



Phyllospheric *Brevibacillus parabrevis* from Wadi Degla reserve: Isolation, molecular identification, GC-MS profiling, and evaluation of antibacterial, antioxidant, and docking studies

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Phyllospheric bacteria are emerging as a promising source of bioactive compounds with antimicrobial and antioxidant properties. These microorganisms, inhabiting the aerial surfaces of plants, have demonstrated significant potential for biotechnological applications. Microbial-derived bioactive compounds have gained significant attention for their potential in developing novel antimicrobial and antioxidant agents. This study investigated the bioactive potential of *Brevibacillus parabrevis* AUMC-B1, a bacterium isolated from the phyllosphere of *Hyoscyamus desertorum* in Wadi Degla Reserve. The bacterium was identified through phenotypic and genotypic characterization, and its ethyl acetate extract exhibited significant antibacterial activity, with the highest inhibition observed at 0.8% against *Escherichia coli* and *Staphylococcus aureus*. In addition, the strong antioxidant potential was assessed using the DPPH radical scavenging assay. GC-MS analysis identified key bioactive metabolites, including cis-13-eicosenoic acid and 12-methyl-E,E-2,13-octadecadien-1-ol, which were further evaluated through molecular docking. Moreover, docking studies revealed strong interactions with bacterial DNA gyrase, suggesting antibacterial mechanisms, while ADME analysis confirmed favorable pharmacokinetic properties such as high oral absorption and lipophilicity. Gene interaction analysis highlighted the compounds' potential role in modulating antioxidant pathways. These findings indicate that *B. parabrevis* AUMC-B1 and its bioactive metabolites have promising antibacterial and antioxidant applications, offering potential for novel therapeutic development.

Keywords: ADME, Antibacterial, Antioxidant, *Brevibacillus parabrevis*, DNA gyrase inhibition, GC-MS, Molecular docking, Peroxiredoxin 5

INTRODUCTION

The phyllosphere, defined as the aerial parts of plants, serves as a dynamic and complex microbial habitat that supports diverse microbial communities, including bacteria, fungi, and archaea. These microorganisms are exposed to fluctuating environmental conditions such as ultraviolet (UV) radiation, temperature variations, nutrient scarcity, and humidity shifts, which drive the evolution of specialized adaptations (Cárdenas-Flores et al., 2025). Phyllospheric bacteria, in particular, play a critical role in plant

health by contributing to nutrient cycling, stress tolerance, and defense against pathogens through the production of bioactive secondary metabolites with antimicrobial, antioxidant, and plant-growth-promoting properties (Rangel & Leveau, 2024). These metabolites have garnered significant attention for their potential in biotechnological applications, including the development of novel antimicrobial and antioxidant agents to address challenges such as antibiotic resistance and oxidative stress-related disorders (Sánchez et al., 2018; Narayanan et al., 2024).

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Desert plants, such as *Hyoscyamus desertorum*, thrive in arid, nutrient-limited environments and often harbor unique microbial communities capable of synthesizing bioactive compounds with pharmaceutical potential (Narayanan et al., 2024). The extreme conditions of desert ecosystems, characterized by high temperatures, low water availability, and intense solar radiation, select for microorganisms with robust biosynthetic pathways that produce metabolites with antimicrobial, antioxidant, and anti-inflammatory activities (Al Makishah, 2021). For instance, *Brevibacillus* species, aerobic, spore-forming Firmicutes originally reclassified from the *Bacillus brevis* group based on 16S rRNA analysis in 1996, have emerged as promising candidates for bioprospecting due to their metabolic versatility and ability to produce diverse bioactive compounds (Zayed et al., 2022). These bacteria are commonly isolated from extreme environments, including desert soils, plant surfaces, and rhizospheres, where they exhibit adaptations to stressors such as desiccation and oxidative stress (Dhanalakshmi & Rajendhran, 2024).

Brevibacillus species are known for producing a wide range of secondary metabolites, including lipopeptides, polyketides, and exopolysaccharides, which exhibit antibacterial, antifungal, and antioxidant properties (Abd-Alla et al., 2018; Yang & Yousef, 2018). For instance, *Brevibacillus laterosporus* has been reported to produce antimicrobial peptides that inhibit pathogenic bacteria, while *Brevibacillus parabrevis* has shown potential in synthesizing compounds with both antimicrobial and antioxidant activities (Zayed et al., 2022). These properties make *Brevibacillus* species valuable for drug discovery and agricultural applications, particularly in combating multidrug-resistant pathogens and mitigating oxidative stress in biological systems (Dhanalakshmi & Rajendhran, 2024). Recent studies have also highlighted the role of phyllospheric bacteria in modulating plant-microbe interactions, enhancing plant resilience, and contributing to sustainable agricultural practices through biocontrol and biofertilization (Legein et al., 2020; Ravimannan & Pushpanathan, 2024).

This study focuses on the isolation and characterization of *B. parabrevis* from the phyllosphere of *H. desertorum* collected from Wadi Degla Reserve, Cairo, Egypt. The bioactive metabolites produced by these

bacteria were extracted using ethyl acetate and characterized utilizing gas chromatography-mass spectrometry (GC-MS) to identify key compounds with pharmacological potential. *In vitro* assays were conducted to evaluate the antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, as well as the antioxidant capacity using the DPPH radical scavenging assay. Additionally, molecular docking and ADME (Absorption, Distribution, Metabolism, and Excretion) analyses were performed to investigate the binding interactions and pharmacokinetic properties of the identified compounds. Gene interaction analysis was employed to elucidate the potential mechanisms underlying their antibacterial and antioxidant activities. These findings underscore the biotechnological potential of phyllosphere-derived *B. parabrevis* as a source of novel bioactive molecules for therapeutic applications, contributing to the growing body of research on microbial bioprospecting in extreme environments.

METHODS

Isolation and characterization of phyllospheric bacteria from *Hyoscyamus desertorum*

Bacteria were isolated from the aboveground tissues of *H. desertorum* collected from desert regions of Wadi Degla, Cairo, Egypt (29°56'57.3"N 31°22'33.1"E) (Figure 1). The identification of the collected *H. desertorum* plant samples was implemented with the valuable assistance of Prof. Dr. Abdel-Aziz Fayed, Professor of Taxonomy at Assiut University (Assiut, Egypt). A voucher plant specimen was preserved at the herbarium of the Faculty of Science at Helwan University in Egypt. The leaves were washed thoroughly with sterilized distilled water to remove surface contaminants and then air-dried at room temperature for seven days. The dried plant material was finely ground using sterilized mortars and pestles, and 0.1 g of the powdered sample was suspended in 10 mL of sterilized distilled water, followed by vortexing for homogenization. Serial dilutions of the homogenized suspension were spread onto Tryptic Soy Agar (TSA, Sigma, Germany) plates supplemented with 30 mg/mL amphotericin B to inhibit fungal growth (Senanayake et al., 2020). The plates were incubated at 28°C for 3 days under aerobic conditions to allow bacterial growth (Lavanya et al., 2021).



