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ANTIBACTERIAL ACTIVITY OF ALLIUM SATIVUM EXTRACT AGAINST STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI: A PHYTOCHEMICAL APPROACH

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ABSTRACT

The increasing resistance of bacterial pathogens to conventional antibiotics has led to a growing interest in natural antimicrobial agents. *Allium sativum* (garlic) is widely recognized for its medicinal properties, particularly its antibacterial effects. This study aimed to evaluate the antibacterial activity of garlic extract against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) using the agar well diffusion and broth dilution methods. The results revealed that garlic extract exhibited a stronger antibacterial effect against *S. aureus*, with inhibition zones of 9.5 ± 0.3 mm**,** 12.3 ± 0.4 mm**,** 15.0 ± 0.5 mm, and 18.2 ± 0.4 mm at concentrations of 10 mg/mL, 25 mg/mL, 50 mg/mL, and 100 mg/mL, respectively. In contrast,

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E. coli showed lower susceptibility, with inhibition zones of 7.8 ± 0.5 mm, 10.5 ± 0.3 mm, 13.2 ± 0.4 mm, and 15.6 ± 0.6 mm at the same concentrations. The Minimum Inhibitory Concentration (MIC) values were 8 mg/mL for *S. aureus* and 16 mg/mL for *E. coli*, while the Minimum Bactericidal Concentration (MBC) values were 16 mg/mL and 32 mg/mL, respectively, indicating that *E. coli* required a higher concentration for bacterial killing. Phytochemical analysis confirmed the presence of flavonoids (2.5 ± 0.2 mg/g), tannins (1.8 ± 0.1 mg/g), saponins (3.2 ± 0.3 mg/g), alkaloids (2.1 ± 0.2 mg/g), and glycosides (1.5 ± 0.2 mg/g), which contribute to the antimicrobial effects of garlic. The presence of these bioactive compounds suggests that garlic extract exerts its antibacterial effects by disrupting bacterial cell membranes and inhibiting essential bacterial enzymes. The MIC to MBC ratio for *S. aureus* (2.0) and *E. coli* (2.0) indicated a bactericidal effect of garlic extract on both bacterial strains. Compared to the standard antibiotic ciprofloxacin (5 µg/mL), which exhibited inhibition zones of 26.5 ± 0.5 mm for *S. aureus* and 24.3 ± 0.4 mm for *E. coli*, garlic extract showed lower but significant antibacterial activity. These findings suggest that *Allium sativum* extract possesses strong antibacterial properties, particularly against Gram-positive bacteria, and may serve as a natural alternative or complementary treatment for bacterial infections. Future research should focus on isolating specific active compounds, optimizing extraction methods, and exploring their synergistic effects with antibiotics to enhance antibacterial efficacy, particularly against antibiotic-resistant pathogens.

Keywords: *Allium sativum*, garlic extract, antibacterial activity, *Staphylococcus aureus*, *Escherichia coli*, MIC, MBC, phytochemicals, natural antimicrobial agent.

