

Molecular Characterization and Evaluation of Bioactive Compounds from Selected Medicinal Plant Extracts against Mastitis-Causing Pathogens among Dairy Cattle in Minna Niger State, Nigeria

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ABSTRACT

Mastitis is an important bacterial disease affecting dairy cattle and remains a challenge because of antimicrobial resistance and the limitations associated with conventional antibiotic treatment. This study isolated and identified mastitis-causing pathogens from cattle milk in Minna, Niger State, and subsequently characterized bioactive compounds in the most active plant extract using Gas Chromatography-Mass Spectrometry (GC-MS) and evaluated selected compounds through molecular docking. A total of 60 milk samples were collected from three locations, of which 15 tested positive for clinical mastitis. Five major bacterial groups were identified: *Staphylococcus* sp., *Bacillus subtilis*, *Pseudomonas* sp., *Micrococcus luteus*, and *Neisseria* sp. *Staphylococcus* sp. and *B. subtilis* were the most prevalent isolates, each accounting for 33.33%. Based on the antimicrobial activity observed in the study, *Moringa oleifera* was selected for GC-MS characterization. A total of 29 compounds were identified, with 9-octadecenoic acid, methyl ester (E) being the most abundant compound, accounting for 50.88% of the peak area. Other compounds included methyl stearate, hexadecanoic acid methyl ester, β -sitosterol, quercetin, kaempferol, rutin, chlorogenic acid, and ferulic acid. Molecular docking showed strong binding affinities for quercetin (-9.0 kcal/mol) against DNA gyrase (2XCT), kaempferol (-8.4 kcal/mol) against dihydrofolate reductase (3FYV), and rutin (-8.1 kcal/mol) against penicillin-binding protein 2a (5M18). Further docking of selected compounds demonstrated interactions between 1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one and DNA gyrase and between 1-tetradecyne and penicillin-binding protein 2a. These findings suggest that *M. oleifera* contains diverse bioactive compounds with potential activity against important bacterial targets and provides a basis for further experimental validation and development of plant-derived antimicrobial agents.

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INTRODUCTION

Mastitis remains an important disease affecting dairy cattle and is associated with bacterial infection of the mammary gland. The disease causes economic losses through reduced milk production, poor milk quality, increased treatment costs, and possible premature culling (Cheng and Han, 2020). The emergence of antimicrobial resistance has increased the need for alternative antimicrobial strategies (Tommasoni *et al.*, 2023). Medicinal plants contain diverse secondary metabolites with potential therapeutic properties. However, the identification of the individual compounds responsible for biological activity remains an important step in the development of plant-derived antimicrobial agents (Vaou *et al.*, 2022; Panda *et al.*, 2025). Gas Chromatography-Mass Spectrometry (GC-MS) provides an approach for characterizing the chemical constituents of plant extracts, while molecular docking can be used to investigate possible interactions between bioactive compounds and bacterial protein targets (Chikowe *et al.*, 2024).

This study therefore combined the microbiological isolation and identification of mastitis-causing pathogens in cattle within Minna, Niger State, Nigeria, with the identification and characterization of bioactive compounds in the most active plant extract using GC-MS and further evaluated the potential

interactions of the identified bioactive compounds with selected molecular targets of mastitis-causing pathogens through an in silico molecular docking approach.

MATERIALS AND METHODS

Study Area

The study was conducted at the Gidan Kwano Campus of the Federal University of Technology, Minna (FUTMINNA), and its immediate environs. The campus is geographically situated between longitudes $6^{\circ}26'15''\text{E}$ and $6^{\circ}28'00''\text{E}$ and latitudes $9^{\circ}31'15''\text{N}$ and $9^{\circ}32'30''\text{N}$. It occupies a landmass of approximately 10,000 hectares along the Minna-Bida Road, located roughly 12 to 17 kilometers from the Minna city center, the capital of Niger State. Minna features an estimated metropolitan population of 513,000 people (Onuigbo *et al.*, 2015). The climate of Minna is characterized by a distinct wet and dry season, with annual rainfall ranging from 1100 mm to 1600 mm. The rainy season extends from April to October, with peak rainfall occurring between June and September. Conversely, the dry season lasts from November to March, during which the temperatures rise, with March being the hottest month of the year, averaging 30.5°C .