Figure 1. Location of collected *H. desertorum* sample from Wadi Degla, Cairo, Egypt and collected *H. desertorum* samples

Phenotypic and genotypic characterization of isolated bacteria

The bacteria were characterized at the genus level using a combination of phenotypic and genotypic analyses. Phenotypic characterization involved microscopic examination, including Gram staining and endospore staining, as well as physiological tests assessing bacterial growth at varying NaCl concentrations, temperatures, and pH levels. Additionally, biochemical assays were conducted to evaluate catalase and oxidase activity, nitrate reduction, starch and casein hydrolysis, Voges-Proskauer reaction, citrate utilization, gelatin liquefaction, and methyl red test. Colony morphology, including size, margin, form, and elevation, was also documented (Ray et al., 2020; Barua et al., 2023).

For genotypic characterization, bacterial cultures were grown in 10mL of sterilized nutrient broth at 28°C for 48 hours (Maghbolli et al., 2022). Genomic DNA was extracted using the Patho-Gene-Spin DNA/RNA extraction kit (Intron Biotechnology, Korea). The extracted DNA was sent to SolGent Company (Daejeon, South Korea) for polymerase chain reaction (PCR) and sequencing. Universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTACGACTT-3') were used to amplify the 16S rRNA gene. PCR products were verified using electrophoresis on a 1% agarose gel with a 100 base pair molecular marker. Purified amplicons were sequenced bidirectionally using dideoxynucleotide (ddNTP) termination. Sequence alignment and phylogenetic analysis were performed using the Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (NCBI) and MegAlign software

(DNA Star, version 5.05).

Isolation and extraction of antibacterial metabolites from *B. parabrevis* AUMC-B1

The extraction of antibacterial metabolites from *B. parabrevis* AUMC-B1 was performed following a modified protocol by Khalil et al. (2021). Bacterial cultures were grown in nutrient broth (NB) for 10 days under optimal conditions. To prepare a colony suspension, the bacterial biomass was resuspended in a sterilized saline solution. A total of 50mL of NB medium was inoculated with 1mL of the bacterial suspension in 250mL Erlenmeyer flasks, while control flasks contained only the culture medium without bacterial inoculation. The cultures were first incubated statically at 28°C for 24h, followed by agitation at 150 rpm for 48h to enhance metabolite production.

Following incubation, the culture broth was separated from bacterial cells using a sterilized syringe filter (0.22µm). The cell-free supernatant was subjected to liquid-liquid extraction using equal volumes of ethyl acetate. After 30min of continuous shaking, the mixtures were left undisturbed for 15min to facilitate phase separation. The organic and aqueous layers were separated using a glass separating funnel, and this extraction process was repeated three times to ensure maximum metabolite recovery. The pooled organic solvent fractions were concentrated using a vacuum rotary evaporator at 40°C to obtain the crude antibacterial extract for further analysis.

Assessment of antibacterial activity using a microtiter plate assay

The antibacterial activity of the powdered extract from *B. parabrevis* AUMC-B1, obtained after evaporation of ethyl acetate (EtOAc), was evaluated against *Escherichia coli* OK087362

(Gram-negative) and *Staphylococcus aureus* LC189114 (Gram-positive) using a 96-well microtiter plate assay. Bacterial suspensions were standardized to 3×10^5 colony-forming units (CFU)/mL in nutrient broth (NB; HiMedia, India). The powdered extract was reconstituted in sterilized DMSO to achieve concentrations of 0.2%, 0.4%, and 0.8% (w/v). Each well received 50 µL of the reconstituted extract solution, with the final volume adjusted to 200 µL using NB containing the bacterial suspension. Negative control wells contained NB with bacterial suspension and sterilized DMSO, while positive control wells included NB with bacterial suspension and ciprofloxacin (Sigma, Germany) at equivalent concentrations. All conditions were tested in triplicate. Plates were incubated at 37°C for 24h under static conditions. Bacterial growth was quantified by measuring optical density (OD) at 600 nm using a microplate reader (ChroMate 4300, Awareness Technology, USA). The OD values were used to calculate the inhibition of bacterial growth relative to the negative control (David et al., 2021).

Evaluation of antioxidant activity using the DPPH radical scavenging assay

The antioxidant activity of *B. parabrevis* AUMC-B1 ethyl acetate (EtOAc) extract was assessed using a modified 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, following the method described by Osamudiamen et al. (2020). The extract was diluted in methanol and mixed with 0.5mL of 0.15mM DPPH solution. The reaction mixture was incubated at room temperature for 30 minutes in the dark to allow interaction between the extract and free radicals. After incubation, the absorbance of the reaction mixture was measured at 517nm using a UV-Vis spectrophotometer. Ascorbic acid was used as a positive control. The antioxidant activity percentage (AA%) or radical scavenging activity was calculated using the following formula: $[(\text{Control absorbance} - \text{Control sample absorbance}) / (\text{Control absorbance})] \times 100$ is the reduction of the DPPH radical (%). This formula quantifies the DPPH radical scavenging ability of the extract, with higher AA% values indicating stronger antioxidant potential.

Gas chromatography-mass spectrometry (GC-MS) analysis of bioactive metabolites for *B. parabrevis* AUMC-B1

The ethyl acetate (EtOAc) extract of *B. parabrevis* AUMC-B1 was analyzed using gas

chromatography-mass spectrometry (GC-MS) to identify its bioactive compounds. The analysis was performed using a Trace GC1310-ISQ mass spectrometer equipped with an HP-5MS capillary column (30m $\times$ 0.25mm, 0.25 µm film thickness). The carrier gas was helium, with a constant flow rate of 1.0mL/min. The injection volume was 1 µL in splitless mode. The oven temperature was initially set at 60°C for 2min, increased to 250°C at a rate of 10°C/min, and held for 5min. The ionization energy was 70eV, and the mass scan range was 50-650m/z. Identification of compounds was performed by comparing mass spectral data with the National Institute of Standards and Technology (NIST) database (Baeshen et al., 2023). The biological activities of the identified compounds were determined based on previous literature.

