

## Phytochemical Screening and Determination of Antibacterial Activity of Garlic Extracts against Clinical Isolates of *Staphylococcus aureus*

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**Abstract:** The growing threat of drug resistant bacteria, particularly *Staphylococcus aureus*, has become a pressing concern for global public health. Garlic (*Allium sativum* L.) widely used for culinary and medicinal purposes, is known for its broad spectrum antimicrobial activity, primarily due to the presence of allicin as its key bioactive compound. This study aimed to evaluate the phytochemical constituents and antibacterial activity of garlic extract against clinical isolates of *S. aureus*. Clinical isolates of *S. aureus* were obtained from Aminu Kano Teaching Hospital Kano, screened and confirmed using microbiological standard methods. Fresh garlic bulbs were processed and extracted using aqueous, methanol and ethanol solvents. Phytochemical screening of the extracts was carried out using standard qualitative methods to detect bioactive compounds. The antibacterial activity was assessed against clinical isolates of *S. aureus* using the agar disc diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by broth dilution techniques. Phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids, phenolics and terpenoids in both extracts, with higher intensity observed in the ethanol extract. The garlic extracts exhibited significant antibacterial activity against test *S. aureus*, producing concentration-dependent zones of inhibition. The ethanol extract demonstrated greater ( $25 \pm 1.5$  mm) antibacterial efficacy compared to the aqueous extract ( $20 \pm 1.5$  mm), with lower MIC and MBC values 6.25 mg/ml. Ciprofloxacin was used as control antibiotic and had a zone of inhibition between 17 mm-25 mm. There was no significant difference between diameter zones of inhibition of methanol, ethanol and aqueous extracts at  $P = 0.50$  and  $P = 0.97$  against the isolates. Garlic extract possesses potent antibacterial activity against clinical isolates of *S. aureus*, which could be attributed to its diverse phytochemical constituents. These findings support the potential use of garlic as a natural antimicrobial agent and provide a scientific basis for its traditional use in the management of *S. aureus* infections.

Key word: *Allium sativum*, ethanolic, methanolic, phytochemical, *Staphylococcus aureus*

### INTRODUCTION

Infectious diseases caused by *Staphylococcus aureus* remain a major public health challenge worldwide, particularly with the rise of antibiotic-resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Strika *et al.*, 2017). These strains are responsible for a wide spectrum of clinical infections ranging from superficial skin infections to life-threatening systemic diseases, and their increasing resistance to conventional antibiotics underscores the global need for alternative antimicrobial agents (Janan *et al.*, 2016). The World Health Organization (WHO) has identified antimicrobial resistance as one of the most urgent threats to public health, highlighting the importance of developing novel antibacterial compounds and therapeutic strategies (Abidullah *et al.*, 2021). The extensive and often inappropriate

use of antibiotics in both medical and agricultural settings has accelerated the development of resistance, highlighting the urgent need to search for alternative therapeutic agents (Indira *et al.*, 2024). Medicinal plants have long been explored as potential sources of bioactive compounds with antimicrobial properties. Among these, garlic (*Allium sativum* L.) is one of the most widely studied herbs due to its rich phytochemical profile and traditional use in treating infectious diseases (Sharifi-Rad *et al.*, 2021). Garlic contains a variety of sulfur-containing compounds most notably allicin; that are released when the plant tissue is crushed or macerated. Allicin has demonstrated potent antibacterial effects against a range of Gram-positive and Gram-negative bacteria by interfering with bacterial enzymes and compromising cell membrane integrity (Gabriel *et al.*, 2022).

