



In Silico Evaluation of the Antibacterial Potential of Methanolic *Lo'i-Keta* Extract Against DNA Gyrase in *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Abstract: This study aimed to evaluate the antibacterial potential of bioactive compounds identified in the methanolic extract of *lo'i-keta* against DNA gyrase from *Staphylococcus aureus* (PDB ID: 3G75) and *Pseudomonas aeruginosa* (PDB ID: 6M1S) using an *in silico* approach. Bioactive compounds were identified by gas chromatography–mass spectrometry (GC–MS). Ligand preparation and molecular docking were subsequently performed using PyRx 0.8, while ligand–protein interactions were visualized using Discovery Studio. Ciprofloxacin was used as the positive control. GC–MS analysis detected 20 compound peaks, of which 13 compounds were validated and included in the docking analysis. Molecular docking results showed that cyclooctacosane exhibited the strongest binding affinity toward the DNA gyrase of *S. aureus* and *P. aeruginosa*, with binding energies of -8.3 and -8.2 kcal/mol, respectively. These values were more favorable than those of ciprofloxacin, which showed binding affinities of -7.7 and -7.4 kcal/mol, respectively. In addition, dioctyl benzene-1,2-dicarboxylate displayed a residue interaction pattern similar to that of ciprofloxacin within the active site of DNA gyrase. Hexadecanoic acid (palmitic acid) exhibited a more moderate binding affinity but was present at a relatively high abundance in the extract. These findings indicate that several bioactive compounds in *lo'i-keta* may form stable interactions with DNA gyrase, supporting its potential as a molecular target for antibacterial activity. Based on the *in silico* results, cyclooctacosane was identified as the leading candidate DNA gyrase inhibitor, while dioctyl benzene-1,2-dicarboxylate and hexadecanoic acid may serve as supporting candidates for the development of *lo'i-keta*-based antibacterial agents. This study provides a scientific basis for the traditional use of *lo'i-keta*; however, further *in vitro* and *in vivo* investigations are required to validate these findings.

Keywords: *Lo'i-keta*; antibacterial activity; *in silico*; *Staphylococcus aureus*; *Pseudomonas aeruginosa*

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INTRODUCTION

Skin diseases remain a major public health concern and contribute substantially to the global disease burden, particularly in developing countries, including Indonesia. These diseases encompass a broad range of infectious and non-infectious conditions that can impair physical health, psychological well-being, quality of life, and socioeconomic productivity. Microorganisms, including bacteria, fungi, viruses, and parasites, are among the major causes of infectious skin diseases (Alfadli & Khairunisa, 2024). Globally, bacterial skin diseases, particularly pyoderma and cellulitis, continue to impose a substantial health burden. An analysis of Global Burden of Disease 2019 data showed that the age-standardized incidence of bacterial skin diseases increased by 7.38% between 1990 and 2019, with the greatest burden occurring in regions with a low-middle sociodemographic index (Yakupu et al., 2023). In Indonesia, the prevalence of pyoderma has been reported to be 1.4% among adults and 0.2% among children. Furuncles are the most frequently identified form of

pyoderma (22.4%), followed by ecthyma, paronychia, hidradenitis, and folliculitis (Arizka & Hervina, 2021). These epidemiological findings highlight the continuing need for effective and accessible strategies to prevent and treat bacterial skin infections.

Among the bacteria responsible for skin infections, *Staphylococcus aureus* is a clinically important Gram-positive pathogen associated with impetigo, folliculitis, furuncles, cellulitis, and abscesses. In contrast, *Pseudomonas aeruginosa* is an opportunistic Gram-negative pathogen frequently associated with burn wound infections, diabetic ulcers, and chronic wounds. Both bacteria can form biofilms and develop resistance to multiple antibiotics, thereby contributing to treatment failure, prolonged hospitalization, increased healthcare costs, and greater morbidity and mortality. Biofilm formation can further reduce antimicrobial susceptibility by limiting drug penetration, altering bacterial metabolic activity, and facilitating the persistence of resistant bacterial populations. Consequently, methicillin-resistant *Staphylococcus aureus* (MRSA) and carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) are included among the high-priority pathogens in the 2024 WHO Bacterial Priority Pathogens List, emphasizing the need for continued research and development of new antibacterial agents (World Health Organization, 2024). These organisms were selected as research targets because they represent clinically important Gram-positive and Gram-negative pathogens associated with difficult-to-treat skin and wound infections.

DNA gyrase represents a promising molecular target for antibacterial discovery in both bacterial species. This enzyme is a bacterial type IIA topoisomerase that regulates DNA topology by introducing negative supercoils and removing torsional stress generated during DNA replication and transcription. It consists of two GyrA and two GyrB subunits assembled as an A₂B₂ heterotetramer. GyrA is primarily involved in DNA cleavage and re-ligation, whereas GyrB contains the ATPase domain that provides the energy required for DNA-strand passage. Because DNA gyrase is essential for bacterial survival, is highly conserved among bacterial pathogens, and is absent from mammalian cells, it is considered a well-validated target for antibacterial drug development (Salman et al., 2022). However, the specific DNA gyrase subunit examined in the present study—GyrA or GyrB—should be stated explicitly because each subunit contains distinct ligand-binding sites and performs different catalytic functions.