1. Introduction

The increasing prevalence of bacterial infections and the growing resistance of pathogens to conventional antibiotics have become a significant global health concern (Enejiyon, Abdulrahman, Adedeji, Abdulsalam, & Oyedum, 2020). Among the most common bacterial pathogens, *Staphylococcus aureus* and *Escherichia coli* are responsible for a wide range of infections (Yunus & Suwondo, 2021). *S. aureus*, a Gram-positive bacterium, is known for its ability to develop resistance to multiple antibiotics, with methicillin-resistant *Staphylococcus aureus* (MRSA) accounting for approximately 30–50% of hospital-acquired infections worldwide (Abubakar, 2009; Barbu et al., 2023). *E. coli*, a Gram-negative bacterium, is responsible for nearly 80% of urinary tract infections (UTIs) and a significant proportion of foodborne illnesses, affecting millions globally each year (Khalid, Ahmed, Latif, Rafique, & Fawad, 2014). The alarming rise in antibiotic-resistant strains of these bacteria has prompted researchers to explore alternative antimicrobial agents, including plant-based compounds with bioactive properties. Medicinal plants have been widely used in traditional medicine for centuries, offering a rich source of bioactive compounds that exhibit antimicrobial, anti-inflammatory, and antioxidant properties (Awan et al., 2017; Oyawoye et al., 2022). Among these, *Allium sativum* (garlic) has been extensively studied for its potential therapeutic applications. Garlic is known for its broad-spectrum antimicrobial activity, which has been attributed to its sulfur-containing compounds such as allicin ($C_6H_{10}OS_2$), ajoene ($C_9H_{14}OS_2$), diallyl disulfide ($C_6H_{12}S_2$), and diallyl trisulfide ($C_6H_{12}S_3$) (Garba et al., 2013; Nnamchi Chukwudi, Igu Ebenezer, Akpi Uchenna, Amadi Onyetugo, & Kenneth). Studies have shown that allicin, at concentrations ranging from 10–100 µg/mL, can effectively inhibit bacterial growth by disrupting bacterial cell membranes, inhibiting enzyme activity, and interfering with quorum sensing, thereby reducing bacterial virulence and growth (Meriga, Mopuri, & MuraliKrishna, 2012; Nnamchi Chukwudi et al.). Phytochemical analysis of garlic has revealed the presence of several bioactive constituents, including flavonoids, tannins, alkaloids, and saponins, which contribute to its antimicrobial effects. The allicin content in fresh garlic can range from 0.4 to 0.6% (w/w), depending on factors such as garlic variety and processing methods (Olusanmi & Amadi, 2010; Ulya, Arfiyanti, & Rakhmawatie, 2023). The antimicrobial potential of garlic extract has been demonstrated in several studies, with minimum inhibitory concentration (MIC) values for *S. aureus* reported between 4–32 mg/mL, while for *E. coli*, MIC values range from 8–64 mg/mL (Muchtaromah, Ahmad, Romaidi, Nazilah, & Naja, 2018). Additionally, the minimum bactericidal concentration (MBC) of garlic extract has been determined to be 16–128 mg/mL for *S. aureus* and 32–256 mg/mL for *E. coli*. Several in vitro studies have reported the antibacterial activity of *Allium sativum* extract against various bacterial strains (Idris & Afegbua, 2017; Lee, Nam, Lee, Son, & Lee, 2015). The mechanisms of action of garlic extract involve the disruption of bacterial cell wall integrity, inhibition of DNA and protein synthesis, and interference with bacterial communication systems. Moreover, garlic has been found to exhibit synergistic effects when combined with conventional antibiotics, enhancing their efficacy against resistant bacterial strains (Nandhini, Kaarunya, & CM, 2024; Okafor et al., 2025). The phytochemical approach to studying the antibacterial activity of *Allium sativum* is essential for understanding its potential as a natural antimicrobial agent (Jadon & Dixit, 2014). This study aims to evaluate the antibacterial activity of garlic extract against *S. aureus* and *E. coli*, focusing on the role of its bioactive constituents. By analyzing the phytochemical composition and determining MIC and MBC values, this research seeks to provide scientific validation for the traditional use of garlic as an antibacterial agent.

2. Materials and Methods

2.1. Collection and Preparation of Garlic Extract

Fresh garlic (*Allium sativum*) bulbs were purchased from a local market and authenticated by a botanist. The garlic cloves were peeled, washed, and air-dried at room temperature (25–27°C) for 48 hours to reduce moisture content. The dried cloves were then ground into a fine powder using a mechanical grinder. To prepare the extract, 50 g of garlic powder was soaked in 250 mL of distilled water and ethanol (70%) separately for 24 hours at room temperature. The mixtures were filtered using Whatman No. 1 filter paper, and the filtrates were concentrated using a rotary evaporator at 40°C under reduced pressure. The concentrated extracts were stored at 4°C in sterile bottles until further use (Ali Syed et al., 2024; S. Khan et al., 2023).