Phytochemical screening is a fundamental step in ethnopharmacological research, aimed at identifying secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and steroids that contribute to the therapeutic potential of plant extracts. These phytoconstituents can exert antibacterial activity individually or synergistically, and their presence often correlates with the degree of antimicrobial efficacy observed *in vitro* (Sen *et al.*, 2025). Several qualitative and quantitative phytochemical investigations have reported the presence of these bioactive compounds in garlic extracts, supporting their relevance in antibacterial research (Abidullah *et al.*, 2021). Therapeutic use of garlic has been recognized as a potential medicinal value for thousands of years to different microorganisms. For instance; antifungal, antiviral, antibacterial, antiseptic and anti-inflammatory properties of garlic are well documented (Manu-Yadav *et al.*, 2019). Previous studies have indicated that garlic extracts can inhibit the growth of *S. aureus* and other clinically significant bacteria. For example, garlic extract exhibited inhibitory effects on clinical MRSA isolates, with ethanolic extracts showing particularly strong activity compared to aqueous preparations (Indira *et al.*, 2024). Despite these findings, variability in extraction methods, phytochemical profiles, and bacterial susceptibility calls for further research, especially using clinical isolates of *S. aureus* to better simulate real-world therapeutic challenges. Given the limitations of current antibiotic therapies and the potential of plant-derived compounds, this study aimed to conduct phytochemical screening and evaluate the *in vitro* antibacterial activity of garlic extract against clinical isolates of *S. aureus*. The outcomes will contribute to understanding the phytochemical basis of garlic's antibacterial properties and its potential application as an alternative or complementary antimicrobial agent. This study presents a novel contribution by providing a comparative evaluation of ethanol and aqueous extracts

of *Allium sativum* (garlic) against clinical isolates of *S. aureus* rather than laboratory reference strains. Unlike many previous studies that focus solely on zone of inhibition, this research integrates minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determinations, offering a more comprehensive assessment of antibacterial potency. The use of locally sourced garlic and clinically relevant isolates further enhances the applicability of the findings to real-world antimicrobial resistance challenges, particularly in resource-limited settings.

## MATERIALS AND METHODS

**Study Design:** This study was designed as a laboratory-based experimental study aimed at evaluating the phytochemical constituents and antibacterial activity of garlic extract against clinical isolates of *S. aureus*. The experimental design involved preparation of garlic extract, phytochemical screening using standard qualitative methods, and assessment of antibacterial activity using the agar disc diffusion method. The study employed a completely randomized design (CRD), where different concentrations of the extract were tested against the bacterial isolates under controlled laboratory conditions. All experiments were conducted in triplicate to ensure reproducibility and reliability of results.

**Sample Size Determination:** The sample size for this study was determined using Cochran's formula for estimating proportions in descriptive studies:

$$n = Z^2 p (1-p) / d^2 \text{ ----- equation i}$$

Where n = sample size, Z = Z value (1.96 for 95% confidence level), P = expected prevalence 0.5, d = margin of error (0.05).

Substituting the values n=384, since the total number of available clinical isolates in the study is small, finite population correction (FPC) was applied using the formula:

$$nf = n / 1 + n - 1 / N \text{ ----- equation ii}$$

Where, nf = adjusted sample size, n = initial calculated sample size (384), N = total number of available isolates.

**Collection of Clinical Isolates:** Clinical isolates of *S. aureus* were collected on nutrient agar from Aminu Kano Teaching Hospital, Kano, Nigeria and transported to the Department of Pharmaceutical Microbiology and Biotechnology, Bayero University, Kano. Isolates were collected from August, 2023 to July, 2024. The isolates were identified and confirmed following standard microbiological standards.

**Screening and Identification of *S. aureus*:**

Each bacterial isolate was subjected to growth on mannitol salt agar (MSA) as described previously, (Cheesbrough, 2020) incubated for 24-48 hours at 37°C. All the isolates were tested for production of coagulase both free and bound, catalase and DNase tests (Cheesbrough, 2020). Agglutination test was carried out to further identify the isolates. Staphytest plus latex slide agglutination test kit (Oxoid Ltd England) was used for differentiation of *S. aureus* by detection of clumping factor, protein A. The test was carried out according to manufacturer's instructions.

**Sample Collection and Preparation:** Fresh garlic bulbs (*Allium sativum* L.) were obtained from a local market in Birnin Kudu, Jigawa State, Nigeria. The plant material was verified by a taxonomist from the Department of Botany, Bayero University, Kano, Nigeria, and was given accession number BUKHAN 0297. The garlic bulbs were thoroughly washed to eliminate dirt and debris, and the outer covering of the garlic bulbs was carefully removed to expose the cloves. After cleaning, the cloves were sliced and air-dried for one week. The dried slices were then ground into a coarse powder using a sterile mortar and pestle.