The exploration of traditional medicinal plants represents a promising strategy for discovering new antibacterial compounds. Lo'i-keta ([insert the accepted botanical name, including the author citation if available]) is a plant traditionally used by local communities to treat skin infections. A previous *in vitro* study using the agar-diffusion method showed that lo'i-keta extract inhibited the growth of *Staphylococcus epidermidis* but produced no inhibition zone against *Pseudomonas aeruginosa*. Although this finding indicates selective antibacterial activity under the conditions tested, the absence of an inhibition zone does not necessarily demonstrate the absence of biologically active compounds. The results of agar-diffusion assays are strongly influenced by the solubility, molecular size, polarity, concentration, and diffusion rate of the tested compounds in the agar matrix. Consequently, compounds with limited agar diffusion may produce small or undetectable inhibition zones despite having relevant interactions with bacterial molecular targets (Hossain, 2024). Furthermore, the bioactive constituents responsible for the previously observed activity of lo'i-keta and their specific molecular targets have not yet been adequately characterized. This unresolved issue constitutes the principal research gap addressed in the present study.

Advances in computational methods have enabled the preliminary evaluation of compound–target interactions through *in silico* approaches. Molecular docking is widely used to predict the preferred orientation of a ligand within a target-protein binding site, identify potential ligand–residue interactions, and estimate relative binding strength using a docking score or predicted binding affinity (Shah et al., 2019). Accordingly, molecular docking can be used to prioritize bioactive compounds that are predicted to interact favorably with the active or binding site of DNA gyrase. Unlike whole-cell diffusion assays, this target-based computational approach is not directly affected by the ability of a compound to diffuse through agar or cross the bacterial cell envelope. Nevertheless, docking results represent predicted molecular interactions and cannot independently confirm antibacterial efficacy, membrane permeability, pharmacokinetic behavior, or biological safety. Experimental validation therefore remains essential.

Based on this rationale, the present study aimed to evaluate the molecular interactions of bioactive compounds identified in the methanolic extract of lo'i-keta with the DNA gyrase [specify GyrA or GyrB] proteins of *Staphylococcus aureus* and *Pseudomonas aeruginosa* using molecular docking. More specifically, the study sought to compare the predicted binding affinities of the investigated compounds, characterize their interactions with key amino acid residues within the selected binding sites, and identify the most promising compounds for subsequent experimental investigation. This study is expected to provide a scientific basis for understanding the potential molecular mechanism underlying the antibacterial properties of lo'i-keta and to support the prioritization of its bioactive compounds as preliminary candidates for the development of antibacterial agents against clinically important skin-infection-associated bacteria.

METHOD

Study Design and Period

This study was conducted from July 2025 to January 2026. It employed a computational (*in silico*) approach using molecular docking to investigate the potential interactions between bioactive compounds identified in the methanolic extract of lo'i-keta and the DNA gyrase proteins of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The bioactive compounds were identified using gas chromatography–mass spectrometry (GC–MS). Ligand and protein preparation was subsequently performed, followed by molecular docking analysis using AutoDock to predict the binding affinities between the ligands and target proteins. The docking results were then used to evaluate the potential antibacterial activity of the bioactive compounds in the lo'i-keta methanolic extract.

Research Objects

The research objects consisted of bioactive compounds identified in the lo'i-keta methanolic extract through GC–MS analysis, which were used as ligands, and the DNA gyrase proteins of *S. aureus* and *P. aeruginosa*, which served as receptors. The identified bioactive compounds were prepared and analyzed using molecular docking to predict their binding affinities and potential antibacterial activities against the target proteins.

Equipment and Software

The equipment used for extraction and compound screening included beakers, a magnetic stirrer, filter paper, a rotary evaporator, and a GC–MS instrument. The *in silico* analysis was performed using an Acer Nitro 5 laptop equipped with an Intel Core

i5 processor, an NVIDIA GeForce RTX 3050 graphics processing unit, and the Windows XP Professional operating system. The software used in this study included PyRx with the Vina Wizard module and Discovery Studio.

Materials

The material used in the extraction process was lo'i-keta, and methanol was used as the extraction solvent. For the *in silico* study, the three-dimensional structures of the compounds identified in the lo'i-keta methanolic extract were downloaded from the PubChem database. In addition, the three-dimensional structures of DNA gyrase from *S. aureus* (PDB ID: 3G75) and *P. aeruginosa* (PDB ID: 6M1S) were obtained from the official Protein Data Bank database.

Methanolic Extraction

Methanolic extraction was performed to isolate polar bioactive compounds from the natural material. Methanol is effective for extracting a broad range of organic compounds and is therefore commonly used to obtain optimal extraction yields. The extraction procedure generally consisted of raw-material preparation through drying and grinding, maceration with methanol for a predetermined period, filtration to separate the liquid extract from the plant residue, and evaporation of the methanol to obtain a concentrated or dried extract.

GC–MS Analysis

Following extraction, the bioactive constituents were further analyzed using GC–MS. The concentrated extract obtained through methanolic extraction was examined to identify the active compounds present in the sample. The extract was dissolved in an appropriate solvent and subsequently injected into the GC–MS instrument. The resulting chromatographic and mass spectral data were used to identify the bioactive compounds contained in the lo'i-keta extract.

Molecular Docking and Interaction Analysis

After completion of the docking simulations, the results generated by PyRx in .csv format were converted into .xlsx format for further analysis. The ligand-binding poses and their interactions with the target proteins were then evaluated. The analysis included selecting the ligand pose with the most favorable binding-affinity score, representing the most stable predicted interaction between the ligand and the active site of the target protein. Parameters such as binding energy, hydrogen-bond interactions, and hydrophobic interactions were assessed to identify the most favorable ligand–protein complexes.