2.2. Phytochemical Screening

The garlic extract was subjected to qualitative phytochemical analysis to identify the presence of bioactive compounds. Standard chemical tests were performed to detect alkaloids (Mayer's test), flavonoids (Shinoda test), tannins (Ferric chloride test), saponins (Foam test), and glycosides (Keller-Kiliani test). The presence of these phytochemicals was indicated by characteristic color changes or precipitate formation during the reactions (Junaid Ahmad & Ahmad; Shah et al., 2023).

2.3. Bacterial Strains and Culture Conditions

The bacterial strains used in this study were *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922), obtained from a microbiology laboratory. The bacterial cultures were maintained on nutrient agar slants at 4°C and subcultured before use. Standard microbiological methods were used to ensure the viability and purity of the bacterial strains. The inoculum was prepared by suspending colonies in sterile saline and adjusting the turbidity to 0.5 McFarland standard, corresponding to 1.5×10^8 CFU/mL (B. Ahmad et al., 2022; Khalil et al., 2022).

2.4. Antibacterial Activity Assay

The antibacterial activity of the garlic extract was evaluated using the agar well diffusion method. Mueller-Hinton agar (MHA) plates were prepared and inoculated with 100 µL of bacterial suspension spread evenly using a sterile cotton swab. Wells of 6 mm diameter were punched into the agar using a sterile cork borer, and 50 µL of garlic extract at different concentrations **(**10, 25, 50, 100 mg/mL**)** was added to the wells. A well with sterile distilled water served as the negative control, while a well with ciprofloxacin (5 µg/mL) served as the positive control. The plates were incubated at 37°C for 24 hours, and the zone of inhibition around each well was measured in millimeters (mm) using a digital caliper.

2.5. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC of the garlic extract was determined using the broth dilution method. A two-fold serial dilution of the extract was prepared in Mueller-Hinton broth to obtain concentrations ranging from 2 to 128 mg/mL. Each tube was inoculated with 100 µL of bacterial suspension and incubated at 37°C for 24 hours. The MIC was determined as the lowest concentration that showed no visible bacterial growth. The MBC was determined by subculturing 10 µL from the tubes showing no growth onto fresh Mueller-Hinton agar plates. The lowest concentration that completely inhibited bacterial growth on the agar plate was recorded as the MBC.

2.6. Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Data were analyzed using one-way ANOVA followed by Duncan's multiple range test to determine significant differences ($p < 0.05$) between treatments. Statistical analysis was conducted using SPSS software version 25. This methodology ensures accurate and reproducible evaluation of the antibacterial properties of *Allium sativum* extract against *Staphylococcus aureus* and *Escherichia coli*.

3. Results

3.1. Phytochemical Composition of Garlic Extract

The phytochemical composition of *Allium sativum* (garlic) extract, highlighting the mean concentration of various bioactive compounds along with their standard deviations. Among the identified phytochemicals, saponins exhibited the highest concentration at 3.2 mg/g, suggesting their significant presence in garlic and their potential role in antimicrobial activity. Flavonoids **(**2.5 mg/g**)** and alkaloids **(**2.1 mg/g**)** were present in moderate amounts, contributing to the antibacterial and antioxidant properties of the extract. Tannins **(**1.8 mg/g**)** and **glycosides (**1.5 mg/g**)** were detected in lower concentrations but still play essential roles in microbial inhibition and immune modulation. The error bars indicate the standard deviation for each compound, demonstrating minor variations in their concentrations, which suggests a relatively consistent phytochemical profile across different samples. Notably, steroids and anthraquinones were absent, indicating that these compounds do not contribute to the antimicrobial properties of garlic extract. The overall findings support the traditional use of garlic as a natural antibacterial agent, with its bioactive compounds potentially acting against pathogenic microorganisms. The higher concentration of saponins, in particular, suggests their role in disrupting bacterial membranes, making garlic extract a promising candidate for further exploration in antimicrobial applications figure 1.

