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RESEARCH ARTICLE

An *in-silico* study of garlic extract compounds targeting DNA gyrase in *Staphylococcus aureus*

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Abstract

The study used in silico molecular docking and *in vitro* validation to assess the antibacterial efficacy of bioactive compounds derived from garlic (*Allium sativum*) against *Staphylococcus aureus*. With a binding energy of -7.4 kcal/mol, Allicin had the greatest binding affinity among the studied chemicals to DNA gyrase, creating three hydrogen bonds with residues Tyr122, Asp81 and Ala68. Mostly engaged in hydrophobic interactions, Ajoene followed with a binding energy of -7.0 kcal/mol. Comparatively to 24.0 ± 0.3 mm for ciprofloxacin, *in vitro* studies showed a concentration-dependent antibacterial activity with a maximal inhibition zone width of 20.0 ± 0.6 mm at 50 mg/mL extract concentration. Garlic extract's minimum inhibitory concentration (MIC) came out to be 25 mg/mL. At the highest measured dose, the extract also reduced 85 % of DNA gyrase activity, thereby approaching the 95 % inhibition threshold of ciprofloxacin. These findings imply that garlic extract-especially Allicin-has great potential as a natural antibacterial agent aiming at bacterial DNA replication machinery.

Keywords: antibacterial activity; DNA gyrase; garlic extract; *Staphylococcus aureus*

Introduction

One of the bacterial infections with the greatest clinical impact in the world is *Staphylococcus aureus*. From benign skin disorders to serious, life-threatening illnesses including pneumonia, endocarditis and septicaemia, it produces a wide spectrum of infections. Its incredible ability to acquire resistance to several medicines, including penicillin, methicillin and vancomycin, complicating treatment and adding to world public health issues, aggravates its clinical effect even more (1, 2). The fast spread and evolution of antibiotic-resistant strains, such as Methicillin-resistant *S. aureus* (MRSA), have raised the necessity to identify fresh, effective antimicrobial treatments with different mechanisms of action (3, 4). Fig. 1 shows a general pathogenic effects and resistance profile of *S. aureus*.

One key bacterial enzyme well known as a target for antibiotic development is DNA gyrase, a type II topoisomerase involved in the maintenance and replication of bacterial DNA (5). Important activities like replication, transcription and chromosomal segregation are made possible in part by negative supercoils produced by DNA gyrase. Reducing DNA gyrase activity disturbs DNA metabolism and finally causes bacterial cell death. Targeting DNA gyrase, fluoroquinolones and other antibiotics especially emphasises the clinical relevance of this enzyme as a therapeutic target (3, 6). Widespread usage of fluoroquinolones, however, has helped to generate resistance and emphasises the critical need of novel inhibitors able to overcome present resistance mechanisms. Low toxicity, few side effects and potential antibacterial qualities of natural chemicals produced from plants have attracted major scientific interest in response to growing antibiotic resistance (7-9). Long utilised in therapeutic purposes, medicinal herbs have shown great success against many types of bacterial infections in several investigations. Particularly garlic (*Allium sativum*), has been extensively examined for its strong antibacterial, antifungal, antiviral and immunomodulating properties.

Mostly responsible for garlic's medical effects are organosulfur compounds like allicin, diallyl disulphide, diallyl trisulfide and ajoene. One of the most studied substances, allicin has broad-spectrum antibacterial action, therefore suppressing many bacterial species including antibiotic-resistant strains. Allicin and similar sulphur compounds have been found to interact with important bacterial enzymes and cell components, therefore upsetting fundamental cellular processes (7, 8). Although garlic has generally well-documented

