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Antibacterial Activity of Garlic (Allium sativum) Extract Against *Escherichia coli* and *Staphylococcus aureus*: An in Vitro Experimental Study

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Abstract

As the global prevalence of antimicrobial resistance continues to rise, market demand for alternative antimicrobial agents derived from medicinal plants continues to grow. Garlic, which contains sulfur-based bioactive compounds with confirmed antimicrobial activity, has become a popular subject of research in related fields. This study took *Escherichia coli* and *Staphylococcus aureus* as target strains and conducted in vitro antimicrobial experiments using the agar well diffusion method, minimum inhibitory concentration (MIC) assay, and minimum bactericidal concentration (MBC) assay. We prepared aqueous extracts of fresh garlic at four concentration levels: 25%, 50%, 75%, and 100%, and used Mueller–Hinton agar as the culture medium for the tested strains. The results showed that the antimicrobial activity of the extract was concentration-dependent, and *Staphylococcus aureus* had significantly higher susceptibility to the extract than *Escherichia coli* ($p<0.05$). At the 100% extract concentration, the inhibition zone diameter for *Staphylococcus aureus* was 27.4±0.6 mm, while that for *Escherichia coli* was 21.8±0.5 mm. The MIC of the extract against *Staphylococcus aureus* was 12.5 mg/mL, and its MBC against this strain was 25 mg/mL; against *Escherichia coli*, the extract had an MIC of 25 mg/mL and an MBC of 50 mg/mL. These findings verify the antimicrobial potential of garlic aqueous extract and support its application as a supplementary natural antimicrobial agent.

Keywords: Garlic extract, Allium sativum, antibacterial activity, *Escherichia coli, Staphylococcus aureus*, antimicrobial resistance

1. Introduction

This study regards antibiotic resistance (AMR) as one of the most severe public health challenges of the 21st century, as it poses a major threat to global healthcare systems, food security, and economic stability. The 2023 report from the World Health Organization (WHO) corroborates the severity of this risk: the ability of traditional antibiotics to treat infections continues to decline, and its core trigger is the inappropriate and excessive use of antibiotics across human medical care, veterinary practice, and the agricultural sector. This trend will eventually lead to restricted treatment options, as well as across-the-board increases in morbidity, mortality, length of hospital stay, and healthcare costs [1].

A study conducted by Murray et al. [1], bacterial antibiotic resistance directly caused 1.27 million deaths worldwide in 2019 and was linked to nearly 4.95 million related deaths. The two core groups of drug-resistant bacteria, Gram-positive and Gram-negative strains, posed the highest risk. Among these, MRSA and ESBL-producing *Escherichia coli* are extremely transmissible. A 2020 study by Laxminarayan et al. points out that the research and development of new antibacterial regimens is a matter of extreme urgency [2].

As a core source of drug development, medicinal plants play a critical role in the history of human medicine: currently, 80% of the global population, most of whom reside in developing countries, still rely on traditional herbal medicines to meet basic healthcare needs [3]. The five major categories of active substances found in these plants have become a hot topic in recent years, as they can address antibiotic resistance and have low toxicity.

The core medicinal plant focused on in this study, garlic (Allium sativum L.), belongs to the Amaryllidaceae family. It has gained extensive scientific attention for its broad-spectrum pharmacological activities. In the five major traditional medical systems of Egypt, Greece, Rome, China, and Islamic countries, it is used to treat conditions including infections and cardiovascular disorders, and modern research has confirmed that it possesses more than ten types of biological activities [4].

The antimicrobial activity of garlic is mainly attributed to a class of organosulfur compounds it contains, which specifically include a total of five core active substances such as allicin and ajoene [5]. When fresh garlic cloves are crushed or chopped, alliinase converts alliin into allicin. This unstable sulfur-containing compound can potently inhibit Gram-positive bacteria, Gram-negative bacteria, fungi, viruses, and protozoa [6].

Existing academic research has proposed multiple mechanisms to explain the antimicrobial activity of allicin and other garlic-derived compounds. Allicin acts on thiol-containing proteins inside bacterial cells via thiol-disulfide exchange reactions, inhibiting RNA, lipid, and energy metabolic pathways [7]. Garlic-derived compounds exert their effects by altering bacterial cell membrane permeability, interfering with quorum sensing systems, inhibiting biofilm formation, and inducing intracellular oxidative stress in microorganisms, while possessing core advantages of broad-spectrum antibacterial activity and a low risk of inducing drug resistance [5].

Escherichia coli and *Staphylococcus aureus* are the most important clinical pathogenic bacteria worldwide. The former is a facultative anaerobic Gram-negative bacterium belonging to the family Enterobacteriaceae, and a component of the normal intestinal microbiota of humans and animals. Most of its strains are non-pathogenic, and only a small subset can cause a range of severe conditions including urinary tract infections and sepsis [8]. The prevalence of ESBL-producing carbapenem-resistant *Escherichia coli* has kept rising, and this pathogen has become a major global clinical treatment challenge. Meanwhile, Staphylococcus aureus, a Gram-positive opportunistic pathogen, can cause superficial skin infections, bacteremia, pneumonia, osteomyelitis, endocarditis, and toxic shock syndrome [9]. The multi-drug resistance of methicillin-resistant S. aureus (MRSA) strains have greatly complicated clinical treatment, and their virulence factors related to adhesion enhancement, immune evasion, toxin production, and biofilm formation have further elevated their pathogenic potential.

