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MOLECULAR DOCKING-BASED EVALUATION OF GC–MS-IDENTIFIED BIOACTIVE METABOLITES FROM *BACILLUS PARAMYCOIDES* ISOLATED FROM SEAWEED AGAINST ESSENTIAL ANTIBACTERIAL TARGETS

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ABSTRACT:
Antimicrobial resistance has forced the search for new antibacterial agents from microbial sources. In this study, the bioactive secondary metabolites of *Bacillus paramycoides* were characterized by Gas Chromatography–Mass Spectrometry (GC–MS), and their binding affinity was assessed by molecular docking analysis. GC–MS profiling revealed a wide variety of metabolites including fatty acids, terpenoids, phenolic compounds, sterols, and fatty acid amides. Of these, 2,6-dihydroxybenzaldehyde, palmitic acid, and phytol were selected for in silico study, and molecular docking was performed against two key antibacterial targets of Escherichia coli, UDP-N-acetylmuramoyl-tripeptide:D-alanyl-D-alanine ligase (MurF; PDB ID: 1GG4) in peptidoglycan biosynthesis and DNA gyrase B (PDB ID: 4DUH) in DNA replication. Binding affinities ranged from -5.2 to -7.7 kcal/mol. 2,6-dihydroxybenzaldehyde showed the highest interaction with MurF (-6.3 kcal/mol), and phytol showed the highest interaction with DNA gyrase B (-7.7 kcal/mol), which suggests the antibacterial activity of these metabolites through inhibition of bacterial cell wall synthesis and DNA replication. The results of this study identify *Bacillus paramycoides* as a potential source of bioactive metabolites and shed molecular light on their putative antibacterial mechanisms.

KEYWORDS: *Bacillus paramycoides*, GC–MS, Molecular Docking, MurF, DNA Gyrase B, Phytol, 2,6-Dihydroxybenzaldehyde, Antibacterial Activity.

INTRODUCTION

Marine environments are a potential, yet largely unexplored, source of bioactive secondary metabolites for drug discovery, as secondary metabolites with significant bioactive potential are produced by microorganisms isolated from the seaweed *Gracilaria edulis* (Sneha et al., 2025). The emergence and dissemination of antimicrobial resistance (AMR) have substantially reduced the effectiveness of conventional antibacterial therapies and created an urgent need for the discovery of new antimicrobial agents. (Aborode et al., 2024; Shtaiwi et al., 2024). Marine ecosystems constitute a highly diverse reservoir of microorganisms capable of producing chemically distinctive secondary metabolites. Marine microorganisms encounter environmental conditions that differ substantially from those of terrestrial habitats, and these ecological pressures can contribute to the production of metabolites with diverse structural and biological properties. Among marine microorganisms, Bacillus species have attracted considerable attention because of their capacity to produce a broad spectrum of bioactive secondary metabolites, including lipopeptides, polyketides, macrolactones, peptides, fatty acids, and other chemically diverse compounds with antimicrobial and other pharmacological activities (Mondol et al., 2013; Xiao et al., 2022). The diversity of metabolites reported from marine-derived Bacillus further supports their importance as promising microbial resources for natural-product discovery.

The genus Bacillus is recognized as a prolific producer of structurally diverse secondary metabolites. Marine-derived Bacillus strains have been reported to produce cyclic and linear lipopeptides, diketopiperazines, polyketides, nonribosomal peptides, macrolactins, and other metabolites displaying antibacterial, antifungal, anticancer, and other biological activities (Xiao et al., 2022). In addition, Bacillus paramycoides has attracted increasing interest because of its demonstrated biological potential. Previous studies have reported antimicrobial activity associated with B. paramycoides-based preparations, including activity against E. coli and other bacterial pathogens (El-Refai et al., 2024). However, compared with extensively investigated Bacillus species, the chemical profile and target-based antibacterial potential of metabolites produced by seaweed-associated B. paramycoides remain comparatively insufficiently characterized. This provides a rationale for investigating this organism as a potential source of bioactive metabolites.

