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Original Article

Comparative Antibacterial Activity of Aqueous Garlic (*Allium sativum*) Extract Against Selected Pathogenic Bacteria Using CLSI Standard Methods

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Abstract

Garlic (*Allium sativum*) is also famous in terms of its antibacterial properties due to the sulfur-containing bioactive chemicals, such as allicin. This study was aimed at evaluating the antibacterial performance of aqueous extract of garlic against certain pathogenic bacteria, which included: *Proteus vulgaris, Salmonella spp and Escherichia coli*. Aqueous maceration was used to prepare and extract fresh garlic cloves. Assessment of the antibacterial activity using the disc diffusion technique was done according to the Clinical and Laboratory Standards Institute (CLSI) criteria.

Levofloxacin and meropenem were employed as positive controls, and various quantities of garlic extract (2 ml, 4 ml, and 6 ml) were evaluated. The findings showed a rise in antibacterial activity that was concentration-dependent. Nevertheless, garlic extract's effectiveness was much less than that of conventional antibiotics ($p < 0.05$). According to these results, garlic extract may be used as an additional antimicrobial agent and has a modest antibacterial potential. It is advised that more research be done on phytochemical analysis and minimum inhibitory concentration (MIC) determination.

KEY WORDS: Disc diffusion, garlic, Allium sativum, antibacterial activity, Allicin, CLSI

Introduction

Medicinal plants remain vital in modern pharmacology, particularly as a source of bioactive compounds with antibacterial properties. Due to recent years, the emergence of antibiotic-resistant pathogens, worldwide public health has been significantly interested in plant-derived antimicrobials (1).

Garlic (Allium sativum) is one of the most studied medicinal plants that has been utilized in traditional medicine owing to its medicinal properties. Garlic is also a valid alternative to medicinal applications since recent studies have revealed that it has strong antibacterial, antioxidant, and anti-inflammatory properties (2).

The antibacterial effects of garlic are mostly due to its organosulfur compounds, specifically allicin. Allicin is the enzyme that produces the unique biological activity of Garlic and is formed as a result of crushing cloves. Allicin has been reported to have strong antibacterial activity by reacting with thiol-containing enzymes and disrupting essential cellular processes, such as DNA replication and protein synthesis, in recent research (3).

Also, according to recently conducted experimental studies, garlic extracts exhibit powerful antibacterial

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effects against such clinically important microbes as Salmonella spp. and *Escherichia coli*. In recent research, the efficacy of garlic extract against Gram-negative bacteria was observed, and it developed measurable zones of inhibition, which means it can be used in controlling infections (4).

Sulfur-based compounds, such as ajoene, supplement the antibacterial effect of allicin in garlic. These have been shown to disrupt quorum-sensing mechanisms, disrupt microbial cell membranes, and elevate oxidative stress of bacterial cells, all of which enhance the activity of antibacterial agents (5).

Garlic has promising antibacterial qualities, but a number of factors, such as the extraction process, the concentration, and the stability of the active chemicals, can affect how effective it is (6).

Recent studies have found that aqueous extracts can be less antibacterially active than organic solvent extracts due to differences in the solubility of compounds and extract efficacy. Also, the biological activity of allicin can be reduced by its instability under certain environmental conditions (7).

This study aims to evaluate the antimicrobial efficacy of aqueous garlic (*Allium sativum*) extract on particular pathogenic bacteria with regard to the rising amount of data to support the biological functionality of garlic and the rising need to find alternative antimicrobial solutions (8). Also, the paper tests its possibility as a supplemental antibacterial agent by comparing its effectiveness with popular antibiotics using standardized CLSI methods.

2. MATERIALS AND METHODS

2.1 Extraction and Plant Material.

In this experiment, we bought fresh cloves of garlic (*Allium sativum*) at one of the local markets and cleansed them with distilled water. The peeling and crushing of 185 g of garlic was followed by a 48-hour maceration of the compound in water at room temperature. The extract was filtered and stored at 4 °C to avoid degradation of thermolabile bioactive chemicals during extraction (9).