The ligand pose with the best binding affinity was generally defined as the pose with the lowest binding energy. Discovery Studio was used to visualize and examine specific molecular interactions, including hydrogen bonds, hydrophobic interactions, and electrostatic contacts. This analysis was performed to elucidate the predicted binding mechanism of each ligand within the active site of the target protein. The resulting data were subsequently compared with relevant experimental findings or previously published literature to assess the consistency and validity of the results.

RESULTS AND DISCUSSION

Identification of Compounds in the Methanolic Lo'i-Keta Extract Using Gas Chromatography–Mass Spectrometry

Gas chromatography–mass spectrometry (GC–MS) was used to characterize the chemical constituents of the methanolic lo'i-keta extract. The chromatographic profile obtained from the analysis is presented in Figure 1.

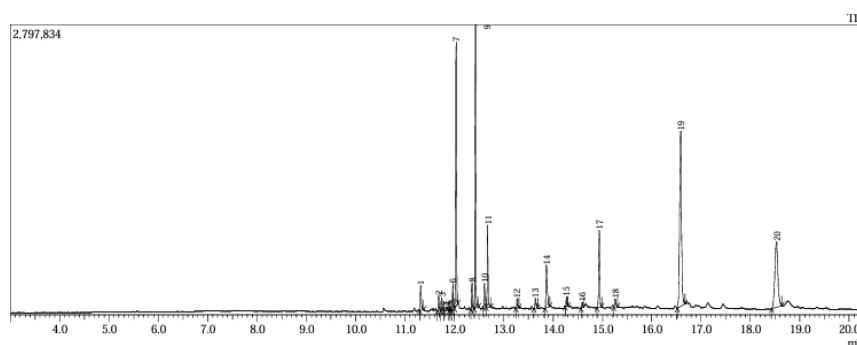


Figure 1. GC–MS chromatogram of the methanolic lo'i-keta extract

The GC–MS chromatogram showed 20 detectable peaks, of which 13 compounds were validated based on their similarity to reference mass spectra. The identified compounds were tetradecanoic acid, pentadecanoic acid, 1-tetradecanol, 9-octadecenoic acid, hexadecanoic acid, 9-hexadecenoic acid, octadecanoic acid, 1-pentadecanol, cyclooctacosane, dioctyl benzene-1,2-dicarboxylate, (E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol, (6E,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracos-2,6,10,14,18,22-hexaene, and icosan-1-ol. Their high similarity-index values indicated good agreement with the reference spectral library (Teoh et al., 2023; Abbas et al., 2025).

The chemical profile was dominated by long-chain aliphatic alcohols, saturated and unsaturated fatty acids, an aromatic ester, and a cyclic hydrocarbon. The compound with the highest relative abundance was icosan-1-ol, with a peak area of 24.41%, followed by 1-pentadecanol at 15.15% and dioctyl benzene-1,2-dicarboxylate at 4.02%. The predominance of long-chain alcohols and fatty acids indicates that the extract contains a substantial proportion of lipophilic metabolites that may contribute to its biological activity (Teoh et al., 2023; Abbas et al., 2025).

Free fatty acids and related lipophilic compounds have been reported to exert antibacterial effects by disrupting membrane organization, increasing membrane permeability, altering membrane fluidity, and promoting the leakage of intracellular constituents. Their antibacterial effects are influenced by carbon-chain length, degree of unsaturation, concentration, and bacterial species (Yoon et al., 2018; Yasir et al., 2020; Casillas-Vargas et al., 2021).

Several biologically relevant fatty acids were detected, including tetradecanoic acid, pentadecanoic acid, hexadecanoic acid, 9-hexadecenoic acid, and 9-octadecenoic acid. In addition to membrane disruption, fatty acids may interfere with essential bacterial enzymes, energy metabolism, and cellular replication processes (Yasir et al., 2020; Casillas-Vargas et al., 2021). Unsaturated fatty acids have also been reported to inhibit biofilm formation and virulence-related phenotypes in *Staphylococcus aureus*, although these effects are dependent on the compound and experimental conditions (Stenz et al., 2020; Kim et al., 2022).

Differences in chromatographic abundance should not be interpreted as direct differences in antibacterial potency. Peak area represents the relative detector response under the analytical conditions used, whereas biological activity depends on several additional factors, including target affinity, solubility, permeability, metabolism, and effective concentration. A compound detected at low abundance may therefore exhibit substantial biological activity, whereas an abundant compound may have limited potency or bioavailability. Accordingly, chromatographic abundance, molecular docking, pharmacokinetic prediction, toxicity prediction, and experimental antibacterial testing should be interpreted as complementary forms of evidence.

All 13 validated compounds were subsequently evaluated as candidate ligands through molecular docking against the DNA gyrase proteins of *Staphylococcus aureus* and *Pseudomonas aeruginosa* to assess their potential antibacterial activity in silico (Singh et al., 2023).

Prediction of Biological Activity and Pharmacokinetic Properties

The potential biological activities of the identified compounds were predicted using PASS Online through the Way2Drug platform, whereas their pharmacokinetic properties were evaluated using SwissADME. The predicted antibacterial activity profile is shown in Figure 2.