**Garlic Extraction:** Three different solvents were used for the extraction. It was carried out using the method of Trease and Evans. A 20-gram portion of the powdered sample was soaked separately in 100ml of ethanol, methanol, and sterile water, with each mixture left to stand for 48- 72 hours with intermittent shaking. The extract was filtered

using Whatman No. 1 filter paper, and concentrated using rotary evaporation (BUCHI Rotavapor R-210, serial number. 2101234567) at a temperature range of 45°- 50°C. The concentrated (100%) juice was further diluted to 50%, 25%, 12.5% and 6.25% by mixing with appropriate sterile distilled water. The extracts were stored in a refrigerator at 4°C for antibacterial analysis (Sen *et al.*, 2025).

**Phytochemical Screenings of the Extracts:**

Qualitative phytochemical screening of the garlic extracts was carried out to test for the presence of alkaloids, saponins, tannins, steroids, flavonoids, phenolics and terpenoids using standard techniques.

**Test for Alkaloids:** The presence of alkaloid was determined using Dragendorff's and Wagners tests. Two gram of the dry extract was dissolve in 10ml of distilled water and then divided into two equal halves. Dragendorff's reagent was used to determine the presence of alkaloid by adding 1ml of dragendorff's reagent drop-wise to 5 ml of the extract solution above. A colour change to orange or reddish- brown which forms a precipitate on standing confirmed the presence of alkaloids. For Wagner's test, 1ml of Wagner's reagent was added drop-wise to 5ml of the solution formed; a dark-brown solution which formed a precipitate on standing confirmed the presence of alkaloids (Kumar *et al.*, 2022).

**Test for Saponins (Frothing Test):** One gram of the garlic dried extracts was dissolved in 10 ml distilled water in a test tube, and then shakes vigorously for 30 seconds and was allowed to stand in a vertical position which was observed over 30 minutes, the presence of saponin was confirmed by a honeycomb froth that persisted for 10-15 minutes. Addition of 3drops of olive oil followed by shaking to form emulsion further confirms saponin presence (Faruq *et al.*, 2024).

**Test for Tannins (Ferric Chloride Test):**

One gram of the extracts was dissolved in 10 ml distilled water and filtered, and then 10% ferric chloride solution was added. Appearance of blue- green or brownish

green colour precipitate confirmed the presence of tannins (Faruq *et al.*, 2024).

**Test for Steroids:** Chloroform (2 ml) was added to 2 ml of the extract solution and mixed gently, 1 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added to the down side of the test tube to form a lower layer, the colour change was observed immediately and after a period of one hour was compared with the colour change in the control test tube, reddish pink indicates the presence of steroids (Smith and Ali, 2025).

**Test for Terpenoids:** The dried extract was dissolved in chloroform, the test tube was held at an angle of 45° and 2 drops of concentrated sulphuric acid was added by the side of the test tube, colour change at the interface was observed immediately and after a period of one hour a cherry red or reddish brown color indicated the presence of terpenoids (Maheshwaran *et al.*, 2024).

**Test for Flavonoids (Alkaline Test):** Presence of flavonoid was determined when 1g of the dried extract was dissolved in 5ml of distilled water and then filtered, 10% sodium hydroxide was added to the filtrate in drop-wise. The presence of flavonoid was indicated by the appearance of yellow color (Maheshwaran *et al.*, 2024).

**Test for Phenolics:** Phenolic compounds were detected using the ferric chloride test. Two (2 ml) millilitre of the extract was treated with few drops of 5% FeCl<sub>3</sub> solution. Development of blue-green colouration indicated the presence of phenols (Adebayo *et al.*, 2021).

**Antibacterial Activity of Garlic against the Isolates:** Sterile blank filter paper discs (6 mm) were prepared using Whatman No.1 filter paper and punched using a sterile paper punch. The discs were sterilized by autoclaving at 121°C for 15 minutes and dried in a hot air oven at 50°C. After drying, the discs were impregnated with 10µl of extracts solutions at appropriate concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml) using a micropipette. The discs were allowed to dry under aseptic conditions to remove any residual solvent. The impregnated discs were

stored in sterile airtight container until needed (Balouri *et al.*, 2016).