These in silico workflows enable the fast screening of enormous chemical libraries to narrow down candidate compounds that can specifically bind to and inhibit crucial metabolic pathways with high binding affinity (Anita & Selvaraj, 2023). This approach relies on targeting conserved subcellular proteins like FtsZ and MreC that are essential for bacterial cell wall synthesis and septum formation during cell division (Wahyuni et al., 2024). Using this approach,

this study focuses on the secondary metabolites produced by *Bacillus paramycoides* that could inhibit key enzymatic functions in *E. coli* by retrieving high-resolution crystal structures of these bacterial targets from the Protein Data Bank and refining them using the Protein Preparation Wizard to atomic accuracy for subsequent docking simulations (Hassan et al., 2024). Ligands that were identified using GC–MS were also prepared by energy minimization to optimize their geometric conformation for strong docking interactions in the protein active sites (Bramki et al., 2024). The three-dimensional structures of the receptor proteins selected were used to define the parameters of the grid, which allowed for the site-specific docking simulations (Tariq et al., 2023). AutoDock 4.2 was used to evaluate molecular interactions, including binding affinities and the formation of hydrogen bonds or hydrophobic contacts within the catalytic pockets, which were compared to established antibiotic standards to determine the relative effectiveness of these natural compounds (Abdelhamid et al., 2025; Altayb et al., 2022). In addition to binding affinity, structural stability of the protein-ligand complexes was assessed by root-mean-square deviation to confirm that these interactions are stable under dynamic physiological conditions.

Lastly, the drug-likeness and toxicity profiles of the most promising lead compounds were assessed using Lipinski's Rule of Five to confirm that these secondary metabolites have favorable pharmacodynamic properties for potential clinical translation (Montecillo & Bae, 2022). Furthermore, using subcellular localization prediction tools, the prioritized proteins were confirmed to be localized to the bacterial cytoplasm or periplasm, which further validated their accessibility for therapeutic targeting. This stringent screening ensures that the selected bioactive compounds have structural specificity for their targeted proteins and desirable physicochemical properties for drug development (Elsaman et al., 2025; Liu et al., 2023). Subsequently, interactions such as pi-pi stacking, metal coordination, and hydrogen bonding were considered to calculate the binding affinities of the complexes, where higher negative binding scores corresponded to better thermodynamic stability of the ligand-protein complexes (Babatunde et al., 2025). Visualization of these molecular poses also enabled characterization of important amino acid residues that stabilize the ligand in the binding site, which is a critical step in the mechanism of enzymatic inhibition (Konappa et al., 2020).

Moreover, the analysis of these binding poses showed that metabolites with the best affinity scores typically form stable hydrogen-bonding networks with key catalytic residues, similar to interactions observed with conventional drugs (Pattapulavar et al., 2025). By incorporating DoGSiteScorer, we were able to conduct a complete analysis of protein cavities and identified druggable sites, along with sites likely to bind ligands with high affinities. Collectively, these multidisciplinary computational approaches form a robust, predictive workflow that accelerates the identification of potential natural product leads against E. coli. This study characterizes the interaction landscape between bioactive secondary metabolites from *Bacillus paramycoides* isolated from seaweed and essential bacterial enzymes, which can guide future experimental validation and therapeutic development.

MATERIALS AND METHODS

GC–MS Analysis of Secondary Metabolites

Secondary metabolites extracted from *Bacillus paramycoides* of epiphytic bacteria from Gracilaria edulis seaweed were subjected to Gas Chromatography–Mass Spectrometry (GC–MS) analysis for compound identification. Compounds identified by Gas Chromatography–Mass Spectrometry (GC-MS) using a Shimadzu GC-MS-QP2010 Plus equipped with an SH-RTX-5MS capillary column (60 m × 0.32 mm × 0.25 µm), and operated under standard conditions, employing helium as the carrier gas; mass spectra were recorded in the range of m/z 40 to 900 compared against entries from NIST Mass Spectral Database.

Selection of Bioactive Compounds

Of the metabolites detected by GC–MS, 2,6-dihydroxybenzaldehyde, palmitic acid, and phytol were chosen for molecular docking studies because of the Metabolites exhibiting the highest relative peak abundance in the GC–MS chromatogram, namely 2,6-dihydroxybenzaldehyde, palmitic acid, and phytol, were prioritized for subsequent molecular docking analysis.. The chemical structures of the chosen compounds were retrieved from the PubChem database and prepared for docking analysis.