Computational docking analysis of selected bioactive compounds from GC-MS

Molecular docking was performed using Maestro 13.4 to investigate the binding interactions of selected bioactive compounds with bacterial DNA gyrase from *S. aureus* and *E. coli*, as well as human peroxiredoxin 5. The crystal structures of the target proteins were retrieved from the Protein Data Bank (PDB IDs: 5CDM, 7Z9C, and 1HD2). The bioactive compounds used for docking were selected based on GC-MS analysis, where compounds with the highest binding affinity and potential biological activity were prioritized. These included 12-Methyl-E,E-2,13-octadecadien-1-ol and cis-13-Eicosenoic acid. Ligand structures were prepared using the LigPrep module of Maestro. The protein structures were preprocessed using the Protein Preparation Wizard, where missing side chains were added, bond orders were assigned, and hydrogen atoms were optimized. The receptor grid was generated around the active site of each protein to ensure optimal ligand docking. Molecular docking simulations were performed using the Glide module in standard precision (SP) mode to predict binding affinities and interactions. Docking scores were calculated in kcal/mol, and hydrogen bond interactions were analyzed to determine the stability and strength of ligand binding (Parveen et al., 2024).

In silico ADME analysis of selected bioactive compounds

The ADME (Absorption, Distribution, Metabolism, and Excretion) properties of selected bioactive compounds were evaluated

using Schrödinger's QikProp module to assess their pharmacokinetic profiles. The compounds chosen for this analysis—cis-13-eicosenoic acid, and 12-Methyl-E,E-2,13-octadecadien-1-ol—were previously identified from GC-MS and molecular docking studies based on their biological relevance. The molecular structures of the selected compounds were prepared using the LigPrep module, optimizing their geometry and protonation states at physiological pH (7.4). Following structure preparation, the QikProp tool was used to calculate several key pharmacokinetic properties, including molecular weight (MW), hydrogen bond donors and acceptors, solvent-accessible surface area (SASA), partition coefficient (QPlogPo/w), blood-brain barrier permeability (QPlogBB), aqueous solubility (QPlogS), and percentage of human oral absorption. The molecular weight (MW) was analyzed to determine the compliance of each compound with Lipinski's Rule of Five, a guideline for drug-likeness. The number of hydrogen bond donors and acceptors was examined to evaluate each compound's potential for forming hydrogen bonds, which influence solubility and permeability. SASA was measured to assess the molecular surface area available for interaction with biological membranes, impacting absorption. QPlogPo/w, a measure of lipophilicity, was analyzed to determine membrane permeability, as highly lipophilic compounds tend to have better absorption but may face metabolic stability challenges. QPlogBB was calculated to predict the likelihood of blood-brain barrier penetration, which is essential for evaluating potential central nervous system (CNS) activity. QPlogS, which represents aqueous solubility, was examined to assess the compounds' dissolution potential, a key factor in drug absorption. Finally, the percentage of human oral absorption was predicted to estimate the likelihood of systemic bioavailability after oral administration. The ADME results were analyzed to determine the drug-likeness, pharmacokinetic feasibility, and potential limitations of the tested compounds (Parveen et al., 2024).

Gene interaction analysis of bioactive compounds in inhibiting *E. coli* DNA gyrase genes and enhancing antioxidant properties

Gene interaction analysis was conducted using GeneMANIA to evaluate the potential role of selected bioactive compounds in inhibiting *E. coli* DNA gyrase genes (*gyrA* and *gyrB*) and enhancing antioxidant properties by interacting with key antioxidant-related genes in humans.

The study focused on understanding the functional and genetic interactions of these genes and how the selected compounds might influence these pathways. For the DNA gyrase inhibition analysis, *Escherichia coli* was selected as the model organism, and *gyrA* and *gyrB* genes were used as query genes. GeneMANIA was used to retrieve information on co-expression, genetic interactions, shared protein domains, and physical interactions that are crucial for DNA gyrase function. The network included top functionally related genes such as *parC*, *parE*, *recF*, and *topB*, which are involved in bacterial DNA replication and repair. For the antioxidant enhancement analysis, human antioxidant genes were queried in GeneMANIA, including *PRDX5*, *SOD1*, *SOD2*, *SOD3*, *CAT*, and *GPX4*, which play critical roles in oxidative stress defense mechanisms. The generated network included interactions related to physical binding, co-expression, shared protein domains, and pathway interactions, which provide insights into how the compounds could modulate antioxidant activity. The data from these analyses were interpreted in relation to the molecular docking and ADME results to understand how the bioactive compounds interact with these genes and their encoded proteins (Zargar et al., 2023).

Statistical analysis

All experiments were conducted in triplicate, and the results are presented as mean $\pm$ standard deviation (SD). The statistical significance of differences between groups was analyzed using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. A p-value < 0.05 was considered statistically significant. Data were processed and analyzed using GraphPad Prism (version 9.5.1) and SPSS (version 27.0) (Midway et al., 2020).

RESULTS

Isolation, characterization, and phylogenetic analysis of *B. parabrevis* AUMC-B1 from the phyllosphere of *Hyoscyamus desertorum*

The isolation and identification of *B. parabrevis* AUMC-B1 from the aboveground portions of *H. desertorum* were confirmed after three days of incubation. Microscopic analysis revealed a rod-shaped, motile, Gram-positive bacterium. On Tryptic Soy Agar (TSA) at 28°C, the isolate exhibited rapid growth with wrinkled, dry, white, uneven, and flat colonies. It demonstrated adaptability to temperatures ranging from 25°C to 50°C, a pH range of 6 to 9, and tolerated up to 10%

NaCl. Biochemical characterization confirmed negative results for gelatin liquefaction, Voges-Proskauer, methyl red, and urea hydrolysis. However, positive results were observed for catalase, oxidase, nitrate reductase, starch hydrolysis, and casein hydrolysis. Molecular identification through 16S rRNA gene sequencing confirmed to *B. parabrevis* AUMC-B1 and submitted in GenBank with accession number PV715975, and phylogenetic analysis showed a close evolutionary relationship with other *Brevibacillus* species, exhibiting 99.79% to 99.93% identity with 100% sequence coverage (Figure 2).

Assessment of antibacterial activity using a microtiter plate assay

The antibacterial properties of *B. parabrevis* AUMC-B1 EtOAc extract demonstrated significant inhibitory activity against *S. aureus* and *E. coli* (Figure 3). A concentration-dependent inhibition was observed, with the highest antibacterial activity at 0.8% concentration, reducing the optical densities to 0.18 for *E. coli* (81.1% inhibition compared to the control) and 0.12 for *S. aureus* (86.7% inhibition compared to the control). Notably, the extract exhibited greater inhibitory effects on *S. aureus* than *E. coli* across all concentrations (35.0% vs. 60.0% reduction at 0.2%; 77.8% vs. 68.4% reduction

at 0.4%; and 86.7% vs. 81.1% reduction at 0.8%, respectively). These results demonstrate the extract's potent antibacterial activity, with particular efficacy against Gram-positive *S. aureus*.

