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Antibiofilm and Antibacterial Activities of Garlic Extract Against Clinical Isolates of Pseudomonas Aeruginosa

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Abstract

Garlic is effective to antibiotic resistant microbes, in garlic there are many natural elements and chemicals, including many enzymes and amino acids. Also the among garlic's highest vital biologically substances is Allicin. Even though allicin is considered the prime antioxidant compound.The antibacterial activity of garlic has been fundamentally attributed to the entity of allicin generated by the enzymatic activity of allinase on allicin. Forty samples of Pseudomonas aeruginosa were isolated from burn where all isolates are identified and cultured on nutrient agar medium, MacConkey agar and Cetrimide agar, and diagnosed by microscopic examination, biochemical tests, and the VITEK2device. Then antibiotic susceptibility testing was completed and biofilm formation for twenty isolate of pseudomonas aeruginosa after that tested three concentration of garlic extract (1, 2, 5 mg/ml) as antibiofilm and antibacterial. The results findings showed a reduction in biofilm formation compared to the control group, confirmed by LSD values at a significance level of $P \leq 0.05$—thereby demonstrating that the reduction in biofilm formation resulted from the effect of the garlic extract Furthermore, demonstrated antibacterial activity, with efficacy varying according to the extract concentration; higher concentrations proved more effective at inhibiting bacterial growth.

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Keywords: Antibiofilm, Antibacterial, Garlic Extract, Pseudomonas aeruginosa

1. Introduction

Plant derived antimicrobials have extremely large therapeutic effects. They are considered as an efficient tool in treating infectious diseases though reducing the complications that connected with synthetic antimicrobials (Saranraj & Sivasakthi, 2014) [23].The changed pharmacological properties of plant products are generally the result of secondary product combinations resulting from plants. Such plant compounds are often secondary metabolites such as steroids, alkaloids, and fatty acid secretions that have a certain functional influence on the body (Shang *et al*., 2019 and Josling, 2005) [24, 13].There are many chemicals and natural elements are found in garlic, in addition to numerous enzymes, sulfur compounds, different vitamins, minerals, and amino acids. Allicin is among garlic's greatest crucial biologically dynamic substances where contain the main antioxidant composite, polar compounds of phenolic and steroidal origin. It is displayed diverse pharmacological countenance without odor and are heat stable (Mohammed *et al*., 2021) [18].The antibacterial activity of garlic has been basically attributed to the entity of allicin generated by the enzymatic activity of allinase on allicin (Alam *et al*., 2018) [1].

Garlic is also effective to antibiotic resistant microbes, and combining garlic extracts with antimicrobial agents results in partial and total synergism (Yadav *et al*., 2015) [26].Pseudomonas aeruginosa is gram negative, rod shape, a aerobic or facultative anaerobic and monoflagellated bacteria. P. aeruginosa is a very invasive microbe that has been discovered in environments, animals, plants, people, and hospital (International Journal of Health & Medical Research, 2025).Furthermore, P. aeruginosa is considered as a globally foodborne pathogens due to its great metabolic adaptability, (Fabre *et al*., 2019) [10] quick reproducibility, and capability to grow at

lower temperature.P. aeruginosa is capable to cause disease include keratitis, otitis, burn, wound, and Garlic can act against antibiotic-resistant microbes, and pairing garlic extracts with antimicrobial agents has produced partial or complete synergistic effects (Yadav *et al*., 2015) [26]. Its metabolic flexibility, rapid reproduction (Fabre *et al*., 2019) [10], and ability to grow at low temperatures have also led researchers to classify P. aeruginosa as a globally important foodborne pathogen. The bacterium can cause keratitis, otitis, burns and wound infections, endocarditis, and urinary tract infections (Montero *et al*., 2020 and Hilliam *et al*. (2020) [19,11]. The growing multidrug resistance of P. aeruginosa poses a serious threat to community health and well-being. Some enzymes alter antibiotics, leaving them ineffective. The bacterium produces aminoglycoside-modifying enzymes, while carbapenemases break down carbapenem antibiotics and give P. aeruginosa resistance to them (Anju *et al*., 2021) [4]. The genes responsible often occur on mobile genetic elements, including plasmids and transposons. Biofilms consist of bacterial cells—either one species or several—enclosed within a polysaccharide matrix that the bacteria secrete (Rajput & Kumar, 2018) [22]. P. aeruginosa produces and releases exopolysaccharides, DNA, and proteins into this matrix (Anju *et al*., 2021) [4]. The extracellular polysaccharide matrix, or EPS matrix, shields biofilm cells from environmental stresses such as UV radiation and sudden shifts in pH (Zhou *et al*., 2013 and Dubois-Brissonnet *et al*., 2016) [28,8].