Preparation of Target Proteins

MurF (PDB ID: 1GG4) and DNA gyrase B (PDB ID: 4DUH) were selected as molecular docking targets because of their essential roles in bacterial cell wall biosynthesis and DNA replication, respectively. Both proteins possess well-resolved experimentally determined crystal structures deposited in the Protein Data Bank (PDB), providing reliable structural models for evaluating ligand–protein interactions and predicting binding modes.

Molecular Docking Analysis

AutoDock Vina was used for molecular docking and binding interactions between the chosen ligands and target proteins were assessed, with the docking grid centered on the active-site region of each protein, binding affinity expressed as docking score (kcal/mol) with lower energy values representing stronger interactions, and several docking conformations generated with the highest-ranked pose (based on binding energy) used for analysis.

Visualization of Protein–Ligand Interactions

Protein–Ligand Interactions were visualized using Discovery Studio Visualizer and the molecular basis of antibacterial activity was explored based on hydrogen bonding, hydrophobic interactions, van der Waals contacts, and amino acid residues involved in ligand binding.

RESULTS

GC–MS Profiling of Secondary Metabolites

A wide range of secondary metabolites, including fatty acids, terpenoids, phenolic compounds, amino acid derivatives, sterols, glycolipids, and fatty acid amides, were detected in the ethyl acetate extract of *Bacillus paramycoides* , 30

compounds were identified by GC-MS (Fig 1; Table 1) based on their retention times and mass spectral matching with the NIST database, including major metabolites 2,6-dihydroxybenzaldehyde, dodecanoic acid (lauric acid), oleic acid, palmitic acid, phytol, neophytadiene, squalene, batilol, erucamide, and palmitoleamide, which have been reported previously to exhibit antimicrobial, antioxidant, anti-inflammatory, and anticancer activities, suggesting that the isolate has pharmaceutical potential.