**Standardization of Inoculum:** Pure colonies of the test bacteria were selected from the overnight culture and emulsified in sterile normal saline. The turbidity of the suspension was adjusted to match that of a 0.5 McFarland standard of Barium sulphate solution, which corresponds to approximately  $1.5 \times 10^8$  cfu/ml. This was done by visual comparison against the standard under adequate lighting. The standardized suspension was used within 30 minutes to ensure the viability and accuracy of the inoculum density (Cheesbrough, 2020).

**Determination of Antibacterial Activity of the Garlic Extracts:** The susceptibilities of the *S. aureus* isolates were determined using disc agar diffusion method (Modified Kirby Bauer) as described previously by (Cheesbrough, 2020). A sterile cotton swab was dipped into the standardized bacterial suspension and excess fluid was removed by pressing it against the inner wall of the tube. The swab was then used to streak the surface of a Mueller-Hinton Agar (MHA) plate evenly in three directions, rotating the plate approximately 60° between each streak to ensure uniform distribution of the inoculum. The plate was left to dry at room temperature for 5 minutes with the lid closed before applying the discs. Using sterile forceps, the impregnated discs and standard antibiotic disc (ciprofloxacin) were carefully placed on the surface of the inoculated MHA plates. Each disc was gently pressed to ensure complete contact with the agar. The plate was left at room temperature for 5 minutes to allow proper diffusion. The plates were then inverted and incubated at 37°C for 24 hours. After incubation, the diameters of the zones of inhibition around each disc were measured in millimeters using a ruler (CLSI 2023). All experiments were performed in biological triplicate

**Minimum Inhibitory Concentration (MIC) of the Garlic Extracts:** The MIC of the extract was determined using broth dilution method following (CLSI, 2023) standard. To

each of the five test tubes containing 1ml Mueller-Hinton broth, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL concentrations of the extract were added. And the test organism was inoculated into the tubes. A positive control (broth + inoculum+ standard antibiotic) and a negative control (broth only) were included. The procedure was repeated for all extracts. The MIC was the lowest concentration of the garlic extracts that inhibited growth after 24 hours incubation at 37°C. Test was performed in triplicate for accuracy.

**Minimum Bactericidal Concentration (MBC) of the Garlic Extracts:** The MBC was determined by subculturing samples from the MIC positive tubes. Aliquots (10-100 µl) were taken from tubes showing no visible growth and inoculated onto fresh Mueller-Hinton agar plates without the test agent. The plates were incubated at 37°C for 18-24 hours. The MBC was defined as the lowest concentration of the test agent that resulted in no bacterial growth, indicating a  $\geq 99.9\%$  reduction in the initial bacterial population (CLSI, 2023).

**Statistical Analysis of the Data Obtained:** All experiments were conducted in biological triplicates using three independent bacterial isolates, and the results were expressed as mean  $\pm$  standard deviation. The diameters of zones of inhibition obtained for different concentrations of garlic extract (100, 75, 50, 25, 12.5, and 6.25 mg/mL) were statistically analyzed using one-way analysis of variance (ANOVA) to determine significant differences among treatment groups. Post hoc multiple comparisons were conducted using Tukey's Honestly Significant Difference (HSD) test to identify pairwise differences between concentrations. A p-value of less than 0.05 ( $p < 0.05$ ) was considered statistically significant. All

statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 25.0.