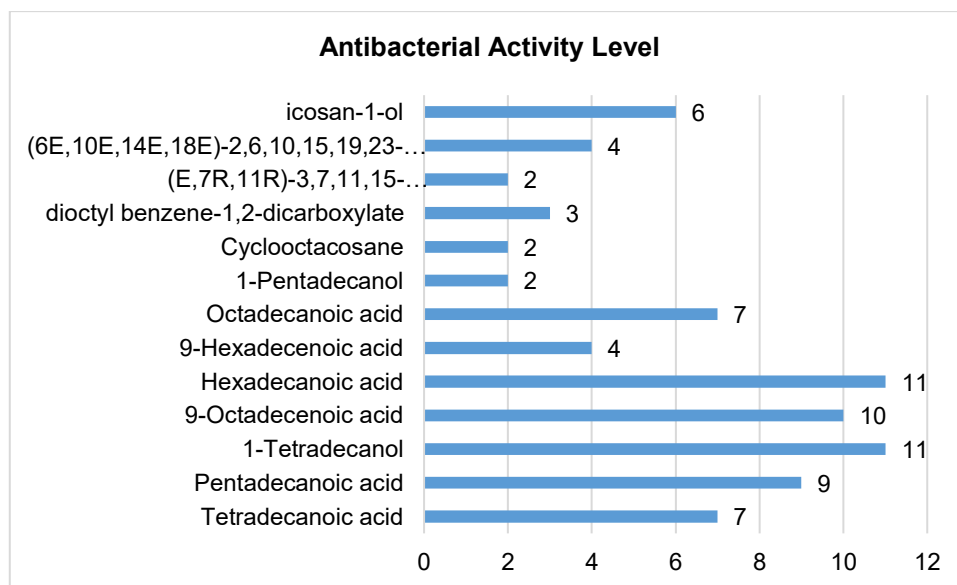


Figure 2. Overall predicted antibacterial activity of compounds in the methanolic lo'i-keta extract

PASS Online uses a structure–activity relationship model to estimate the probability that a compound will exhibit a specific biological activity. The results are expressed as the probability of activity (Pa) and probability of inactivity (Pi). A higher Pa value and lower Pi value indicate a greater predicted likelihood of the corresponding biological activity (Lagunin et al., 2021; Filimonov et al., 2023).

However, PASS predictions are probability-based estimates derived from chemical structure and should not be interpreted as direct evidence that a compound inhibits a specific enzyme or bacterial pathway. The predicted mechanisms must be confirmed through target-specific assays and antibacterial susceptibility testing.

Table 1. Predicted antibacterial activities of compounds in the methanolic lo'i-keta extract

No.	Predicted target or mechanism	Pa	Pi	Proposed antibacterial effect
1	Inhibition of peptidoglycan biosynthesis through a Lipid II-dependent pathway	0.814	0.002	Inhibits enzymes involved in peptidoglycan polymerization and Lipid II utilization, thereby impairing bacterial cell-wall synthesis.
2	UDP-GlcNAc L64-epimerase inhibition	0.810	0.005	Disrupts the formation of UDP-GalNAc required for cell-wall and peptidoglycan-associated structures.

3	Alanine-tRNA ligase inhibition	0.711	0.004	Prevents the attachment of alanine to tRNA and consequently disrupts bacterial protein synthesis.
4	Disruption of bacterial membrane integrity and permeability	0.911	0.008	Damages the bacterial membrane, leading to leakage of intracellular components and cell death.
5	Ubiquinol–cytochrome c reductase inhibition	0.937	0.003	Disrupts the bacterial electron-transport chain and reduces ATP production.
6	Pyruvate dehydrogenase inhibition	0.830	0.002	Inhibits the conversion of pyruvate to acetyl-CoA and impairs bacterial energy metabolism.
7	Inhibition of the undecaprenyl-phosphate recycling pathway	0.969	0.001	Prevents regeneration of undecaprenyl phosphate required for the transport of peptidoglycan precursors.
8	AP-site DNA lyase inhibition	0.833	0.004	Impairs bacterial base-excision repair and promotes the accumulation of DNA damage.

The highest predicted activity was inhibition of the undecaprenyl-phosphate recycling pathway, with $P_a = 0.969$ and $P_i = 0.001$. Undecaprenyl phosphate is an essential lipid carrier that transports precursors required for peptidoglycan and other bacterial cell-surface polymers. Because its intracellular pool is limited and shared among several biosynthetic pathways, disruption of its synthesis, translocation, or recycling may impair bacterial cell-wall formation and reduce bacterial viability (Jorgenson & Young, 2016; Müller et al., 2022; Sit et al., 2023; Qin et al., 2025).

The predicted inhibition of ubiquinol–cytochrome c reductase also showed a high P_a value of 0.937. Inhibition of the bacterial electron-transport chain may reduce proton-gradient formation and ATP production, thereby impairing essential energy-dependent cellular processes. Nevertheless, this predicted mechanism should be verified experimentally because the PASS result does not identify which compound is principally responsible for the activity.

Disruption of bacterial membrane integrity and permeability had a P_a value of 0.911. This mechanism is biologically plausible because several identified compounds contain long hydrophobic chains that may associate with membrane lipids. Fatty acids and long-chain alcohols have been reported to disturb bacterial membranes and induce the leakage of intracellular components (Yoon et al., 2018; Yasir et al., 2020; Desbois & Smith, 2021). However, membrane-disrupting activity should be confirmed using membrane-permeability assays, measurements of membrane potential, intracellular leakage assays, or microscopic examination.

The predicted AP-site DNA lyase inhibitory activity suggests a possible effect on bacterial base-excision repair. AP-site DNA lyases participate in the repair of apurinic and apyrimidinic sites generated by endogenous damage and oxidative stress. Inhibition of this pathway may increase the accumulation of DNA lesions and strand breaks (Wallace, 2022; Pavlov et al., 2023). In theory, impairment of DNA repair could complement DNA gyrase inhibition; however, such a synergistic mechanism was not experimentally evaluated in the present study and should therefore be regarded as hypothetical.