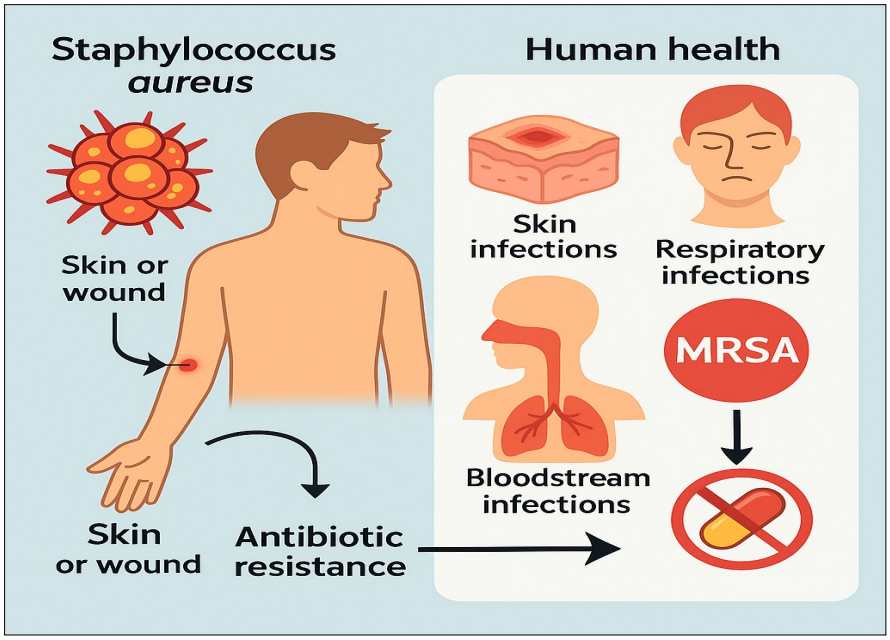

Fig. 1. Pathogenic effects of *Staphylococcus aureus* on human health, including skin, respiratory and bloodstream infections. The diagram also highlights the threat of antibiotic resistance, particularly Methicillin-resistant *Staphylococcus aureus* (MRSA).

antibacterial properties, the specific molecular pathways especially its interaction with DNA gyrase in *Staphylococcus aureus* remain poorly investigated. By means of natural antibacterial agents as feasible substitutes or adjuncts to conventional antibiotics, investigating these interactions can help to encourage their development. Especially molecular docking studies (in silico approaches), computational techniques have lately become quite effective tools for early screening and assessment of possible treatment candidates. An effective, reasonably priced stage before experimental confirmation, molecular docking helps scientists to forecast interactions between bioactive compounds and target enzymes (10, 11). This method speeds up drug discovery pipelines and offers important new perspectives on the fundamental molecular processes of drug action. Given the urgent need to find novel antimicrobial medicines able to inhibit bacterial enzymes involved in replication and DNA maintenance, this work aims to undertake an in-silico study of garlic-derived compounds against the DNA gyrase enzyme of *S. aureus*. By means of computational molecular docking, the present work aims to identify garlic compounds with strong inhibitory potential against DNA gyrase, so clarifying the molecular basis for their antibacterial action and providing important new directions for the development of natural substitutes to conventional antibiotics. The results of this study should provide insightful data that could open the path for fresh treatment approaches and the creation of safe, efficient, plant-derived antimicrobial medicines to control antibiotic-resistant bacterial illnesses.

Materials and Methods

The experimental strategies used to evaluate the inhibitory ability of several garlic-derived bioactive compounds against DNA gyrase enzyme of *Staphylococcus aureus* are thoroughly described in this chapter. Two primary elements make up the study: an in-silico computer study then confirmatory *in vitro* laboratory (*in vitro*) testing.

In-silico analysis

Target protein selection and preparation (DNA gyrase)

Selected from *Staphylococcus aureus*, the target enzyme DNA gyrase is important as a verified antibiotic target. Retaken from the Protein Data Bank (PDB) (https://www.rcsb.org) The three-dimensional crystal structure of *Staphylococcus aureus* DNA gyrase (PDB ID: 6Z1A) was selected from the PDB due to its high-resolution quality (2.2 Å), complete representation of the GyrB subunit and clear annotation of active site residues, making it highly suitable for accurate molecular docking simulations.