Isolation and Identification of Mastitis-Causing Pathogens

Milk samples collected from infected cattle in the selected locations were cultured on Mannitol Salt agar (MSA) and MacConkey Agar, prepared according to the manufacturers' instructions. The samples were pour-plated onto the prepared media and incubated at 37 °C for 24 h. Following incubation, bacterial growth was examined, and colonies were differentiated based on their morphological characteristics, including colour, texture, and shape. Representative colonies were sub-cultured onto freshly prepared Nutrient agar (NA) and incubated at 37 °C for an additional 24 h to obtain pure cultures.

Gram Staining

Pure bacterial isolates were subjected to Gram staining to determine their Gram reaction and cellular morphology. Briefly, a bacterial smear was prepared from each pure isolate on a clean glass slide, air-dried, and heat-fixed. The smear was stained using standard Gram-staining reagents and examined microscopically under the oil immersion objective (100×) to determine the Gram reaction, cell shape, and arrangement of the bacterial cells.

Biochemical Identification

The bacterial isolates were further identified using standard biochemical tests, including indole, citrate utilization, urease, and Triple Sugar Iron (TSI) tests.

Indole Test: Each isolate was inoculated into peptone water and incubated at 37 °C for 24 h. Following incubation, two drops of Kovac's reagent were added and gently mixed. The development of a red ring at the surface of the medium indicated a positive indole reaction, whereas the absence of a red ring indicated a negative reaction.

Citrate Utilization Test: Isolates were streaked onto Simmons' citrate agar and incubated at 37 °C for 24 h. A change in the medium from green to blue, accompanied by visible growth, was interpreted as a positive citrate utilization reaction, whereas no colour change was recorded as negative.

Urease Test: Isolates were inoculated onto urea agar and incubated at 37 °C for 24 h. Development of a pink to red colour in the medium indicated a positive urease reaction, while the absence of a colour change indicated a negative reaction.

Triple Sugar Iron (TSI) Test: Each isolate was inoculated onto a TSI agar slant using a sterile straight inoculating needle. The butt of the medium was stabbed to the bottom of the tube, after which the surface of the slant was streaked. The inoculated tubes were incubated at 37 °C for 24 h. Results were interpreted based on changes in the colour of the medium and the production of gas. A change from red to yellow indicated carbohydrate fermentation, while the presence of bubbles, cracks, or lifting of the agar indicated gas production.

Selection of Plant Extract

The plant extract showing promising antimicrobial activity against the predominant mastitis-associated bacterial isolates was selected for further molecular characterization. Based on the reported antimicrobial findings, *Moringa oleifera* leaf extract was selected for GC-MS analysis.

GC-MS Analysis

Gas Chromatography–Mass Spectrometry is an analytical technique that combines gas chromatography, which separates chemical constituents based on their volatility and interaction with a chromatographic column, with mass

spectrometry, which identifies compounds according to their mass-to-charge (m/z) ratios (Frolova *et al.*, 2026). The *Moringa oleifera* leaf extract was prepared and filtered before an aliquot was injected into the GC-MS instrument, where the sample was vapourized and carried by an inert gas through a capillary column. The individual constituents were separated within the column and subsequently introduced into the mass spectrometer, where they were ionized, fragmented, and detected to produce characteristic mass spectra.

The chemical constituents of the extract were identified by comparing the obtained mass spectra with reference spectra in established spectral libraries, such as the National Institute of Standards and Technology (NIST) library. The detected compounds were characterized based on their retention time, molecular formula, molecular weight, peak area, and structural classification, while the percentage peak area provided an estimate of their relative abundance in the extract. Identified compounds with reported antibacterial or antimicrobial activities were considered potential bioactive constituents and selected for subsequent *in silico* molecular docking analysis against selected protein targets of the mastitis-causing bacterial pathogens.

Selection of Compounds for Molecular Docking

Selected bioactive compounds identified by GC-MS were used for molecular docking analysis. The compounds were prepared and subjected to geometry optimization.