Pharmacokinetic properties were predicted using SwissADME, which estimates physicochemical characteristics, absorption, distribution, drug-likeness, and medicinal-chemistry properties of small molecules (Daina et al., 2017). Relevant parameters include molecular weight, lipophilicity, aqueous solubility, gastrointestinal absorption, blood–brain barrier permeability, P-glycoprotein substrate status, cytochrome P450 inhibition, Lipinski-rule violations, and bioavailability score.

These parameters are particularly important for cyclooctacosane, dioctyl benzene-1,2-dicarboxylate, and long-chain alcohols because their high hydrophobicity may favor non-polar contacts during molecular docking while simultaneously reducing aqueous solubility, absorption, formulation feasibility, and systemic bioavailability. The complete SwissADME results should therefore be presented in a separate table to support the selection of lead compounds.

Prediction of Compound Toxicity

The acute toxicity of the identified compounds was predicted using ProTox-3. The SMILES representation of each compound was submitted to the platform to obtain its predicted oral median lethal dose (LD₅₀) and toxicity class. The results are presented in Table 2.

Table 2. Predicted toxicity of compounds in the methanolic lo'i-keta extract

No	Compound	SMILES	Predicted LD ₅₀	Toxicity class
1	<i>Tetradecanoic acid</i>	<chem>CCCCCCCCCCCCCCCC(=O)O</chem>	900mg/kg	4
2	<i>Pentadecanoic acid</i>	<chem>CCCCCCCCCCCCCCCCC(=O)O</chem>	900mg/kg	4
3	<i>1-Tetradecanol</i>	<chem>CCCCCCCCCCCCCCCCO</chem>	1000mg/kg	4
4	<i>9-Octadecenoic acid</i>	<chem>CCCCCCCC=CCCCCCCCC(=O)O</chem>	48mg/kg	2
5	<i>Hexadecanoic acid</i>	<chem>CCCCCCCCCCCCCCCCC(=O)O</chem>	900mg/kg	4
6	<i>9-Hexadecenoic acid</i>	<chem>CCCCCCC=CCCCCCCCC(=O)O</chem>	48mg/kg	2
7	<i>Octadecanoic acid</i>	<chem>CCCCCCCCCCCCCCCCC(=O)O</chem>	900mg/kg	4
8	<i>1-Pentadecanol</i>	<chem>CCCCCCCCCCCCCCCCO</chem>	1000mg/kg	4
9	<i>Cyclooctacosane</i>	<chem>C1CCCCCCCCCCCCCCCCC CCCCCCCCCCC1</chem>	750mg/kg	3
10	<i>dioctyl benzene-1,2-dicarboxylate</i>	<chem>CCCCCCCCOC(=O)C1=CC =CC=C1C(=O)OCCCCCCC</chem>	1340mg/kg	4
11	<i>(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol</i>	<chem>C[C@@H](CCC[C@@H](C)CCC/C(=C/CO)/C)CCCC(C)C</chem>	5000mg/kg	5
12	<i>(6E,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracos-2,6,10,14,18,22-hexaene</i>	<chem>CC(=CCC/C(=C/CC/C(=C/CC/C(=C/CC/C(=C/CCC=C(C)C)\C)\C)/C)C</chem>	5000mg/kg	5
13	<i>icosan-1-ol</i>	<chem>CCCCCCCCCCCCCCCCCCCCO</chem>	1000mg/kg	4

Acute oral toxicity is commonly expressed as LD₅₀, which represents the dose expected to cause death in 50% of a test population. Under the toxicity classification described by Banerjee et al. (2018), Classes 1 and 2 indicate the highest acute toxicity, whereas Classes 5 and 6 indicate comparatively lower toxicity.

The compounds 9-octadecenoic acid and 9-hexadecenoic acid were assigned predicted LD₅₀ values of 48 mg/kg and toxicity Class 2. These predictions indicate potentially severe acute toxicity at relatively low doses. However, the high predicted toxicity of these long-chain unsaturated fatty acids should be interpreted cautiously. The submitted SMILES structures, double-bond positions, stereochemistry, and ionization states should be verified because incorrect structural input or selection of an unintended isomer may substantially affect the prediction.

Cyclooctacosane had a predicted LD₅₀ value of 750 mg/kg and was listed as toxicity Class 3. This result is internally inconsistent with the toxicity intervals described in the manuscript because an LD₅₀ of 750 mg/kg would ordinarily fall within Class 4. The original ProTox-3 output should therefore be rechecked, and the reported class should be reconciled with the classification criteria.

Most compounds, including tetradecanoic acid, pentadecanoic acid, hexadecanoic acid, octadecanoic acid, 1-tetradecanol, 1-pentadecanol, icosan-1-ol,

and dioctyl benzene-1,2-dicarboxylate, had predicted LD₅₀ values of 900–1,340 mg/kg and were assigned to toxicity Class 4. These compounds may therefore exhibit moderate acute toxicity, particularly at high doses.

The compounds (E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol and (6E,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracos-2,6,10,14,18,22-hexaene had predicted LD₅₀ values of 5,000 mg/kg and were assigned to toxicity Class 5, indicating comparatively lower predicted acute toxicity.

Overall, the identified compounds showed predicted toxicity ranging from Classes 2 to 5. These computational results provide only an initial indication of safety because they do not fully account for formulation, absorption, metabolism, repeated exposure, organ-specific toxicity, or interactions among compounds within the extract. Selected compounds should therefore undergo concentration-dependent cytotoxicity testing in mammalian cells and, where appropriate, subsequent in vivo toxicity evaluation.