Evaluation of antioxidant activity using the DPPH radical scavenging assay

Antioxidant activity was assessed using the DPPH radical scavenging assay (Figure 4), with ascorbic acid as a positive control. The extract displayed significant antioxidant potential, with radical scavenging activity increasing in a concentration-dependent manner. The IC_{50} value for ascorbic acid was 20.78 $\mu\text{g/mL}$, while the IC_{50} value for the EtOAc extract was 55.65 $\mu\text{g/mL}$, indicating that the extract exhibited strong antioxidant activity.

Gas chromatography-mass spectrometry (GC-MS) analysis of bioactive metabolites for *B. parabrevis* AUMC-B1

The GC-MS analysis of *B. parabrevis* AUMC-B1 EtOAc extract identified several bioactive compounds, as shown in Figure 6. The retention times (RT), molecular formulas, molecular weights, and biological activities of the identified compounds are summarized in Table 1 and Figure 5.

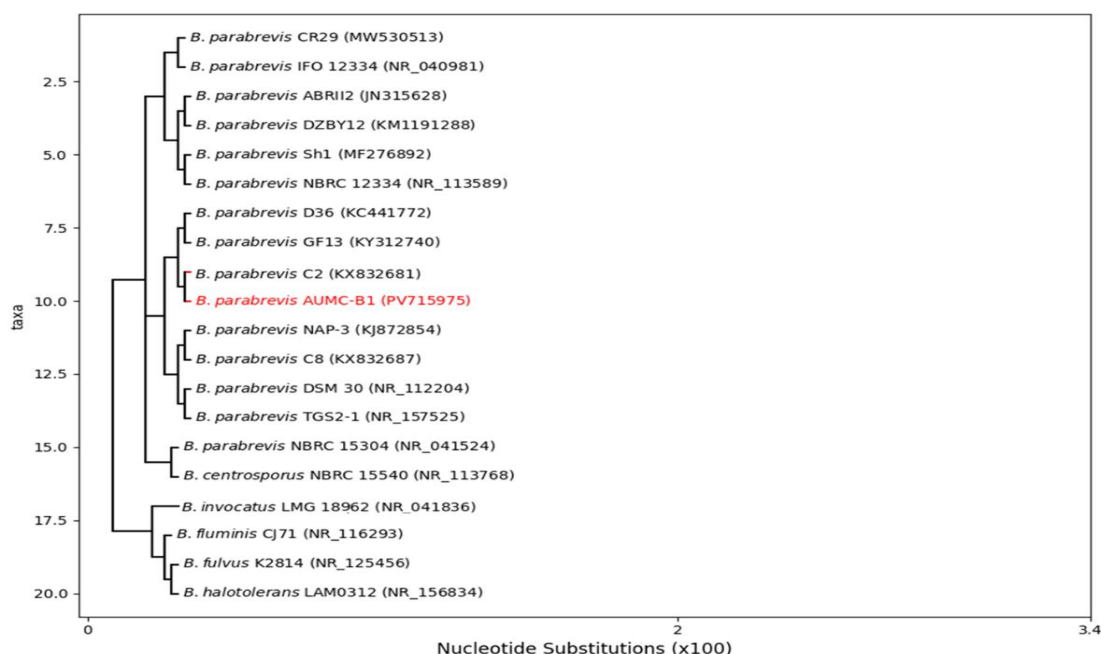


Figure 2. Phylogenetic analysis of *B. parabrevis* AUMC-B1. Phylogenetic tree constructed based on 16S rRNA gene sequencing, showing the evolutionary relationship of *B. parabrevis* AUMC-B1 with closely related *Brevibacillus* species retrieved from GenBank [The sequenced strain exhibited 99.79% to 99.93% identity with *Brevibacillus parabrevis*, with 100% sequence coverage]

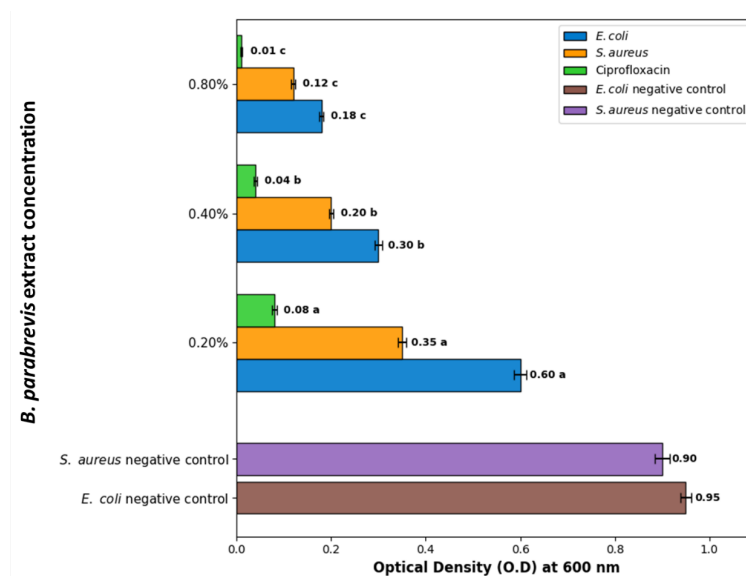


Figure 3. Effect of varying concentrations (0.2%–0.8% w/v) of *B. parabrevis* AUMC-B1 ethyl acetate extract on the optical density of *E. coli* and *S. aureus*, indicating concentration-dependent antibacterial activity [Ciprofloxacin (control antibiotic) was tested at equivalent concentrations (0.2%–0.8% w/v) in liquid culture via microtiter plate assay, measuring OD-based growth inhibition. Error bars represent standard deviation from triplicate experiments, reflecting consistent assay reproducibility. Optical density values are presented as mean $\pm$ SD. Different letters (a, b, c) denote statistically significant differences ($P < 0.05$) among groups]

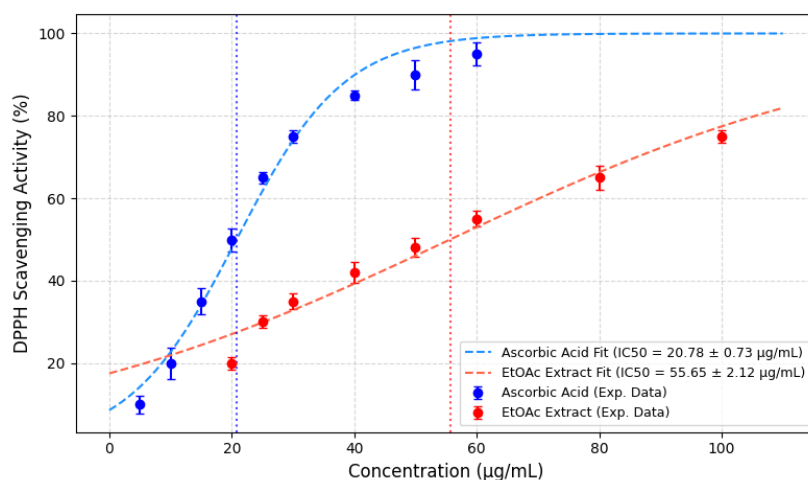


Figure 4. Evaluation of the antioxidant potential of *B. parabrevis* AUMC-B1 EtOAc extract using the DPPH radical scavenging assay, demonstrating a concentration-dependent increase in antioxidant activity