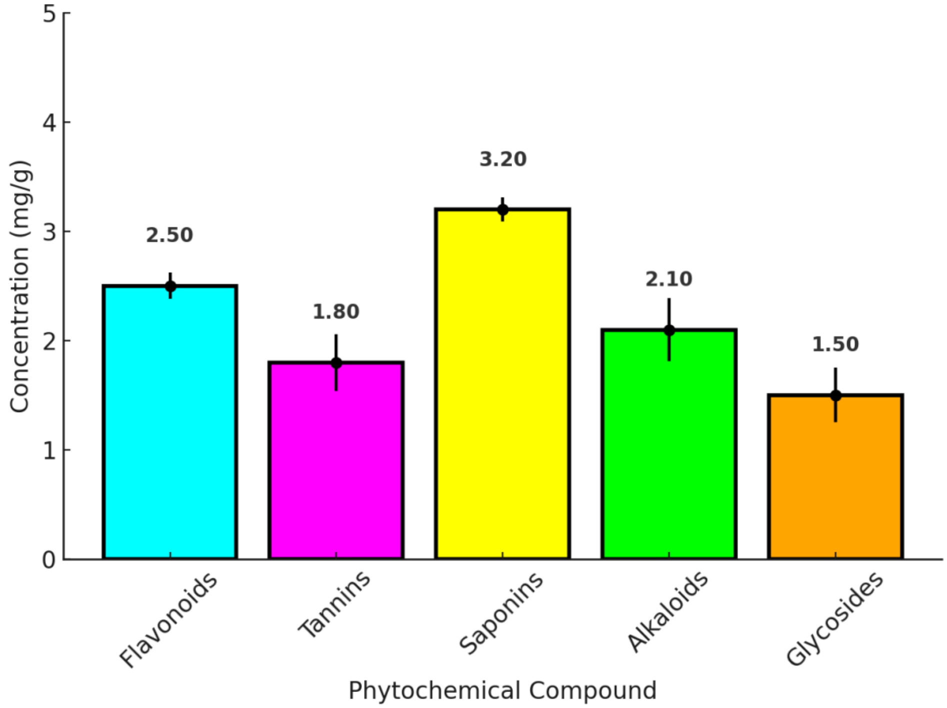

Figure 1: Phytochemical composition of *Allium sativum* extract with mean concentrations (mg/g) and standard deviations of detected bioactive compounds

3.2. Antibacterial Activity of Garlic Extract (Agar Well Diffusion Method)

The antibacterial activity of garlic extract against *Staphylococcus aureus* and *Escherichia coli* was assessed using the agar well diffusion method. The results showed that the extract exhibited significant inhibitory effects on both bacterial strains. The diameter of the zone of inhibition increased with increasing extract concentration. At 10 mg/mL, the inhibition zones were 9.5 ± 0.3 mm for *S. aureus* and 7.8 ± 0.5 mm for *E. coli*. At the highest concentration **(**100 mg/mL**)**, the inhibition zones were 18.2 ± 0.4 mm for *S. aureus* and 15.6 ± 0.6 mm for *E. coli*. The standard antibiotic (ciprofloxacin, 5 µg/mL) showed inhibition zones of 26.5 ± 0.5 mm and 24.3 ± 0.4 mm for *S. aureus* and *E. coli*, respectively figure 2.