Molecular Docking Against DNA Gyrase

Molecular docking against DNA gyrase was conducted using PyRx. The target proteins and ligands were prepared using Discovery Studio, and the ligands were converted into .pdbqt format before docking. The resulting binding-affinity values were used to estimate the relative favorability of ligand–protein interactions. The docking results are summarized in Table 3.

Table 3. Molecular docking results for the DNA gyrase proteins of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

No.	Compound	S. Aureus	P. Aeruginosa
		Binding Affinity	
1.	Ciprofloxacin (Kontrol)	-7.7	-7.4
2.	Tetradecanoic acid	-5.1	-4.8
3.	Pentadecanoic acid	-4.9	-4.7
4.	1-Tetradecanol	-4.6	-4.4
5.	9-Octadecenoic acid	-5.1	-5.2
6.	Hexadecanoic acid	-5	-4.2
7.	9-Hexadecenoic acid	-5.4	-4.6
8.	Octadecanoic acid	-5.5	-5
9.	1-Pentadecanol	-4.8	-4.5
10.	Cyclooctacosane	-8.3	-8.2
11.	dioctyl benzene-1,2-dicarboxylate	-5.7	-5.7
12.	(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	-5.5	-5.3
13.	(6E,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracos-2,6,10,14,18,22-hexaene	-5.6	-5.3
14.	icosan-1-ol	-4.8	-4.3

DNA gyrase is an essential bacterial type II topoisomerase involved in the regulation of DNA supercoiling, replication, and transcription. It is also a validated target for antibacterial drug development, particularly for fluoroquinolone antibiotics (Collin et al., 2011; Aldred et al., 2014; El-Hossary et al., 2022).

Cyclooctacosane exhibited the most negative predicted binding affinities, with values of -8.3 kcal/mol against *S. aureus* DNA gyrase and -8.2 kcal/mol against *P. aeruginosa* DNA gyrase. These values were more negative than those obtained for ciprofloxacin, which showed binding affinities of -7.7 and -7.4 kcal/mol, respectively.

Nevertheless, a more negative docking score does not necessarily indicate stronger experimental enzyme inhibition or superior antibacterial activity. Docking scores are approximations influenced by protein preparation, binding-site definition,

ligand protonation, conformational sampling, solvent treatment, scoring algorithms, and assumptions regarding protein flexibility (Ma et al., 2024).

The favorable docking score of cyclooctacosane may partly result from extensive hydrophobic contacts with the binding pocket. Large non-polar molecules can obtain favorable van der Waals scores despite poor aqueous solubility, limited target specificity, or inadequate cellular penetration. Cyclooctacosane should therefore not be considered superior to ciprofloxacin without confirmation of its binding mode, solubility, pharmacokinetic properties, DNA gyrase inhibitory activity, and antibacterial potency.

Diocetyl benzene-1,2-dicarboxylate exhibited identical binding-affinity values of -5.7 kcal/mol against both bacterial DNA gyrases. This consistency suggests that the compound may accommodate structural differences between the two target proteins. However, its affinity was weaker than that of ciprofloxacin. Similar docking scores across two targets provide only preliminary evidence of cross-target compatibility and do not independently establish broad-spectrum antibacterial activity (Mandal et al., 2022; Singh et al., 2023).

Hexadecanoic acid exhibited moderate affinity toward *S. aureus* DNA gyrase at -5.0 kcal/mol and weaker affinity toward *P. aeruginosa* DNA gyrase at -4.2 kcal/mol. This difference may result from variations in amino acid sequence, binding-pocket geometry, ligand orientation, or docking parameters. It should not be attributed directly to adaptation of DNA gyrase to the Gram-negative outer membrane because DNA gyrase is a cytosolic enzyme.

In living *P. aeruginosa* cells, the outer membrane, porins, and multidrug-efflux systems may restrict the intracellular accumulation of antibacterial compounds. However, such permeability barriers are not represented in conventional molecular docking simulations.

The potential antibacterial role of hexadecanoic acid is likely multifactorial. In addition to its predicted interaction with DNA gyrase, saturated fatty acids may disrupt membrane integrity, alter membrane lipid organization, and interfere with bacterial metabolism (Yasir et al., 2020; Casillas-Vargas et al., 2021). Therefore, its biological activity should not be attributed primarily to DNA gyrase inhibition.

Validation of the Molecular Docking Protocol

The docking protocol was evaluated through redocking of the native ligand into the target binding site. The root-mean-square deviation (RMSD) between the redocked pose and crystallographic pose was used as the principal validation parameter. An RMSD value of ≤ 2 Å is commonly considered acceptable because it indicates that the docking protocol can reproduce the experimentally observed binding orientation (Kherunnisa et al., 2020).