The protein preparation involved several meticulous steps, as follows:

- Removal of non-essential molecules, such as water molecules, ions and ligands unrelated to the active site, was performed using UCSF Chimera software (12).
- Hydrogen atoms were added and partial charges were computed using AutoDock Tools 1.5.7 (13).
- The final optimized structure was saved in PDBQT format, compatible with molecular docking software.

Ligand preparation

Four active garlic-derived compounds were selected based on previous reports documenting their antimicrobial potential. These compounds, with their corresponding ZINC IDs, include:

- **Allicin** (ZINC1530846)
- **Diallyl sulfide** (ZINC1531083)
- **Diallyl disulfide** (ZINC1531082)
- **Ajoene** (ZINC5193906)

Structural files for these selected compounds were downloaded in SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). Subsequent steps included:

- Conversion of ligand files from SDF to PDB format using Open Babel software.
- Optimization and energy minimization of ligand geometries were performed using Avogadro software.
- Preparation of ligands, including the addition of polar hydrogens, determination of flexible bonds and assignment of partial charges using AutoDock tools.
- Final ligands were saved in PDBQT format for subsequent docking analysis.

Molecular docking procedure

Molecular docking was performed using AutoDock Vina 1.2.3 software to evaluate interactions between garlic-derived compounds and DNA gyrase enzyme. Docking steps included:

- Defining the enzyme's active site based on published literature and crystal structure data (PDB: 6Z1A).
- Creating a precise Grid Box (25×25×25 Å) encompassing the active site with exact central coordinates.
- Running docking simulations with an exhaustiveness level set at 12 to increase docking accuracy and reliability.
- Each compound underwent three independent docking runs to confirm reproducibility and consistency.

Docking results were evaluated primarily according to binding energies (kcal/mol), with lower binding energies indicating stronger inhibitory interactions. Additionally, molecular interactions such as hydrogen bonds, hydrophobic interactions and ionic bonds were analyzed using Discovery Studio Visualizer software.

In vitro experimental study (Laboratory confirmation)

Preparation of garlic extract

Fresh garlic cloves (*Allium sativum*) were collected, thoroughly washed and dried at 40 °C for 48 hrs in an electric drying oven. The dried samples were then ground into a fine powder. Ethanolic extraction (70 % ethanol) was performed by macerating garlic powder for 72 hrs at room temperature with periodic shaking. The resulting extract was filtered using Whatman No.1 filter paper, concentrated using a rotary evaporator at 40 °C under vacuum and stored refrigerated until further use.

Bacterial strain and culture preparation

A standard strain of *Staphylococcus aureus* (ATCC 25923) was obtained from a certified microbiology laboratory. The bacteria were grown on nutrient agar medium and incubated at 37 °C for 24 hrs. Subsequently, a bacterial suspension equivalent to 0.5 McFarland standard (~1.5×10^8 CFU/mL) was prepared in sterile saline solution for antibacterial assays.

Antimicrobial activity assay (Disc diffusion method)

The antibacterial potential of garlic extract was evaluated using the disc diffusion method as described by Clinical and Laboratory Standards Institute (14):

- Different concentrations of garlic extract (10, 20, 30 and 50 mg/mL) were prepared.
- Sterile paper discs (6 mm diameter) were impregnated with the prepared extracts and placed onto agar plates inoculated uniformly with the prepared bacterial suspension.
- Positive controls (ciprofloxacin antibiotic discs) and negative controls (discs impregnated with ethanol only) were included for comparison.
- Plates were incubated at 37 °C for 24 hrs and inhibition zones were measured precisely in millimeters to assess antibacterial effectiveness. Each treatment was performed in triplicate (n = 3) and the results were expressed as mean ± standard deviation.

