temperature was initially held at 40°C for 2 min, increased to 150°C and subsequently to 250°C at 8°C/min, and finally maintained at 280°C for 20 min. The relative abundance of each compound was determined from its peak area relative to the total chromatographic peak area (Keerthiga & Anand, 2015). Chromatographic data and mass spectra were processed using TurboMass v5.2.0, and compound identification was performed by comparison with the NIST mass spectral library.

## RESULTS

**Purification of Isolates**

The actinomycete colonies showing distinct morphological characteristics were selected and purified by repeated streaking on Starch Casein Agar (SCA) supplemented with the required salt concentration. The plates were incubated at 28 ± 2°C for 7–14 days, and colonies were repeatedly subcultured until pure cultures with uniform morphological characteristics were obtained. The purified isolates were maintained on SCA slants under appropriate saline conditions and used for subsequent antimicrobial screening and secondary metabolite production studies. The isolate HA8 was selected for antibacterial activity and further investigations.

**Screening of antibacterial activity**

The antibacterial activity of crude solvent extracts from the HA8 strain was initially screened against selected multidrug-resistant bacterial pathogens using the agar well diffusion method. Four solvent extracts, namely petroleum ether, hexane, chloroform, and ethyl acetate, were evaluated to identify the extract exhibiting the highest antibacterial activity. Among the tested extracts, the ethyl acetate extract showed the broadest and strongest antibacterial activity at 100 µL, producing maximum zones of inhibition of 20 mm against *Acinetobacter baumannii*, 18 mm against *Pseudomonas aeruginosa*, 17 mm against *Enterococcus faecium*, and 16 mm against *Escherichia coli* (Table 1). Based on these preliminary screening results, the ethyl acetate extract was selected for further concentration-dependent antibacterial evaluation.

| MDR Pathogens | Zone of Inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | HA8 | | | | | |
| | Hexane | Chloroform | Ethyl Acetate | Petroleum ether | Positive | Negative |

| | | | | | | |
|---|---|---|---|---|---|---|
| *A. baumannii* | - | 9±1.00 | **20 ±0.58** | - | 21±1.00 | - |
| *S. pyogenes* | - | 10±1.53 | **15±1.53** | 11±1.00 | 23±1.00 | - |
| *E. coli* | 11±1.00 | 13±1.00 | **16±1.53** | 12±1.53 | 22±1.00 | - |
| *S. aureus* | 15±1.53 | - | **14±1.53** | - | 25±1.00 | - |
| *E. faecium* | 10±1.53 | 16±1.53 | **17±1.53** | - | 24±1.00 | - |
| *P. aeruginosa* | - | 10±1.00 | **18±1.00** | 14±1.00 | 26±0.58 | - |

**Table 1. Antibacterial activity of crude extracts from HA8**

Value represents mean ±SD; n=3, - No zone

**Antibacterial activity of ethyl acetate crude extract of HA8 Strain**

To further evaluate the antibacterial potential of the selected ethyl acetate extract, different volumes (25, 50, 75, and 100 µL) were tested against the bacterial pathogens using the agar well diffusion method. The increasing inhibition zones observed with increasing extract volume indicated a concentration-dependent antibacterial response. The maximum inhibition was observed at 100 µL, with zones of 21 mm against *A. baumannii* and 17 mm against *Enterococcus faecium*, while *P. aeruginosa* showed an inhibition zone of 19 mm. The results are presented in (Table 2).

| **Bacterial pathogens** | **Zone of inhibition mm** | | | | | |
|---|---|---|---|---|---|---|
| | **25 µl** | **50 µl** | **75 µl** | **100 µl** | **Positive** | **Negative** |
| *Staphylococcus aureus* | 10±0.66 | 11±1.03 | 13±0.54 | 16±0.68 | 25±1.00 | - |
| *Streptococcus Pyogenes* | 11±0.36 | 12±0.63 | 15±0.74 | 17±0.58 | 27±1.00 | - |
| *Pseudomonas aeruginosa* | 10±0.34 | 12±0.53 | 17±0.63 | 19±0.68 | 29±1.00 | - |
| *Acinetobacter baumannii* | 11±0.76 | 13±1.83 | 16±0.74 | **21±0.58** | 29±1.00 | - |
| *Escherichia coli* | 9±1.53 | 11±0.24 | 12±1.00 | 16±0.58 | 28±1.00 | - |
| *Enterococcus faeciu m* | 10±1.00 | 12±1.12 | 14±0.58 | 17±1.15 | 29±0.58 | - |