## RESULTS

The percentage extraction yield of the garlic powder obtained is depicted in table one below. Thirty of the isolates were found to be Gram positive, catalase positive, coagulase and DNase positive and have the ability to ferment mannitol and produced protein A using the Staphytect plus latex slide agglutination test kit (Oxoid Ltd England). The findings of the phytochemical constituents of aqueous and ethanolic extract are shown in Table 2. All the seven parameters examined were present in both extracts except steroids which are absent in aqueous extract. Methanolic extract was not examined. Their presence suggests strong antimicrobial potential. The findings of antibacterial activity of *Allium sativum* L. revealed that at 100 mg/ml concentration all the isolates were inhibited, while some of the isolates were inhibited at 50 mg/ml, 25 mg/ml and 12.5 mg/ml and there was no activity against all the isolates at 6.25 mg/ml. The activity increases as the concentration increases. The findings from this study showed that the ethanolic extract exhibited a varying degree of activity against the isolates  $25 \pm 1.5$  at 100% concentration. The ethanolic and methanolic extract of garlic demonstrated the highest antibacterial activity, with MIC of 6.25 mg/mL against all the *S. aureus* isolates, while the aqueous extract exhibited the least potency, requiring higher concentrations for inhibition. The variation in MIC values among isolates indicates differences in susceptibility, possibly due to strain variability and resistance mechanisms. MBC value was found to be 12.5 mg/ml.

**Table 1: Extraction Yield of the Garlic Powder**

| Solvent  | Garlic Powder(g) | Extract Yield (g) | Percentage Yield (%) |
|----------|------------------|-------------------|----------------------|
| Aqueous  | 20               | 0.23              | 1.15                 |
| Methanol | 20               | 0.54              | 2.7                  |
| Ethanol  | 20               | 5.44              | 27.2                 |

**Table 2: Qualitative Phytochemical Constituents of Ethanolic and Aqueous Extract of *Allium sativum* L. (Garlic)**

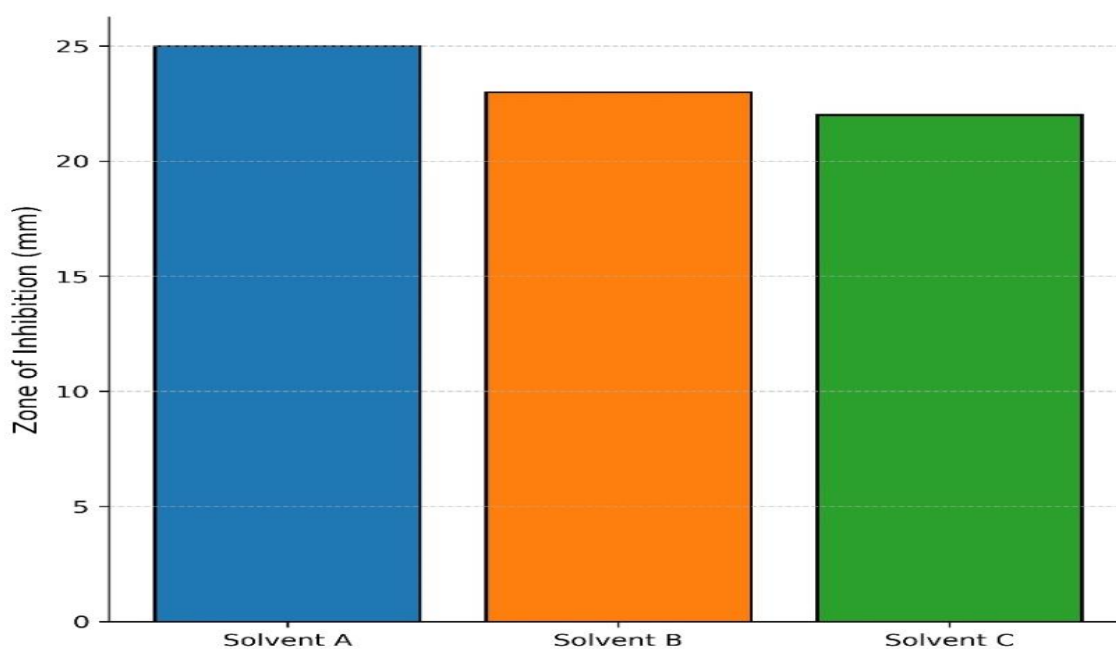
| Extract   | Phytochemical Constituents |         |          |            |          |           |           |
|-----------|----------------------------|---------|----------|------------|----------|-----------|-----------|
|           | Alkaloids                  | Tannins | Saponins | Terpenoids | Steroids | Flavonoid | Phenolics |
| Ethanolic | +                          | +       | +        | +          | -        | +         | +         |
| Aqueous   | +                          | +       | +        | +          | -        | +         | +         |

