


# Isolation and Characterization of Antibacterial Substances Produced by Bacteria Isolated from the Dead Sea Region, Jordan

Ghufraan H. Mahmood<sup>1,2</sup>, Suha M. Abudoleh<sup>3\*</sup> , Nisreen Dahshan<sup>1</sup> , Dima N. Lafi<sup>1</sup>  and Ahmad A. Talhouni<sup>4</sup> 

<sup>1</sup>Department of Applied Pharmaceutical Sciences and Clinical Pharmacy, Faculty of Pharmacy, Isra University, Amman, Jordan.

<sup>2</sup>Department of Pharmaceutics, Faculty of Pharmacy, University of Baghdad, Baghdad, Iraq.

<sup>3</sup>Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, Middle East University, Amman, Jordan.

<sup>4</sup>Department of Anesthesia, Faculty of Allied Sciences, Isra University, Amman, Jordan.

## Abstract

The increasing prevalence of multidrug-resistant (MDR) pathogens necessitates the discovery of novel antibacterial agents. In this study, culturable soil bacteria from the Dead Sea region (Jordan) were isolated and the antibacterial activity of their extracellular metabolites were evaluated. Twenty-one isolates were screened using a perpendicular-streak primary assay; three stable producers (GH-15, GH-20, GH-21) were prioritized. Cell-free supernatants were extracted with n-hexane and ethyl acetate, tested using agar well diffusion, and profiled using thin-layer chromatography (TLC) coupled with bioautography (GH-15). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by broth microdilution against *Bacillus subtilis* ATCC 11774, *B. cereus* ATCC 10876, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Proteus mirabilis* ATCC 29906. The ethyl acetate extract of GH-15 showed the lowest MIC against *E. coli* (0.073 mg/mL). The MIC values were equal to MBC for several strains, indicating bactericidal activity. For GH-15 hexane extract, the lowest MIC was 2.625 mg/mL (*E. coli* and *B. cereus*), and the lowest MBC was 5.25 mg/mL (*E. coli* and *B. cereus*). Dead Sea soils yielded bacterial isolates with broad antibacterial activity; solvent-dependent activity and TLC-bioautography suggested multiple bioactive metabolites, supporting further purification and chemical identification.

**Keywords:** Antibiotics, Soil, Dead Sea, Bacteria, Bioautography, MIC, MBC

\*Correspondence: sabudoleh@meu.edu.jo

**Citation:** Mahmood GH, Abudoleh SM, Dahshan N, Lafi DN, Talhouni AA. Isolation and Characterization of Antibacterial Substances Produced by Bacteria Isolated from the Dead Sea Region, Jordan. *J Pure Appl Microbiol.* 2026;20(2):1782-1790. doi: 10.22207/JPAM.20.2.67

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## INTRODUCTION

Antibiotics are natural or synthetic substances that are primarily produced as secondary metabolites by microorganisms with antimicrobial properties.<sup>1</sup> A major source of antibiotics is the bacterial community, which is widely used in human healthcare. Each year, approximately 500 antibiotics are discovered, most of which are obtained from soil bacteria.<sup>1-3</sup> Antibiotics have revolutionized pharmacology and medicine and have successfully treated many infectious diseases.<sup>4</sup> However, over time, many microbes have developed resistance to these drugs due to excessive use,<sup>5-7</sup> which has severely reduced treatment options, making multidrug-resistant (MDR) pathogens a global public health problem.<sup>8</sup> The misuse of antibiotics can result in the spread of resistant strains, posing public health risks worldwide; antibiotic-resistant bacteria (ARB) could lead to 10 million deaths by 2050.<sup>9,10</sup>

The Dead Sea is unique in comparison to other extremely salty environments. Its salinity exceeds 34%, and, in addition to other harsh conditions, such as low pH and a distinct ionic composition different from seawater, the Dead Sea supports a diverse ecosystem of halophilic microorganisms that have adapted to its extreme environment.<sup>11,12</sup> The ability of these microorganisms to thrive under harsh conditions has led to the production of natural microbial products with considerable potential for developing new antibiotics to address the growing issue of antibiotic resistance.<sup>13</sup> In particular, bacteria have shown promise as a source of bioactive compounds and are likely to continue to be an important resource.<sup>14</sup>