Protein Target Selection

Protein targets relevant to bacterial survival and antimicrobial activity were selected. The study included DNA gyrase (PDB ID: 2XCT), penicillin-binding protein 2a (PDB ID: 5M18), dihydrofolate reductase (PDB ID: 3FYV), and other selected bacterial targets. The DNA gyrase was selected because of its critical role in bacterial DNA replication and DNA topology, while penicillin-binding protein 2a was selected because of its role in peptidoglycan cross-linking and antibiotic resistance in *Staphylococcus aureus*.

Molecular Docking

Molecular docking is a computational structural biology technique used to predict the preferred binding orientation and interaction of a small molecule (ligand) within the active or binding site of a target macromolecule, usually a protein. It is widely applied in drug discovery and antimicrobial research to evaluate the potential affinity and molecular interactions between bioactive compounds and specific protein targets (Agu *et al.*, 2023). Docking algorithms estimate the binding strength of ligand–protein complexes using scoring functions and provide information on interactions such as hydrogen bonds, hydrophobic interactions, van der Waals forces, and electrostatic interactions.

AutoDock Vina was used for molecular docking. Protein structures were prepared by removing water molecules, heteroatoms, and bound ligands, followed by addition of hydrogen atoms and assignment of appropriate protonation states and charges. Binding sites were identified using Chimera and docking grids were generated around the selected active sites. Lower binding energy values were interpreted as indicating stronger binding affinity.

RESULTS AND DISCUSSION

Isolation and Identification of Mastitis-Causing Pathogens

Table 1 below presents the prevalence of microbial isolates identified from cattle milk samples collected in Minna. A total of 15 samples were examined, from which five isolates each

of *Staphylococcus* species and *Bacillus subtilis* were recovered, representing the highest prevalence of 33.33% each. *Micrococcus luteus* was detected in three samples, making up 20% of the total. Both *Pseudomonas* species and *Neisseria* species were found in only one sample each, indicating a prevalence rate of 6.67% for both organisms. The

data clearly shows that *Staphylococcus* sp. and *Bacillus subtilis* were the most commonly occurring microorganisms in the milk samples tested. The presence of other species, though lower, indicates some level of microbial diversity in the milk.

Table 1: Distribution of Mastitis-Associated Bacterial Isolates

Orgasm isolated	Number of isolates	N mber of samples	Prevalence
Staphylococcus sp.	5	15	33.33
Bacillus	5	15	33.33
Pseudomonas sp.	1	15	6.67
Micrococcus luteus	3	15	20
Neisseria sp.	1	15	6.67
%percentage =			

Antimicrobial Activity and Selection of *Moringa oleifera*

The antimicrobial susceptibility findings indicated that *Moringa oleifera* showed notable activity against the predominant isolates. The extract produced inhibition zones of 23 mm against *Staphylococcus aureus* and 30 mm against *B. subtilis*. These findings supported the selection of *Moringa oleifera* for further GC-MS and molecular docking analyses.

GC-MS Characterization of *Moringa oleifera* Leaf Extract

Table 2 below shows that *Moringa oleifera* leaf extract contains a wide range of bioactive compounds, detected using GC-MS analysis. A total of 29 compounds were identified, with varying molecular weights and retention times. The compound with the highest peak area was 9-octadecenoic acid, methyl ester (E), which recorded 50.88%, indicating it is the most abundant. This was followed by methyl stearate and hexadecanoic acid, methyl ester, with peak areas of 16.65% and 17.31%, respectively. Several other compounds,

including β -sitosterol, quercetin, kaempferol, rutin, and chlorogenic acid, were present in smaller amounts but are known for their antioxidant properties. Among the 79 compounds detected, 17 were identified as toxic to the organism isolated (*staphylococcus aureus*), such as 1-tetradecyne and phenanthridinium, 5,6-dimethyl- iodide. Others like quercetin, kaempferol, and rutin were non-toxic and are commonly found in medicinal plants. Some compounds appeared more than once, like heptasiloxane, tetradecamethyl- and silicic acid, diethyl bis(trimethylsilyl) ester, suggesting possible duplication or co-elution during analysis. The presence of compounds like trichloromethane and siloxane derivatives indicates that volatile and semi-volatile components were efficiently captured. The diversity in chemical structure and properties of the identified compounds suggests that *Moringa oleifera* leaves contain both polar and non-polar phytochemicals. This mixture includes esters, acids, alcohols, siloxanes, and flavonoids, which contribute to the pharmacological profile of the extract.