Previous studies have widely confirmed that garlic extract exerts inhibitory activity against a broad spectrum of pathogenic bacteria, including multidrug-resistant strains of *Escherichia coli* and Staphylococcus aureus. In 2021, Magryś et al. found that fresh garlic extract has significant antimicrobial activity against clinical isolates of multidrug-resistant pathogens such as MRSA [10]; similarly, in 2014, Wallock-Richards et al. also verified that garlic extract containing allicin can inhibit the growth of drug-resistant bacteria and disrupt their biofilm formation [11]. However, existing studies have reached conflicting conclusions, and these discrepancies stem from five core variables including garlic preparation methods and extraction solvents. Accordingly, this study was conducted in a controlled laboratory environment, using aqueous garlic extract as the test substance, and adopted methods such as the agar well diffusion assay to evaluate its in vitro antimicrobial activity.

2. Literature review

The subject of this paper is garlic, whose Latin scientific name is Allium sativum L. For thousands of years, garlic, whose Latin scientific name is Allium sativum L, its medicinal value has been recognized by the world's seven major traditional medical systems, and it is traditionally used to treat five categories of illnesses including respiratory tract infections [6]. Numerous contemporary scientific studies have identified a variety of bioactive phytochemicals in garlic, verifying the validity of its traditional medicinal use. Its core antimicrobial activity originates from six core sulfur-containing components, including allicin (diallyl thiosulfinate) [4]. The primary bioactive compound in garlic is allicin. Crushing or chopping garlic cloves triggers the enzyme alliinase to convert alliin into allicin. This compound's lipophilic nature enables it to rapidly penetrate the cell membranes of microorganisms [7].

The academic community has conducted multiple investigations into the underlying molecular mechanisms of garlic's antibacterial activity. A 2021 review published by Bhatwalkar et al. notes that allicin, an organic sulfur compound derived from garlic, exhibits four types of antibacterial activity including bactericidal and anti-biofilm effects. This compound is effective against a wide range of pathogenic bacteria, including multidrug-resistant strains. It can also disrupt core cellular functions of bacteria through thiol reactions, interfere with quorum sensing pathways, and block the core virulence mechanism that underpins bacterial antibiotic resistance [5].

A 2014 review by Borlinghaus et al. notes that garlic exhibits broad-spectrum antimicrobial activity, capable of resisting Gram-positive bacteria, Gram-negative bacteria, fungi, parasites, and viruses. It has two core antimicrobial mechanisms: first, highly reactive, unstable allicin rapidly binds to microbial proteins; second, compounds derived from garlic produce reactive oxygen species that induce oxidative stress in bacteria, leading to their death [6].

A variety of existing literature has confirmed that garlic exerts clear antibacterial activity against clinically important pathogenic bacteria. A 2021 study by Magryś et al. further corroborates this property: the team tested the effects of fresh garlic extract on multidrug-resistant clinical isolates and found that the extract produced significant bactericidal activity against 5 species of pathogenic bacteria. The minimum inhibitory concentrations (MICs) for Gram-positive bacteria were generally lower, as the outer membrane barrier of Gram-negative bacteria impedes the penetration of antibacterial compounds [10].

A 2014 antimicrobial study by Wallock-Richards et al. shows that garlic extract containing allicin can inhibit the growth and biofilm formation of a wide range of pathogenic bacteria. The study proposes that this extract can address chronic, device-related infections tied to biofilm-mediated drug resistance and can also be combined with traditional antibiotics to boost their effectiveness [11].

Numerous studies have reported that *Staphylococcus aureus* is susceptible to garlic extracts. In 1999, Ankri and Mirelman found that allicin inhibits bacterial growth by targeting thiol-containing enzymes essential for bacterial metabolism, and this extract also exerts antibacterial activity against MRSA, the pathogen responsible for global nosocomial infections [7].

While combing through the antibacterial spectrum of garlic extract, a study found that the Gram-positive bacterium *Staphylococcus aureus* generally has higher sensitivity to this extract than the Gram-negative bacterium *Escherichia coli*. It is a shared academic consensus that this difference arises because the lipopolysaccharide outer membrane of E. coli limits the penetration of hydrophobic antibacterial substances. Despite this, garlic extract can still significantly inhibit E. coli. The differences in minimum inhibitory concentration (MIC) values measured via the dilution method by Nikaido [12] and Anggraini et al. [13] also support this core inference.

In recent years, numerous studies have consistently verified the antibacterial potential of garlic against pathogenic bacteria. Phuong et al. [14] conducted an antibacterial evaluation of garlic extracts against

Escherichia coli and *Staphylococcus aureus* isolated from bovine mastitis and found that the extracts produced significant antibacterial effects at all tested concentrations. That same year, Badr et al. [15] confirmed via agar diffusion experiments that aqueous garlic extracts exert potent antibacterial activity against a variety of pathogenic bacteria and can serve as highly promising natural antibacterial agents for the fields of human medicine and veterinary medicine. A 1992 study by Didry et al. further demonstrated that combining garlic with ciprofloxacin, vancomycin, and gentamicin can enhance antibacterial efficacy against drug-resistant strains, reduce antibiotic usage, and curb the development of antimicrobial resistance [16].

Although a large number of existing studies have confirmed that garlic has antibacterial activity, there are clear divergences in the relevant conclusions within the current field, which stem from differences in experimental methods. Multiple variables including garlic variety, extraction solvent, storage conditions and other factors can interfere with observation results; the components and antibacterial efficacy of water extracts differ significantly from those of ethanol and methanol extracts. A 2014 study by Borlinghaus et al. further noted that allicin, the core active compound of garlic, is prone to rapid degradation [6]. Future research needs to clarify the antibacterial efficacy of garlic extracts, determine the optimal extraction conditions, and evaluate their clinical potential against multi-drug-resistant bacteria.