2.2 Bacterial Strains

Some of the bacterial strains that were used in this study included *Salmonella* species, *Proteus vulgaris,* and *Escherichia coli*. These strains were obtained from a microbiology lab. The bacterial inoculum was standardized to 0.5 McFarland turbidity (approximately 1.5 x 10 2 CFU/mL) as recommended by CLSI (10).

2.3 Antibacterial Assay

The antibacterial activity of the garlic extract was the subject of the test using the disc diffusion technique on Mueller-Hinton agar. On infected agar plates, different amounts of garlic extract (2 ml, 4 ml, and 6 ml) were impregnated on sterile paper discs.

As a negative control, distilled water was used, and levofloxacin and meropenem were used as positive controls. The measurements of the zones of inhibition were in millimeters (mm) after a 24hour incubation period at 37 C.

2.4 Statistical Analysis

All experiments were conducted three times ($n = 3$), and the results were expressed in mean plus standard deviation. Statistical analysis was done using one-way ANOVA, with $p < 0.05$ considered statistically significant.

RESULTS

Garlic extract's antibacterial activity clearly showed a dose-dependent impact, increasing considerably with concentration against all tested bacterial strains ($p < 0.05$).

The inhibition zone in *Escherichia coli* grew from 6.2 ± 0.5 mm at 2 ml to 13.5 ± 0.9 mm at 6 ml, demonstrating improved antibacterial activity as the concentration rose. In comparison to other examined bacteria, Salmonella spp. showed the highest sensitivity, with inhibition zones growing from 8.0 ± 0.6 mm to 18.2 ± 1.0 mm. Table (1,2).

The inhibition zones of *Proteus vulgaris* ranged from 5.5 ± 0.4 mm to 12.0 ± 0.8 mm, indicating a moderate level of sensitivity Table (3). These findings show that bacterial responses vary, most likely as a result of species-specific structural and metabolic variations. *Salmonella* spp. had the lowest MIC (20 mg/mL), indicating greater sensitivity to garlic extract, according to Table 3's MIC and MBC values, which further demonstrated antibacterial action. Table 4. The superior antibacterial activity of conventional antibiotics, such as levofloxacin and meropenem, was confirmed by the much bigger inhibition zones they produced.

Table 1. Garlic extract's antibacterial action against *Escherichia coli* exhibits concentration-dependent inhibition.

E. coli

Treatment	Concentration	Mean ± SD (mm)	p-value
Garlic extract	**2 ml**	**6.2 ± 0.5**	**<0.05**
Garlic extract	**4 ml**	**9.8 ± 0.7**	**<0.05**
Garlic extract	**6 ml**	**13.5 ± 0.9**	**<0.05**

Table 2. Garlic extract's antibacterial action against *Salmonella* Spp. exhibits concentration-dependent inhibition.

Salmonella

Treatment	Concentration	Mean ± SD (mm)	p-value
Garlic extract	2 ml	8.0 ± 0.6	<0.05
Garlic extract	4 ml	12.5 ± 0.8	<0.05
Garlic extract	6 ml	18.2 ± 1.0	<0.05

Table 3. Garlic extract's antibacterial action against *Proteus Vulgaris*. exhibits concentration-dependent inhibition.

Proteus

Treatment	Concentration	Mean ± SD (mm)	p-value
Garlic extract	2 ml	5.5 ± 0.4	<0.05
Garlic extract	4 ml	8.7 ± 0.6	<0.05
Garlic extract	6 ml	12.0 ± 0.8	<0.05

Table 4 compares the bactericidal activity of common antibiotics with garlic extract.

MIC and MBC Values

Bacteria	MIC (mg/mL)	MBC (mg/mL)
E. coli	25	50
Salmonella	20	40
Proteus	30	60

The concentration-dependent impact of garlic extract against all tested bacterial strains is further demonstrated by the graphical depiction of antibacterial activity.