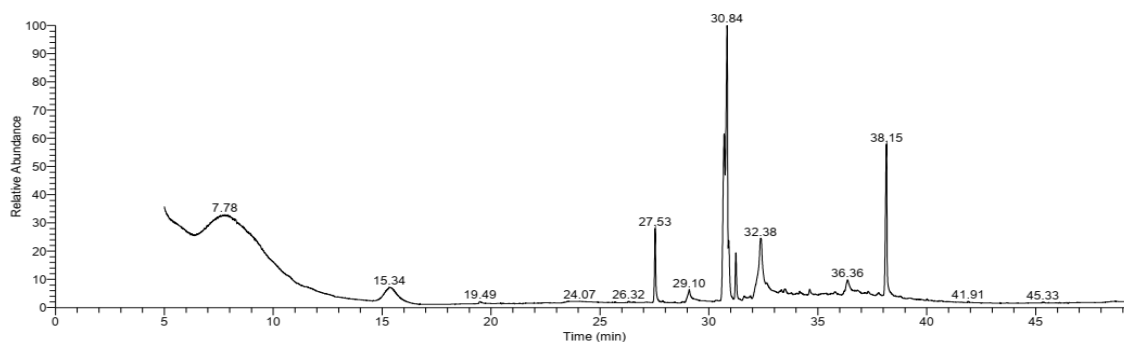


Figure 5. Gas chromatography-mass spectrometry chromatogram of *B. parabrevis* AUMC-B1 ethyl acetate extract

Table 1. Bioactive compounds identified in the ethyl acetate extract of *B. parabrevis* AUMC-B1 using GC-MS analysis

RT (min)	Compound name	Area (%)	Molecular formula	Molecular weight	Biological activity
27.53	Hexadecanoic acid, methyl ester	6.73	C ₁₇ H ₃₄ O ₂	270	Antibacterial, anti-inflammatory (Shaaban et al., 2021)
29.10	Hexadecanoic acid	1.52	C ₁₆ H ₃₂ O ₂	256	Antioxidant, antimicrobial (Ganesan et al., 2024)
30.70	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	20.19	C ₁₉ H ₃₄ O ₂	294	Antioxidant, anti-inflammatory (Umoh et al., 2024)
30.83	11-Octadecenoic acid, methyl ester	29.17	C ₁₉ H ₃₆ O ₂	296	Antimicrobial, lipid-lowering (Kosasih et al., 2020)
30.92	9-Octadecenoic acid (Z)-, methyl ester	1.06	C ₁₉ H ₃₆ O ₂	296	Antioxidant, antimicrobial (Ghareeb et al., 2022)
31.24	Octadecanoic acid, methyl ester	4.57	C ₁₉ H ₃₈ O ₂	298	Antioxidant, antibacterial (Farhan et al., 2021)
31.62	7-Methyl-Z-tetradecen-1-ol acetate	0.52	C ₂₁ H ₂₂ O ₁₁	268	Anticancer, anti-inflammatory (El-Naggar et al., 2023)
32.39	9,12-Octadecadienoic acid (Z,Z)-	5.64	C ₁₈ H ₃₂ O ₂	280	Anti-inflammatory, antioxidant (Elwekeel et al., 2023; Menachery et al., 2025)
33.49	Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate	0.53	C ₁₆ H ₂₈ O ₃	268	Antioxidant, antibacterial (Duraishamy & Selvaraju, 2020)
34.17	cis-13-Eicosenoic acid	0.40	C ₁₈ H ₃₄ O ₂	282	Antioxidant, antibacterial (Ayoola et al., 2020; Al-Juhaimi et al., 2022)
34.63	2,2,3,3,4,4 Hexadeutero octadecanal	0.59	C ₁₈ H ₃₀ D ₆ O	274	Antimicrobial (Ali et al., 2024)
36.36	12-Methyl-E,E-2,13-octadecadien-1-ol	0.59	C ₁₉ H ₃₆ O	280	Antioxidant, antibacterial (Karthik et al., 2023; Asgari Nematian, 2025)
38.15	Bis(2-ethylhexyl) phthalate	16.40	C ₂₄ H ₃₈ O ₄	390	Antibacterial (Javed et al., 2022)

Computational docking analysis of selected bioactive compounds from GC-MS

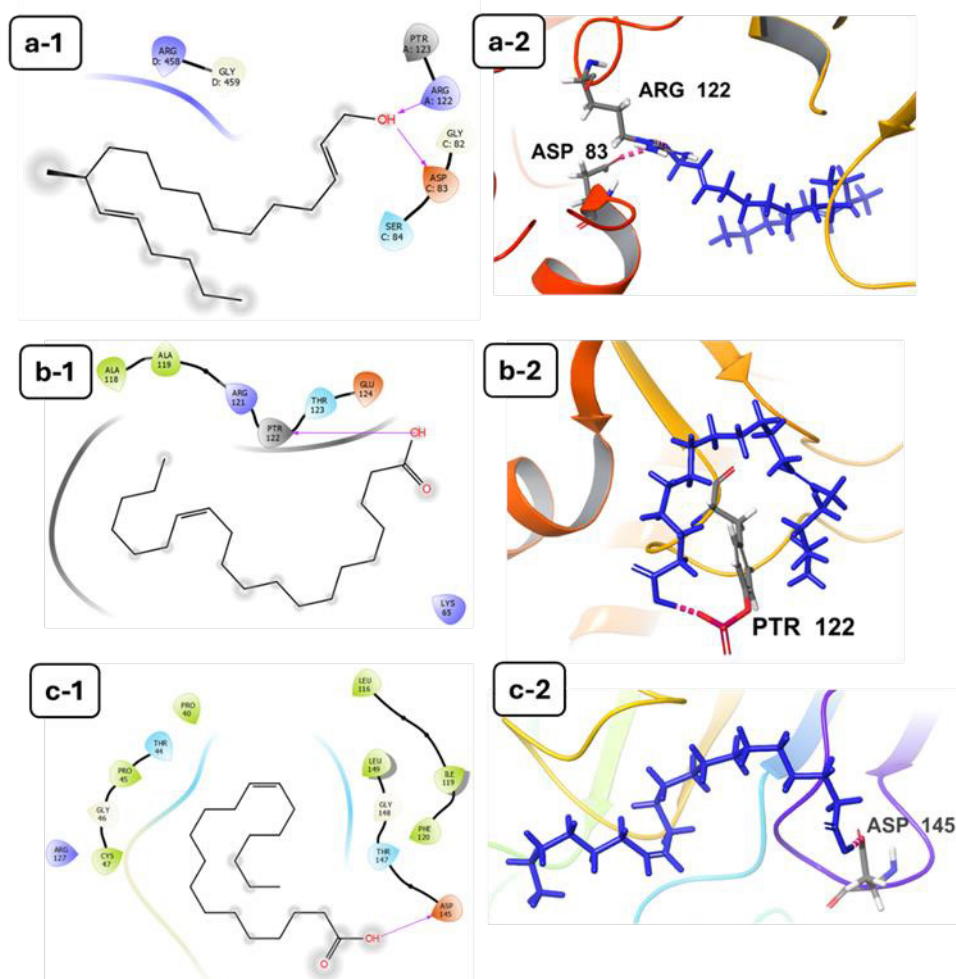
Molecular docking simulations were performed to evaluate the binding interactions of selected bioactive compounds with DNA gyrase from *S. aureus* and *E. coli*, as well as human peroxiredoxin 5. The docking scores and hydrogen bond interactions were analyzed to determine binding affinities and stability. The results are summarized in Table 2 and Figure 6. Among the tested compounds, 12-Methyl-E,E-2,13-octadecadien-1-ol interacted with *S. aureus* DNA gyrase with a binding affinity of -4.307 kcal/mol, forming hydrogen bonds with ASP 83 and ARG 122. In contrast, cis-13-Eicosenoic acid exhibited the weakest binding interactions across all tested proteins, with docking scores of -1.671kcal/mol for *E. coli* DNA gyrase and -1.495kcal/mol for human peroxiredoxin 5.