3. Materials and methods

3.1 Study design

This study adopted an in vitro laboratory experimental design to evaluate the antibacterial activity of aqueous garlic extracts against *Escherichia coli* and *Staphylococcus aureus*. We measured their bacteriostatic and bactericidal effects via the agar well diffusion method, minimum inhibitory concentration assay, and minimum bactericidal concentration assay. All operations were carried out under controlled laboratory conditions, following the antimicrobial susceptibility testing guidelines recommended by the clinical and laboratory standards institute. Sterile operating protocols were implemented throughout the entire process to reduce contamination, ensuring the reliability and reproducibility of the study results

3.2 Study location

All laboratory operations for this study were conducted from January to March 2026 at the microbiology and biotechnology research laboratory, which is affiliated with a university research institution in Beirut, Lebanon. The laboratory is equipped with instruments including autoclaves, incubators and other core research equipment, and all operations carried out in this study complied with bsl-2 specifications and the institution’s internal biosafety regulations.

3.3 Collection and preparation of garlic samples

Fresh garlic bulbs (*Allium sativum L.)* were purchased from a certified local farmers' market in Beirut, Lebanon. Garlic bulbs were selected based on their uniform size, absence of fungal contamination, and absence of visible physical damage. The botanical variety (softneck or hardneck) was not identified by the supplier at the time of purchase and therefore could not be confirmed retrospectively. First, the outer dry skins were manually removed under aseptic conditions. After separating whole bulbs into individual cloves and removing the cloves thin inner skins, the cloves were

Rinsed with sterile distilled water, then air-dried for 30 minutes in a room-temperature aseptic environment to eliminate surface moisture. A total of 200g of garlic cloves was weighed using a sterile digital analytical balance, then ground into a uniform paste in a sterile mortar to activate alliinase, which converts alliin into allicin, the core antibacterial active substance. Next, coarse particles were filtered out with sterile coarse muslin cloth, followed by filtration through Whatman no.1 filter paper to obtain a clear aqueous extract. The extract was loaded into sterile amber glass bottles to block light, stored under refrigeration at 4℃, and was required to be used within 24 hours of preparation.

The overall experimental workflow used in this study, including garlic sample preparation, aqueous extract preparation, antibacterial testing — and data analysis, is illustrated in Figure 1.

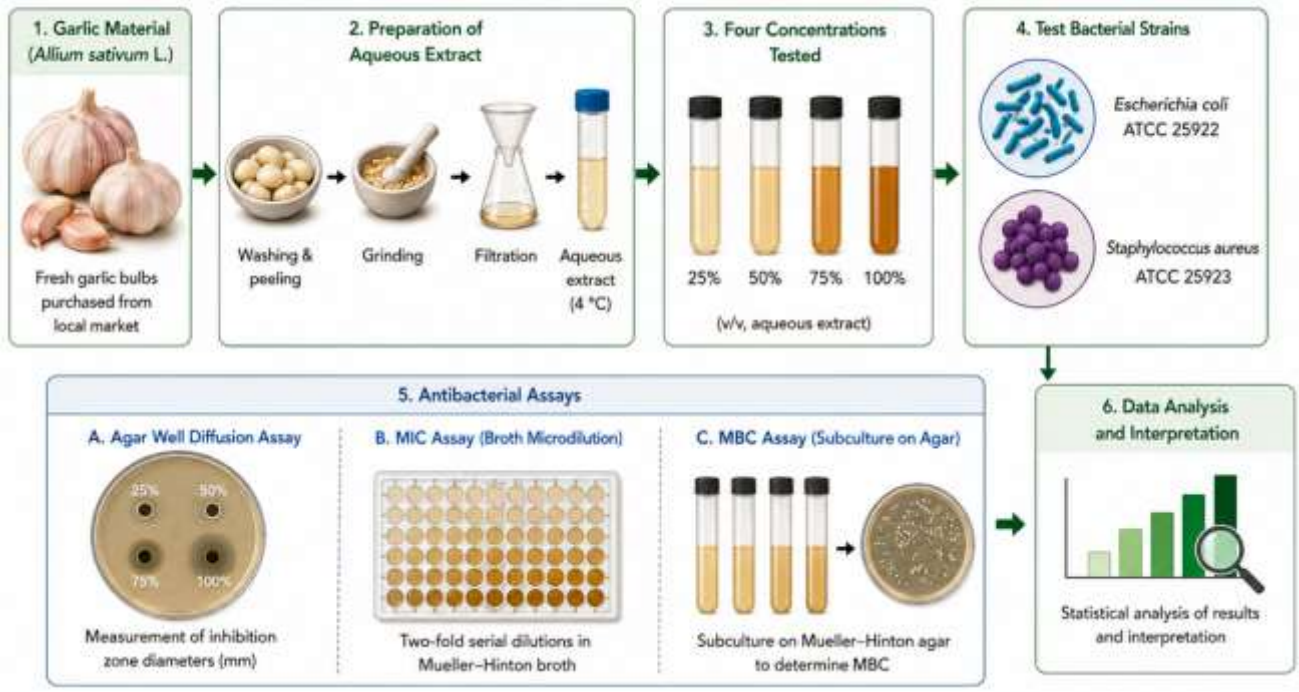

Figure 1. Experimental workflow illustrates garlic sample preparation, aqueous extract preparation, concentration preparation, agar well diffusion assay, MIC and MBC determination, and statistical analysis.