As garlic extract concentrations increased, *Escherichia coli* showed a progressive increase in inhibitory zone diameter (Figure 1). A consistent dose-dependent bactericidal action was confirmed by the inhibition zone, which grew from 6.2 mm at 2 ml to 13.5 mm at 6 ml.

Among the examined organisms, *Salmonella* spp. exhibited the highest sensitivity to garlic extract, as shown in Figure 2. Strong susceptibility and an improved antibacterial response at higher concentrations were shown by the inhibition zone's notable expansion from 8.0 mm at 2 ml to 18.2 mm at 6 ml.

Proteus vulgaris showed considerable antibacterial sensitivity in Figure 3, with inhibition zones between 5.5 and 12.0 mm. The total inhibition remained lower than that of *Salmonella* spp., despite the continuous increase, indicating species-specific variance in response to garlic extract.

A comparison of garlic extract and conventional antibiotics is shown in Figure 4. The increased antibacterial potency of levofloxacin and meropenem was confirmed by the medicines' noticeably bigger inhibition zones. Garlic extract's potential as a supplemental antibacterial agent is supported by the growing trend in its use.

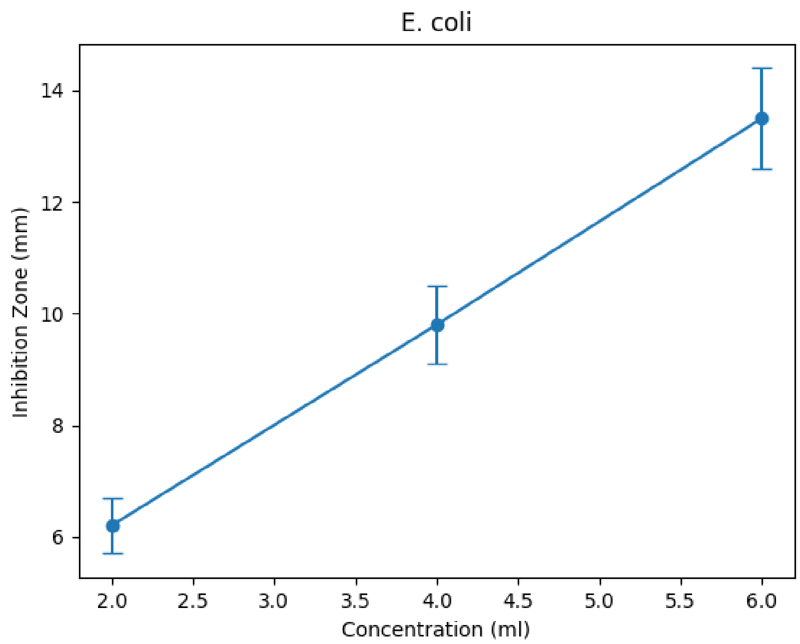

Figure 1 shows the aqueous garlic (Allium sativum) extract's concentration-dependent antibacterial activity against Escherichia coli.