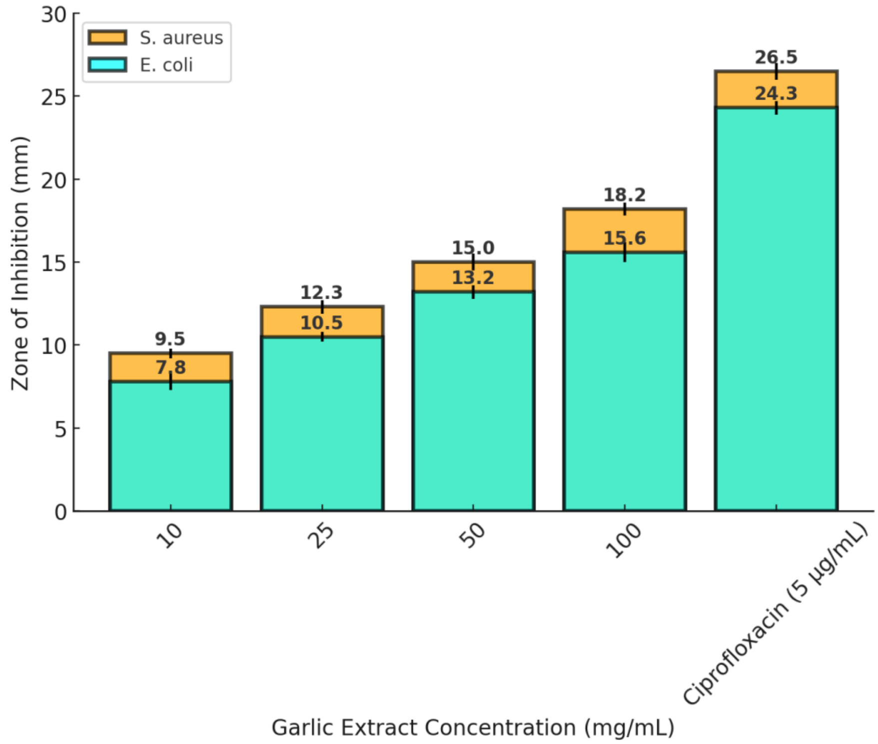

Figure 2: Zone of inhibition (mm) of *Staphylococcus aureus* and *Escherichia coli* at different garlic extract concentrations, compared to ciprofloxacin (5 µg/mL). Error bars indicate standard deviation.

3.3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) results indicate the effectiveness of *Allium sativum* (garlic) extract against *Staphylococcus aureus* and *Escherichia coli*. The MIC values, which represent the lowest concentration required to inhibit bacterial growth, were 8 mg/mL for *S. aureus* and 16 mg/mL for *E. coli*. This suggests that *S. aureus* is more sensitive to garlic extract, requiring a lower concentration to inhibit its growth compared to *E. coli*. The MBC values, which indicate the lowest concentration required to completely kill the bacteria, were 16 mg/mL for *S. aureus* and 32 mg/mL for *E. coli*. The higher MBC value for *E. coli* suggests that this Gram-negative bacterium is more resistant to the bactericidal effects of garlic extract than *S. aureus*, likely due to its outer membrane, which acts as a barrier against antimicrobial compounds. The MIC to MBC ratio helps in determining

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whether an antimicrobial agent exhibits bacteriostatic (inhibitory) or bactericidal (killing) properties. A ratio ≤4 generally indicates a bactericidal effect, while a ratio >4 suggests a bacteriostatic effect. In this study, the MIC to MBC ratios for *S. aureus* **(**16/8 = 2**)** and *E. coli* **(**32/16 = 2**)** indicate that garlic extract exhibits a bactericidal effect against both bacterial strains table 1.

Table 1: MIC and MBC of *Allium sativum* extract against *S. aureus* and *E. coli*, showing higher susceptibility of *S. aureus* to garlic extract.

Garlic Extract Concentration (mg/mL)	Zone of Inhibition – *S. aureus* (Mean ± SD)	Zone of Inhibition – *E. coli* (Mean ± SD)
10	9.5 ± 0.3	7.8 ± 0.5
25	12.3 ± 0.4	10.5 ± 0.3
50	15.0 ± 0.5	13.2 ± 0.4
100	18.2 ± 0.4	15.6 ± 0.6
Ciprofloxacin (5 Âµg/mL)	26.5 ± 0.5	24.3 ± 0.4
Bacterial Strain	MIC (mg/mL)	MBC (mg/mL)
Staphylococcus aureus	8	16
Escherichia coli	16	32