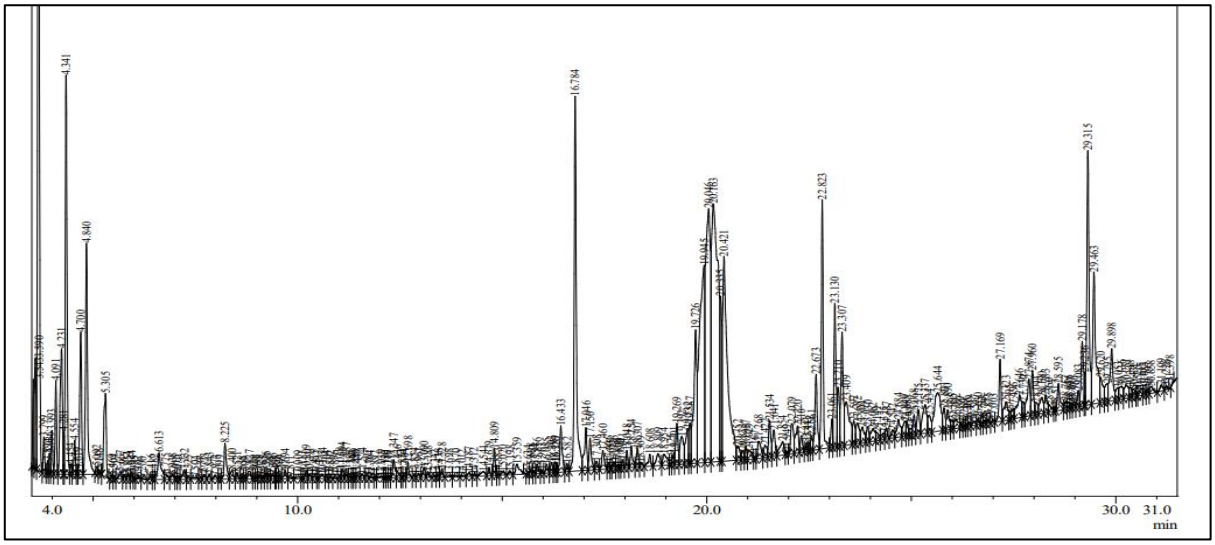


Fig 1: GC-MS Chromatograph

Table 1. GC–MS identified metabolites from *Bacillus paramycoides*

S. No.	RT (min)	Compound Name	Molecular Formula	Molecular Weight (g/mol)	Chemical Class	Reported Biological Activities
1	6.613	Glycerin	$C_3H_8O_3$	92.09	Polyol	Humectant, antioxidant, wound healing, moisturizing
2	6.775	Undecanoic acid	$C_{11}H_{22}O_2$	186.29	Fatty acid	Antifungal, antimicrobial, anti-inflammatory
3	10.604	Octanoic acid	$C_8H_{16}O_2$	144.21	Fatty acid	Antibacterial, antifungal, antiviral
4	12.347	Caprolactam	$C_6H_{11}NO$	113.16	Lactam	Antimicrobial, industrial intermediate
5	13.342	4-Fluoro-1-methyl-5-carboxylic acid, ethyl ester*	Verify by NIST	Verify by NIST	Carboxylic ester	Limited biological reports; synthetic intermediate
6	14.541	L-Proline, 5-oxo-, methyl ester	$C_6H_9NO_3$	143.14	Amino acid derivative	Antioxidant, metabolic intermediate
7	14.883	2,6-Dihydroxybenzaldehyde	$C_7H_6O_3$	138.12	Phenolic aldehyde	Antioxidant, antimicrobial, anti-inflammatory
8	15.359	Guanosine	$C_{10}H_{13}N_5O_5$	283.24	Purine nucleoside	Antioxidant, antiviral, immunomodulatory
9	17.460	Dodecanoic acid (Lauric acid)	$C_{12}H_{24}O_2$	200.32	Fatty acid	Antibacterial, antiviral, antifungal
10	19.945	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (DEHP)	$C_{24}H_{38}O_4$	390.56	Phthalate ester	Reported antimicrobial activity; commonly considered an environmental contaminant
11	20.421	9-Octadecenoic acid (Oleic acid)	$C_{18}H_{34}O_2$	282.46	Unsaturated fatty acid	Antioxidant, anti-inflammatory, antimicrobial, wound

						healing
12	21.534	Neophytadiene	$C_{20}H_{38}$	278.52	Diterpenoid	Antioxidant, antimicrobial, anti-inflammatory
13	23.130	n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	256.42	Saturated fatty acid	Antioxidant, antimicrobial, anti-inflammatory
14	23.409	13-Docosenamide (Z) (Erucamide)	$C_{22}H_{43}NO$	337.58	Fatty acid amide	Antimicrobial, antioxidant, anti-inflammatory
15	24.684	1-Nonadecene	$C_{19}H_{38}$	266.51	Alkene	Antioxidant, antimicrobial
16	25.068	Phytol	$C_{20}H_{40}O$	296.53	Diterpene alcohol	Antioxidant, antimicrobial, anticancer, anti-inflammatory
17	25.644	Squalene	$C_{30}H_{50}$	410.73	Triterpene	Antioxidant, anticancer, chemoprotective, skin protective
18	25.890	Hexadecanamide	$C_{16}H_{33}NO$	255.44	Fatty acid amide	Antimicrobial, antioxidant, anti-inflammatory
19	26.547	Docosanoic acid, docosyl ester	$C_{44}H_{88}O_2$	649.17	Long-chain fatty ester	Antioxidant, antimicrobial, emollient
20	27.874	Butyric acid, 2-methyl-, pentadecyl ester	$C_{20}H_{40}O_2$	312.53	Fatty acid ester	Antimicrobial, antioxidant
21	27.960	Palmitoleamide	$C_{16}H_{31}NO$	253.43	Fatty acid amide	Anti-inflammatory, neuroprotective, antimicrobial
22	28.283	Batilol	$C_{21}H_{44}O_3$	344.57	Glycolipid	Immunomodulatory, antioxidant, antitumor, anti-inflammatory
23	28.360	Docosapentaenoic acid methyl ester	$C_{23}H_{38}O_2$	346.55	Polyunsaturated fatty acid	Anti-inflammatory, cardioprotective, antioxidant
24	28.767	8,11,14-Docosatrienoic acid methyl ester	$C_{23}H_{40}O_2$	348.56	Polyunsaturated fatty acid	Antioxidant, antimicrobial, anti-inflammatory
25	29.178	(Z)-3-(Pentadec-8-en-1-yl)phenol	$C_{21}H_{34}O$	302.49	Alkylphenol	Antioxidant, antimicrobial, anti-inflammatory
26	29.315	Diethylene glycol dibenzoate	$C_{18}H_{18}O_5$	314.33	Aromatic ester	Plasticizer; limited biological activity reported
27	29.463	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Monopalmitin)	$C_{19}H_{38}O_4$	330.50	Glyceride	Antimicrobial, antioxidant, emulsifying agent
28	29.898	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.56	Phthalate ester	Reported antimicrobial activity; possible environmental contaminant
29	30.858	Cholest-8-en-3β-ol, acetate	$C_{29}H_{48}O_2$	428.69	Sterol derivative	Antioxidant, anti-inflammatory, membrane stabilizing
30	31.109	Cholesta-3,5-diene	$C_{27}H_{44}$	368.64	Sterol derivative	Antioxidant, anti-inflammatory