Despite the unique environmental conditions of the Dead Sea, few studies have investigated the antibacterial activity of Dead Sea isolates. Accordingly, the current study aimed to isolate antibacterial agents from the Dead Sea soil bacteria and test the antibacterial activity of their extracts. Moreover, the activity of the secondary metabolites produced by a single isolate was investigated against different Gram-positive and Gram-negative bacteria. This study prioritized the isolation of stable antibacterial-producing bacteria from multiple Dead Sea sites and combined solvent extraction, TLC bioautography, and MIC/

MBC determination to provide an initial bioactivity profile of Dead Sea-derived bacterial metabolites against both Gram-positive and Gram-negative reference strains. Hypothesizing that soil bacteria isolated from Dead Sea region would provide a source for secondary metabolites with promising antibacterial activity.

## MATERIALS AND METHODS

Nutrient broth was obtained from Sachar Lau (Spain), and nutrient agar was obtained from Biolab (Hungary). Additionally, soybean casein digestion medium (tryptone broth) and yeast malt broth (YM broth) were obtained from HiMedia (India). For antibacterial activity testing, reference strains including *B. subtilis* ATCC (11774), *B. cereus* ATCC (10876), *S. aureus* ATCC (29213), *E. coli* ATCC (25922), *P. aeruginosa* ATCC (27853), and *P. mirabilis* ATCC (29906) were obtained from Kwikstik (France). Tween 80 was purchased from SIGMA (France). For antibiotic extraction, n-hexane was purchased from ROMIL (Jordan) and ethyl acetate was purchased from Carlo Erba (France).

### Methods

#### Soil sample collection

Nineteen samples were collected from nine different locations in the Dead Sea, Jordan (December 2019) according to a previously described method.<sup>13</sup> Approximately 30 g of soil was collected from a 5-15 cm depth and preserved in a sterile disposable cup at room temperature (25 °C).

#### Bacterial isolation from soil samples

Rubble from the soil samples was removed before weighing 1 g of each collected sample. Serial dilutions of soil samples were prepared using normal saline (0.9% NaCl). From each dilution, (100 µL) was incubated in a nutrient agar plate for 24 hrs at 37 °C. Colonies with different morphologies were selected and streaked onto a new nutrient agar plate to obtain a pure isolate, according to the method described previously, with some modifications.<sup>14,2</sup>

#### Primary screening

The isolated bacteria were tested to

determine their ability to produce antimicrobial activity against a group of pathogenic bacterial reference strains: *B. subtilis* ATCC (11774), *B. cereus* ATCC (10876), *E. coli* ATCC (25922), *P. aeruginosa* ATCC (27853), *P. mirabilis* ATCC (29906), and *S. aureus* ATCC (29213), according to the perpendicular streaking method.<sup>15</sup> Each isolate was streaked in the middle of a nutrient agar plate and then incubated for 48 hrs at 37 °C. Pathogenic bacterial suspensions were adjusted to match 0.5 McFarland standard and streaked perpendicularly around the soil isolates. All plates were incubated again at 37 °C for another 24 hrs. Each experiment was repeated three times.

### Morphological characterization

The colony morphology of the isolated bacteria with antimicrobial production potential was studied, including colony color and shape. The microscopic features of the isolated bacteria, including Gram staining and spore formation, were examined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

### Secondary screening

#### Production and downstream isolation of antibacterial substances

Three potential bacterial isolates that produced promising antibacterial activity were chosen for further production and testing, and they were coded as GH-15, GH-20, and GH-21. These isolates were chosen because of their stability in producing antimicrobial agents during primary screening. Each potential isolate was cultivated in a 500 ml flask containing 200 ml of tryptone soy broth medium and then incubated for up to 21 days at 37 °C in an orbital shaker. Cultured bacterial suspensions were then centrifuged at 11,000 rpm at 5 °C for > 5 min. The supernatant was filtered through sterile 0.45-µm cellulose membrane filters.<sup>16-18</sup>

#### Extraction of supernatant filtrate and testing of antimicrobial activity using the agar well diffusion method

Extraction was conducted according to a previously described method, with some modifications.<sup>19,20</sup> The filtrates were extracted using a separating funnel with two organic solvents: hexane, followed by ethyl acetate.