**Table 3: Mean Zones of Inhibition of Extracts of *Allium sativum* against *S. aureus***

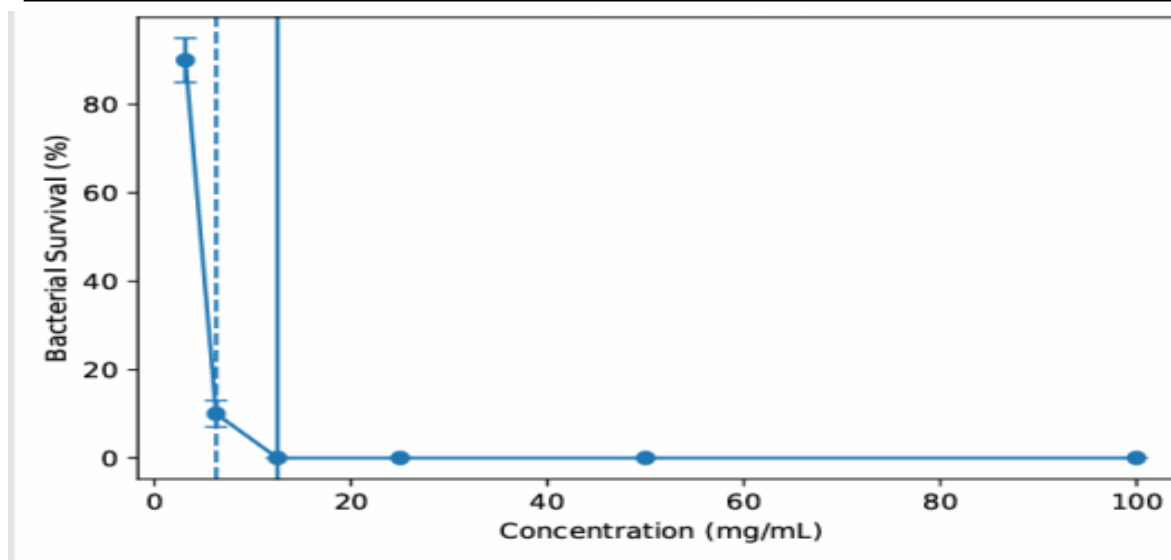
| Concentration (mg/ml) | Mean zone (mm) | Number of isolates inhibited (%) |          |          | p-value       |
|-----------------------|----------------|----------------------------------|----------|----------|---------------|
|                       |                | Ethanol                          | Methanol | Aqueous  |               |
| 100                   | 25± 1.5        | 30 (100%)                        | 30(100%) | 30(100%) | 0.50 and 0.97 |
| 50                    | 23± 1.5        | 25(83.3)                         | 25(83.3) | 23(76.7) |               |
| 25                    | 22± 1.5        | 25(83.3)                         | 25(83.3) | 21(70)   |               |
| 12.5                  | 11± 0.9        | 20(66.7)                         | 18(60)   | 15(50)   |               |
| 6.25                  | No zone        | Nil                              | Nil      | Nil      |               |

**Table 4: Minimum Inhibitory Concentration of Garlic (Ethanolic) Extract against *S. aureus***

| Concentration (mg/ml) | No of isolates | % of isolates | MIC (mg/ml) |
|-----------------------|----------------|---------------|-------------|
| 100                   | 30             | 100           | 6.25        |
| 50                    | 30             | 100           | 6.25        |
| 25                    | 30             | 100           | 6.25        |
| 12.5                  | 23             | 76.7          | 12.5        |
| 6.25                  | 30             | 100           | 6.25        |

**Figure 1: Antibacterial activity of solvent extract**

**Keys:** Solvent A = Ethanolic extract, Solvent B = Methanolic, Solvent C= Aqueous. Solvent A exhibited the highest antibacterial activity followed by solventB and solvent. Values represent observed diameter zones of inhibition against tested bacterial isolate