**Table 2: Antibacterial activity of ethyl acetate extract of HA8 strain**

Value represents mean ±SD; n=3, - No zone

**Antioxidant activity of HA8**

The active fraction from HA8 strain were checked inhibition of DPPH radical at different concentrations (20- 100 µg/ml) and ascorbic acid used as Standard. The scavenging activity shows the 227.30±0.1 µg/g at 100 (µg/ml) concentration **Fig.2**

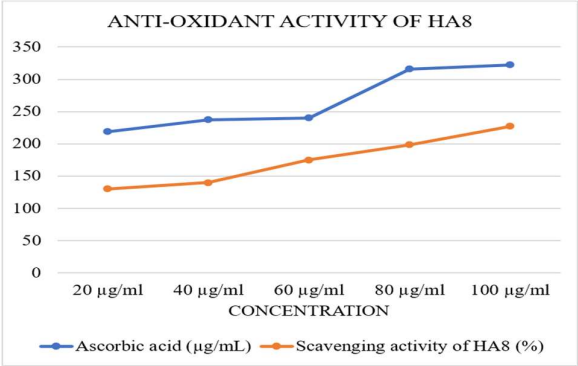


**Fig.2: DPPH scavenging assay of HA8**

**Table 3. Anti-oxidant scavenging activity of HA8**

| **Concentration (µg/ml)** | **Ascorbic acid (µg/mL)** | **Scavenging activity of HA8 (%)** |
|---|---|---|
| **20** | 219.15 ± 3.1 | 130.45±0.2 |
| **40** | 237.25 ± 2.9 | 140.02±0.4 |
| **60** | 240.34 ± 3.5 | 175.34±1.3 |
| **80** | 315.78 ± 2.5 | 198.51±1.3 |
| **100** | 322.40 ± 1.6 | 227.30±0.1 |

**FT- IR Spectrum**

The FT-IR spectrum of the HA8 ethyl acetate extract revealed several characteristic absorption bands in the range of approximately 4000–400 $cm^{-1}$, indicating the presence of diverse functional groups in the extract (Fig. 3). A broad absorption band was observed in the higher-wavenumber region around 3200–3300 $cm^{-1}$, which may be associated with O–H or N–H stretching vibrations. Absorption bands observed in the region of approximately 2900–2850 $cm^{-1}$ can be attributed to aliphatic C–H stretching vibrations. Additional bands in the fingerprint region, particularly around 1600–1400 $cm^{-1}$, may correspond to C=C, C–H bending, or other functional-group vibrations.

Further absorption bands in the lower-wavenumber region indicate the presence of C–O, C–N, and other related functional groups. Overall, the FT-IR profile indicates the presence of multiple functional groups in the HA8 ethyl acetate extract, supporting the chemical complexity of the bioactive fraction.

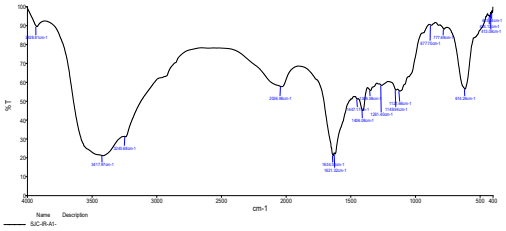

**Fig.3. FITR Spectrum**

**GC-MS Characterization**

The chemical compositions of isolates' secondary metabolites were identified, and the separated chromatogram was compared using the NIST library. The HA8 ethyl acetate GC-MS chromatogram showed the presence of 30 compounds with peak area and retention time. All the compounds were summarized with their molecular formula, molecular weight, peak area, and retention time. The major compounds are n-Hexadecanoic acid, 9-Octadecenamide, (Z) (CAS) OLEOAMIDE, 6-Octadecenoic acid, (Z), Undecanoic acid (CAS), and Undecylic acid, which are found in HA8 ethyl acetate extract (Fig.4**).**