Biofilms create a serious problem for the host because, once established, they can resist antibiotics, phagocytosis, and surfactants, which makes them difficult to remove (Olivares *et al*., 2019) [20]. The same resistance has also been reported by de Sousa *et al*. (2023) [7]. In P. aeruginosa, biofilm formation depends on genetic regulation and environmental conditions. Quorum sensing is one of the main systems involved: as cell density increases, it enables bacteria to communicate and coordinate their behavior through signals such as acyl-homoserine lactones (AHLs) and 2-heptyl-3-hydroxy-4-quinolone (PQS). Bacteria secrete extracellular polymeric substances (EPS), which create a matrix that anchors them to surfaces and shields them from environmental stress; this process is another key part of biofilm formation (de Sousa *et al*., 2023) [7]. This study evaluates garlic extract's antibacterial activity against P. aeruginosa and examines its effect on biofilm formation.

2. Methodology

2.1. Collection Bacteria Isolation

Forty samples of Pseudomonas aeruginosa were isolated from burn where all isolates are identified and cultured on nutrient agar medium,MacConkey agar and Cetrimide agar, and diagnosed by microscopic examination, biochemical tests, and the VITEK2device.

2.2. Antibiotic Susceptibility test

Antibiotic susceptibility testing was performed on twenty of the forty bacterial isolates with the VITEK® 2 Compact system, following the manufacturer's instructions. Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Yang *et al*., 2009). The antibiotics tested were Cefixime (CFM), Erythromycin (E), Chloramphenicol (C), Trimethoprim (TR), Piperacillin (PC), and Amikacin (AK).

2.3. Detection Biofilm Formation

Biofilm formation of *P. aeruginosa* was measured spectrophotometrically with a microtiter-plate assay (Li *et al*., 2019 and Borlinghaus *et al*., 2021) [16,5]. Bacterial colonies were inoculated into 10 mL of Trypticase soy broth containing 1% glucose and incubated at 37C° for 24 h. The cultures were then diluted 1:100 in TSB, and 200 µL of the diluted suspension was added to each well of a sterile 96-well, flat-bottom plate. Wells containing only sterile broth served as negative controls, allowing sterility to be checked and non-specific binding to be excluded. After incubation, the liquid was removed. Each well was washed four times with phosphate-buffered saline to eliminate non-adherent cells (Lee & Zhang, 2021) [15]. The attached biofilms were then fixed for 30 min with 2% sodium acetation. Fixed biofilms were stained with 0.1% crystal violet for 30 min. The walls were washed with deionized water to remove excess stain, and the plates were then left to air-dry at room temperature. Biofilm formation was quantified by measuring the optical density (OD) of the stained adherent cells with a microplate ELISA reader at 600 nm. Each assessment was performed in triplicate, and the mean OD was calculated for every isolate. Based on these values, the isolates were classified as non-, weak, moderate, or strong biofilm producers, as described in previous studies. (Panda *et al*., 2016) [21].

Table 1: Classification of biofilm formation by tissue culture plate method

Optical densities values	Adherence	Biofilm formation
> 0.240	Strong	Strong
0.120- 0.240	Moderate	Moderate
< 0.120	Non/weak	Non/weak

2.4. Preparation of crude extracts

Garlic extract was performed by using maceration technique. 100-gram sample of fresh garlic cloves was peeled and ground. Then, it is divided to prepare both aqueous and 70% ethanolic extracts (Borlinghaus *et al*., 2021) [5].Whilst aqueous extract is prepared with 100 grams of ground garlic by soaking it in 100 mL of hot distilled water for four hours with continuous stirring. Then the mixture was cool and filtered to remove impurities. consequently, by using a freeze dryer,the water was removed,to obtain the dry extract. For ethanolic extract, 100 grams of ground garlic were soaked in 70% ethanol for five days with room temperature and with daily stirring to ensure extraction efficiency (Mohammed *et al*., 2021) [18] Yield percentage for both extracts was calculated by using formula underneath:

$$\text{Yield (\%)} = (\text{Weight of dry extract} \div \text{Initial weight of plant material}) \times 100$$

Finally, these extracts were stored in tightly sealed and sterilized glass vials at 4°C until that is used in subsequent experiments (Fadwa *et al*., 2021) [9].