Each organic phase was collected separately and allowed to dry completely under a fume hood. Stock solutions (84 mg/mL hexane and 75 mg/mL ethyl acetate) were prepared and tested against *B. subtilis* ATCC (11774), *B. cereus* ATCC (10876), *S. aureus* ATCC (29213), *E. coli* ATCC (25922), and *P. aeruginosa* ATCC (27853) using the agar-well diffusion method.

### Thin-layer chromatography (TLC)

TLC was conducted on hexane and ethyl acetate extracts of one isolate (GH-15) that showed the highest antibacterial activity during secondary screening, using (8 cm × 4 cm) aluminum sheet TLC plates under a ultraviolet-visible range of 250-366 nm.<sup>21</sup> Extracts were applied to the sheets using Minicaps capillary pipettes (10 µL). The sheets were dipped into a mobile phase (toluene:acetone; 1:1) for separation. All TLC separations were conducted at room temperature (25 °C) and dried for 5 min to completely remove the solvent. The sheets were observed under UV light (366 nm). The cells were then stained with iodine.

#### Detection of the antibacterial activity by using the bioautography technique

Direct bioautography was conducted as described previously.<sup>22</sup> Semi-solid nutrient agar was prepared and inoculated with bacterial suspension matching 0.5 McFarland standard for *B. subtilis* ATCC (11774) and *E. coli* ATCC (25922). Each TLC plate (8 × 4 cm) was transferred to an empty Petri dish. The inoculated agar was poured into each TLC plate and incubated at 37 °C for 24 hrs. Inhibition zones were examined, and the  $R_f$  values were used to determine the position of the active spots.

$$R_f = \frac{\text{Distance moved by extract}}{\text{Distance moved by solvent}} \dots 1$$

#### Minimum Inhibitory Concentration (MIC) and minimum Bactericidal Concentration (MBC)

The MIC was determined using the two-fold broth microdilution method in a 96-well plate.<sup>23</sup> Each experiment was conducted in triplicate. A stock solution of the GH-15 extract was prepared by dissolving the extract in five drops of Tween 80 and distilled water to obtain a final concentration of 84 mg/mL for the hexane and 75 mg/mL for the ethyl acetate extract. The

lowest concentration of the extract with no visible turbidity was considered as the MIC. Each extract with no visible growth was cultured on nutrient agar plates and incubated for 24 hrs at 37 °C to determine the MBC. The lowest concentration with no bacterial growth was considered as the MBC.

## RESULTS

### Collection of soil samples

One hundred different types of bacteria were successfully isolated from soil samples based on their colony morphology (Figure 1).

### Primary screening

Each soil-isolated bacterium was cultured on the middle surface of the nutrient agar plates, and the test bacteria were cultured perpendicularly to evaluate their ability to produce antimicrobial agents (Table 1). Twenty-one of the 100 bacterial isolates successfully produced antibacterial substances against the reference strains. Most isolates were active against *S. aureus*

and *B. cereus* whereas no activity was detected against *P. aeruginosa* or *P. mirabilis*.

### Morphological characterization

Morphological determination was conducted for bacterial isolates that had the potential to produce antibacterial substances during primary screening (Table 2).

### Secondary screening

Bacterial isolates with promising antibacterial activities (GH-15, GH-20, and GH-21) were selected for secondary screening. Bacterial cultures were grown for 21 days. Five milliliters of the culture flask was collected daily to test the antibacterial activity of the supernatant. Activity was recorded as the diameter of the inhibition zone (Table 3).