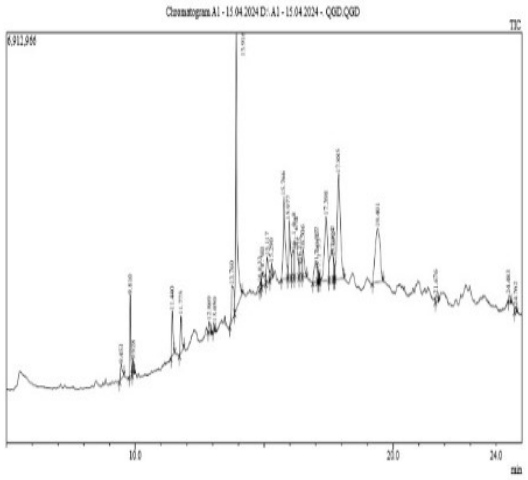

**Fig.4. GC-MS spectrum of Ethyl acetate extract HA8**

**Table.4. Chemical compositions of HA8 Ethyl acetate crude extract**

| S. No | Compound Name | Area % | Retention Time | Molecular Weight |
|---|---|---|---|---|
| 1 | Dodecanoic acid (CAS) Lauric acid | 1.09 | 9.453 | 200.32 |
| 2 | Davanone | 3.15 | 9.810 | 236.35 |
| 3 | 1H Cycloprop[e]azulen-7-ol, decahydro-1,1,7 | 0.51 | 9.928 | 220.3505 |
| 4 | Lilac alcohol B | 2.28 | 11.440 | 170.25 |
| 5 | Tetradecanoic acid | 2.20 | 11.775 | 228.3709 |
| 6 | Pentadecanoic acid | 0.58 | 12.869 | 242.40 |
| 7 | 1-Hexadecanol (CAS) Cetal | 0.36 | 13.050 | 242.44 |
| 8 | Cyclopentadecanone, 2-hydroxy- | 3.63 | 13.760 | 240.3816 |
| 9 | n-Hexadecanoic acid | 16.56 | 13.916 | 256.4241 |
| 10 | 9H-Purine, 9-(trimethylsilyl)-2,6-bis[(trimethyl | 0.47 | 14.833 | 280.47 |
| 11 | Heptadecanoic acid (CAS) Margaric acid | 1.56 | 14.933 | 270.5 |
| 12 | n-Nonadecanol-1 | 2.17 | 15.117 | 284.5203 |
| 13 | 9-Octadecenoic acid (Z)-, methyl ester (CAS) | 0.86 | 15.290 | 296.4879 |
| 14 | 6-Octadecenoic acid, (Z)- | 7.18 | 15.766 | 282.4614 |
| 15 | Octadecanoic acid | 4.18 | 15.977 | 284.5 |
| 16 | 2-{[2'-(3"-isopropenyl-1"-cyclopropenyl)-2'-m | 2.67 | 16.108 | 124.18 |
| 17 | 1,3-Dioxolane-4-methanol, 2-pentadecyl-, acet | 4.48 | 16.216 | 356.5 |
| 18 | Hexadecanamide | 1.79 | 16.375 | 255.44 |
| 19 | 5-N-PENTADECYL-1,2,3,4-TETRAHYDRO | 1.99 | 16.506 | 342.6010 |
| 20 | 6-N-DECYL-1,2,3,4-TETRAHYDRONAPHT | 2.32 | 17.022 | 272.5 |
| 21 | (-)-Caryophyllene oxide | 0.41 | 17.092 | 220.35 |
| 22 | DESACYL-KONDURANGOGENINS A | 0.27 | 17.133 | 1149.3 |
| 23 | Undecanoic acid (CAS) Undecylic acid | 8.63 | 17.398 | 186.29 |

| | | | | |
|---|---|---|---|---|
| 24 | 1,5-Hexadiene, 3,4-diethyl-1,6-diphenyl-, (E,E | 3.61 | 17.642 | 290.45 |
| 25 | Hexadecanoic acid, 1-(hydroxymethyl)-1,2-eth | 1.08 | 17.692 | 568.9 |
| 26 | 9-Octadecenamide, (Z)- (CAS) OLEOAMIDE | 14.19 | 17.885 | 281.4766 |
| 27 | 5(4H)-Oxazolone, 4-methyl-2-phenyl-(CAS) | 10.47 | 19.401 | 295.33 |
| 28 | 1,5-UNDECADIENE, 6,7,7,8,8,9,9,10,10,11, | 0.57 | 21.676 | 152.28 |
| 29 | 5-Hepten-3-one, 2-(5-ethenyltetrahydro-5-met | 0.45 | 24.483 | 236.3499 |
| 30 | Scillarenin | 0.30 | 24.762 | 384.5 |