Determination of Minimum Inhibitory Concentration (MIC)

The broth dilution method was applied to identify the MIC values of garlic extract:

- Serial dilutions of garlic extract were prepared in Mueller-Hinton broth.
- Each dilution was inoculated with bacterial suspension equivalent to 0.5 McFarland.
- Tubes were incubated at 37 °C for 24 hrs.
- The MIC was recorded as having the lowest concentration of garlic extract at which no visible bacterial growth was observed.

DNA gyrase enzyme assay (*In vitro* confirmation)

To confirm direct inhibitory action on DNA gyrase enzyme, an enzymatic assay was performed using a commercial DNA gyrase assay kit (TopoGEN, Inc., USA):

- Purified DNA gyrase enzyme from *S. aureus* was incubated with selected concentrations of garlic extract compounds according to kit instructions (incubation conditions: 37 °C, 30 min, pH 7.4).
- The enzyme activity was assessed by evaluating its ability to convert relaxed plasmid DNA to supercoiled DNA.
- Results were compared to a positive control (known DNA gyrase inhibitor such as Ciprofloxacin) and a negative control (enzyme reaction without inhibitor).

Results and Discussion

Overview of the experimental workflow

To facilitate a better understanding of the study's structure and progression, a flow diagram was created to illustrate the overall experimental workflow. It summarizes the two main phases of the investigation: the in silico molecular docking of garlic-derived compounds targeting *Staphylococcus aureus* DNA gyrase and the *in vitro* assays evaluating antibacterial activity. This visual representation highlights the sequence of steps and the corresponding key results, offering readers a clear view of the entire process at a glance (Fig. 2).

Molecular docking of garlic extract compounds with DNA gyrase

The binding potential of selected compounds from garlic extract to the active site of the DNA gyrase enzyme of

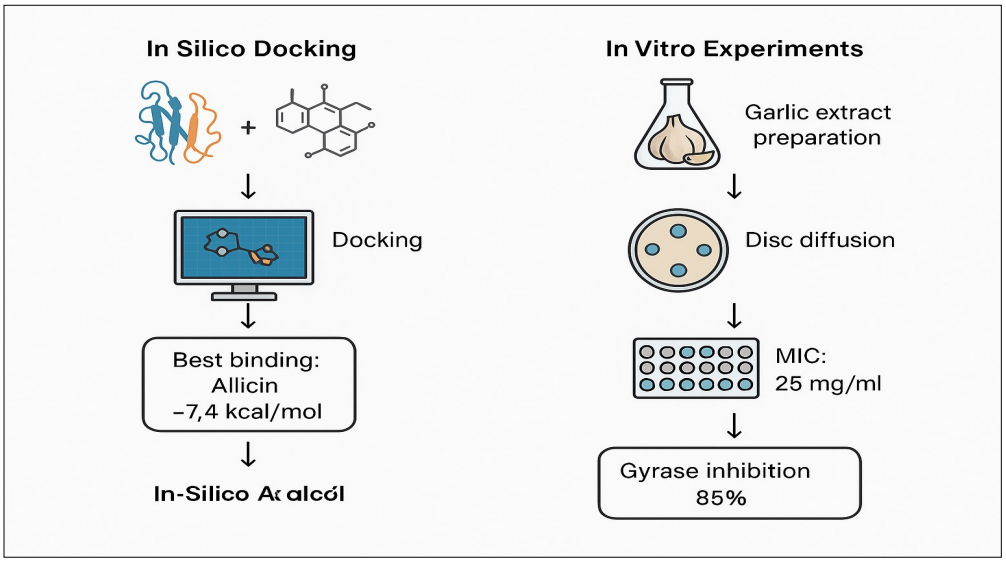

Fig. 2. Summary of the experimental workflow and main results from in-silico and *in vitro* assays.