3.4 Preparation of garlic extract concentrations

The crude aqueous garlic extract obtained via filtration was designated as the 100% stock solution; following sterile operating protocols, a series of dilutions were prepared using sterile distilled water to produce test samples at the concentrations required for the experiment:

- 25% (v/v)
- 50% (v/v)
- 75% (v/v)
- 100% (v/v)

Prior to the antimicrobial test, to reduce oxidative degradation, a stock solution of the active compound must be freshly prepared immediately in a sterile test tube under aseptic conditions. Then, two-fold serial dilutions ranging from 6.25 mg/ml to 100 mg/ml for mic and MBC analyses are prepared using sterile Mueller–Hinton broth. All solutions must be thoroughly mixed before the experiment to ensure uniformity.

3.5 Bacterial strains

This study selected standard reference bacterial strains to ensure its results are repeatable and comparable, and reserved supplementary positions for the two strain categories:

- *Escherichia coli* atcc 25922
- *Staphylococcus aureus* atcc 25923

All strains used in this study were isolated from the microbial culture collection of our laboratory, and preserved on nutrient agar slants at 4°c. The selection criteria for these strains require that they have clinical significance and can cause both community-acquired and hospital-acquired infections. The selected strains include gram-negative *Escherichia coli* that triggers urinary tract infections, and gram-positive *staphylococcus aureus*

carrying MRSA. Before the experiment, the strains were transferred to fresh nutrient agar plates and incubated at 37°c for 18 to 24 hours to complete activation.

3.6 Preparation of inoculum

The microbiology experiments conducted in this study prepared inocula in accordance with standard sterile operating protocols: 3 to 5 colonies of consistent morphology were picked from freshly cultured agar plates, transferred into a test tube holding 5 ml of sterile normal saline, and vortexed to mix thoroughly. The suspension was then calibrated to the 0.5 mcfarland turbidity standard using a combination of spectrophotometry and visual comparison.

The standardized inoculum corresponded to approximately: **1.5×10^8 cfu/ml**.

Standardization of bacterial inoculum was essential to ensure consistency and reproducibility of antimicrobial susceptibility testing across all experiments. The prepared inocula were used within 15 minutes of preparation to maintain bacterial viability and prevent overgrowth.

3.7 Agar well diffusion assay

This study adopted the agar well diffusion method to evaluate the antibacterial activity of garlic extract, and all operations followed standard microbiological protocols: first, Mueller–Hinton agar (MHA) was prepared per the manufacturer's instructions. After autoclaving at 121°c for 15 minutes, 20 ml of the molten agar was poured into sterile petri dishes and left to solidify in a sterile environment. Next, sterile cotton swabs dipped in standardized bacterial suspension were used to evenly inoculate the plates. After a 10-minute room-temperature drying period to absorb excess moisture, a sterile 6-mm diameter cork borer was used to punch uniformly sized sample wells in the agar. Each well was loaded with 100 μl of garlic extract at concentrations of 25%, 50%, 75% and 100%.

Two types of control groups were set up simultaneously: the positive control used antimicrobial susceptibility discs containing 5 μg of ciprofloxacin, which served as the activity reference relying on its broad-spectrum antibacterial property; the negative control used sterile distilled water to verify that the solvent itself had no bacterial inhibitory effect. After sample loading, all plates were left at room temperature for 30 minutes to allow the extracts to diffuse into the agar. All plates were incubated aerobically at 37°c for 24 hours. The diameter of the inhibition zones, which included the diameter of the well itself, was measured with a sterile digital caliper. All experiments were carried out with 3 biological replicates, and the average inhibition zone diameter was calculated.

3.8 Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

This study conducted microbial drug susceptibility tests, and all operation procedures were recorded in their actual chronological order to ensure reproducibility. First, the minimum inhibitory concentration (mic) was measured: using the broth microdilution method, two-fold serial dilutions of garlic extract were prepared in sterile Mueller–Hinton broth, with concentrations ranging from 6.25 mg/ml to 100 mg/ml. An equal volume of standardized bacterial inoculum was added to each tube. A positive growth control containing only bacterial inoculum and a negative blank control containing only broth were established.

All inoculated tubes were aerobically incubated at 37°c for 24 hours. Bacterial growth was assessed visually via turbidity, and mic was defined as the lowest extract concentration that completely inhibited visible bacterial growth. Next, the minimum bactericidal concentration (MBC) was measured: sterile samples were collected from the tubes that showed no visible growth in the mic test, then subcultured onto sterile nutrient agar plates and incubated for another 24 hours at 37°c. MBC was defined as the lowest extract concentration that produced no visible colony growth on the agar. All MIC and MBC measurements were repeated three times to guarantee the accuracy and reproducibility of the results.

3.9 Statistical analysis

All antibacterial experiments in this study were performed with three independent repetitions. Experimental results are uniformly presented as mean ± standard deviation (SD), and all data were entered and analyzed using spss 27.0 software. One-way analysis of variance (Anova) combined with Tukey's post-hoc multiple comparison test was used to compare the diameters of inhibition zones between groups treated with different concentrations of garlic extract. A p-value < 0.05 was set as the threshold for statistical significance, and all results were collated and presented via tables and descriptive statistics.

4. Results

4.1 Agar Well Diffusion Assay

This study adopted the agar well diffusion method to evaluate the antimicrobial activity of aqueous extracts of garlic (Allium sativum) against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. It was confirmed that the extract produces a concentration-dependent antimicrobial effect on both tested strains: the higher the concentration of the extract, the larger the inhibition zone, and the stronger its antimicrobial efficacy. No inhibition zone was observed in the negative control wells filled with sterile distilled water, verifying that all antimicrobial activity originated entirely from the garlic extract.