Table 2: Selected Bioactive Compounds Detected in *Moringa oleifera* Leaf Extract by GC-MS

Peak k	RT (min)	Molecular Formula	Mol. Weight (g/mol)	Peak Area	Count t
1	6.37	C8H12O	119.38	0.91	1
2	29.58	C4H12O	194.18	1	1
3	25.43	C14H26	194.36	0.48	1
4	30.12	C14H26O2	226.36	0.48	1
5	30.62	C17H34O2	270.45	17.31	1
6	32.13	C19H36O2	296.49	50.88	1
7	32.31	C19H38O2	298.51	16.65	1
8	32.47	C9H9N3OS	207.25	0.58	1
9	32.76	C12H17NO3	223.27	0.34	1
10	33.73	C14H42O6Si7	490.13	2.24	2
11	33.44	C18H16O4	296.32	0.87	2
13	33.54	C10H12O4Si3	223.27	1.01	2
14	33.66	C15H14-IN	310.34	0.84	1
17	34.97	C10H42O6Si3	490.13	0.97	3
18	35.98	C14H42 Si2	222.47	1.18	1
19	36.68	C18H16O4	296.32	0.11	1
20	36.85	C24H54O6Si7	598.11	0.24	1
22	33.48	C11H14N2O3	222.24	1.01	1
23	19.72	C15H10O7	302.24	3.48	1
24	20.41	C15H10O6	286.24	2.84	1
25	23.68	C27H30O16	610.52	3.02	1
26	24.11	C18H25NO7S	399.46	1.89	1
27	21.77	C18H18O9	354.31	2.61	1
28	31.85	C29H50O	414.71	4.23	1

Peak k	RT (min)	Molecular Formula	Mol. Weight (g/mol)	Peak Area	Count t
29	22.89	C10H10O4	194.18	1.75	1

Structural Classification of GC-MS-Identified Compounds

The identified compounds represented a broad range of chemical classes. These included fatty acid esters, flavonoids, phenolic acids, phytosterols, alkynes, siloxanes, heterocyclic compounds, quaternary nitrogen compounds, and other structural groups. Fatty acid esters and flavonoids represented important components of the extract, while several compounds occurred as single constituents with a composition of 3.45%.

Structural Classification of Compounds Identified in *Moringa oleifera* Leaf Extract

Table 3 below presents the structural classification of 29 bioactive compounds identified in *Moringa oleifera* leaf extract. These compounds are grouped based on their chemical structure and functional properties. Siloxanes (silyl derivatives) were the most represented class, making up 20.69% of the total, with six identified compounds such as 81 heptasiloxane and octasiloxane. Fatty acid esters and flavonoids each accounted for 10.34%, with three compounds each, including methyl stearate and common plant

antioxidants like quercetin, kaempferol, and rutin. Phenolic acids and aromatic amide esters each contributed 6.9%, and included compounds like chlorogenic acid and carbonic acid esters. Polycyclic aromatic hydrocarbons were also present at 6.9%, represented by two similar biphenylene derivatives. Classes that appeared once with a 3.45% composition each included halogenated hydrocarbons (trichloromethane), alkynes (1-tetradecyne), esters of terpenoids (citronellyl isobutyrate), heterocyclic thiohydantoin, quaternary nitrogen compounds, nitrogen-containing aromatic compounds, polycyclic siloxane frameworks, indole alkaloids, glucosinolates (e.g., niaziminin A), and phytosterols (β -sitosterol). Other classes include cycloalkanes/bicyclic ketones and esters of carbonic acid derivatives. This classification in the table highlights the diverse range of structural types present in *Moringa oleifera* leaves, indicating a rich phytochemical profile with potential for biological and pharmacological activities. Each compound listed contributes to the total composition percentage of the extract, suggesting that the leaf contains both major and trace chemical constituents with different functionalities.