2.5. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined by broth microdilution in a 96-well microtiter plate. The substance was serially diluted in Mueller-Hinton Broth (MHB), and 100 µL of each dilution was placed into the wells before adding 100 µL of standardized bacterial suspension (≈1.5×10^8 CFU/mL).

Plates were incubated at 37°C for 24 hours, then examined for bacterial growth in each well. (De Marco *et al*., 2017) [6].

2.6.Effect garlic extract on biofilm formation.

An *in vitro* biofilm assay was performed in a 96-well microtiter plate, following the method described by Al-Fridawy *et al*. (2020) [29] with minor modifications. Overnight cultures of P. aeruginosa were diluted 1:100 in 15 mL of Tryptic Soy Broth (TSB) supplemented with 2% sucrose, either with or without the tested extracts at the concentrations listed below. The cultures were incubated at 37∘C for 24 h. Each well was then washed twice with 200μL of distilled water and allowed to dry for 45 min. Next, 100μL of 0.4% crystal violet was added to each well and left for 30–45 min. The wells were washed four times with distilled water, then decolorized with 200μL of 95% ethanol. After 45 min, 100μL of the decolorized solution was transferred to a new plate, and crystal violet staining was measured at 570 nm using a microplate reader. Biofilm formation was measured by comparing absorbance in wells treated with the compounds against that in untreated control wells. Each biofilm assay was run in triplicate across at least three separate experiments for every concentration. (Abdel-Moneim *et al*., 2021) [2].

2.7. Antibacterial Testing for Garlic Extract

An *in vitro* biofilm assay was performed in a 96-well microtiter plate, following the method described by Al-Fridawy *et al*. (2020) [29] with minor modifications. Overnight cultures of P. aeruginosa were diluted 1:100 in 15 mL of Tryptic Soy Broth (TSB) supplemented with 2% sucrose, either with or without the tested extracts at the concentrations listed below. The cultures were incubated at 37∘C for 24 h. Each well was then washed twice with 200μL of distilled water and allowed to dry for 45 min. Next, 100μL of 0.4% crystal violet was added to each well and left for 30–45 min. The wells were washed four times with distilled water, then decolorized with 200μL of 95% ethanol. After 45 min, 100μL of the decolorized solution was transferred to a new plate, and crystal violet staining was measured at 570 nm using a microplate reader. Biofilm formation was measured by comparing absorbance in wells treated with the compounds against that in untreated control wells. Each biofilm assay was run in triplicate across at least three separate experiments for every concentration. (IBM Corp., 2019) [12].

2.8. Statistical analysis

The Statistical Packages of Social Sciences -SPSS (2019) program was used to detect the effect of difference groups parameters. Least significant difference (LSD) was used to significant compare between means in this study(Khadim & Al Marjani, 2019) [14].

3. Result and discussion

3.1. Collection Bacteria Isolation

Pseudomonas aeruginosa isolates were diagnosed based on microscopic characteristics and biochemical tests and their growth on Cetrimide agar,MacConkey agar and nutrient agar medium.The diagnosis was also confirmed by using VITEC device, as shown in figure 1:

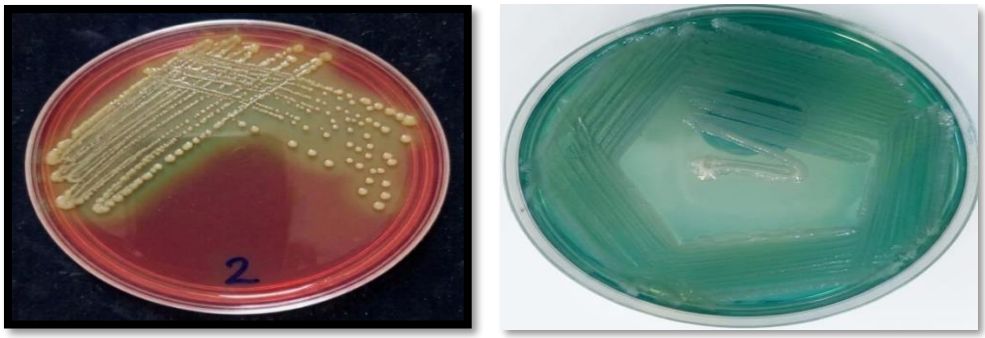


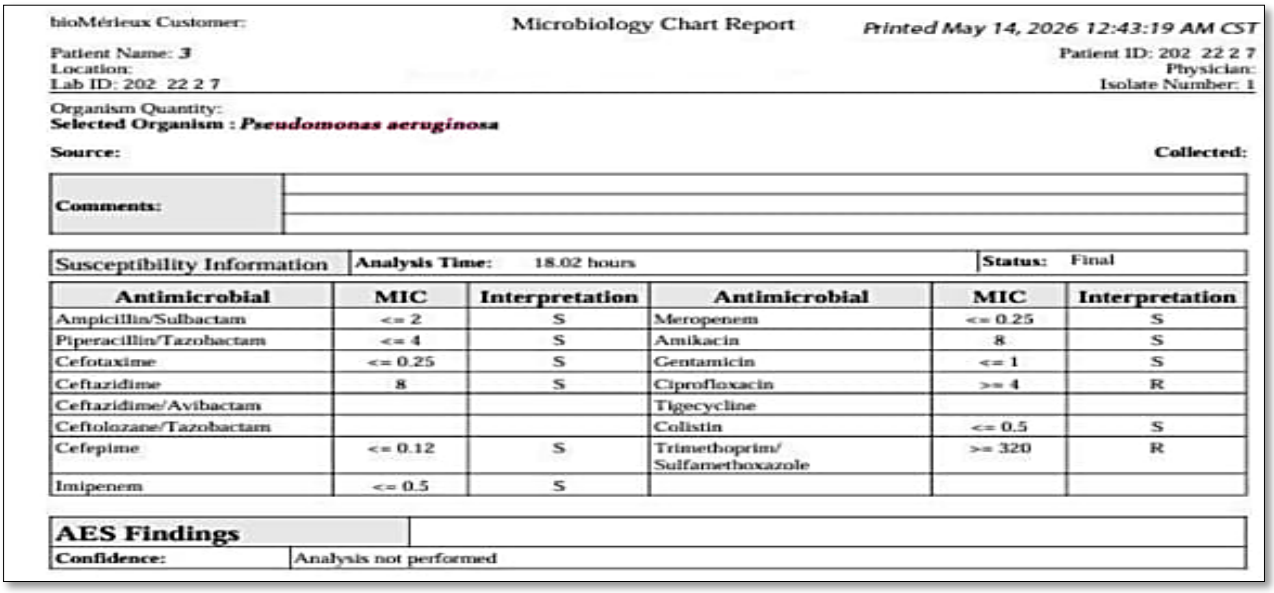
Fig1: Pseudomonas aeruginosa on Cetrimide agar

In this study, a total of forty P. aeruginosa clinical isolates collected from burn samples.Seven types of antibiotic were tested against twenty bacterial isolates, as shown in table 2 and figure 2.

Table 2: Antimicrobial susceptibility testing in clinical isolates of Pseudomonas aeruginosa

Antibiotics	NO. of isolate Resistance(%)	NO. of isolate Intermediate(%)	NO. of isolate Sensitive
Cefixime(CFM)	13(65%)	7(35%)	0(0%)
Erythromycin (E)	20(100%)	0(0%)	0(0%)
Chloramphenicol(C)	17(85%)	3(15%)	0(0%)
Trimethoprim (TR)	20(100%)	0(0%)	0(0%)
Piperacillin (PC)	19(95%)	1(5%)	0(0%)
Amikacin (AK)	0(0%)	10(50%)	10(50%)
Ciprofloxacin (CTR)	14(70%)	6(30%)	0(0%)