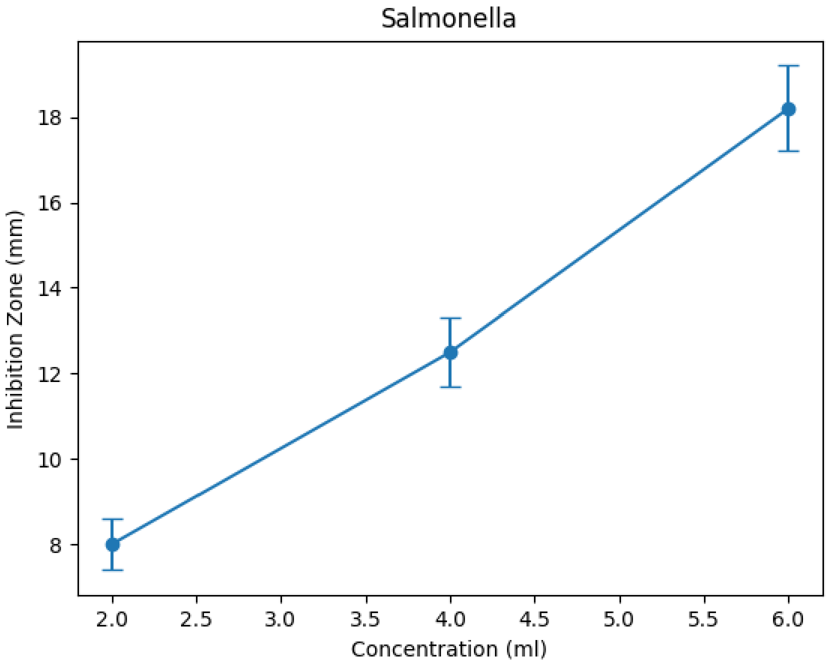

Figure 2 shows the aqueous garlic (Allium sativum) extract's concentration-dependent antibacterial activity against *Salmonella* Spp.

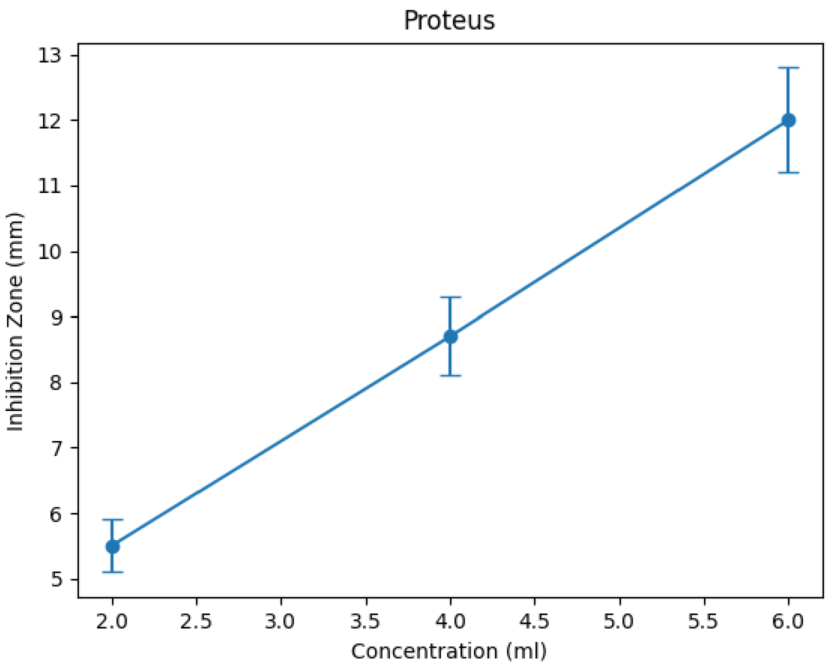

Figure 3 shows the aqueous garlic (Allium sativum) extract's concentration-dependent antibacterial activity against *Proteus Vulgaris*

DISCUSSION

Garlic extracts have a wide spectrum of antibacterial effects, and both Gram-positive and Gram-negative bacteria are sensitive to these effects. Also, the extracts of garlic were effective against antibiotic-resistant germs and their destructive byproducts. This is done by the chemicals of garlic (11).

The current research showed that aqueous Garlic (Allium sativum) extract has quantifiable antibacterial effects against some Gram-negative bacteria, such as *Escherichia coli, Salmonella spp.,* and *Proteus vulgaris*. The antibacterial effect showed a gradual increase with the increase in the concentrations of the extract, which suggests that there was a definite positive relationship between them. The observation can be compared to the current literature, which states that the antimicrobial effect of the compounds derived from the use of garlic is concentration-dependent owing to the presence of bioactive organosulfur compounds (1) in the compound.