### Production and downstream isolation of target antibacterial compounds

#### Extraction of supernatant filtrate and testing of antimicrobial activity

The bacterial isolates were grown for

**Table 1.** Antibacterial activity of 21 soil isolates against a group of Gram-positive and Gram-negative bacteria-primary screening using perpendicular streak

Isolates	<i>B. subtilis</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>P. mirabilis</i>
GH-1	+	-	+	-	-	-
GH-2	+	-	+	-	-	-
GH-3	+	-	+	±	-	-
GH-4	+	-	+	-	-	-
GH-5	+	-	+	-	-	-
GH-6	+	-	+	-	-	-
GH-7	-	-	-	±	-	-
GH-8	+	-	+	-	-	-
GH-9	+	-	+	-	-	-
GH-10	+	-	±	±	-	-
GH-11	+	-	-	-	-	-
GH-12	+	-	+	-	-	-
GH-13	+	-	+	-	-	-
GH-14	+	-	+	-	-	-
GH-15	+	-	+	-	-	-
GH-16	+	-	+	-	-	-
GH-17	+	-	+	-	-	-
GH-18	+	-	+	-	-	-
GH-19	+	+	+	±	-	-
GH-20	+	+	+	-	-	-
GH-21	+	+	+	-	-	-

(+) Active against tested bacteria; (-) Inactive against tested bacteria; ( ± ) Reduced activity against test bacteria

the required time before centrifugation. The supernatant was extracted using hexane and ethyl acetate. The reconstituted extracts (84 mg/mL for hexane and 75 mg/mL for ethyl acetate) were active against the tested Gram-positive and Gram-negative bacteria (Table 4). All tested extracts showed activity against *B. cereus* and *E. coli*. However, not all extracts showed activity against *B. subtilis*, *S. aureus*, and *P. aeruginosa*.

### Thin layer chromatography (TLC)

TLC was conducted for the GH-15 extract to determine the number of bacterial metabolites. Both the hexane and ethyl acetate extracts were separated using the same mobile phase. Three spots were detected in the ethyl acetate extract and two spots were detected in the hexane extract.

**Table 2.** Morphology of Potential Bacterial Soil Isolates

Isolate	Gram Stain	Morphological Description
GH-1	Gram-positive <i>bacillus</i>	Cream-colored, round colony with raised margins; flat, moist, and adherent to the surface.
GH-2	Gram-positive <i>bacillus</i>	Cream-colored, irregular-shaped colony; flat with curled edges, shiny, and mucoid.
GH-3	Gram-positive <i>bacillus</i>	Light yellow-colored, punctiform, round colony with entire edges; highly adherent to the surface.
GH-4	Gram-positive <i>bacillus</i>	Cream-colored, round colony with soft texture and entire edges.
GH-5	Gram-positive <i>bacillus</i>	Cream-colored, mucoid, flat colony with irregular shape and lobate edges.
GH-7	Gram-positive <i>bacillus</i>	Shiny white-colored, round colony with entire edges; highly viscous.
GH-9	Gram-positive (spore-forming)	Cream-colored, irregular-shaped, flat colony with undulate edges; matte and moist.
GH-10	Gram-negative <i>bacillus</i>	Light green-colored, circular colony with entire edges; undulate, shiny, viscous, and transparent.
GH-11	Gram-positive <i>bacillus</i>	White-colored colony with smooth margins; convex-raised, small, round, shiny, and viscous.
GH-12	Gram-positive <i>bacillus</i>	Cream-colored, wrinkled, flat colony with lobate edges, curled appearance, irregular shape, slimy, and moist.
GH-13	Gram-positive <i>bacillus</i>	Yellowish-white colored, wrinkled colony with wavy edges; irregular-shaped, flat, matte, and dry.
GH-15	Gram-positive <i>bacillus</i>	Brownish cream-colored, punctiform, smooth, round colony with entire edges.
GH-16	Gram-positive (spore-forming)	Milky-colored colony with entire edges; raised, round, smooth, moist, and matte.
GH-17	Gram-positive (spore-forming)	Milky-colored colony with entire edges; convex, round, moist, and matte.
GH-18	Gram-positive (spore-forming)	Milky-colored colony with entire edges; convex, smooth, round, matte, and moist.
GH-19	Gram-positive <i>bacillus</i>	White-colored, punctiform, convex colony with undulate margins.
GH-20	Gram-positive <i>bacillus</i>	Yellow-colored, punctiform, flat colony with entire margins.
GH-21	Gram-positive <i>bacillus</i>	Yellow-colored, punctiform, flat colony with entire margins.