All isolates showed resistance to erythromycin and trimethoprim. Ciprofloxacin resistance occurred in 70% of the isolates, which is consistent with the findings of Mansouri *et al*. (2013) [17]. That study reported ciprofloxacin resistance in 55.55% of isolated P. aeruginosa and cefotaxime resistance in 87.3%; the strains came from different infection sites. This high level of multidrug resistance may result from hydrolytic enzyme production and the acquisition of resistance mechanisms by P. aeruginosa. In the present study, resistance to β-lactams varied among the isolates, with particularly high resistance to third-generation cephalosporins. Several isolates were also ESBL producers. Clinically, antibiotic-resistant bacteria prolong hospitalization and increase mortality. (Zhang *et al*., 2024) [27



bioMérieux Customer: Microbiology Chart Report Printed May 14, 2026 12:43:19 AM CST

Patient Name: 3 Patient ID: 202 22 2 7
Location: Physician:
Lab ID: 202 22 2 7 Isolate Number: 1

Organism Quantity:
Selected Organism : *Pseudomonas aeruginosa*

Source: Collected:

Comments:

Susceptibility Information | Analysis Time: 18.02 hours | Status: Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin/Sulbactam	<= 2	S	Meropenem	<= 0.25	S
Piperacillin/Tazobactam	<= 4	S	Amikacin	8	S
Cefotaxime	<= 0.25	S	Gentamicin	<= 1	S
Ceftazidime	8	S	Ciprofloxacin	>= 4	R
Ceftazidime/Avibactam			Tigecycline		
Ceftolozane/Tazobactam			Colistin	<= 0.5	S
Cefepime	<= 0.12	S	Trimethoprim/ Sulfamethoxazole	>= 320	R
Imipenem	<= 0.5	S			

AES Findings
Confidence: Analysis not performed

Fig 2: Antimicrobial susceptibility testing of Pseudomonas aeruginosa

3.2. Biofilm formation

Twenty isolates were assessed for biofilm formation and then placed into three groups according to their production levels: 25% (n=5) formed strong biofilms, 75% (n=15) produced moderate biofilms, and 0% (n=0) were weak biofilm producers. Burn isolates showed a greater capacity for strong and moderate biofilm formation than isolates collected from other sources, as illustrated. in table 3,4.

Table3: Optical densities values of biofilm formation in Pseudomonas aeruginosa

NO. OF Isolates	Optical densities values	Biofilm formation
Ps1	0.339	Strong
Ps 2	0.232	Moderate
Ps 3	0.207	Moderate
Ps 4	0.196	Moderate
Ps 5	0.212	Moderate
Ps 6	0.156	Moderate
Ps 7	0.259	Strong
Ps 8	0.193	Moderate
Ps 9	0.218	Moderate
Ps 10	0.320	Strong
Ps 11	0.176	Moderate
Ps 12	0.352	Strong
Ps 13	0.329	Strong
Ps 14	0.315	Strong
Ps 15	0.299	Strong
Ps 16	0.319	Strong
Ps 17	0.341	Strong
Ps 18	0.287	Strong
Ps 19	0.303	Strong
Ps 20	0.267	Strong

Table 4: Classification of biofilm formation by tissue culture plate method in P. aeruginosa

Biofilm formation	NO. of isolate	(Percentage)
Strong	5	(25 %)
Moderate	15	(75%)
None /Weak	0	(0%)
Total	20	(100%)

All isolates examined in this study formed biofilms. This ability can make bacterial infections harder to control: the biofilm shields cells from host immune defenses and limits the penetration and effectiveness of antimicrobial agents, which may lead to persistent or recurrent infection. Because none of the isolates showed weak or absent biofilm formation, biofilm production appears to be a conserved trait among them and may contribute to their pathogenicity and antimicrobial resistance.

3.3 Determination of Minimum Inhibitory Concentration (MIC)

The MIC—the lowest concentration showing no visible bacterial growth—was recorded at 2 mg/mL, while the sub-inhibitory concentration was 1 m g/mL. To determine the

minimum bactericidal concentration, samples from wells without visible growth were cultured on Mueller Hinton Agar (MHA) plates and incubated at 37°C for 24 hours. The lowest concentration of crude garlic extract that prevented bacterial growth on the solid medium was recorded as the MBC: 5 mg/mL.