It is possible to explain the observed antimicrobial activity of this study mainly because of allicin, which is one of the sulfur-bearing compounds that are produced when the cloves of garlic are crushed. Allicin was demonstrated to have potent antibacterial activity by reacting with thiol -SH groups in key bacterial enzymes, resulting in the interference with key metabolic processes, including DNA replication, protein production, and cellular respiration (12). This mechanism answers the inhibitory effects that are observed against all the strains of bacteria tested.

Salmonella spp. was the most sensitive organism tested to garlic extract, and this could be due to variation in membrane permeability and metabolic vulnerability. Recently, researchers have proposed that even though Gram-negative bacteria have an outer membrane that may serve as a barrier to antimicrobial agents, small molecules and reactive molecules can penetrate the barrier and have intracellular effects (allicin) (13). This could explain why the inhibition level in Salmonella spp. is relatively high when compared to other bacteria tested.

The effect of the antibacterial activity, though observed, was much lower in effectiveness than that of conventional antibiotics like levofloxacin and meropenem. This fact is consistent with the latest studies that show that plant extracts have antimicrobial effects, but they are mostly moderate in terms of activity in comparison with traditional antibiotics because of the inconsistency in concentrations and stability of active substances (14).

Antibiotics are very pure and highly specific to target bacterial structures, and plant extracts have complex mixtures of compounds that may be synergistic but less potent (15)

The extraction technique is also important in establishing the antibacterial activity of garlic. Aqueous extraction method was used in this study, which is classified as safe, and the method is appropriate for the preservation of thermolabile compounds (16). Nevertheless, recent research has indicated that aqueous extracts can have less active compounds than organic solvent extracts, which can decrease the antibacterial activity. In addition, allicin is also reportedly chemically unstable and can degrade easily due to environmental factors like heat, light, and changes in pH, among others, which could further reduce its antimicrobial activity (17).

The differences in antibacterial activity of the various bacterial species could also be explained by inherent differences in cell structure, metabolic activity, and resistance mechanisms (18). *Proteus vulgaris* was less sensitive than *Salmonella spp*. e.g., possibly because of dissimilarities in the membrane structure or enzyme defense mechanisms. Recent researches have highlighted that bacterial resistance mechanisms, like efflux pumps and enzymatic degradation, can have a role in determining the susceptibility of microorganisms to plant-derived compounds (19).

Clinically, the results of this paper indicate that the use of garlic extract might not directly replace the traditional antibiotics, but it has the potential to be a complementary antimicrobial agent. Recent studies have shown the potential of the application of plant-derived compounds in combination with antibiotics to improve antimicrobial activity and possibly decrease the emergence of resistance (20). This synergy strategy could be a good idea in solving the international dilemma of antimicrobial resistance.

The perspectives to study in the future include the isolation and characterization of active constituents of garlic, the optimization of extraction, and the evaluation of antimicrobial activity through new analytical methods. Also, there should be in vivo research and clinical trials to establish the safety and therapeutic efficacy of garlic-based antimicrobial agents.

In general, the findings of the given research contribute to the recent evidence, according to which garlic has biologically relevant antibacterial effects; however, the usage of this herb in clinical practice necessitates additional research and confirmation.

CONCLUSION

This paper has revealed that aqueous garlic extract has moderate antibacterial effects that are concentration-dependent against chosen pathogenic bacteria. Nevertheless, its efficiency is much lower than that of regular antibiotics. Garlic extract can be used as a complementary antimicrobial agent instead of substituting normal antibiotics. Isolation of active compounds, determination of MIC values, and clinical use should be further investigated.

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Conflict of Interest Disclosure

The authors state that they do not have any conflicts of interest when publishing this paper.

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Authors' Contribution

The study design and conception, data collection, and the work on the experiment, as well as the preparation of the manuscript, were done by Yasmeen Ali Hussien. Data analysis and interpretation of the results were done by Soroor Mohammed Hadi.

Data Availability Statement

The information used to validate the results of this research can be found in the article. The necessary author may provide additional information at his or her reasonable request.

Ethical Approval Statement

No human subjects or animals were used in this study. Hence, there was no need for ethical approval.

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