**Table 3.** Antibacterial activity of GH-15, GH-20, GH-21 supernatant as inhibition zone (cm ± SD) against tested Gram-positive bacteria

Isolate	Cultivation Time (h)	<i>S. aureus</i>	<i>B. subtilis</i>	<i>B. cereus</i>
GH-15	72	1.95 ± 0.05	2.0 ± 0.16	2.1 ± 0.3
GH-20	408	1.50 ± 0.01	1.85 ± 0.05	1.95 ± 0.05
GH-21	408	1.30 ± 0.01	1.80 ± 0.1	1.85 ± 0.15

### Bioautography

TLC plates were inoculated with test bacteria grown on semi-solid agar to determine the active spots. Spots with  $R_f$  (0.727) for hexane and  $R_f$  (0.818) for ethyl acetate indicated the active compounds (Figure 2).

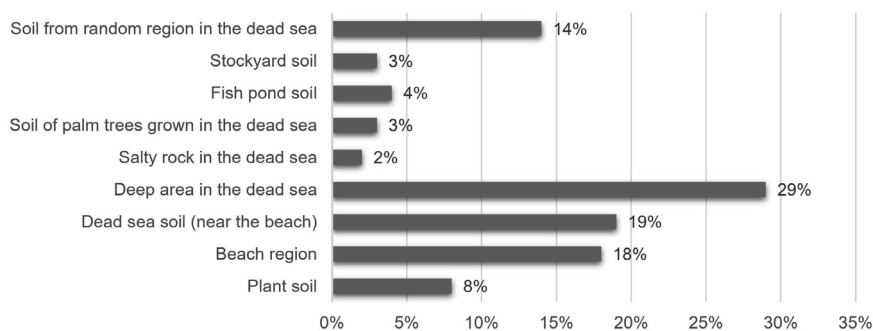
### MIC

The MIC values were examined against *B. subtilis* ATCC (11774), *B. cereus* ATCC (10876), *S. aureus* ATCC (29213), *E. coli* ATCC (25922), *P. aeruginosa* ATCC (27853), and *P. mirabilis* ATCC (29906) (Table 5). The lowest MIC of the acetate

**Table 4.** Antibacterial activity of GH-15, GH-20, and GH-21 extract as inhibition zone (cm ± SD) against Gram-positive and Gram-negative bacteria

Extract (Bacteria)	<i>B. subtilis</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
Ethyl acetate extract (GH-15)	4.05 ± 0.07	3.73 ± 0.17	4.15 ± 0.07	3.95 ± 0.07	2.6 ± 0.22
Hexane extract (GH-15)	3 ± 0.01	4.78 ± 0.22	4.65 ± 0.21	5.15 ± 0.2	1.2 ± 0.14
Ethyl acetate extract (GH-20)	4.3 ± 0.3	3.9 ± 0.17	4 ± 0.12	4.1 ± 0.1	2.4 ± 0.4
Hexane extract (GH-20)	-	3.25 ± 0.35	-	3.5 ± 0.01	-
Ethyl acetate extract (GH-21)	3.85 ± 0.12	3.15 ± 0.2	3.35 ± 2.35	3.56 ± 0.2	-
Hexane extract (GH-21)	-	3.65 ± 0.2	3.33 ± 0.2	3.93 ± 0.3	-

(-) No activity



**Figure 1.** Percentage of bacteria isolated (100 isolates) from each collection site in the Dead Sea region



**Figure 2.** Bioautography. E: Ethyl acetate extract, H: Hexane extract, stained with iodine

extract was against *E. coli* (0.073 mg/mL), whereas the hexane extract had the lowest MIC (2.625 mg/mL) against *E. coli* and *B. cereus*.

