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Research Article

Antibacterial and Antifungal Activities of Garlic (*Allium sativum*) against Common Pathogens

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Abstract

Garlic (*Allium sativum*) has been used worldwide to fight microbial infections. *Allium sativum*, in vegetable form, exhibits a high potential against pathogenic bacteria and fungi. In the current study, antimicrobial effects of pure juice, aqueous extract, ethanolic extract, and dried powder of garlic in various concentrations (100%, 75%, 50%, and 25%, respectively) against *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Bacillus cereus, Aspergillus parasiticus,* and *Aspergillus flavus* were studied using the well diffusion technique. The results revealed that all extracts of garlic successfully inhibited the progression of *E. coli, S. aureus, S. typhi, A. parasiticus,* and *A. flavus*, while *B. cereus* was found to be resistant to garlic at all concentrations. The minimum inhibitory concentration (MIC) observed against *E. coli*, *S. typhi* and *A. parasiticus* was 15 mg/ml, against *S. aureus* it was 05 mg/ml, and against *A. flavus* it was 20 mg/ml. Among the extracts, the antimicrobial efficacy of garlic juice was found to be better than its ethanolic extract, dried powder, and aqueous extract. It was concluded that garlic exhibits a high potential against various types of bacterial and fungal pathogens. Moreover, it also fulfills the criteria to be used as an antimicrobial agent.

1. Introduction

Garlic (*Allium sativum L.*) is a herbaceous plant which belongs to the family of arcs (*Aliaceae*) and has a height of 20-40 cm. The bulb of garlic has a pungent taste and comes with a strong odor [1]. Various studies revealed that it is a native plant of Asia but has long been cultivated all over the world [2-4]. Garlic is counted among those herbs which have been widely known and used for centuries to treat infections, as well as to maintain and improve physical and mental health [5]. The growing research interest in medicinal plants is due to the emergence of resistance against antibiotics [6]. Antimicrobial activity in garlic is credited mostly to the presence of organosulfur compounds, including allicin and diallyl sulfides [7]. Allicin was reported to show antimicrobial activities and is responsible for most of garlic's pharmacological activities [8]. Fresh juice of garlic is effective against many disease-producing bacteria, antibiotic-resistant bacteria, and toxin [9]. Garlic is a strong antimicrobial agent which is successful against various pathogenic microbes, such as methicillin-resistant *Staphylococcus aureus* (MRSA), *S. aureus*, *Escherichia coli*, *Klebsiella*, *Salmonella*, *Streptococcus*, *Proteus mirabilis*, *Shigella senteriae*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Helicobacter pylori*, *Bacillus*, *Clostridia*, yeasts, and moulds [8-11]. The emergence of bacterial resistance to modern antibiotics poses a risk to the successful treatment of communicable diseases [12]. Therefore, the current study was aimed to explore the *in vitro* antimicrobial activity of garlic (pure juice, aqueous, ethanolic, and powder) extracts against *E. coli*, *S. typhi*, *S. aureus*, *B. cereus*, *A. parasiticus*, and *A. flavus*.

2. **Materials and Methods**

2.1 Procurement of Plant Materials (Garlic) *Allium Sativum*

Garlic bulbs were obtained from a local vegetable market of Peshawar, Pakistan and processed in the Food Microbiology Laboratory, PCSIR Labs Complex, Peshawar, Pakistan.

2.2 Preparation of Extracts

2.2.1 Garlic Juice Extract Preparation

Garlic (100 gm) bulbs were peeled and sterilized using ethanol. Garlic cloves were homogenized aseptically using mortar and pestle. Sterile cheesecloth was used to filter the homogenized mixture. The filtered (approximately 3000 mg/ml) garlic extract was considered as 100% pure. Furthermore, 75%, 50%, and 25% dilutions were prepared by using sterile distilled water and kept at 4°C until used.

2.2.2 Aqueous Extract Preparation

A crude aqueous extract of 500 mg/ml was obtained by homogenizing 50 g of peeled sterile garlic cloves in sterile 100 ml water in a Laboratory Warring blender at 3000 rpm and filtered through a 0.45 m filter (Sartorius stedim, Germany). The filtrate was taken in a sterile container and considered as 100% pure. Furthermore, 75%, 50%, and 25% dilutions were prepared using sterile distilled water and stored at 4°C until used.

2.2.3 Ethanolic Extract Preparation

Garlic bulbs were peeled, weighed (500 g), surface sterilized, and then placed for drying in a hot air oven for 2-3 days at 35-40°C. After drying, garlic peel was converted into powder using a blender. To make the garlic ethanolic extract, 100 g of garlic was macerated thoroughly and steeped in 95% ethanol for 30 minutes using a soxhellet device. Sterile distilled water was used for making 75%, 50%, and 25% concentrations.