## DISCUSSION

Actinomycetes are particularly important because of their established capacity to produce structurally diverse secondary metabolites with antimicrobial and other pharmacological properties (Barka *et al*., 2016; Newman & Cragg, 2020). In the present study, the halophilic actinomycete strain HA8 was evaluated for antibacterial and antioxidant activities, together with preliminary chemical characterization. The antibacterial screening revealed clear differences among the solvent extracts of HA8, with the ethyl acetate extract showing the strongest activity. It produced inhibition zones of 20 mm against *Acinetobacter baumannii*. The comparatively lower or selective activity of the other solvent extracts indicates that solvent polarity may influence the recovery of antibacterial constituents. Similar antimicrobial activity of ethyl acetate extracts from actinomycetes has been reported previously (Janardhan *et al*., 2014; Rathinam *et al*., 2023). The activity of HA8 against MDR-associated pathogens is particularly relevant to the continuing global antimicrobial resistance crisis. The World Health Organization has reported widespread resistance to commonly used antibiotics and emphasized the continuing global threat posed by AMR. The observed inhibition of *A. baumannii*, *P. aeruginosa*, *E. coli*, and *Enterococcus* spp. supports further investigation of HA8 as a potential source of antimicrobial metabolites. Similar antimicrobial potential has been reported among unexplored halophilic actinobacteria (Rathinam *et al*., 2023). The active HA8 fraction also demonstrated concentration-dependent DPPH radical-scavenging activity, with the highest activity observed at 100 µg/mL. This finding indicates the presence of constituents capable of donating hydrogen atoms or electrons to the DPPH radical. Antioxidant activity among actinomycetes and rare actinobacteria has been reported previously, supporting their potential as sources of natural antioxidant compounds (Janardhan *et al*., 2014; Mohammadipanah & Momenilandi, 2018). The lower activity of HA8 compared with ascorbic acid is expected because the HA8 fraction contains a complex mixture of metabolites rather than a purified antioxidant. FT-IR analysis of the HA8 ethyl acetate extract showed characteristic absorption bands at 3069, 1406, 1346, and 1212 $cm^{-1}$, indicating the presence of diverse chemical functionalities. FT-IR provides preliminary information about functional groups but cannot establish the identity of individual compounds. Similar spectroscopic approaches have been used for the preliminary characterization of actinomycete-derived bioactive extracts (Janardhan *et al*., 2014). GC–MS analysis revealed 30 chromatographic components tentatively assigned through comparison with the NIST mass spectral library. The major constituents included *n*-hexadecanoic acid (16.56%), 9-octadecenamide or oleamide (14.19%), undecanoic acid (8.63%), 6-octadecenoic acid (7.18%), and octadecanoic acid (4.18%). The predominance of fatty acids and related lipid-derived compounds indicates the chemical complexity of the HA8 extract. GC–MS profiling has similarly been employed for preliminary characterization of actinomycete metabolites (Janardhan *et al*., 2014).

## CONCLUSION

The present study demonstrated the promising biotechnological potential of the halophilic actinomycete strain HA8 as a source of bioactive secondary metabolites with antibacterial and antioxidant properties. These findings highlight HA8 as a valuable source of chemically diverse metabolites with potential applications in the development of novel antimicrobial and antioxidant agents. In future, purification and structural elucidation of the active metabolites, together with MIC/MBC, antibiofilm, anti-inflammatory, cytotoxicity, mechanistic, and in vivo studies, will be essential to validate their therapeutic potential. Genome sequencing and biosynthetic gene-cluster analysis may further facilitate the discovery of novel metabolites and provide opportunities for developing HA8-derived compounds as future pharmaceutical and biotechnology products.

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