In silico ADME analysis of selected bioactive compounds

The ADME analysis of the selected bioactive compounds revealed significant variations in their pharmacokinetic properties. 12-Methyl-E,E-2,13-octadecadien-1-ol exhibited the highest human oral absorption, indicating strong potential for systemic bioavailability. This compound, along with cis-13-Eicosenoic acid, demonstrated high lipophilicity, suggesting efficient membrane permeability but potentially lower aqueous solubility. Differences in hydrogen bond donors and acceptors were observed, influencing the interaction potential with biological membranes and transport proteins. The solvent-accessible surface area (SASA) varied among the compounds, affecting their permeability characteristics. Furthermore, blood-brain barrier permeability predictions indicated that none of the compounds showed strong CNS penetration potential (Table 3).

Table 2. Molecular docking scores and hydrogen bond interactions of bioactive compounds against target proteins

Protein	Compound	Docking Score (kcal/mol)	H-Bond Interactions
<i>S. aureus</i> DNA gyrase	12-Methyl-E,E-2,13-octadecadien-1-ol	-4.307	ASP 83, ARG 122
<i>E. coli</i> DNA gyrase	Cis-13-Eicosenoic acid	-1.671	PTR 122
Human peroxiredoxin 5	Cis-13-Eicosenoic acid	-1.495	ASP 145

**Figure 6.** (a) Docking pose of 12-Methyl-E,E-2,13-octadecadien-1-ol with *S. aureus* DNA gyrase, (b) Docking pose of cis-13-Eicosenoic acid with *E. coli* DNA gyrase; (c) Docking pose of cis-13-Eicosenoic acid with human peroxiredoxin 5**Table 3.** *In silico* ADME analysis of selected bioactive compounds

Compound	MW	Hydrogen bond donor	Hydrogen bond acceptor	SASA	QPlogPo/w	QPlogBB	QPlogS	% Human oral absorption
Cis-13-Eicosenoic acid	310.519	1	2	777.074	6.678	-1.707	-6.984	95.774
12-Methyl-E,E-2,13-octadecadien-1-ol	280.493	1	1.7	720.373	6.062	-0.891	-6.370	100.000

Gene interaction analysis of bioactive compounds in inhibiting *E. coli* DNA gyrase genes and enhancing antioxidant properties

The GeneMANIA analysis for *gyrA* and *gyrB* genes revealed extensive genetic and co-expression interactions with genes involved in bacterial DNA replication and repair, including *parC* and *parE* (DNA topoisomerase IV subunits), *recF* (gap repair protein), and *topB* (DNA topoisomerase III). The network analysis showed that *gyrA* and *gyrB* share functional pathways with multidrug resistance genes such as *acrA*, indicating that disrupting DNA gyrase function may enhance bacterial susceptibility to antibiotics. The presence of 12-Methyl-E,E-2,13-octadecadien-1-ol and *cis*-13-Eicosenoic acid in molecular docking suggests their potential binding to DNA gyrase, which could interfere with the co-expressed network genes and reduce bacterial DNA replication efficiency. This illustrates that these compounds could enhance the bactericidal effect by disrupting genetic regulatory networks associated with gyrase function. For the antioxidant gene interaction analysis, GeneMANIA identified strong physical and pathway interactions among the antioxidant genes, particularly between *PRDX5*, *SOD1*, *SOD2*, and *GPX4*, which are essential for neutralizing reactive oxygen species (ROS). The network also highlighted interactions with nuclear factor *NFE2L2* (*NRF2*), a key regulator of antioxidant response. The results suggested that 12-Methyl-E,E-2,13-octadecadien-1-ol and *cis*-13-Eicosenoic acid, which demonstrated strong binding with antioxidant proteins in molecular docking, may act by modulating the expression or activity of *PRDX5* and *SOD* enzymes. This could enhance cellular resistance to oxidative stress and contribute to improved antioxidant defense mechanisms (Figure 7).

DISCUSSION

Phyllospheric bacteria play a crucial role in plant-microbe interactions, often exhibiting antimicrobial and antioxidant properties. Among these, *B. parabrevis* is known for its ability to produce bioactive metabolites with therapeutic potential. This study investigates the antibacterial and antioxidant activities of *B. parabrevis* AUMC-B1, highlighting its pharmacological significance. Phyllospheric bacteria have been widely reported in various plant species, including *Pseudomonas putida* in carrots (Ravimannan & Pushpanathan, 2024), *Serratia marcescens* in

rice (Ali et al., 2021), and *Stenotrophomonas* sp. in sweet potatoes (Marković et al., 2023). Our findings suggest that *H. desertorum* leaves can serve as a habitat for beneficial bacteria, such as *B. parabrevis* AUMC-B1.