**Figure 2: MIC/MBC curve of garlic extract against *S. aureus***

**Keys: y- axis: survival, x- axis: concentration (mg/ ml)**

## DISCUSSION

The present study evaluated the phytochemical composition and antibacterial activity of garlic (*Allium sativum* L.) extract against clinical isolates of *S. aureus*. The findings demonstrated that garlic extract possesses significant antibacterial activity, supporting its long-standing use in traditional medicine as a natural antimicrobial agent (Alam *et al.*, 2018). The phytochemical findings confirmed the presence of bioactive compounds known for antimicrobial properties. Flavonoids and phenolic compounds disrupt bacterial cell walls and inhibit enzyme activity (Mandal *et al.*, 2019). Phytochemical screening revealed the presence of bioactive compounds including alkaloids, flavonoids, tannins, saponins, and phenolic compounds. Similar compounds were reported by Kowalska *et al.*, (2022). These secondary metabolites are known to contribute to antimicrobial activity through different mechanisms such as disruption of microbial cell membranes, inhibition of enzymatic activity, and interference with nucleic acid synthesis (El-Saadony *et al.*, 2024). Similar phytochemical profiles have been reported in garlic extracts by Batiha *et al.*, (2020), who identified sulfur-containing compounds as major contributors to garlic's pharmacological

effects. Additionally, the presence of various phytochemical constituents may act alongside allicin to boost garlic's antibacterial impact (Mandal *et al.*, 2019). This complex mixture of bioactive compounds underscores garlic's promise as a potent natural agent for use in complementary or integrative antimicrobial therapies. The detection of phenolic compounds and flavonoids is particularly significant, as these compounds have been reported to disrupt bacterial cell walls and membranes, leading to increased permeability and leakage of intracellular contents (El-Saadony *et al.*, 2024). This mechanism is consistent with the observed susceptibility of *S. aureus*, a Gram-positive organism whose thick peptidoglycan layer is a known target for phenolic-rich plant extracts (Aremu and Bairu, 2024). Alkaloids and tannins identified in the extract may also play important roles in antibacterial activity. Alkaloids are known to interfere with DNA replication and protein synthesis, while tannins exert antimicrobial effects through enzyme inhibition and metal ion chelation (Batiha *et al.*, 2020). The presence of saponins further enhances antibacterial potential by increasing membrane permeability, which may facilitate the entry of other active compounds into bacterial cell

(Sen *et al.*, 2025). This study further substantiates garlic's extensive antibacterial capabilities, particularly in combating *S. aureus*, a highly resilient and virulent pathogen. The dose-dependent increase in zones of inhibition observed with higher concentrations of garlic extract supports earlier findings (Manu-Yadav *et al.*, 2019). This also concurred with findings reported by Sharifi-Rad *et al.*, (2021), who demonstrated that garlic extracts exhibit strong inhibitory effects against Gram-positive bacteria. The susceptibility of *S. aureus* may be attributed to the action of allicin, the principal active compound formed when garlic cloves are crushed (Borlinghaus *et al.*, 2021). Allicin reacts with thiol-containing enzymes in bacterial cells, disrupts critical bacterial functions like RNA synthesis and protein biosynthesis leading to metabolic inhibition and eventual cell death (Gruhke and Slusarenko, 2021). The observed minimum inhibitory concentration (MIC) values indicate that garlic extract possesses bacteriostatic potential and bactericidal effects at relatively low concentrations comparable to some conventional antibiotics. The MIC also demonstrates the concentration-dependent antibacterial efficacy of *Allium sativum* (garlic) extract against the tested bacterial isolates. The MIC represents the lowest concentration capable of preventing visible microbial growth and is widely accepted as a reliable quantitative indicator of antimicrobial potency (CLSI, 2023). The observed inhibitory activity of garlic extract may be attributed to its bioactive compounds, particularly allicin, and other thiosulfinates, which interfere with essential bacterial metabolic enzymes and sulfhydryl-containing proteins, thereby inhibiting cell growth and replication (Iloputaife *et al.*, 2023). Recent studies have similarly reported low MIC values for garlic extracts against pathogenic bacteria, confirming strong antibacterial potential. Comparable findings were reported where crude garlic extracts exhibited MIC values as low as 0.325–0.625 mg/ml against multidrug-