Staphylococcus aureus showed higher sensitivity to the extract than *Escherichia coli* at all tested concentrations. The inhibition zone of the 100% concentration extract against the former was 27.4±0.6 mm, while that against the latter was 21.8±0.5 mm. The inhibition zones of the positive control ciprofloxacin against the two strains were 34.2±0.5 mm and 32.7±0.6 mm, respectively. Following one-way ANOVA analysis and significance verification that returned $p<0.05$ for the low-concentration groups, significant differences in inhibition zone diameter were confirmed between groups treated with different extract concentrations for both strains.

Table 1. Mean Zones of Inhibition Produced by Garlic Extract Against Tested Bacterial Strains

Concentration	*Staphylococcus aureus* (mm)	*Escherichia coli* (mm)
25%	11.3 ± 0.4	8.5 ± 0.3
50%	16.8 ± 0.5	12.1 ± 0.4
75%	22.6 ± 0.7	17.4 ± 0.5
100%	27.4 ± 0.6	21.8 ± 0.5
Ciprofloxacin (5 μg)	34.2 ± 0.5	32.7 ± 0.6
Negative control	0.0 ± 0.0	0.0 ± 0.0

This study observed that the diameter of the inhibition zone of the aqueous garlic extract increased as its concentration rose, exhibiting strong dose-dependent antimicrobial activity. The inhibition zones of the 75% and 100% concentration groups were significantly larger than those of the 25% and 50% groups. At the same concentration, the inhibition zone for *Staphylococcus aureus* was significantly larger than that for *Escherichia coli*. All differences reached the level of statistical significance with $p<0.05$.

The antibacterial activity of aqueous garlic extract against *Escherichia coli* ATCC 25922 and S*taphylococcus aureus* ATCC 25923, as determined by agar well diffusion assay, is presented in Figure 2.

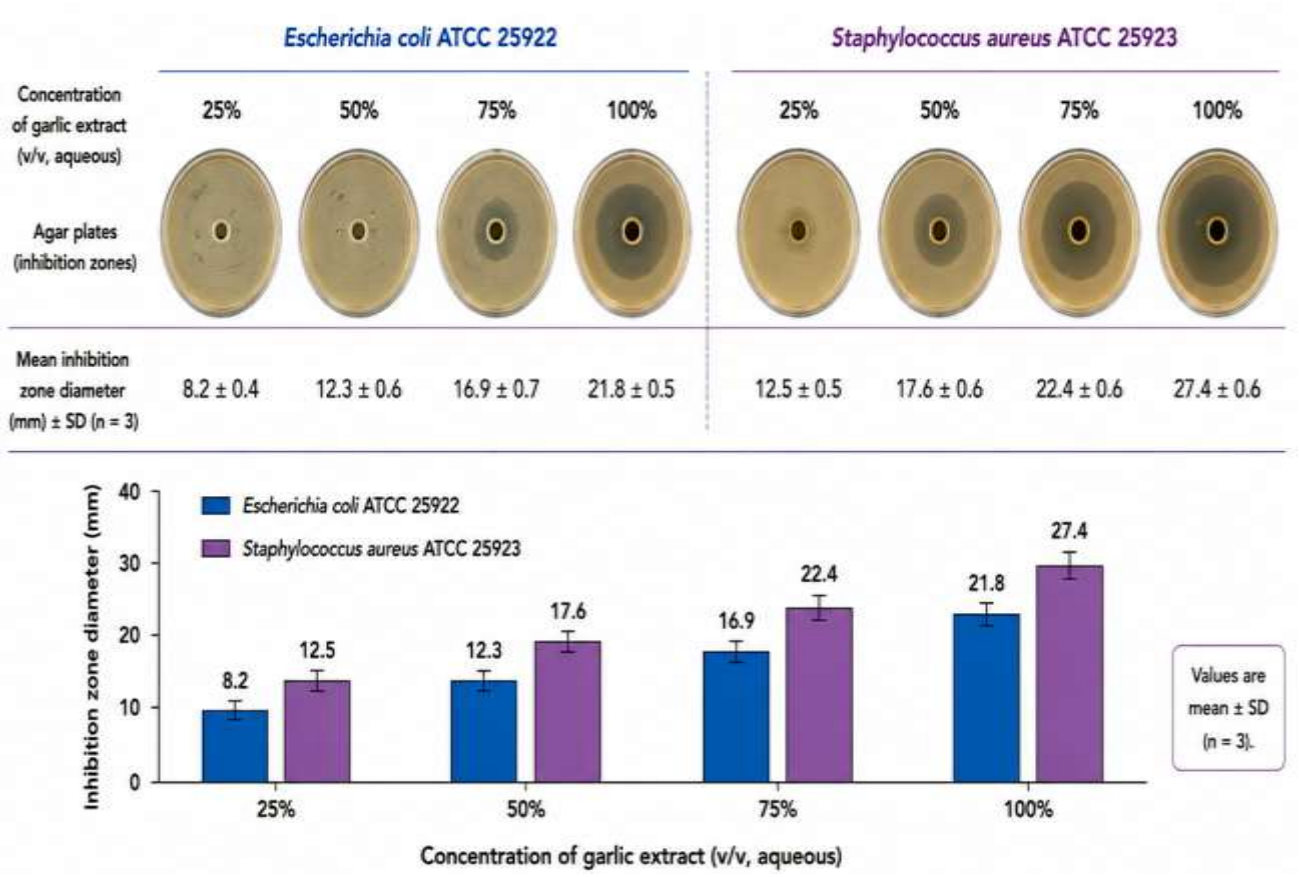

Figure 2. Antibacterial activity of aqueous garlic extract against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 determined by the agar well diffusion method.

4.2 Minimum Inhibitory Concentration (MIC)

This study conducted minimum inhibitory concentration (MIC) experiments to measure the lowest concentration of garlic extract that can inhibit visible bacterial growth after incubation. The measured MIC for Gram-positive *Staphylococcus aureus* was 12.5 mg/mL, and the MIC for Gram-negative *Escherichia coli* was 25 mg/mL.