### MBC

All clear wells in the MIC test were cultured in nutrient agar to determine the MBC (Table 6). Ethyl acetate MIC was equal to the MBC against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*. The lowest MBC for the hexane extract was 5.25 mg/mL against *E. coli* and *B. cereus*.

### DISCUSSION

The rapid increase in antibiotic resistance by pathogenic bacteria has led to the detection of novel antibacterial agents.<sup>15</sup> Soil bacteria represent one of the most promising frontiers

**Table 5.** MIC values for hexane and ethyl acetate extracts of GH-15 extracts against pathogenic bacteria

Bacterial Reference strains	Ethyl acetate extract (mg/mL)	Hexane extract (mg/mL)
<i>B. subtilis</i>	0.292	5.25
<i>B. cereus</i>	0.292	2.625
<i>S. aureus</i>	0.292	5.25
<i>E. coli</i>	0.073	2.625
<i>P. aeruginosa</i>	9.375	10.5
<i>P. mirabilis</i>	1.17	10.5

**Table 6.** MBC values for hexane and ethyl acetate extracts of GH-15 extracts against pathogenic bacteria

Pathogenic strain	Ethyl acetate extract (mg/mL)	Hexane extract (mg/mL)
<i>B. subtilis</i>	0.292	10.5
<i>B. cereus</i>	0.585	5.25
<i>S. aureus</i>	0.292	10.5
<i>E. coli</i>	0.073	5.25
<i>P. aeruginosa</i>	9.375	10.5
<i>P. mirabilis</i>	4.687	10.5

for antibacterial agents discovery.<sup>16,17</sup> Harsh environmental conditions, including high salinity and elevated temperatures, in the Dead Sea can lead to the dominance of tolerant bacteria that can be a source of promising secondary metabolites with antibacterial activity.<sup>13</sup> The total number of different bacteria isolated was 100, which were selected based on morphological differences. Of the isolates, 21% passed the primary screening against pathogenic bacteria, a finding similar to that reported previously (Table 1).<sup>18,19</sup> The 21 isolates were tested against six types of pathogenic bacteria using the agar-well diffusion method to evaluate the antibiotic activity produced in the supernatant. Potential isolates in the primary screening were examined according to colony shape, Gram staining, and cell shape. All isolates were Gram-positive bacteria (Table 2). The observed dominance of Gram-positive bacteria likely reflects environmental factors.

Secondary screening aimed to test the activity of the potential antibacterial agents produced in the supernatant.<sup>20</sup> GH-15 exhibited maximal antibacterial activity after 72 h of culture, whereas GH-20 and GH-21 required prolonged incubation (408 hrs) (Table 3). This delayed activation of secondary metabolite production could be attributed to stress-induced changes in the metabolic pathways.<sup>21</sup> Marked differences were observed in the antibacterial profile of the solvent extraction. Both the hexane and ethyl acetate extracts of GH-15 exhibited activities against *B. subtilis*, *B. cereus*, *S. aureus*, *E. coli*, and *P. aeruginosa*. The ethyl acetate extract of GH-20 demonstrated activity against all tested pathogenic bacteria, whereas the hexane extract

showed activity against *B. cereus* and *E. coli*. The hexane extract of GH-21 exhibited activity against *B. cereus*, *E. coli*, and *S. aureus*. The ethyl acetate extract of GH-21 was active against all tested bacteria, except *P. aeruginosa*. This indicated that each isolate of (GH-20 and GH-21) produced at least two types of secondary metabolites with different antibacterial activities. The diversity of the antibacterial profile was consistent with that observed in other Dead Sea isolates.<sup>12,22</sup>

Thin-layer chromatography coupled with bioautography confirmed that the antibacterial activity was related to the separation of secondary metabolites. Three different spots were detected in the ethyl acetate extract and two spots were detected in the hexane extract under UV light. A bioautography test was conducted to determine the  $R_f$  of spots with antibacterial activity. The hexane extract of G15 demonstrated an active spot at  $R_f = 0.727$ , whereas the ethyl acetate extract showed an active spot at  $R_f = 0.818$ . MIC and MBC were determined for both G15 extracts. The ethyl acetate extract showed better antibacterial activity than the hexane extract against all the tested bacterial strains. Superior activity of the ethyl acetate extract of silage isolated from *Bacillus*, compared to a hexane extract has been reported.<sup>23</sup> *Bacillus* antimicrobial metabolites include polyketides, lipopeptides, and volatile compounds that are more soluble in ethyl acetate than in hexane.<sup>24,25</sup>