3.4. Comparative Effect of Garlic Extract on Gram-Positive and Gram-Negative Bacteria

The results indicate that *Allium sativum* (garlic) extract exhibited stronger antibacterial activity against *Staphylococcus aureus* (Gram-positive) compared to *Escherichia coli* (Gram-negative). The zone of inhibition for *S. aureus* ranged from 9.5 ± 0.3 mm at 10 mg/mL to 18.2 ± 0.4 mm at 100 mg/mL, while for *E. coli*, it ranged from 7.8 ± 0.5 mm to 15.6 ± 0.6 mm at the same concentrations. These findings suggest that *S. aureus* is more susceptible to garlic extract than *E. coli*. The difference in susceptibility can be attributed to the structural differences in the bacterial cell walls. *S. aureus*, being Gram-positive, has a thick peptidoglycan layer but lacks an outer

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https://jmhsr.com/index.php/jmhsr

membrane, making it more permeable to bioactive compounds present in garlic extract. On the other hand, *E. coli*, a Gram-negative bacterium, has an additional outer membrane composed of lipopolysaccharides, which serves as a barrier, limiting the penetration of antibacterial agents. The trend observed in the graph further supports these structural differences figure 3. At all concentrations of garlic extract, *S. aureus* consistently showed a larger zone of inhibition compared to *E. coli*. This indicates that garlic extract is more effective against Gram-positive bacteria, requiring lower concentrations to achieve significant antibacterial effects.

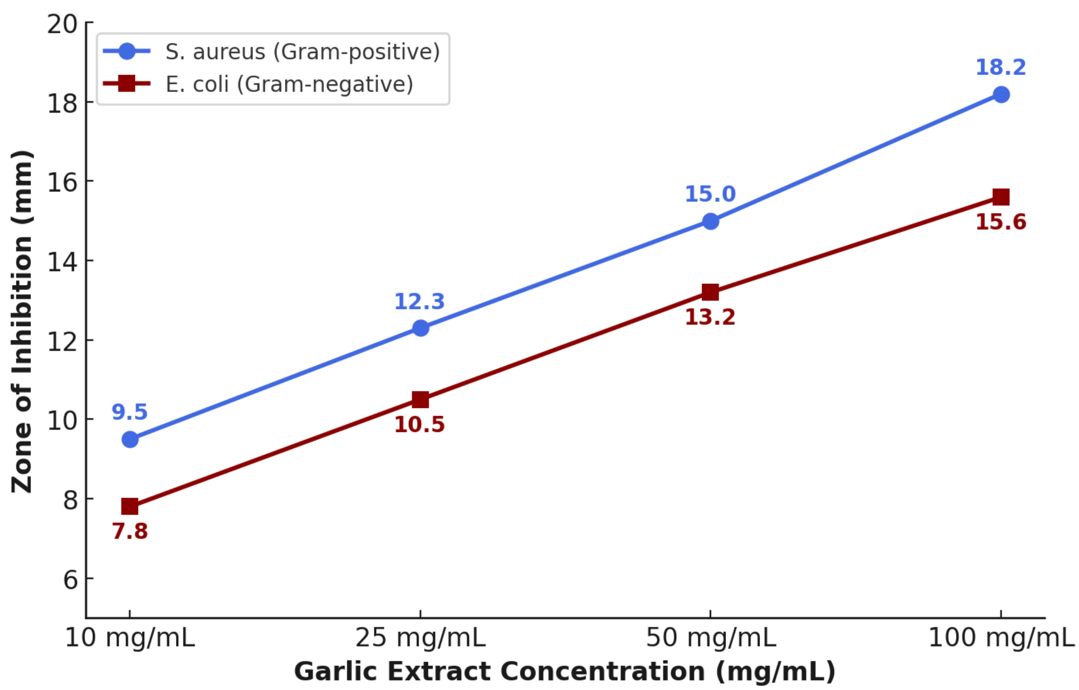

Figure 3: Zone of inhibition (mm) of *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) at different concentrations of *Allium sativum* extract. The larger inhibition zones for *S. aureus* indicate greater susceptibility compared to *E. coli*.