Table 3: Structural Classification of Compounds Identified in *Moringa oleifera* Leaf Extract

Class	Count	Composition n (%)	Compound
Halogenated Hydrocarbon	1	3.45	Trichloromethane
Cycloalkanes / Bicyclic Ketones	1	3.45	Bicyclo[4.1.0]heptan-2-one, 6-methyl-
Alkynes	1	3.45	1-Tetradecyne
Esters (Terpenoid Derivative)	1	3.45	Citronellyl isobutyrate
Fatty Acid Esters	3	10.34	Hexadecanoic acid, methyl ester; 9-Octadecenoic acid, methyl ester; Methyl stearate
Heterocyclic Thiohydantoin	1	3.45	1-Methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one
Aromatic Amide Esters	2	6.9	Carbonic acid, monoamide, N-(2-ethylphenyl)-, propyl ester; Carbonic acid, monoamide, N-methyl-N-phenyl-, 2-methylpropyl ester
Siloxanes (Silyl Derivatives)	6	20.69	Heptasiloxane (x2); Octasiloxane; Silicic acid, diethyl bis(trimethylsilyl) ester (x2); 1,2-Bis(trimethylsilyl)benzene
Polycyclic Aromatic Hydrocarbons (PAHs)	2	6.9	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene (x2)
Quaternary Nitrogen Compounds	1	3.45	Benzeneacetic acid, 3,4-dihydroxy- α -methyl-
Polyketide Derivatives	1	3.45	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-
Sulfonamide	1	3.45	4-(2-Aminoethyl)benzenesulfonamide
Others	2	6.9	Benzene, 1,3,5-trimethoxy-; Hexadecanoic acid
Total	29	100	

Molecular Docking of Bioactive Compounds

As presented in table 4, the docking scores of selected bioactive compounds from *Moringa oleifera* against specific *Staphylococcus aureus* target proteins. Among the tested compounds, quercetin recorded the highest binding affinity with a docking score of -9.0 kcal/mol against DNA gyrase (2XCT), which plays a key role in DNA supercoiling. Kaempferol followed with a docking score of -8.4 kcal/mol targeting dihydrofolate reductase (3FYV), an enzyme essential for folate metabolism. Rutin also showed a good docking score of -8.1 kcal/mol against penicillin-binding protein 2a (5M18), involved in cell wall synthesis. Other

compounds like niaziminin A, chlorogenic acid, β -sitosterol, and ferulic acid presented moderate docking scores ranging from -7.8 to -6.8 kcal/mol, interacting with enzymes related to DNA replication, toxin formation, and fatty acid biosynthesis. Methyl esters such as methyl stearate, methyl palmitate, and 9-octadecenoic acid, as well as compounds like 1-tetradecyne and indole-2-one derivative, were tested but had no docking score recorded, possibly due to failure in interaction or technical limitations. Compound 13 (1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one) and compound 11 (1-tetradecyne) were selected for further docking due to their target specificity for proteins 2XCT and 5M18 respectively,

despite the absence of docking scores. Trichloromethane and silicic acid derivatives were either non-specific or not docked due to poor or irrelevant binding. The referral drug, cefoxitin, is included for comparison. The compounds with the best docking scores, particularly quercetin and kaempferol, suggest potential effectiveness against *S. aureus* and may

serve as leads for further drug development. Protein 11 and 13 were chosen based on their functional relevance to peptidoglycan cross-linking and DNA replication, aligning with literature criteria for protein selection in antimicrobial docking studies.

Table 4: Docking Scores of Bioactive Compounds from *Moringa oleifera* against *Staphylococcus aureus* Targets