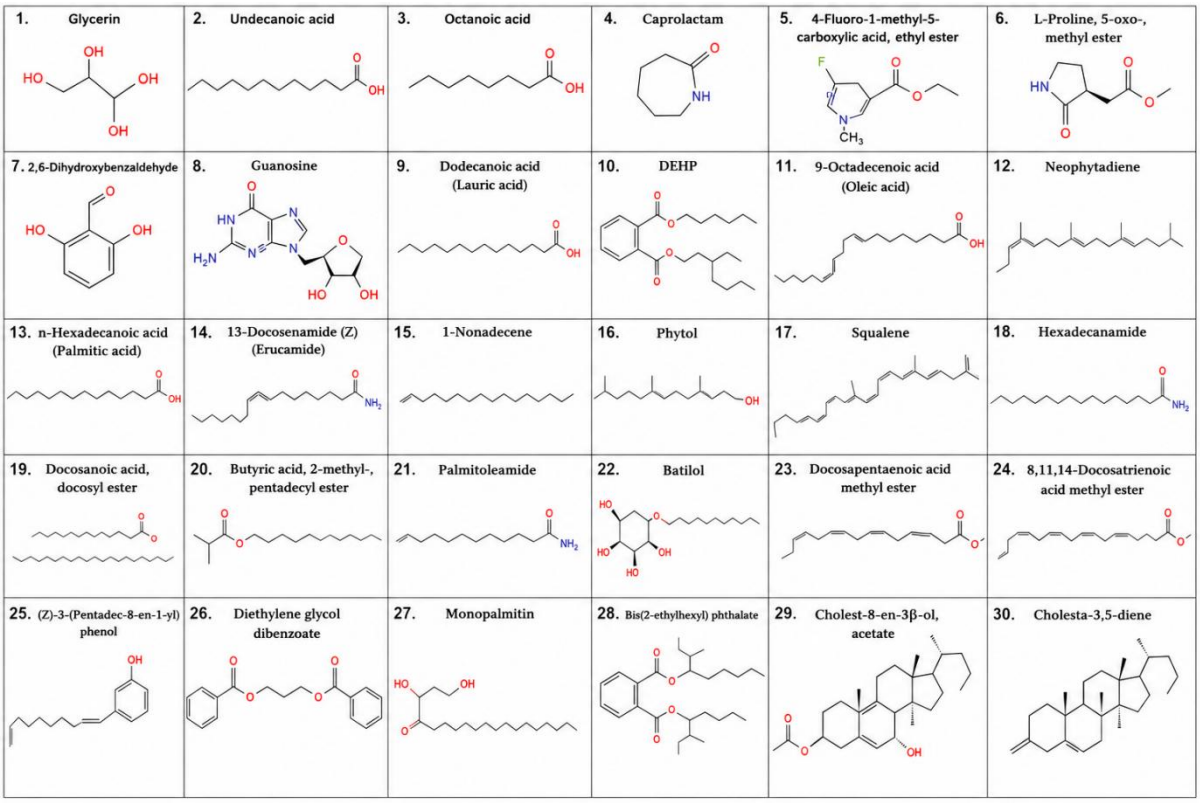
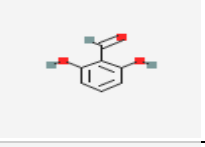
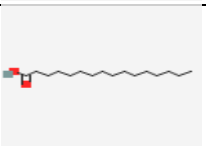
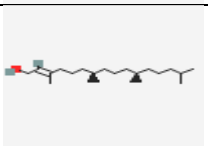


Fig 2: 2D Structures of GCMS compounds

Molecular Docking Analysis

GC–MS analysis identified 2,6-dihydroxybenzaldehyde, palmitic acid, and phytol as three biologically important metabolites, and molecular docking studies were performed on these compounds against two essential antibacterial targets of Escherichia coli: MurF (PDB ID: 1GG4) (Fig 2) and DNA gyrase B (PDB ID: 4DUH) (Fig 3). The docking scores are presented in Table 1.