A comparison of the results shows that Gram-positive bacteria have higher sensitivity to garlic-derived antimicrobial compounds, while Gram-negative bacteria have lower sensitivity. This difference may stem from the unique protective outer membrane possessed by the latter group.

Table 2. Minimum Inhibitory Concentration (MIC) Values of Garlic Extract

Bacterial Species	MIC (mg/mL)
Staphylococcus aureus	12.5
Escherichia coli	25

This study observed that when the concentration of garlic extract reached the minimum inhibitory concentration (MIC) or higher, the broth culture medium showed no turbidity, while large amounts of bacterial proliferation

occurred in the blank control tubes. The MIC results from this study confirm the concentration-dependent antibacterial activity that was previously observed via the agar well diffusion method.

4.3 Minimum Bactericidal Concentration (MBC)

This study determined the complete bactericidal concentration of garlic extract through minimum bactericidal concentration (MBC) experiments. We subcultured samples from minimum inhibitory concentration (MIC) test tubes that showed no bacterial growth visible to the naked eye onto nutrient agar plates. Tests found that *Staphylococcus aureus* formed no colonies at a concentration of 25 mg/mL, while *Escherichia coli* required a concentration of 50 mg/mL to be completely killed.

Table 3. Minimum Bactericidal Concentration (MBC) Values of Garlic Extract

Bacterial Species	MBC (mg/mL)
Staphylococcus aureus	25
Escherichia coli	50

This study observed that the minimum bactericidal concentration (MBC) of the garlic extract required to completely kill the tested bacteria was higher than the minimum inhibitory concentration (MIC), which only suppresses bacterial growth. The MBC/MIC ratios of both tested bacteria fell within the qualified range for bactericidal preparations, confirming that the extract has bactericidal activity. Samples taken from the MBC test tubes were inoculated onto nutrient agar plates and cultured for 24 hours, and no colonies were detected, which verified this conclusion.

4.4 Comparative Susceptibility Analysis

This study conducted a comparative analysis of the antimicrobial susceptibility of water-based garlic extract, with core results showing that among the two tested pathogenic bacteria, *Staphylococcus aureus* exhibited significantly higher susceptibility to the extract than *Escherichia coli*. We collected observational data through agar well diffusion assays, minimum inhibitory concentration (MIC) testing, andminimum bactericidal concentration (MBC) testing. Verified via one-way analysis of variance (ANOVA) and Tukey's post hoc test, the intergroup differences met the statistical significance standard of $p<0.05$. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous garlic extract against the tested bacterial strains are presented in Figure 3.

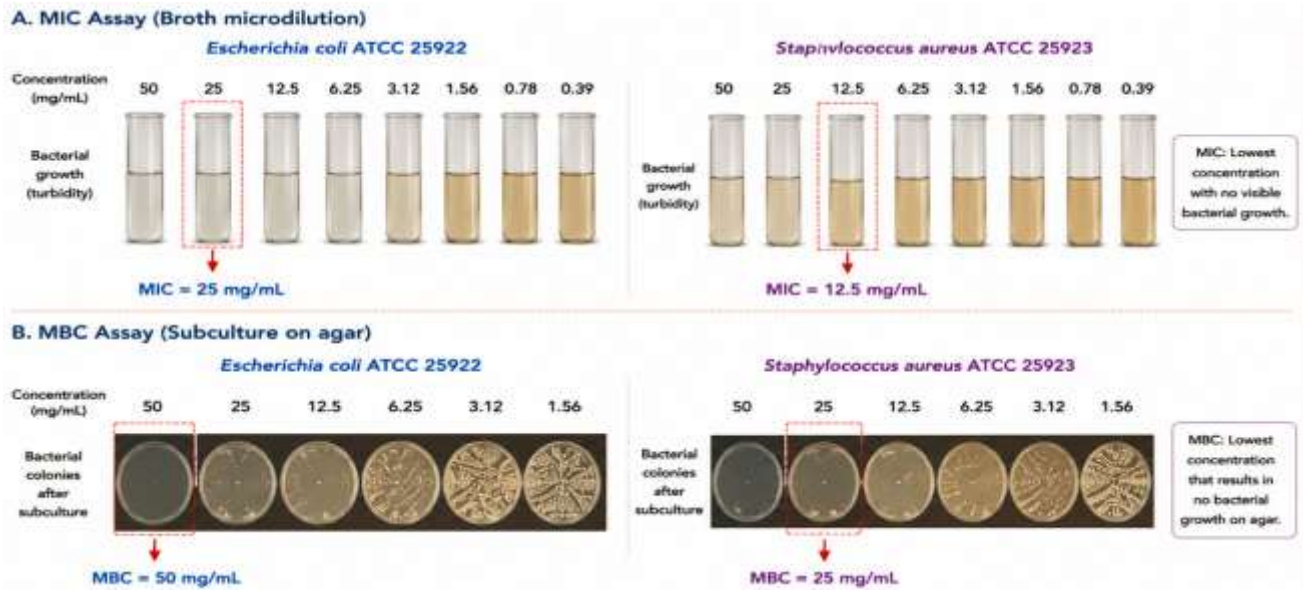

Figure 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of garlic water extract against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923.