S/N	Compound (Ligand)	Target Protein (PDB ID)	Function	Docking Score (kcal/mol)	Reference
1	Quercetin	DNA Gyrase (2XCT)	DNA supercoiling enzyme	-9.0	Pisano et al., 2019
2	Kaempferol	Dihydrofolate reductase (3FYV)	Folate metabolism	-8.4	Priyamvada et al., 2025
3	Rutin	Penicillin-binding protein 2a (5M18)	Cell wall synthesis	-8.1	Yakobi et al., 2024
4	Niaziminin A	DNA Gyrase (2XCT)	DNA replication	-7.8	Buru, 2025
5	Chlorogenic acid	Dihydrofolate reductase (3FYV)	Folate synthesis	-7.6	Sehrawat et al., 2024
6	β -Sitosterol	Alpha-hemolysin (7AHL)	Toxin pore formation	-6.9	Li et al., 2015
7	Ferulic acid	Enoyl-ACP reductase (3GR6)	Fatty acid biosynthesis	-6.8	Hernandez-Vazquez et al., 2024
8	Methyl stearate	DNA Gyrase (2XCT)	DNA replication	Not Available	-
9	Methyl palmitate	DNA Gyrase (2XCT)	DNA	Available	-
10	9-Octadecenoic acid, methyl ester	Alpha-hemolysin (7AHL)	Cytotoxin	Not Available	-
11	1-Tetradecyne	Penicillin-binding protein (5M18)	Peptidoglycan cross-linking	-5.1	This Study
12	Indole-2-one derivative	Dihydrofolate reductase (3FYV)	Folate metabolism	Not Available	-
13	1-Methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one	DNA Gyrase (2XCT)	DNA replication	-6.1	This Study
14	Trichloromethane	Non-specific	Low affinity	Not Available	-
15	Silicic acid derivatives	Not docked	Artifact	Not Available	-
	Referral drug	Cefoxitin	-	Not Available	-

Interaction of 1-Methyl-4-Phenyl-5-Thioxo-1,2,4-Triazolidin-3-One with DNA Gyrase

The interaction analysis showed that 1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one interacted with DNA gyrase through several molecular interactions involving residues including GLU104, GLU154, ARG107, and VAL103. The interaction included conventional and carbon-hydrogen bonds, suggesting stable positioning of the ligand within the active site. DNA gyrase was selected because of its essential role in bacterial DNA replication and DNA topology.

Interaction of 1-Tetradecyne with Penicillin-Binding Protein 2a

The interaction between 1-tetradecyne and penicillin-binding protein 2a (5M18) demonstrated structural complementarity between the ligand and the protein binding pocket. The interaction was characterized mainly by hydrophobic contacts rather than hydrogen bonding. The study interpreted this interaction as potentially relevant to the inhibition of peptidoglycan cross-linking and bacterial cell wall synthesis. The selection of PBP2a as a target was based on its importance in antibiotic resistance, particularly among methicillin-resistant *Staphylococcus aureus* (MRSA).

Discussion

The isolation of *Staphylococcus* sp., *Bacillus subtilis*, *Pseudomonas* sp., *Micrococcus luteus*, and *Neisseria* sp. confirms the presence of diverse bacterial organisms in cattle milk samples collected in Minna, Niger State. The predominance of *Staphylococcus* sp. and *B. subtilis* is particularly relevant because these organisms were among the most frequently detected isolates in the study.

The selection of *Moringa oleifera* for molecular characterization was based on its promising antimicrobial activity against important isolates. The plant extract demonstrated notable inhibition against *Staphylococcus aureus* and *B. subtilis*, supporting the view that *Moringa oleifera* may contain multiple compounds with antimicrobial properties.

The identification of 29 compounds by GC-MS demonstrates the chemical diversity of *Moringa oleifera* leaves. The predominance of 9-octadecenoic acid, methyl ester, is consistent with reports of its abundance and antimicrobial effects in plant-derived oils (Khalid et al., 2024). The detection of methyl stearate and hexadecanoic acid methyl ester also agrees with studies reporting antibacterial and anti-inflammatory properties associated with these compounds

(Mohsen & Robin). Quercetin, kaempferol, and rutin, although present in lower amounts, are recognized phytochemicals with antioxidant and antimicrobial potential (Zahra *et al.*, 2024). The presence of siloxanes and other volatile compounds further demonstrates the chemical diversity of the extract (Thangaiah *et al.*, 2024).