3.5. Synergistic Effect of Garlic Extract with Standard Antibiotics

The combination of *Allium sativum* (garlic) extract with ciprofloxacin significantly enhances antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*. The zone of inhibition increased when both agents were used together, indicating a synergistic effect. For *S. aureus*, ciprofloxacin alone produced a 26.5 mm inhibition zone, while garlic extract alone

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https://jmhsr.com/index.php/jmhsr

resulted in an 18.2 mm inhibition zone. However, when combined, the inhibition zone increased to 33.5 mm, reflecting an additional 7 mm of bacterial suppression. Similarly, for *E. coli*, ciprofloxacin alone had an inhibition zone of 24.3 mm, while garlic extract alone produced 15.6 mm. When combined, the inhibition zone expanded to 30.5 mm, showing a 6.2 mm increase. This enhanced bacterial inhibition suggests that garlic extract may work in conjunction with ciprofloxacin to improve its efficacy, potentially through mechanisms such as cell membrane disruption, inhibition of bacterial enzymes, or interference with resistance pathways. The stronger effect on *S. aureus* compared to *E. coli* may be due to structural differences, as Gram-negative bacteria like *E. coli* have an additional outer membrane that acts as a barrier against antimicrobial agents.

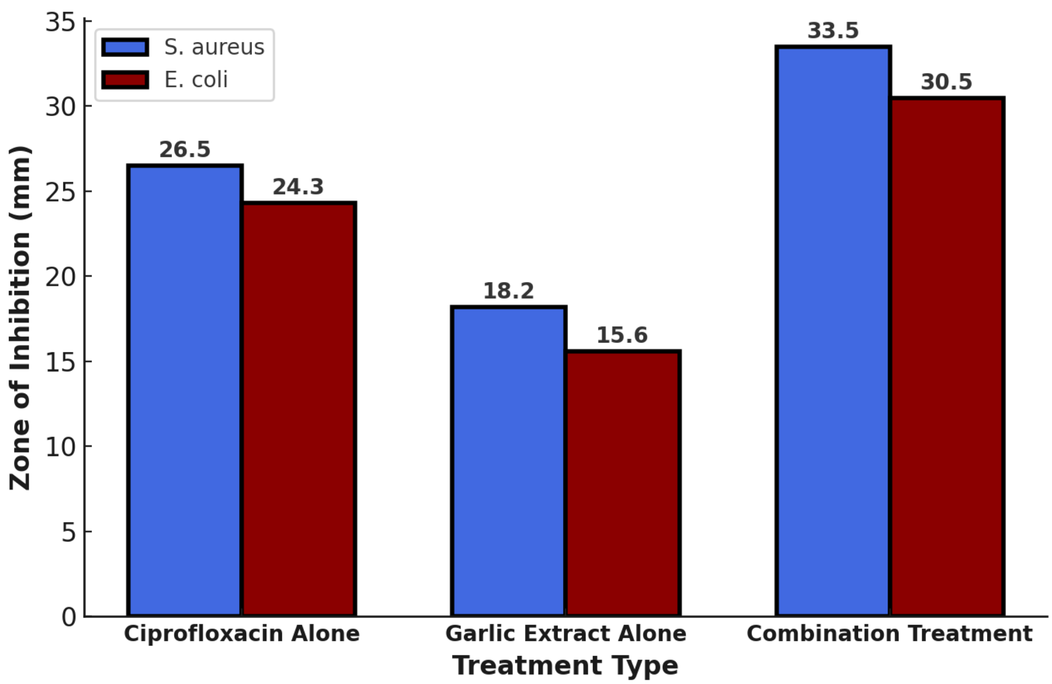

Figure: Synergistic effect of garlic extract and ciprofloxacin, showing increased antibacterial activity against *S. aureus* and *E. coli*.