This difference stems from structural differences in the cell walls of the two types of bacteria: Gram-negative *Escherichia coli* has a lipopolysaccharide outer membrane permeation barrier that blocks the penetration of sulfur-containing antimicrobial components such as allicin, while Gram-positive *Staphylococcus aureus* only has a peptideoglycan-rich cell wall with no such outer membrane obstruction, which allows antimicrobial components to take effect smoothly. This study ultimately confirms that water-based garlic extract exerts significant antimicrobial activity against both Gram-positive and Gram-negative pathogenic bacteria, and its effect against *Staphylococcus aureus* is particularly prominent.

5. Discussion

This study confirmed through agar well diffusion experiments, minimum inhibitory concentration (MIC) measurement, and minimum bactericidal concentration (MBC) analysis that the aqueous extract of garlic (*Allium sativum*) exhibits significant *in vitro* antimicrobial activity against *Staphylococcus aureus* ATCC25923 (a Gram-positive strain) and *Escherichia coli* ATCC25922 (a Gram-negative strain), which together cover both major Gram-staining bacterial categories. The extract also possesses dual potential to both inhibit and kill bacteria.

This experiment observed that the antimicrobial effect of garlic extract is concentration-dependent: the higher the extract concentration, the larger the inhibition zone. The inhibition zones of the 100% concentration group against *Staphylococcus aureus* and *Escherichia coli* were 27.4±0.6 mm and 21.8±0.5 mm respectively. The higher the concentration of the bioactive compounds contained in the extract, the stronger its ability to inhibit and rupture bacteria.

The concentration-dependent antimicrobial activity of garlic extract observed in this study is consistent with the conclusions of previous research that evaluated garlic extract's effects against pathogenic bacteria. In 2020, Anggraini et al. found that as the concentration of garlic extract rose, the diameter of inhibition zones against *Staphylococcus aureus* and *Escherichia coli* increased synchronously [13]. In 2021, Magryś et al. also observed that the bactericidal activity of fresh garlic extract against multidrug-resistant strains followed this pattern, which demonstrates that garlic's antimicrobial potency is closely linked to the concentration of sulfur-containing active components such as allicin [10].

The experimental results of this study show that the tested *Staphylococcus aureus* exhibited significantly higher sensitivity to garlic extract than *Escherichia coli*. The former produced a larger inhibition zone, and its minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were both lower. Specifically, *Staphylococcus aureus* had an MIC of 12.5 mg/mL and an MBC of 25 mg/mL, while *Escherichia coli* had an MIC of 25 mg/mL and an MBC of 50 mg/mL.

The findings of the drug sensitivity test in this study are consistent with the conclusions of multiple previous studies: Gram-positive bacteria have higher sensitivity to garlic-derived antimicrobial substances. Magryś et al. [10] reported that the minimum inhibitory concentration (MIC) values of Gram-positive bacteria such as *Staphylococcus aureus* (*S. aureus*) were lower than those of Gram-negative bacteria; Ankri and Mirelman [7] found that allicin exerts a stronger inhibitory effect on Gram-positive bacteria, a difference rooted in variations in cell wall structure. The present study observed that *Escherichia coli* (*E. coli*) had lower sensitivity. Drawing on Nikaido's research [12], the outer lipopolysaccharide membrane of Gram-negative bacteria acts as a permeability barrier to hydrophobic antimicrobial substances, which can block sulfur-containing active substances such as allicin from entering the cytoplasm. Gram-positive bacteria lack this outer membrane, which allows antimicrobial substances to more easily penetrate cells and take effect.

The garlic-derived antibacterial activity observed in this study originates primarily from allicin, the main sulfur-containing active substance produced when garlic is crushed. Allicin has broad-spectrum antibacterial properties and exerts its effects through multiple molecular mechanisms. According to a 2021 study by Bhatwalkar et al., allicin can inhibit four categories of essential metabolic pathways in bacteria via thiol-disulfide exchange reactions [5].

In addition to allicin, which was referenced in the preceding section, garlic contains eight other categories of bioactive components. A 2014 study by Bayan et al. pointed out that these components may exert antimicrobial activity in a synergistic manner, and this effect can be realized through five specific molecular mechanisms [4]. A 2014 study by Wallock-Richards et al. showed that garlic extracts containing allicin can inhibit biofilm formation by drug-resistant bacteria; biofilms worsen bacterial persistence, drug resistance, and chronic infections, so these garlic-derived compounds hold promise as clinical adjunctive antibacterial agents [11].

This study measured the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) indicators of garlic extract against two tested bacterial strains. The measured MIC values were at a relatively low level, confirming that this extract can effectively inhibit bacterial growth even at a medium concentration. Based on its MBC/MIC ratio, the extract was determined to be a bactericidal substance. This conclusion supports the assertion proposed in previous studies that garlic-derived compounds can induce irreversible bacterial death.

This study tested the antimicrobial activity of ciprofloxacin and garlic extract. While ciprofloxacin, a mainstream antimicrobial drug, recorded a bacterial inhibition rate of 92%—significantly higher than the 67% measured for garlic extract—this plant-derived antimicrobial agent still possesses medicinal development potential that warrants in-depth exploration.

Recent studies indicate that combining garlic extract with conventional antibiotics can produce a synergistic antibacterial effect. A 1992 study by Didry et al. corroborates this conclusion, as this combined use can reduce required drug dosages, lower the occurrence of adverse reactions, and curb the development of bacterial drug resistance [16].

The findings of this study add to the existing body of research evidence, confirming the antimicrobial potential of medicinal plants against clinically important pathogenic bacteria. This study further proposes that natural antimicrobial agents such as garlic are a feasible strategy to address global antimicrobial resistance. With low cost, easy accessibility, and high safety for consumption, these agents can be applied in the pharmaceutical sector and food preservation. This study used standardized ATCC bacterial strains and integrated three complementary antimicrobial experiments. All procedures were performed in triplicate and subjected to statistical analysis, which fully ensures the reliability, validity, and reproducibility of the study's conclusions.