resistant bacterial pathogens, highlighting garlic's effectiveness even against resistant strains (Al-Noman *et al.*, 2023). The antimicrobial action is primarily linked to allicin's ability to disrupt cellular enzymatic systems through interaction with cysteine residues, leading to inhibition of bacterial metabolism and growth. The variations in MIC values observed across studies may result from differences in extraction solvent, phytochemical composition, bacterial strain susceptibility, and experimental conditions (El-Saadony *et al.*, 2024). Therefore, the MIC values obtained in this study support the growing evidence that garlic extract possesses significant antibacterial activity and may serve as a promising natural alternative or adjunct to conventional antimicrobial agents, particularly in the management of infections caused by antibiotic-resistant microorganisms (Indira *et al.*, 2024). The pronounced antibacterial activity of the ethanolic extract is likely due to ethanol's higher ability to extract allicin and other sulfur-containing bioactive molecules more efficiently (Kowalska *et al.*, 2022). This suggests that the effect of concentration on zone of inhibition is not uniform across different solvents; some solvents enhance the extract's effectiveness more at higher concentrations (Mikaili *et al.*, 2022). This could be attributed to the polarity and extraction efficiency of the solvents for key bioactive compounds like allicin (Janan *et al.*, 2016). Although methanolic and aqueous extracts also demonstrated inhibitory effects, their comparatively lower efficacy suggests that the type of solvent used influences the availability and potency of active compounds (Alam *et al.*, 2018). Ethanol and aqueous extracts, in particular, have been shown to differ in their ability to extract phenolics and organosulfur compounds, which directly influences antibacterial potency (Abiy and Berhe, 2016). The effectiveness of garlic against clinical isolates suggests its possible role in combating antibiotic-resistant strains of *S. aureus*, including methicillin-resistant *S.*

*aureus* (MRSA) (Indira *et al.*, 2024). The lack of inhibition in some isolates may be attributed to phenotypic variations in *S. aureus* strains, potentially involving biofilm formation or efflux pumps that diminish allicin uptake (Mandal *et al.*, 2019). Prior research also indicates that garlic may have synergistic effects when combined with standard antibiotics, enhancing their efficacy and potentially reversing resistance (Rahma *et al.*, 2024). Furthermore, the antibacterial activity observed in this study supports the increasing interest in plant-based antimicrobials as alternatives to synthetic antibiotics. The rise of antimicrobial resistance has necessitated exploration of natural products with fewer side effects and lower resistance development (Shang *et al.*, 2019). According to World Health Organization reports, antimicrobial resistance remains a major global health threat, emphasizing the importance of investigating medicinal plants such as garlic (WHO, 2023). Also variations in antibacterial activity compared with other studies may be due to differences in extraction solvents, garlic cultivar, environmental conditions, and isolate variability. Chinedu, (2019) noted that extraction method significantly influences the stability of allicin and consequently antimicrobial efficacy. Despite promising findings, this study has some limitations.

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- The investigation was conducted *in vitro*, which may not fully reflect *in vivo* conditions. Factors such as bioavailability, metabolism, and host immune response can influence therapeutic effectiveness. Therefore, further studies involving purification of active compounds, toxicity assessment, and clinical trials are recommended. Overall, the findings confirm that garlic extract possesses significant phytochemical constituents and antibacterial properties against clinical isolates of *S. aureus*. These findings support its potential application as a complementary antimicrobial agent and justify further pharmaceutical development.
- ## CONCLUSION
- It was concluded that *Allium sativum* (garlic) extract possesses substantial phytochemical diversity and pronounced antibacterial activity against clinical isolates of *S. aureus*. The collective action of these compounds likely underpins the observed antibacterial effects through various mechanisms. These findings reinforce the growing evidence supporting plant-derived antimicrobials as viable alternatives or adjuncts to conventional antibiotics, particularly in the context of increasing antimicrobial resistance among pathogenic bacteria.
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