2.2.4 Garlic Powder Preparation

About 1.0 kg of garlic bulb was properly cleaned under running tap water, aseptically peeled and chopped into small pieces, shade dried, and pulverized with a Kenwood electric blender. Then, different dilutions, that is, 75%, 50%, and 25% were prepared by adding edible starch.

2.3 Test Microorganisms

To determine the antimicrobial activity of garlic extract, test microorganisms were collected from the Food Microbiology Laboratory, PCSIR Labs Complex, Peshawar, Pakistan. These microbes included two Gram-positive bacteria (*B. cereus* and *S. aureus*), two Gram-negative bacteria (*S. typhi* and *E. coli*), and two fungal strains (*A. flavus* and *A. parasiticus*). The microbes were re-cultured on nutrient agar (oxide) and potato dextrose agar (oxide) media.

2.4 Antimicrobial Activity

Antimicrobial activity was screened using well diffusion technique [8]. Test microbes were enriched in nutrient broth (oxide) and potato dextrose broth (Hi media) for making the necessary concentration of microbes with 0.5 MacFarlent standards and the National Committee for Clinical Laboratory standards, which corresponds to approximately 1.0 x 10^8 CFU/ml volume of 100 µl microbial suspension transferred to the prepared plates of Muller Hinton Agar (MHA) (oxide) and spread. A sterilized 6 mm cork borer was used to create agar wells. Using a micropipette, about 50 µl of each one of the dilutions (100%, 75%, 50%, and 25%) of garlic extracts was added into the agar well. Two antibiotics (ciproxcin 05 µg and fluconazole 25 µg) were used as a standard (positive control). For bacterial growth, the plates were incubated at 37°C for 24 hrs. For fungal growth, they were incubated at 28°C for 3 to 5 days. Following the same method, three repetitive analyses were performed. The diameters of the inhibitory zones were measured in millimeters (mm) and the findings were documented. Inhibition zones with diameters smaller than 12 mm were deemed to be devoid of antibacterial action.

2.5 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of pure garlic extracts was determined using the tube dilution method. The extracts were diluted at concentrations of 100, 75, 50, 25, 20, 15, 10, and 05 mg/ml in nutrient broth media using serial dilution and incubated for 24 hrs at 37ºC. In each nutrient broth tube, 1 ml (of 24 hours old) culture of test organism diluted at the ratio of 1:1000 was inoculated and thoroughly mixed in a vortex mixer. The dilution with the lowest concentration did not show any growth in three repetitive analyses and was recorded as the MIC (with no detectable growth). [13, 14].

1.6 Statistical Analysis

The mean of all the obtained results of the inhibition zone and MIC was analyzed using a single ANOVA test on SPSS (version 23.0) software [15].

3. Results

3.1 Antimicrobial Activity of Garlic Juice

Garlic juice showed sufficient sensitivity to the selected pathogens. It showed a maximum inhibition zone in comparison with standard antibiotics (ciproxcin 05 µg and fluconazole 25 µg). *S. aureus* inhibited a zone almost the same as that of standard antibiotics with 50% of juice. Furthermore, 75% of juice showed the greatest extent of inhibition by *S. typhi*, followed by *A. flavus* and *E. coli*. *B. cereus* showed no sensitivity for all the concentrations of garlic juice (Table 1).

3.2 Antimicrobial Activity of Garlic Aqueous Extract

The selected pathogens showed adequate sensitivity to the garlic aqueous extract. *E. coli* showed 50% sensitivity to 100% extract with the standard dose (ciproxcin 05 µg and fluconazole 25 µg), while a specific trend of decreased activity from standard to low concentration was observed in case of *S. typhi* and *A. flavus*. The 100% garlic aqueous extract showed a greater zone of inhibition, while 50% extract demonstrated the same zone of inhibition to *A. parasiticus* as the standard antibiotic fluconazole. Effective results were delivered at all concentrations (max 28 mm) against *S. aureus*. On the contrary, *B. cereus* was found to be resistant to all concentrations (Table 2)

Table 1. Garlic Juice Zone of Inhibition (mm) in Various Concentrations against Pathogens

Microorganisms	Mean Zone of Inhibition (mm)				Standards (Positive Control)
	100%	75%	50%	25%	Ciproxcin 05 µg / Fluconazole 25 µg
Escherichia coli	28	24	20	15	35
Salmonella typhi	33	29	24	22	27
Staphylococcus aureus	44	38	34	31	35
Bacillus cereus	05	00	00	00	35
Aspergillus parasiticus	26	22	17	14	13
Aspergillus flavus	20	16	14	12	18