3.4 Effect of garlic extract on biofilm formation

The results show that garlic extract caused a significant decrease ($P \leq 0.05$) in biofilm formation across all clinical isolates (Ps1, Ps10, Ps12, Ps13, and Ps14) of P. aeruginosa, as illustrated in table 5 and figure 3:

Table 5: Biofilm formation before and after treatment with garlic extract in difference isolates of pseudomonas aeruginosa

No. of Isolates	Biofilm formation before treatment with garlic extract	Biofilm formation after treatment with garlic extract			
		Sub-MIC =1mg/ml	MIC=2mg/ml	MBC=5mg/ml	L.S.D.
1	0.339 ±0.04 a	0.236 ±0.03 ab	0.137 ±0.03 bc	0.078 ±0.01 c	0.118 *
10	0.320 ±0.03 a	0.272 ±0.03 ab	0.195 ±0.04 bc	0.095 ±0.02 c	0.107 *
12	0.352 ±0.04 a	0.266 ±0.05 ab	0.168 ±0.03 b	0.092 ±0.02 b	0.156 *
13	0.329 ±0.04 a	0.244 ±0.03 ab	0.159 ±0.03 bc	0.079 ±0.01 c	0.115 *
14	0.315 ±0.02 a	0.239 ±0.03 ab	0.133 ±0.02 bc	0.072 ±0.01 c	0.106 *

Means having with the different letters in same row differed significantly. * ($P \leq 0.05$).

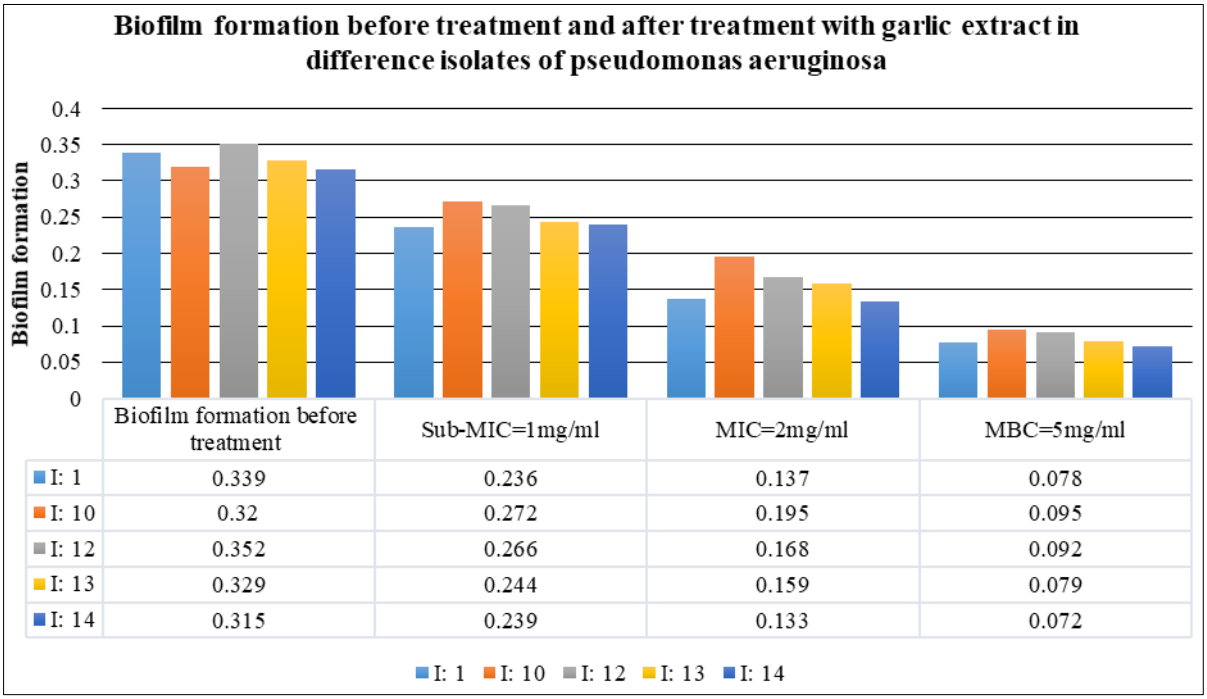


Fig 3: Biofilm formation before and after treatment with garlic extract in difference isolates of pseudomonas aeruginosa