Staphylococcus aureus was studied using in silico molecular docking approaches. Reflecting diverse possible values for enzymatic inhibition, the results in Table 1 showed variability in the compounds' capacity to form stable complexes with the enzyme (15, 16). With the lowest binding energy of -7.4 kcal/mol, the chemical allicin performed the best among the investigated compounds. Apart from a remarkable hydrophobic interaction with Ala68, allicin developed three strong hydrogen bonds with important amino acid residues Tyr122 and Asp81, therefore boosting the stability of the resulting complex (17). With a binding energy of -7.0 kcal/mol, ajoene placed second. It engaged in multiple hydrophobic interactions with residues such as Ile78 and Gly85 within the active site, suggesting considerable binding stability even without hydrogen bonding. In contrast, diallyl disulfide and diallyl sulfide showed weaker binding affinities of -6.5 and -6.2 kcal/mol, respectively. Diallyl disulfide formed a single hydrogen bond with Asn46, while diallyl sulfide depended solely on a weak hydrophobic interaction with Glu50, suggesting less stable binding compared to allicin and ajoene (18, 19).

Types of molecular interactions

Regarding kinds of interactions, allicin and diallyl disulfide primarily depended on forming strong hydrogen bonds with the active site residues. In contrast, ajoene and diallyl sulfide mainly engaged in hydrophobic interactions; notably, none of the tested compounds demonstrated electrostatic (charged) interactions (20, 21). The detailed interaction profile of each compound is summarized in Table 2. A bar chart displaying the measured binding energies for every compound was created to help grasp the variations between the molecules, as shown in Fig. 3. The results indicate that allicin holds the highest inhibitory potential against DNA gyrase due to its ability to form stable hydrogen bonds with key enzyme residues, reinforcing earlier hypotheses about its enzyme-disruptive action (22). On the other hand, ajoene, through strong hydrophobic contacts, may exert inhibition primarily via steric hindrance rather than electrostatic binding, despite lacking hydrogen bonding. The weaker performance of diallyl disulfide and diallyl sulfide likely stems from their simpler structures and lower ability to establish stable interaction networks with the enzyme's active site (23). These results highlight that specific garlic-derived molecules can directly inhibit vital bacterial enzymes, offering promising leads for developing plant-inspired natural antibacterial agents. However, while the docking results are compelling, biological validation through vitro gyrase inhibition and live-cell antibacterial assays is essential to establish the clinical relevance of these findings.

Table 1. Binding energies and number of hydrogen bonds with key residues of DNA gyrase

Compound	Binding energy (kcal/mol)	Number of hydrogen bonds	Key interacting residues
Allicin	-7.4	3	Tyr122, Asp81, Ala68
Ajoene	-7.0	0	Ile78, Gly85
Diallyl disulfide	-6.5	1	Asn46
Diallyl sulfide	-6.2	0	Glu50

Table 2. Garlic compound-DNA gyrase molecular interactions

Compound	Hydrogen bonds	Hydrophobic interactions	Electrostatic (charged) interactions
Allicin	Yes	Yes	No
Ajoene	No	Yes	No
Diallyl disulfide	Yes	Weak	No
Diallyl sulfide	No	Weak	No

Disc diffusion bacterial inhibition assay

Following Clinical and Laboratory Standards Institute (14), the antibacterial activity of the ethanolic garlic extract was assessed against the reference strain *Staphylococcus aureus* (ATCC 259) using the disc diffusion test. The results in Table 3 showed the formation of clear and increasingly larger inhibition zones with the increase in the concentration of the garlic extract. At the lowest concentration (10 mg/mL), the mean inhibition zone diameter reached 11 mm with a standard deviation of ±0.5 mm. With the increase in concentration to 20 mg/mL, the diameter increased to 14 mm, while the concentration of 30 mg/mL recorded a diameter of 17 mm. At the highest concentration (50 mg/mL), the greatest inhibitory effect was observed with a mean diameter of 20 mm, which is a noticeable difference approaching the efficacy of the positive control (ciprofloxacin