Table 1. Binding affinity of selected metabolites against antibacterial targets

Compound	Structure	MurF (1GG4) (kcal/mol)	DNA Gyrase B (4DUH) (kcal/mol)
2,6-Dihydroxybenzaldehyde		-6.3	-5.5
Palmitic Acid		-5.2	-6.1
Phytol		-5.5	-7.7

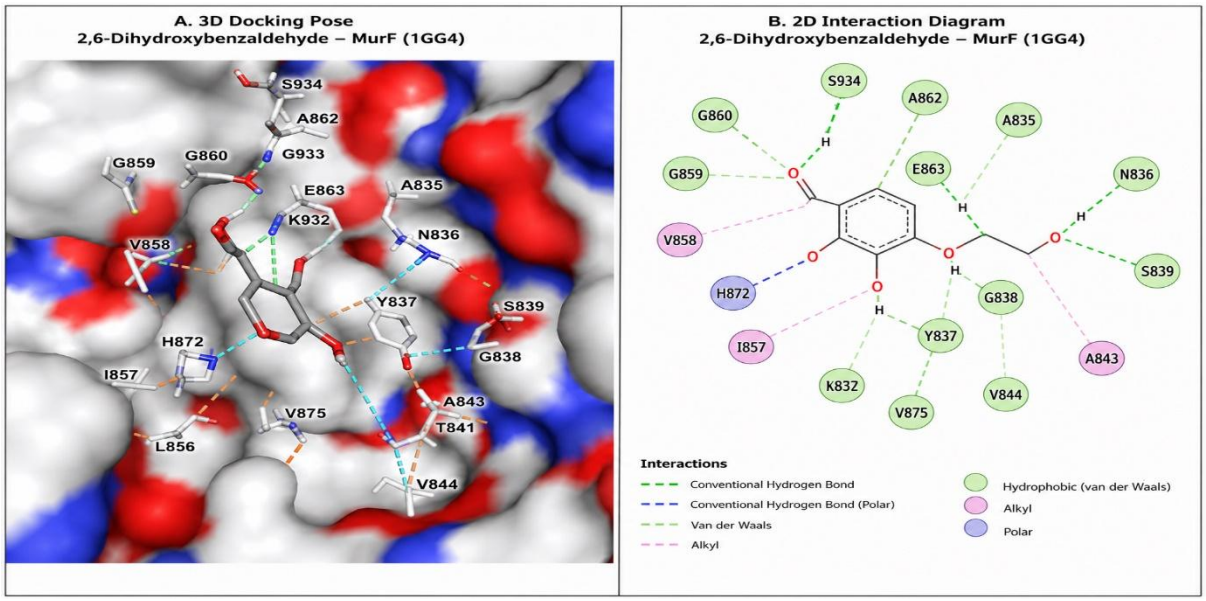


Fig 3: 3D docking pose and 2D interaction map of 2,6-Dihydroxybenzaldehyde–MurF (1GG4)