Table 4. Validation results for docking against the DNA gyrase proteins of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Replicate	<i>S. aureus</i>		<i>P. aeruginosa</i>	
	Binding Affinity	RMSD	Binding Affinity	RMSD
1	-6.4	0	-9.2	0
2	-6.3	0	-9	0
3	-6.2	0	-9.1	0
Mean	-6.3	0	-9.1	0

All three replicates produced RMSD values of 0 Å. An RMSD of exactly 0 Å across independent redocking replicates is unusual and may indicate that the original ligand coordinates were retained, that RMSD was not calculated between independent

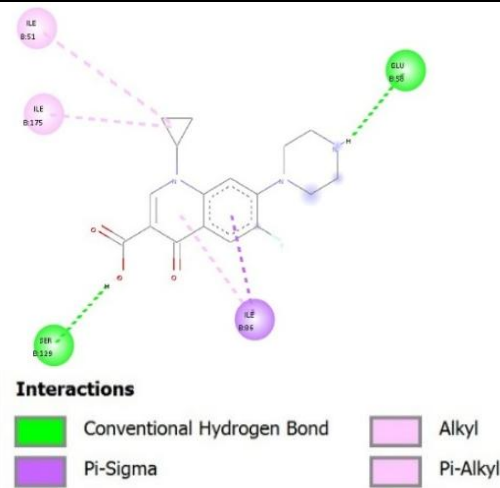
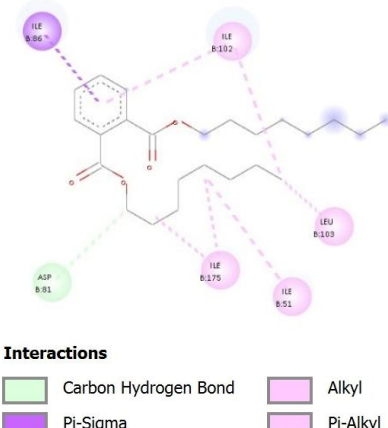
poses, or that a different output parameter was reported. The validation procedure should therefore be repeated before the docking protocol is considered fully validated.

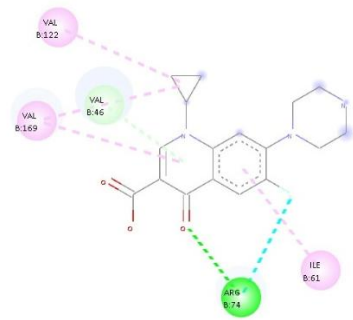
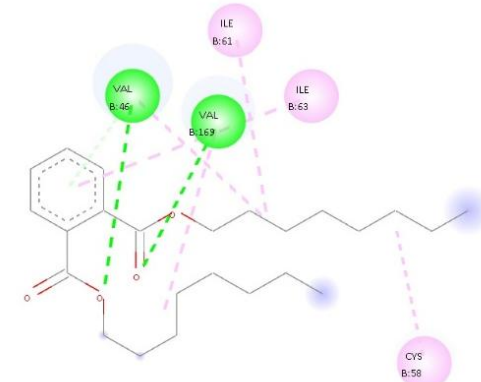
A valid redocking procedure should include removal of the crystallographic ligand, independent docking into the predefined binding site, alignment of the redocked and experimental structures, and calculation of the heavy-atom RMSD between the predicted and crystallographic ligand poses. The manuscript should also report the Protein Data Bank identifiers, native ligand identities, grid-box coordinates and dimensions, exhaustiveness values, protonation states, treatment of cofactors and water molecules, and the software used to calculate RMSD (Ma et al., 2024).

Visualization of Ligand–Protein Interactions

Ligand–protein interactions were visualized using Discovery Studio. The analysis focused on the amino acid residues involved and the types of contacts formed between the ligands and the DNA gyrase binding pockets. Similarity to the control ligand was evaluated based on shared residues and interaction patterns. However, sharing one or more residues with ciprofloxacin does not necessarily indicate an identical inhibitory mechanism because ligand orientation, interaction geometry, contact strength, and residue function must also be considered.

Table 5. Predicted interactions of ciprofloxacin and dioctyl benzene-1,2-dicarboxylate with the DNA gyrase proteins of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Staphylococcus aureus			
Compound	Interacting residues	Predicted interaction types	Visualization
Ciprofloxacin (Control)	SER129 GLU58 ILE51 ILE86 ILE175	Hydrogen konvensional Pi-Sigma Alkyl Pi-Alkyl	
dioctyl benzene-1,2-dicarboxylate	ILE86 ILE102 ILE175 ILE51 LEU103 ASP81	Pi-Alkyl Alkyl Carbon Hydrogen Bond	

<i>Pseudomonas aeruginosa</i>			
Compound	Interacting residues	Predicted interaction types	Visualization
Ciprofloxacin (Control)	ARG74 ILE61 VAL46 VAL122 VAL169	Hydrogen konvensional Halogen (Fluorine) Alkyl Pi-Alkyl Pi Donor Hydrogen	
dioctyl benzene-1,2-dicarboxylate	VAL46 VAL169 ILE61 ILE63 CYS58	Hydrogen konvensional Pi Donor Hydrogen Pi Sigma Alkyl Pi Alkyl	

1. Interaction with *Staphylococcus aureus* DNA Gyrase

The predicted interaction pattern of dioctyl benzene-1,2-dicarboxylate with *S. aureus* DNA gyrase was dominated by hydrophobic contacts. Alkyl and π -alkyl interactions were observed with ILE B:51, ILE B:86, ILE B:102, ILE B:175, and LEU B:103. These residues form a non-polar environment that may accommodate the ligand's long alkyl chains through van der Waals interactions.

A π -sigma interaction involving the aromatic ring may contribute to stabilization of the ligand orientation within the binding pocket. A carbon-hydrogen bond with ASP B:81 represented the principal polar contact. Because this interaction is weaker than a conventional hydrogen bond, the overall binding mode appears to be driven primarily by non-specific hydrophobic contacts.

Hydrophobic interactions may lower the predicted binding free energy and stabilize a ligand-protein complex. However, the limited number of strong directional polar contacts suggests that dioctyl benzene-1,2-dicarboxylate may exhibit lower target specificity than ciprofloxacin. It should therefore be considered a preliminary ligand candidate rather than a confirmed DNA gyrase inhibitor.