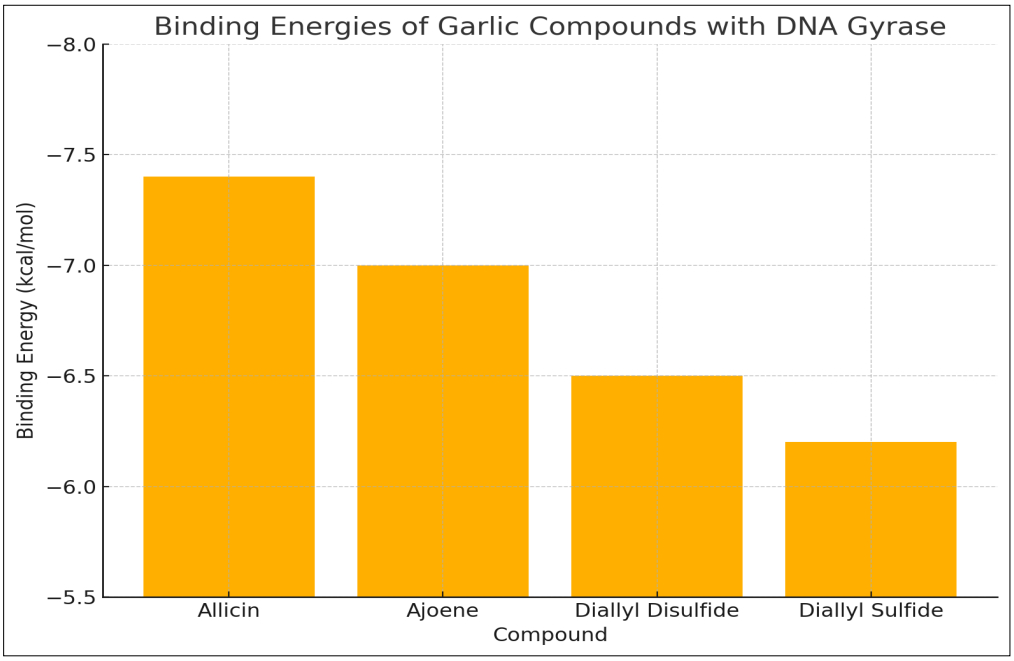

Fig. 3. Garlic compound-DNA gyrase binding energies comparison.

Table 3. Effect of garlic extract concentration on the inhibition zone against *Staphylococcus aureus*

Treatment	Inhibition zone (mm) ± SD	Significance
Garlic extract 10 mg/mL	11.0 ± 0.5	e
Garlic extract 20 mg/mL	14.0 ± 0.8	d
Garlic extract 30 mg/mL	17.0 ± 0.5	c
Garlic extract 50 mg/mL	20.0 ± 0.6	b
Ciprofloxacin (control)	24.0 ± 0.4	a
Ethanol (negative control)	0.0 ± 0.0	f

Values are presented as mean ± standard deviation (n = 3). Different letters indicate statistically significant differences between treatments ($p < 0.001$) according to one-way ANOVA followed by Tukey's post hoc test.

disc), which achieved an inhibition zone diameter of 24 mm. On the contrary, the discs treated with ethanol alone (negative control) showed no inhibition zone, indicating that the activity is due to the bioactive components of garlic and not to the solvent effect. These results demonstrate the direct relationship between the concentration of garlic extract and the increase in its antibacterial activity, supporting previous hypotheses about the presence of active compounds at different concentrations in the extract (24). These findings aligned with recent reports (25, 26) confirming that plant extracts rich in organosulfur compounds exert significant inhibitory effects against both gram -negative and gram-positive bacteria. Moreover, the comparable efficacy of crude garlic extract to that of ciprofloxacin highlights its promising potential as a natural source of effective antibacterial agents (27).