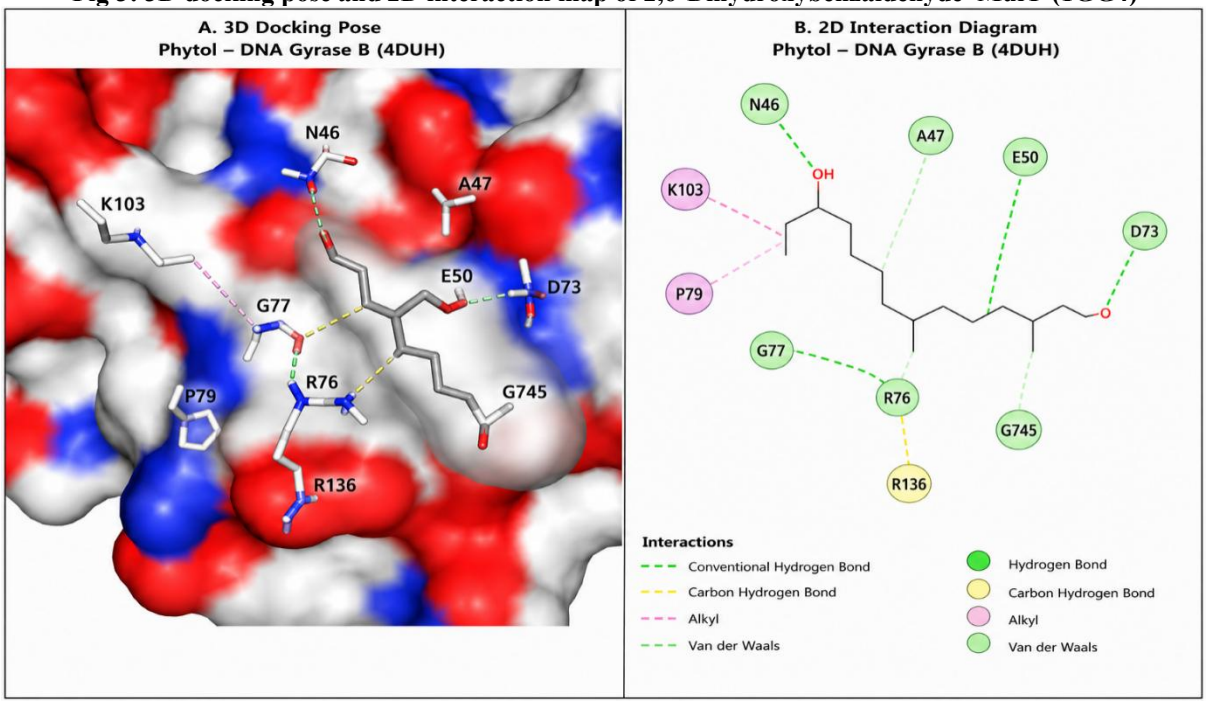


Fig 4: 3D docking pose and 2D interaction map of Phytol–DNA Gyrase B (4DUH)

The compound 2,6-dihydroxybenzaldehyde bind most strongly to MurF (-6.3 kcal/mol) among the two tested protein and was accommodated within the active-site cavity of the enzyme, where it formed several stabilizing interactions with amino acid residues surrounding the binding site, indicating an inhibitory effect on the biosynthesis of peptidoglycan, which is essential to the bacterial cell wall. Palmitic acid also showed moderate binding affinity to MurF (-5.2 kcal/mol) and DNA gyrase B (-6.1 kcal/mol), suggesting that it interacts with proteins involved in cell wall synthesis and DNA replication. Phytol had the highest binding affinity to DNA gyrase B, with a docking score of -7.7 kcal/mol, occupying the active-site pocket and forming extensive hydrophobic interactions that stabilized the complex. The high binding affinity of phytol suggests that it may inhibit DNA gyrase-mediated DNA replication and transcription.

Comparative Analysis of Docking Affinity

Docking scores for the compounds were compared, and target preference was observed: MurF, 2,6-Dihydroxybenzaldehyde (-6.3 kcal/mol) > Phytol (-5.5 kcal/mol) > Palmitic Acid (-5.2 kcal/mol); DNA gyrase B, Phytol (-7.7 kcal/mol) > Palmitic Acid (-6.1 kcal/mol) > 2,6-Dihydroxybenzaldehyde (-5.5 kcal/mol), which suggests that 2,6-dihydroxybenzaldehyde may be a primary inhibitor of bacterial cell wall biosynthesis through MurF inhibition, while phytol may be a more effective inhibitor of DNA gyrase B and disrupt bacterial DNA replication. Taken together, these results provide molecular evidence for the antibacterial activity of *Bacillus paramycoides* metabolites and indicate that these compounds may have multiple antibacterial mechanisms of action.

DISCUSSION

In the present study, the antibacterial potential of secondary metabolites produced by *Bacillus paramycoides* was evaluated by integrating GC–MS profiling and molecular docking approaches. GC–MS analysis showed a chemically diverse metabolite profile, including fatty acids, terpenoids, phenolic compounds, sterols, glycolipids, and fatty acid amides, which are known sources of biologically active compounds with antimicrobial and pharmaceutical applications (Rahman et al., 2022; Konappa et al., 2020). Among the metabolites identified, 2,6-dihydroxybenzaldehyde, palmitic acid, and phytol were selected for molecular docking studies due to their reported biological activities and abundance in the GC–MS profile.