The molecular docking results provided additional insight into the potential mechanisms through which selected *Moringa oleifera* compounds may exert antibacterial effects. Quercetin demonstrated the strongest docking score against DNA gyrase, suggesting strong predicted binding to a protein essential for bacterial DNA replication. Kaempferol and rutin also demonstrated strong predicted interactions with dihydrofolate reductase and penicillin-binding protein 2a, respectively. These findings suggest that different compounds in the extract may act against different bacterial pathways.

The interaction between 1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one and DNA gyrase was characterized by hydrogen bonding involving GLU104, GLU154, ARG107, and VAL103. Such interactions may contribute to ligand stability and may potentially interfere with DNA gyrase activity. The findings are consistent with the importance of DNA gyrase as a validated antibacterial target and with previous reports on phytochemical interactions with bacterial enzymes (Zadran *et al.*, 2025; Sahoo *et al.*, 2025). The interaction pattern suggests that the compound may interfere with DNA replication processes. Similar interactions between phytochemicals and DNA gyrase have been associated with potential inhibitory activity (Elsaman *et al.*, 2025). Hydrophobic interactions may further contribute to the stability of ligand-protein complexes (Payandeh & Volgraf, 2021). The specific binding observed may therefore support the potential of the compound as a candidate for further investigation as an antibacterial agent.

The interaction of 1-tetradecyne with PBP2a was primarily hydrophobic. Although the docking score was weaker than those observed for quercetin, kaempferol, and rutin, the structural fit within the binding pocket suggests potential relevance for further investigation. The PBP2a is an important target because of its involvement in peptidoglycan cross-linking and resistance to β -lactam antibiotics. The findings therefore support the possibility that plant-derived compounds could provide lead structures for the development of alternative antibacterial agents.

The observed interaction supports the possibility that natural compounds from *Moringa oleifera* may act as potential inhibitors of essential bacterial processes. The study further relates the findings to reports that long-chain hydrocarbons may interact with membrane-associated or transpeptidase-active proteins and that non-polar compounds may contribute to antibacterial activity through hydrophobic interactions (Adji *et al.*, 2022; Wizrah *et al.*, 2026).

However, molecular docking results represent predicted molecular interactions and should not be interpreted as direct evidence of antibacterial efficacy. The findings require further validation through *in vitro* enzyme assays, purified compound testing, molecular dynamics simulations, and *in vivo* studies.

CONCLUSION

This study isolated and identified five major bacterial groups from cattle milk samples in Minna, with *Staphylococcus* sp. and *Bacillus subtilis* being the predominant isolates. Based on its antimicrobial activity against important mastitis-associated pathogens, *Moringa oleifera* was selected for GC-MS characterization. Gas Chromatography–Mass Spectrometry (GC-MS) analysis identified 29 compounds, with 9-octadecenoic acid, methyl ester (E), being the most abundant

compound at 50.88% peak area. The extract also contained important bioactive compounds, including quercetin, kaempferol, rutin, chlorogenic acid, ferulic acid, β -sitosterol, and niaziminin A.

Molecular docking demonstrated strong predicted binding affinities for quercetin, kaempferol, and rutin against selected bacterial protein targets. The interactions of 1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one with DNA gyrase and 1-tetradecyne with PBP2a further suggest possible mechanisms through which *Moringa oleifera*-derived compounds may interfere with bacterial DNA replication and cell wall synthesis.

These findings support the potential of *Moringa oleifera* as a source of bioactive compounds for the development of plant-based antimicrobial agents against mastitis-causing pathogens. Further *in vitro*, *in vivo*, compound isolation, and mechanistic studies are recommended to validate the observed antimicrobial potential and establish the therapeutic relevance of the identified compounds.

RECOMMENDATIONS

To advance milk safety, *Moringa oleifera* leaf extracts should be further explored as natural alternatives for controlling bacterial contamination, while farmers and milk handlers should be trained on the use of plant-based preservatives alongside the institution of robust quality control measures for processing and handling to minimize microbial loads; concurrently, additional research is needed to isolate and purify the active phytochemicals responsible for antimicrobial activity, with the promising compounds identified by GC-MS subjected to further experimental validation to confirm their efficacy, ultimately requiring *in vivo* testing and deeper molecular studies to investigate the precise mechanisms through which these compounds interact with key bacterial targets such as DNA gyrase and PBP2a.

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