2. Interaction with *Pseudomonas aeruginosa* DNA Gyrase

Ciprofloxacin formed a combination of predicted polar and hydrophobic interactions with *P. aeruginosa* DNA gyrase. A conventional hydrogen bond was identified with ARG B:74, while hydrophobic contacts involved VAL B:46, VAL B:122, VAL B:169, and ILE B:61. A fluorine-associated interaction was also predicted with ILE B:61. The combination of directional and hydrophobic contacts supports the use of ciprofloxacin as a positive-control ligand.

Diethyl benzene-1,2-dicarboxylate was predicted to interact with VAL B:46, VAL B:169, ILE B:61, ILE B:63, and CYS B:58. Its interaction pattern consisted of conventional hydrogen bonding, π -donor hydrogen bonding, π -sigma, alkyl, and π -alkyl contacts. The presence of both polar and non-polar interactions suggests that the ligand can occupy the selected binding pocket.

Nevertheless, interaction diagrams generated from a static docking pose should be interpreted cautiously. Weak contacts detected in a two-dimensional diagram may not remain stable after energy minimization, solvent relaxation, or molecular-dynamics simulation. More reliable evaluation would require molecular-dynamics analysis, including protein and ligand RMSD, residue fluctuation, hydrogen-bond occupancy, and binding free-energy estimation.

Identification of Potential Antibacterial Candidates

The selection of potential antibacterial candidates should be based on integrated criteria rather than docking affinity alone. Relevant criteria include GC–MS identification confidence, relative abundance, binding affinity, plausibility of the binding pose, similarity to control interactions, solubility, predicted pharmacokinetics, toxicity, and experimental feasibility.

Cyclooctacosane was the highest-ranked compound based on docking affinity. However, its pronounced hydrophobicity, possible solubility limitations, and inconsistency in the reported toxicity class reduce confidence in its immediate selection as the principal lead compound. Its activity should be verified through DNA gyrase enzyme-inhibition assays and antibacterial susceptibility testing.

Diethyl benzene-1,2-dicarboxylate showed moderate but consistent affinity across the two target proteins. This result supports further evaluation of its cross-species binding potential, although its affinity was weaker than that of ciprofloxacin and its interaction pattern was dominated by hydrophobic contacts.

Hexadecanoic acid and other fatty acids exhibited moderate docking affinities but may contribute to antibacterial activity through additional membrane-associated and metabolic mechanisms. Icosan-1-ol was the most abundant identified compound, with a peak area of 24.41%, but showed relatively weak docking affinities of -4.8 kcal/mol against *S. aureus* and -4.3 kcal/mol against *P. aeruginosa*. Its contribution may therefore be more closely associated with membrane-related effects than with direct DNA gyrase inhibition.

The reported peak area of 16.5% for hexadecanoic acid should be verified against the original GC–MS output because it differs from the abundance values emphasized earlier in the Results and Discussion. A complete GC–MS table containing retention time, peak area, similarity index, molecular formula, and library match should be included to ensure internal consistency.

Overall, no single compound can yet be identified as the definitive antibacterial constituent of the methanolic lo'i-keta extract. Cyclooctacosane may be prioritized for confirmation of target binding; diethyl benzene-1,2-dicarboxylate for evaluation of cross-species interactions; and hexadecanoic acid and icosan-1-ol for experimental analysis of membrane-associated antibacterial effects.

CONCLUSION

GC–MS analysis of the lo'i-keta methanolic extract identified 13 bioactive compounds, predominantly long-chain aliphatic alcohols, saturated and unsaturated fatty acids, hydrocarbons, and aromatic esters. The compound with the highest relative abundance was icosan-1-ol, with a peak area of 24.41%, followed by pentadecanol at 15.15% and dioctyl benzene-1,2-dicarboxylate at 4.02%.

Molecular docking analysis demonstrated that several bioactive compounds in the lo'i-keta methanolic extract exhibited binding affinity for the DNA gyrase proteins of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Dioctyl benzene-1,2-dicarboxylate showed the strongest binding affinity toward *S. aureus* DNA gyrase, with a binding energy of -8.3 kcal/mol, while its binding affinity toward *P. aeruginosa* DNA gyrase was -8.2 kcal/mol. In addition, hexadecanoic acid showed a binding affinity of -1.2 kcal/mol and formed stable interactions with both target proteins, suggesting its potential as an antibacterial candidate.

This study contributes to the scientific understanding of the bioactive compounds present in the lo'i-keta methanolic extract and their potential to inhibit the DNA gyrase proteins of *S. aureus* and *P. aeruginosa* through an *in silico* approach. Unlike previous studies, which have generally reported only the phytochemical composition or overall antibacterial activity of lo'i-keta, the present study integrates GC–MS analysis with molecular docking to identify candidate compounds that may support the development of natural antibacterial agents derived from lo'i-keta. These findings provide a basis for further *in vitro* and *in vivo* investigations to confirm the biological activities of the identified compounds.

RECOMMENDATION

Future studies should standardize the composition of lo'i-keta through a collaborative approach involving traditional practitioners. Such collaboration may facilitate the development of a more consistent formulation that can be scientifically replicated while respecting cultural values and local knowledge. Further research should quantitatively determine the mass of each ingredient and implement appropriate quality-control procedures for the raw materials to improve the validity, reproducibility, and reliability of the findings. The results of this *in silico* study should be further evaluated through *in vitro* and *in vivo* experiments to confirm the biological and potential clinical antibacterial activity of lo'i-keta. Such evidence is essential to support its development as a scientifically validated antibacterial candidate.

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