The lowest MIC for the hexane extract was detected against *B. cereus* and *E. coli* (2.625 mg/mL), and the highest MIC value was against *P. mirabilis* and *P. aeruginosa* (10.5 mg/mL) (Table 5). The ethyl acetate extract exhibited the highest

antimicrobial activity against *E. coli* (0.073 mg/mL), with an MIC of 0.292 mg/mL against *B. subtilis*, *B. cereus*, and *S. aureus*. Antimicrobial activity was reduced against *P. mirabilis* (1.17 mg/mL) and demonstrated the weakest activity against *P. aeruginosa*. Ethyl acetate extract of marine-isolated *Bacillus* demonstrated greater antibacterial activity, according to MIC values, against *E. coli* (15.62 µg/mL) than its antimicrobial activity against *B. subtilis*, *B. cereus*, and *S. aureus* (31.25 µg/mL).<sup>26</sup> However, MIC values of the ethyl acetate extract of marine-isolated *Bacillus* against *P. aeruginosa* and *E. coli* were equal.<sup>26</sup> Ethyl acetate extract of silage-isolated *Bacillus* demonstrated the same antibacterial activity against *B. subtilis* and *S. aureus* (25 µg/mL).<sup>23</sup>

The MBCs of ethyl acetate extracts against *B. subtilis*, *E. coli*, and *S. aureus* and hexane extracts against *P. mirabilis* and *P. aeruginosa* were equal to their MIC (Table 6). The similarity between the MIC and MBC values against some bacterial strains indicates bactericidal activity. This is consistent with the results reported previously by Balogun et al. who tested *Bacillus* isolates from soil samples collected from a river bank and reported equal MIC and MBC values of the bacterial ethyl extract against *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*.<sup>27</sup> Six promising antibacterial agents were identified from the three bacterial isolates. This paves the way for further evaluation of the extracted secondary metabolites for their identification and characterization.

## CONCLUSION

Several bacterial isolates obtained from the Dead Sea were rich sources of antibacterial agents. Three bacterial isolates (GH-15, GH-20, and GH-21) showed superior activity against Gram-positive and Gram-negative bacteria. Secondary metabolite diversity was indicated by differences in antibacterial activity related to different extraction solvents. The antibacterial activity of (GH-15) extract against the tested bacteria was determined using MIC and MBC. Compound identity and purity were not established, and the activity may reflect a mixture of metabolites. Future studies should include molecular identification of the producing isolates (e.g., 16S rRNA sequencing), bioassay-guided fractionation, structural elucidation (liquid

chromatography with tandem mass spectrometry, nuclear magnetic resonance), assessment of cytotoxicity, and expanded testing against clinical MDR isolates to confirm translational relevance. The ecological importance of the source of the isolates (representing extreme environmental conditions) and the broad activity of the isolates against different bacterial strains justify further investigation of the secondary metabolites produced by the bacterial isolates, which highlights the Dead Sea soil microbiome as a promising reservoir of antibacterial bacteria. The solvent-dependent variation in activity, low MIC values observed in certain extracts, and ecological uniqueness of the isolates collectively support further investigation into their bioactive metabolites.

## ACKNOWLEDGMENTS

The authors would like to thank Isra University, Jordan, for their support.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

SMA and ND conceptualized the study. GHM applied methodology. DNL and AAT performed data validation. GHM performed formal analysis. SMA supervised the study. DNL wrote the original draft. SMA, AAT and ND wrote, reviewed and revised the manuscript. All authors read and approved the final manuscript for publication.

## FUNDING

This research was funded by Isra University, Amman, Jordan.

## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

## ETHICS STATEMENT

Not applicable.

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