The antibacterial activity of *B. parabrevis* AUMC-B1 was confirmed through its EtOAc extract, which exhibited strong inhibitory effects against *E. coli* and *S. aureus*. A concentration-dependent reduction in bacterial growth was observed, with the highest antibacterial activity recorded at 0.8%, where the optical densities of *S. aureus* and *E. coli* decreased to 0.12 and 0.18, respectively. These findings align with those of Abo-Elyousr et al. (2022), who reported that *B. parabrevis*, as an endophytic bacterium, exerts potent antibacterial effects by producing bioactive metabolites that inhibit pathogenic microorganisms. The antimicrobial potential of *B. parabrevis* can be attributed to its ability to produce lipopeptides, enzymes, and other secondary metabolites that disrupt bacterial cell membranes and metabolic pathways (Yang & Yousef, 2018). Moreover, the antagonistic activity of phyllospheric bacteria like *B. parabrevis* is linked to mechanisms such as competitive exclusion for nutrients and space and quorum sensing interference, which disrupts bacterial communication and virulence factor production (Legein et al., 2020). These properties highlight the potential of *B. parabrevis* AUMC-B1 as a promising candidate for developing natural antimicrobial agents.

In addition to its antibacterial potential, *B. parabrevis* AUMC-B1 exhibited strong antioxidant properties, as confirmed by the DPPH radical scavenging assay. Consistent with previous studies, the radical scavenging activity increased in proportion to the extract concentration, which is consistent with previous studies. The calculated IC_{50} values further highlight the extract's antioxidant capacity, with an IC_{50} of 55.56 µg/mL. Its activity suggests the presence of bioactive metabolites with significant radical-neutralizing potential. Similarly, Abd-Alla et al. (2018) reported that *B. parabrevis* produces exopolysaccharides with strong antioxidant properties, highlighting its role as a promising bioactive metabolite producer. The ability of *B. parabrevis* to enhance antioxidant activity may be attributed to its secretion of extracellular polysaccharides, which can scavenge free radicals and protect against oxidative stress.

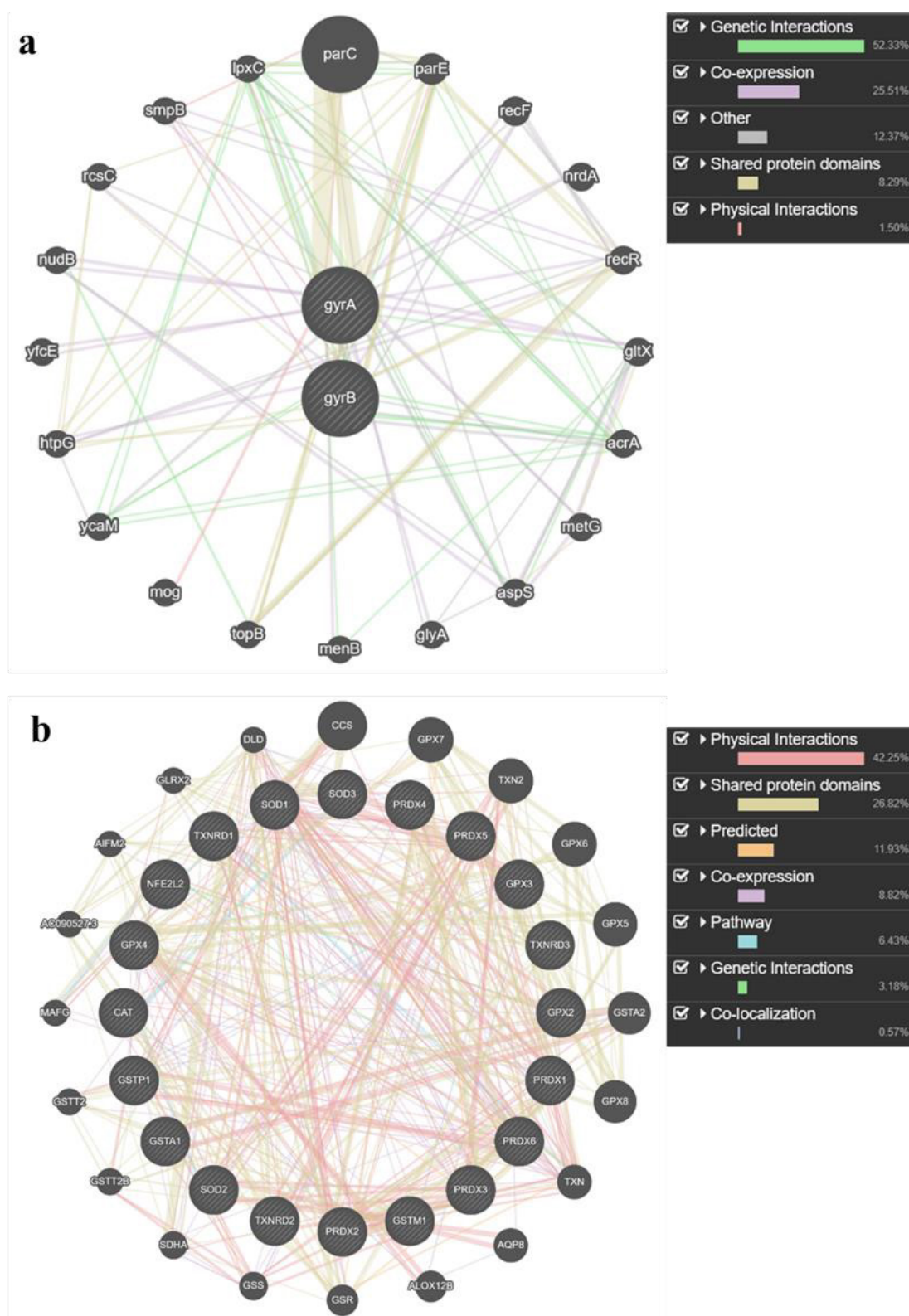


Figure 7. (a) Functional enrichment analysis of *E. coli* DNA gyrase-associated genes, emphasizing co-expression and protein interaction networks. (b) Antioxidant gene network interactions with key regulatory pathways, emphasizing potential enhancement of oxidative stress response

The GC-MS analysis of the ethyl acetate extract from *B. parabrevis* AUMC-B1 revealed a diverse array of bioactive compounds with significant pharmacological properties. The detection of hexadecanoic acid, methyl ester and hexadecanoic acid underscores the extract's antibacterial and anti-inflammatory potential, corroborating the work of Shaaban et al. (2021) and Ganesan et al. (2024). These saturated fatty acids remarkably participate in membrane integrity and inflammatory response modulation (Mukerjee et al., 2021). Unsaturated fatty acid derivatives, such as 9,12-octadecadienoic acid (Z,Z)-, methyl ester and 9-octadecenoic acid (Z)-, methyl ester, were also identified. These compounds possess antioxidant and anti-inflammatory properties, as reported by Compant et al. (2021). Their presence enhances the extract's potential to mitigate oxidative stress and inflammation-related disorders. The identification of 7-methyl-Z-tetradecen-1-ol acetate introduces a compound with reported anticancer and anti-inflammatory activities (El-Naggar et al., 2023). This finding suggests a broader therapeutic scope for the extract, potentially extending to cancer treatment modalities. In addition, bis(2-ethylhexyl) phthalate was detected, known for its antibacterial properties (Javed et al., 2022). While phthalates are commonly used as plasticizers, their presence in microbial extracts indicates possible biosynthetic pathways for these compounds in bacteria. The diverse bioactivities of the identified compounds align with the known capabilities of *Brevibacillus* species to produce antimicrobial peptides and other bioactive substances. For instance, *B. parabrevis* WGTm-23, isolated from a termitarium, contains unique biosynthetic gene clusters potentially coding for novel antimicrobial agents (Dhanalakshmi & Rajendhran, 2024).