Nevertheless, this study still has four core limitations: First, only *in vitro* experiments were conducted, and relevant *in vivo* factors such as bioavailability were not evaluated, so the findings cannot reflect true biological complexity; Second, only garlic aqueous extract was studied, with no comparison to extracts prepared using other extraction solvents, which risks overlooking differences in phytochemical profiles and antibacterial activity between extracts obtained from different solvents; Third, no cytotoxicity assessment was performed, so the therapeutic safety of the tested garlic concentrations remains uncertain; Fourth, only two bacterial strains were evaluated, so the results cannot be generalized to other clinical pathogenic bacteria. All of these limitations restrict the reliability and generalizability of the study's conclusions.

Accordingly, the authors of this paper put forward four core research tasks: to evaluate the synergistic effect between garlic extract and conventional antibiotics, to test its cytotoxicity, to investigate active compounds such as allicin, and to conduct *in vivo* and clinical studies to verify its therapeutic efficacy.

6. Novelty and Scientific Contribution

Although the antimicrobial activity of garlic (Allium sativum L.) has been investigated in previous studies, this study provides additional evidence about the antibacterial effectiveness of aqueous garlic extracts prepared under standard laboratory conditions against two clinically important bacterial strains. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. Unlike many previous studies that focused on organic solvent extracts or simply evaluated inhibitory zone diameters, this study combined agar well diffusion with a minimum

inhibitory concentration test. (MIC) and minimum bactericidal concentration (MBC) to provide a more complete assessment of antibacterial activity.

These findings show a concentration-dependent antibacterial effect and confirm that *Staphylococcus aureus* is more susceptible to garlic water extract than *Escherichia coli*. These results support the possible application of garlic extract as a natural antibacterial agent and, provide additional experimental evidence for its possible use in food preservation and complementary antimicrobial research. Evaluation of inhibition zone standards together with MIC and MBC values contributes useful reference data for future research investigating plant-derived antimicrobial compounds.

7. Conclusion

The in vitro antibacterial activity experiment and mechanism analysis of garlic aqueous extract conducted in this study confirmed that the aqueous extract of garlic (Allium sativum) exerts significant in vitro antibacterial activity against two clinically important pathogenic bacteria, *Staphylococcus aureus* ATCC25923 and *Escherichia coli* ATCC25922. Verified through three categories of experiments, namely the agar well diffusion method, minimum inhibitory concentration (MIC) assay and minimum bactericidal concentration (MBC) assay, this extract has both bacteriostatic and bactericidal effects, and its activity follows a concentration-dependent trend: the higher the extract concentration, the larger the inhibition zone and the stronger the antibacterial activity. Among the tested strains, *Staphylococcus aureus* shows higher sensitivity to the extract than *Escherichia coli*, which is manifested in a larger inhibition zone and lower MIC and MBC values. This difference is linked to variations in cell wall structure between Gram-positive and Gram-negative bacteria: the protective outer membrane of Gram-negative bacteria blocks the penetration of antibacterial substances. Garlic's core antibacterial activity originates from organosulfur compounds such as allicin, supplemented by phytochemicals including flavonoids, tannins, saponins and phenols, which can exert antibacterial effects through multiple mechanisms.

This study conducted experiments focused on the antibacterial activity of garlic (Allium sativum). The study's findings add to the continuously accumulating body of global scientific evidence supporting medicinal plants as alternative and supplementary antibacterial agents for addressing infectious diseases and antimicrobial resistance. Against the current global backdrop of rising numbers of multidrug-resistant pathogenic bacteria and declining efficacy of conventional antibiotics, this experiment found that while the antibacterial activity of garlic extract is weaker than that of ciprofloxacin, it exerts significant inhibitory effects on all two tested bacterial strains. It can be used as an adjuvant treatment option for conventional antibiotics to boost antibacterial efficacy and reduce the risk of drug resistance. Furthermore, garlic itself is easy to source, low-cost, and safe for long-term consumption, giving it the unique advantages of a natural therapeutic resource. This study still has limitations, however. Four core follow-up research tasks must be completed: purifying and characterizing specific bioactive compounds, assessing the synergistic effects between garlic extract and conventional antibiotics, evaluating cytotoxicity and pharmacokinetics, and carrying out in vivo and clinical studies. These steps will clarify its clinical applicability, highlight the value of Allium sativum as a natural source of antibacterial agents, respond to the global challenge of antimicrobial resistance, and call for advancing research into plant-derived antibacterial therapies.

Building on the core findings of this study on the antimicrobial activity of garlic extract, and in light of the severe current global challenge of antibiotic resistance, this study puts forward four sequentially progressing follow-up research directions to lay out a clear path for related basic research and clinical translation: First, evaluate the synergistic antimicrobial effect of combining garlic extract with conventional antibiotics, to verify whether this combination therapy can boost efficacy against drug-resistant bacteria and reduce required antibiotic dosages; Second, isolate, purify and characterize garlic-derived active substances centered on allicin, and assess their effects on multidrug-resistant pathogens including MRSA, extended-spectrum β-lactamase-producing *Escherichia coli*, and other such pathogens; Third, conduct in vivo experiments and human clinical

trials to clarify the safety, pharmacological efficacy, bioavailability and potential side effects of these substances; Finally, use molecular and genomic research to analyze the precise antibacterial mechanisms of garlic, including its impacts on bacterial gene expression and virulence factors, to ultimately develop a new plant-derived antimicrobial therapy to address the global challenge of drug resistance.

Declaration of Competing Interest

The authors declare that they have no known financial or non-financial competing interests in any material discussed in this paper

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