Molecular docking is a widely used rapid and cost-effective strategy to predict ligand–protein interactions and identify potential antibacterial candidates before experimental validation (Yuliandra & Syafni, 2024; Serral et al., 2021). Docking analysis showed that all three compounds interacted favorably with MurF and DNA gyrase B, two key proteins for bacterial survival, where MurF catalyzes peptidoglycan biosynthesis and bacterial cell wall formation, and DNA gyrase B facilitates ATP-dependent DNA supercoiling, which is necessary for DNA replication and transcription (Fields et al., 2016; Mondal et al., 2015). Therefore, targeting these proteins has become an attractive approach for antibacterial drug development (Shtaiwi et al., 2024; Alotaibi et al., 2023). The compound with the best binding affinity for MurF was 2,6-dihydroxybenzaldehyde (docking score of −6.3 kcal/mol), and its interaction within the active-site cavity suggested it could interfere with peptidoglycan biosynthesis and thus compromise bacterial cell wall formation (Rakhi et al., 2024; Pattapulavar et al., 2025). Palmitic acid showed moderate binding affinities to MurF and DNA gyrase B, although its docking scores were comparatively lower; the ability to bind to two different antibacterial targets indicates a possible multi-target mode of action, which may complement other inhibitory effects against essential cellular pathways (Perera and Lawan 2024; Tegegn et al. 2022). The highest binding affinity was observed for phytol (docking score: −7.7 kcal/mol), which is the most notable result as DNA gyrase B is a validated antibacterial target, and inhibition of its ATPase activity leads to inhibition of the DNA replication and transcription processes required for bacterial proliferation (Alotaibi et al., 2023; Abdelrahman et al., 2024). Strong binding observed for phytol may indicate that this diterpene alcohol can occupy the catalytic pocket of DNA gyrase B and inhibit its biological function; natural metabolites with high binding affinity to DNA gyrase B have been reported as potential lead compounds for antibacterial drug development (Elsaman et al., 2025; Zhao et al., 2025). Comparative analysis of the docking scores showed clear target preferences for the tested metabolites, with the ranking order for MurF being 2,6-dihydroxybenzaldehyde > phytol > palmitic acid, and for DNA gyrase B being phytol > palmitic acid > 2,6-dihydroxybenzaldehyde, indicating that different metabolites may act against bacteria via different molecular mechanisms. The potential to target proteins implicated in cell wall biosynthesis and DNA replication may confer additional advantages in terms of overcoming bacterial resistance mechanisms and increasing the efficacy of the selected compounds (Zayed et al., 2023; Eze et al., 2025). Collectively, the outcomes indicate that *Bacillus paramycoides* synthesizes bioactive metabolites that interact with key antibacterial targets of Escherichia coli, highlighting the possibility of using *Bacillus paramycoides* -derived metabolites as antibacterial lead compounds (Heena et al., 2024; Elsaman et al., 2025).

CONCLUSION

This study showed that *Bacillus paramycoides can* be a source of bioactive secondary metabolites with possible antibacterial activity. GC–MS analysis identified a wide array of metabolites, such as fatty acids, terpenoids, phenolic compounds, sterols, and fatty acid amides, indicating the metabolic versatility of the isolate. Of these identified compounds, 2,6-dihydroxybenzaldehyde, palmitic acid, and phytol were chosen for molecular docking studies against two important antibacterial targets of Escherichia coli, MurF and DNA gyrase B. Molecular docking analysis indicated that the selected metabolites had positive binding interactions with the target proteins, and 2,6-dihydroxybenzaldehyde had the highest binding affinity with MurF (-6.3 kcal/mol), indicating it could inhibit peptidoglycan biosynthesis and bacterial cell wall formation, while phytol had the highest binding affinity with DNA gyrase B (-7.7 kcal/mol), which may indicate that it can interfere with bacterial DNA replication and transcription, and palmitic acid interacted moderately with both targets, which may suggest that it could contribute to antibacterial activity through multiple mechanisms. The comparative docking results suggested that these metabolites may work through different but related antibacterial pathways that target both cell wall biosynthesis and DNA replication. Phytol and 2,6-dihydroxybenzaldehyde showed the best antibacterial activity, and these results provided molecular evidence for the antibacterial potential of *B. paramycoides*-derived metabolites and set the stage for further in vitro, in vivo, and pharmacological studies to establish their antibacterial efficacy and suitability for development as novel antimicrobial agents.

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