GC-MS analysis identified several bioactive compounds, with 11-octadecenoic acid, methyl ester (29.17%) and 9,12-octadecadienoic acid (Z,Z)-, methyl ester (20.19%) as the predominant constituents. Although cis-13-eicosenoic acid (0.40%) and 12-methyl-E,E-2,13-octadecadien-1-ol (0.59%) were minor components, their inclusion in docking studies was justified by their established bioactivities and potential synergistic effects within the extract. DNA gyrase is a crucial bacterial enzyme responsible for introducing negative supercoils into DNA, facilitating essential processes such as replication and transcription. Its unique role in bacteria makes it an attractive target for antibacterial agents. Inhibiting DNA

gyrase disrupts DNA replication and transcription, leading to bacterial cell death (Spencer & Panda, 2023). Peroxiredoxin 5 (PRDX5) is a member of the peroxiredoxin family of antioxidant enzymes, widely expressed across various tissues and involved in reducing peroxides such as hydrogen peroxide and organic hydroperoxides, thereby protecting cells from oxidative stress (Rahaman et al., 2024). The interaction of cis-13-Eicosenoic acid with PRDX5 suggests that it may modulate antioxidant activity. The current study, 12-Methyl-E,E-2,13-octadecadien-1-ol demonstrated a notable binding affinity to *S. aureus* DNA gyrase, interacting with ASP 83 and ARG 122, while cis-13-Eicosenoic acid interacted with *E. coli* DNA gyrase and human peroxiredoxin 5. These findings suggest that 12-Methyl-E,E-2,13-octadecadien-1-ol could potentially inhibit bacterial DNA gyrase, while cis-13-Eicosenoic acid may have an impact on PRDX5 function.

The ADME analysis suggests that 12-Methyl-E,E-2,13-octadecadien-1-ol and cis-13-Eicosenoic acid exhibit high lipophilicity and strong oral absorption, making them promising candidates for oral drug formulations. Studies have shown that high lipophilicity can lead to increased plasma protein binding, reducing the free drug concentration available for therapeutic action, which may affect efficacy (Han et al., 2021). However, their high oral absorption indicates potential systemic bioavailability, supporting their application in non-CNS-related therapies, such as anti-inflammatory or antimicrobial drugs (Wasai et al., 2025). Despite these favorable properties, their low aqueous solubility (QPlogS values below -6) could pose a formulation challenge, requiring solubility enhancement strategies. This analysis highlights 12-Methyl-E,E-2,13-octadecadien-1-ol as the most pharmacokinetically favorable compound, with high oral absorption and lipophilicity, albeit with solubility limitations. The inhibition of gyrA and gyrB by 12-Methyl-E,E-2,13-octadecadien-1-ol and cis-13-Eicosenoic acid suggests a possible disruption of DNA supercoiling, leading to bacterial DNA damage and reduced replication efficiency. Previous studies have indicated that targeting DNA gyrase with small-molecule inhibitors can lead to bacterial cell death (Alfonso et al., 2022). The interaction of gyrA and gyrB with essential DNA repair proteins such as recF and topB, as revealed in the GeneMANIA network, further supports the hypothesis that interfering with gyrase activity could have a

cascading inhibitory effect on bacterial survival (Marchese & Debbia, 2016). For antioxidant properties, the enhancement of PRDX5, SOD1, and GPX4 activity through bioactive compounds suggests a potential protective mechanism against oxidative stress. The strong physical interaction between PRDX5 and SOD family members suggests that compounds capable of binding PRDX5 may modulate redox homeostasis and enhance antioxidant defense (Andelova et al., 2023). The GeneMANIA results support previous reports indicating that compounds interacting with NRF2 pathways can enhance endogenous antioxidant responses (Thiruvengadam et al., 2021). These findings indicate that the tested bioactive compounds exhibit dual therapeutic potential, both as antibacterial agents targeting DNA gyrase function and as antioxidants capable of enhancing cellular defense against oxidative damage.

CONCLUSION

This study remarkably isolated and characterized *B. parabrevis* AUMC-B1, revealing its significant antibacterial and antioxidant potential. The bacterial extract exhibited notable antimicrobial activity for *E. coli* and *S. aureus*, as well as potent antioxidant activity with a DPPH radical scavenging activity. GC-MS analysis identified bioactive compounds that were further examined through molecular docking and ADME profiling, confirming their strong interactions with bacterial DNA gyrase and human peroxiredoxin 5. The docking results suggest that these compounds may inhibit bacterial DNA replication and enhance antioxidant defense mechanisms, supporting their dual therapeutic potential. ADME analysis indicated high oral absorption and favorable lipophilicity, though solubility enhancement strategies may be necessary for improved bioavailability. Additionally, gene interaction analysis using GeneMANIA provided insights into their mechanistic roles in antibacterial and antioxidant pathways. These findings highlight the potential of *B. parabrevis* AUMC-B1 and its bioactive metabolites as promising candidates for novel antimicrobial and antioxidant therapies, future research need to focus on purifying active compounds to express concentrations in molarity (mM) for precision, incorporating ethyl acetate dilution controls to further validate antimicrobial specificity, and conducting broth microdilution MIC assays to quantify antibacterial potency, with results to be reported in a follow-up study. These efforts will enhance the therapeutic applicability

of *B. parabrevis* AUMC-B1 metabolites, positioning them as promising candidates for novel antimicrobial and antioxidant therapies.

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List of abbreviations

16S rRNA	16S Ribosomal Ribonucleic Acid
ADME	Absorption, Distribution, Metabolism, and Excretion
ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
CFU	Colony-Forming Unit
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EtOAc	Ethyl Acetate
GC-MS	Gas Chromatography-Mass Spectrometry
IC ₅₀	Half Maximal Inhibitory Concentration
MW	Molecular Weight
NB	Nutrient Broth
NCBI	National Center for Biotechnology Information
OD	Optical Density
PCR	Polymerase Chain Reaction
PRDX5	Peroxiredoxin 5
SASA	Solvent-Accessible Surface Area
SD	Standard Deviation
SP	Standard Precision
TSA	Tryptic Soy Agar
UV	Ultraviolet

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