- Garlic juice significantly inhibited the growth of selected pathogens excluding *B. cereus* ($p<0.005$)

Table 2. Aqueous Garlic Extract Zone of Inhibition (mm) in Various Concentrations against Pathogens

Microorganisms	Mean Zone of Inhibition (mm)				Standards (Positive Control)
	100%	75%	50%	25%	Ciproxcin 05 µg / Fluconazole 25 µg
Escherichia coli	17	15	13	12	35
Salmonella typhi	26	24	23	20	27
Staphylococcus aureus	28	25	22	20	35
Bacillus cereus	00	00	00	00	35
Aspergillus parasiticus	20	16	10	10	13
Aspergillus flavus	16	13	09	09	18

- Aqueous garlic extract significantly inhibited the growth of selected pathogens excluding *B. cereus* ($p<0.005$)

3.3 Antimicrobial Activity of Garlic Ethanolic Extract

Garlic ethanolic extract showed a greater sensitivity to the selected pathogens as compared to the antibiotics ciproxcin 05 µg and fluconazole 25 µg. Ciproxcin (standard) manifested a greater sensitivity to *E. coli* than the extract, while in the case of *S. typhi*, *S. aureus*, *A. parasiticus*, and *A. flavus* the extract showed a greater activity as compared to the standard. *B. cereus* showed no sensitivity at all (Table 3).

Table 3. Garlic Ethanolic Extract Zone of Inhibition (mm) in Various Concentrations against Pathogens

Microorganisms	Mean Zone of Inhibition (mm)				Standards (Positive Control)
	100%	75%	50%	25%	Ciproxcin 05 µg / Fluconazole 25 µg
Escherichia coli	25	23	20	17	35
Salmonella typhi	35	34	32	30	27
Staphylococcus aureus	38	34	31	29	35
Bacillus cereus	00	00	00	00	35
Aspergillus parasiticus	29	27	26	24	13
Aspergillus flavus	22	20	19	17	18

❖ Garlic ethanolic extract significantly inhibited the growth of selected pathogens excluding *B. cereus* ($p<0.005$)

3.4 Antimicrobial Activity of Garlic Powder Extract

Garlic powder was found to be the most effective at 100% concentration, while its activity decreased with the decrease in its concentration. The 100% pure powder showed the maximum zone of inhibition for *S. typhi* and *A. parasiticus*, as compared to the standard antibiotics (ciprofloxacin and fluconazole). *A. flavus* showed the same effects for 100% pure powder and fluconazole. Standard ciprofloxacin strongly inhibits *E. coli*, while the different concentrations of garlic powder also showed varying degrees of inhibition. *B. cereus* was again found to be resistant at all concentrations of powder (Table 4).

3.5 Minimum Inhibitory Concentration (MIC) of Garlic Extract

The mean MIC of pure garlic extract for *E. coli*, *S. typhi*, and *A. parasiticus* was observed at 15 mg/ml, for *S. aureus* it was 05 mg/ml, and for *A. flavus* it was 20 mg/ml. *B. cereus* was found to be resistant to each concentration (Figure 1

Table 4. Garlic Powder Extract Zone of Inhibition (mm) in Various Concentrations against Pathogens

Microorganisms	Mean Zone of Inhibition (mm)				Standards (Positive Control)
	100%	75%	50%	25%	Ciproxcin 05 µg / Fluconazole 25 µg
Escherichia coli	22	19	17	15	35
Salmonella typhi	30	26	23	20	27
Staphylococcus aureus	34	31	27	24	35
Bacillus cereus	00	00	00	00	35
Aspergillus parasiticus	24	21	18	14	13
Aspergillus flavus	18	16	13	10	18

- Garlic powder extract significantly inhibited the growth of selected pathogens excluding *B. cereus* ($p<0.005$)