Before treatment, biofilm formation values were high, ranging from 0.315–0.352. They declined as the concentration increased: at the sub-MIC concentration of 1 (mg/ml), values fell to 0.236–0.272; at the MIC of 2 (mg/ml), they reached 0.133–0.195; and at the MBC of 5 (mg/ml), they dropped further to 0.072–0.095. The pattern shows that garlic extract inhibited biofilm formation more effectively at higher concentrations.

These results agree with Alvi *et al*. (2021) [3], who found that garlic-derived sulfur compounds, including diallyl disulfide, inhibit quorum sensing in P. aeruginosa. That interference reduces biofilm formation and weakens bacterial virulence by disrupting cell communication, adhesion, and the production of extracellular polymeric substances (EPS) required for biofilm maturation. It may also explain the marked decrease in optical absorbance values observed in the present study.

These findings agree with recent studies suggesting that allicin and other sulfur derivatives from garlic can inhibit genes involved in quorum sensing. As the dose increases, virulence-factor production and biofilm formation decline, matching the progressive reduction observed here from sub-MIC to MIC and then to MBC levels.

3.5Antibacterial effects of garlic extract

The antibacterial activity of garlic extract for three isolates of bacteria P. aeruginosa has been tested by using the agar well diffusion (34). At different concentration (1, 2 and 5 mg /ml) of garlic extract, as indicated by the formation of inhibition zones, as shown in table (6) and figure (4):

Table 6: Antibacterial activity of garlic extract against Pseudomonas aeruginosa at different concentrations

No. of isolates	MIC=1 mg /ml	Sub-MIC=2 mg /ml	MBC=5 mg /ml
Ps1	9mm	12mm	15mm
Ps2	9mm	11mm	13mm
Ps3	8mm	10mm	9mm

At 1 mg/mL, inhibition zones measured 8–9 mm. Raising the concentration to 2 mg/mL increased them to 10–12 mm, while 5 mg/mL produced zones between 9 and 15 mm. Ps1 was most affected, with a 15 mm zone, followed by Ps2 at 13 mm and Ps3 at 9 mm. The stronger concentrations generally produced greater inhibition, although the isolates responded differently: Ps1 was most susceptible to the garlic extract, whereas Ps3 showed the weakest response at 5 mg/mL

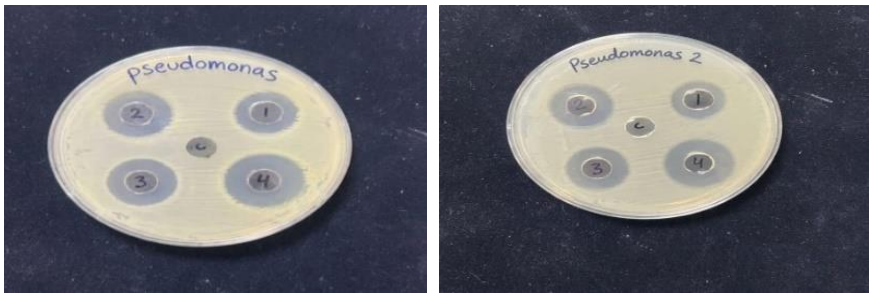


Fig5: Antibacterial Effects of Garlic Extract

5. Conclusion

This study examined the capacity of P. aeruginosa isolates to form biofilms and produce pyocyanin. Biofilm formation is linked to greater antibiotic resistance and bacterial colonization in burn infections. Although the isolates produced moderate or weak biofilms, they still showed high biofilm-associated resistance, suggesting that biofilm formation and pyocyanin production may heighten the bacterium's toxicity and pathogenicity. Garlic extract inhibited P. aeruginosa biofilm formation in a dose-dependent manner: mean optical density (OD) values fell progressively as the extract concentration rose from Sub-MIC to MIC and then to MBC.

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