Determination of the Minimum Inhibitory Concentration (MIC)

A serial dilution test was conducted in Mueller-Hinton broth to determine the Minimum Inhibitory Concentration (MIC) of the garlic extract against *Staphylococcus aureus*. While bacterial growth was seen at lower concentrations (12.5 mg/mL and below), the garlic extract completely inhibited bacterial growth at a concentration of 25 mg/mL or above. The low MIC value (25 mg/mL) of garlic extract against *S. aureus* is considered a promising result, especially when compared to other plant extracts that often require higher concentrations to achieve the same effect (28). Table 4 summarizes the bacterial growth response observed at various concentrations of garlic extract. Notably, no visible growth was detected at concentrations of 25 mg/mL and above, confirming the MIC value obtained in this study. This finding aligns with recent studies demonstrating that the organosulfur compounds in garlic particularly allicin can target fundamental bacterial processes such as DNA replication and protein synthesis (29). Such results support the feasibility of using natural garlic extracts at practical concentrations as alternatives to synthetic antibiotics, helping reduce dependence on chemical antimicrobials (30).

Inhibition of DNA gyrase enzyme activity

The impact of garlic extract on DNA gyrase, a key enzyme required for bacterial DNA replication, was assessed using a specific enzymatic assay. Table 5 shows the results: at a concentration of 50 mg/mL, garlic extract inhibited 85 % of the enzyme's activity compared to the 95 % inhibition achieved by ciprofloxacin. These results confirm that one of the principal biological mechanisms by which garlic extract exerts antibacterial effects is through the inhibition of DNA gyrase, a pivotal enzyme for maintaining the supercoiled functional structure of bacterial DNA (31). This suggests that garlic-derived compounds interact directly with the active site of the enzyme, thereby blocking DNA replication and halting bacterial proliferation. Considering the increasing resistance of pathogens to traditional antibiotics, including beta-lactams, this mechanism

Table 4. MIC determination results for garlic extract against *Staphylococcus aureus*

Garlic extract concentration (mg/mL)	Bacterial growth observation
50	No growth
25	No growth
12.5	Growth
6.25	Growth
3.125	Growth

Table 5. DNA gyrase inhibition by garlic extract compared to positive control

Treatment	Gyrase inhibition (%) ± SD	Significance
Garlic extract 25 mg/mL	65.0 ± 2.1	b
Garlic extract 50 mg/mL	85.0 ± 1.8	a
Ciprofloxacin	95.0 ± 1.2	a

Values are expressed as mean ± standard deviation (n = 3). Different letters indicate statistically significant differences at $p < 0.05$ according to ANOVA.

is of considerable significance (32). Furthermore, these findings demonstrate that garlic acts not only on metabolic pathways or membrane integrity but also targets essential molecular processes, positioning it as a promising multi-target antibacterial agent (33).

Conclusion

This study demonstrated the antibacterial potential of garlic-derived compounds against *Staphylococcus aureus* using both computational and experimental approaches. In silico molecular docking revealed that allicin had the strongest binding affinity to DNA gyrase (-7.4 kcal/mol), forming stable interactions with key active site residues. *In vitro* validation confirmed these findings, with allicin-rich garlic extract achieving a MIC of 25 mg/mL and 85 % inhibition of DNA gyrase activity. These integrated results suggest that garlic compounds particularly allicin could serve as natural enzyme -targeting antibacterial agents. Further research on purified compounds and resistant strains is recommended.

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Authors' contributions

RAA performed the *in vitro* antibacterial assays, including the disc diffusion method and MHI determination and participated in drafting the manuscript. NARM carried out the DNA gyrase enzyme inhibition assay and assisted with the preparation of the garlic extract. BHAM contributed to the design of the experimental workflow and supervised the laboratory protocols. AHT conducted the statistical analysis and participated in the critical revision of the manuscript. ANF conducted the in- silico molecular docking analysis, interpreted the computational results and coordinated the research work across institutions. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interests to disclose.

Ethical issues: None

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