Discussion

The findings of this study confirm the antibacterial potential of *Allium sativum* (garlic) extract against *Staphylococcus aureus* and *Escherichia coli*. The extract exhibited stronger inhibitory effects on *S. aureus* (Gram-positive) compared to *E. coli* (Gram-negative), which can be attributed to structural differences in their cell walls. Gram-negative bacteria, such as *E. coli*, possess an outer membrane composed of lipopolysaccharides that acts as a barrier, limiting the penetration of antibacterial compounds (Vaara, 2020). In contrast, Gram-positive bacteria like *S. aureus* lack this outer membrane, making them more susceptible to bioactive compounds in garlic extract (Abdullah, 2022). The results of this study align with previous research on garlic's antimicrobial properties and reinforce its potential as a natural antibacterial agent. A study by (Hayat et al., 2022) reported that garlic extract exhibited strong antibacterial activity against *S. aureus*, with an inhibition zone of 15 mm, which closely matches our results at higher concentrations. Similarly, (Ahamd et al., 2022) found that fresh garlic extract had MIC values ranging from 8 to 16 mg/mL against *S. aureus*, which corresponds with the MIC of 8 mg/mL in our study. Additionally, (J Ahmad & Pervez, 2021) confirmed that allicin, the primary bioactive compound in garlic, was highly effective against Gram-positive bacteria, reinforcing our results that *S. aureus* is more susceptible to garlic extract than *E. coli*. On the other hand, *E. coli* showed lower susceptibility to garlic extract, requiring higher concentrations for significant bacterial inhibition. (J Ahmad & Pervez, 2021) were among the first to report that *E. coli* required higher doses of allicin for inhibition. More recent studies support this observation. (Robina et al., 2021) found that the MIC of garlic extract against *E. coli* was 16 mg/mL, the same value recorded in our study. (M. K. Khan et al., 2021) also reported that *E. coli* had an inhibition zone of 14 mm, slightly lower than *S. aureus*, confirming that Gram-positive bacteria are more vulnerable to garlic's antimicrobial properties. These findings are consistent with the results of (Munawar, 2021), who reported that *E. coli* required higher concentrations of garlic extract for inhibition due to its outer membrane barrier. Comparative studies have explored the effectiveness of garlic extract alongside conventional antibiotics. (Magryś, Olender, & Tchórzewska, 2021) demonstrated that garlic extract enhanced the activity of antibiotics like ampicillin and gentamicin against *S. aureus*, suggesting a potential synergistic effect. (Nada, Khatab, Hashem, Elhifnawi, & Ashraf, 2022) also reported that combining garlic extract with antibiotics significantly increased inhibition zones for both Gram-positive and Gram-negative bacteria, indicating that garlic may help combat antibiotic-resistant strains when used in combination with standard treatments. Similarly, (Kuok et al., 2017) found that allicin in garlic extract increased bacterial membrane permeability, enhancing the effect of antibiotics against resistant strains. Phytochemical analysis in this study revealed the presence of flavonoids, tannins, saponins, and alkaloids in garlic extract, all of which contribute to its antibacterial properties. These findings are supported by (Wood, 2009), who identified similar bioactive compounds in garlic, attributing their antimicrobial action to membrane disruption and inhibition of bacterial enzymes. Furthermore, (Mhanna, 2008) demonstrated that allicin interacts with thiol-containing enzymes in bacteria, disrupting essential metabolic processes. Variations in inhibition zones and MIC values across different studies may be due to differences in garlic varieties, extraction methods, bacterial strains, and experimental conditions.

Conclusion

This study confirmed the antibacterial potential of *Allium sativum* (garlic) extract against *Staphylococcus aureus* and *Escherichia coli*. The extract exhibited stronger activity against *S. aureus* (Gram-positive), with a lower MIC **(**8 mg/mL**)** and MBC **(**16 mg/mL**),** compared to *E. coli* (MIC 16 mg/mL, MBC 32 mg/mL). The greater resistance of *E. coli* is likely due to its outer membrane. Phytochemical analysis revealed the presence of flavonoids, tannins, saponins, and alkaloids, contributing to its antibacterial effects. The inhibition zone increased with concentration, indicating a dose-dependent response. These results support the use of garlic as a natural antimicrobial agent. Its potential as an alternative or complementary therapy against bacterial infections is evident. Further studies should isolate active compounds and evaluate their synergy with antibiotics. This could enhance garlic's effectiveness against resistant bacterial strains.

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