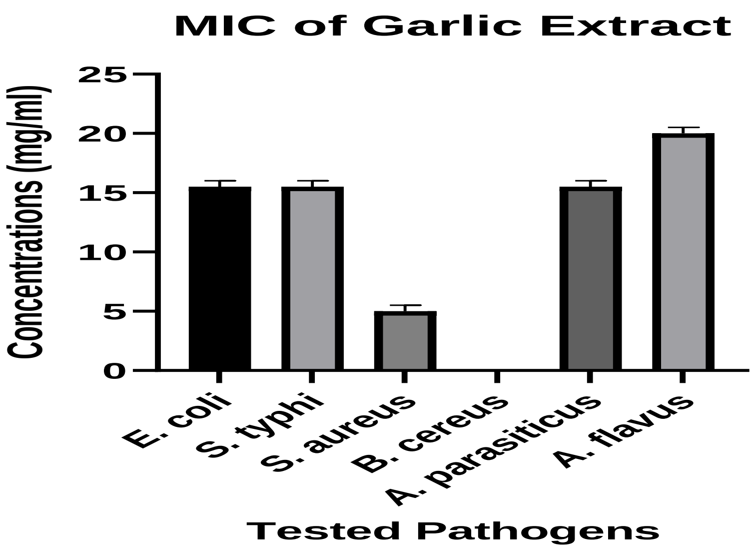

Figure 1. Minimum Inhibitory Concentration (MIC) of the Various Concentrations of Garlic Extracts against Pathogens

4. Discussion

Garlic (*Allium sativum*) phytoconstituents have long been known for their antibacterial effects. Their extracts are known to suppress the growth of pathogenic fungus, as well as Gram-positive and Gram-negative bacteria [2]. These ingredients help defend the plants against bacterial, fungal, and viral diseases [6]. The existence of previously known bioactive chemicals may be connected to the antibacterial activity of these plant extracts (Table 5).

Table 5. Previously Reported Bioactive Compounds of Garlic

Bioactive Compounds	References
Organosulfur Compounds	[7]
Allicin	[8]
Ajoene	[16]
Diallyl Sulfides	[7]

The current study demonstrated the antimicrobial efficacy of pure juice, aqueous extract, ethanolic extract, and dried powder against selected pathogenic bacterial (*E. coli S. typhi, S. aureus,* and *B. cereus*) and fungal (*A. parasiticus,* and *A. flavus*) isolates. The isolates tested in this study are responsible for many diseases including bacterial meningitis, diarrheal diseases caused by *E.coli, B. cereus,* and *S. typhi* [17], nosocomial infections and bacteremia by multidrug-resistant (MDR) *Staphylococci* [18], aspergillosis of the lungs, corneal, otomycotic, and naso-orbital infections. The observed zones of growth inhibition by these isolates were compared to those elicited by ciprofloxacin and fluconazole, showing the susceptibility exhibited by these isolates. The results indicated broad-spectrum antimicrobial activity of garlic extracts and opened a wide therapeutic window for the treatment of infections caused by these organisms.

Our results are in line with previous studies [19-21], which demonstrated the antibacterial potency of aqueous extracts (AE) and ethanolic extracts (EE) against *E. coli*, *S. aureus S. pneumoniea, P. aeruginosa, Vibrio parahaemolyticus, Klebsiella* spp., and *Proteus* spp., and anticandidal effects against *Candida* spp. [10]. Gram-negative bacteria have an outer lipid membrane in addition to a peptidoglycan layer. For an organic substance to have some antibacterial effect on these bacteria, the lipid membrane must be partially dissolved or should become porous to enhance its permeability, enabling molecules and ions from bacterial cells to leak out, ultimately cracking the bacteria [1].

In our study, we determined *B. cereus* as the most resistant strain. This finding is contradictory to another study [22], which determined garlic extracts as sensitive to Gram-positive isolates, including *B. cereus*. The results of our experiments showed that different microbial species exhibited sensitivity towards the pure juice of garlic, which is far better than using the standard antibiotics (ciproxcin and fluconazole). The data also showed that the efficacy pattern observed was pure juice > ethanol extract > dried powder > aqueous extract. An antimicrobial agent with low activity against an organism usually gives a high MIC, while highly effective antimicrobial agents typically yield low MIC [23]. Our results also conform to the conclusion reached by Acheampong et al. [23]. The mean MIC of pure garlic extract for *E. coli*, *S. typhi* and *A. parasiticus* was observed as 15 mg/ml, for *S. aureus* it was

05 mg/ml, and for *A. flavus* it was 20 mg/ml. *B. cereus* was found to be resistant to each concentration. The results of our study provide scientific justification for the use of garlic and its extract in health products and herbal remedies against multidrug-resistant (MDR) bacterial and fungal infections.

4. Conclusion

The current study concludes that garlic is more effective against *S. aureus*, *S. typhi*, *A. parasiticus*, and *A. flavus*, as compared to standard antibiotics (ciproxcin / fluconazole). The results suggest that garlic can be used in both traditional and modern medicine for the treatment of bacterial and fungal infections. Tentatively, it can also be used to protect food from the risk of contamination by pathogenic microorganisms.

Conflicts of Interest

The authors declare no conflict of interest.

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