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Effect of *Allium sativum* (Garlic) Extract on Virulence Factors of *Pseudomonas aeruginosa*

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Abstract

Due to the importance of garlic extract in medicine, it contains many effective compounds that act as antibacterial agents, effective against many types of negative and positive bacteria, and given that the *Pseudomonas aeruginosa* bacteria are characterized by their resistance to many antibiotics, so it requires finding materials that are safe in terms of their use and are anti-bacterial. Methodology Garlic extract is usually prepared by crushing or squeezing garlic cloves and then extracting the active compounds using solvents or other methods. There are several ways to prepare garlic extract, including steam distillation, Soxhlet extraction, maceration, and ultrasonic extraction. The plant extract was employed in the following concentrations: 10, 20, 30, and 40%. The biofilm production was measured over the course of two separate 24-48-hour incubation periods. The biofilm production significantly decreased as the plant extract concentration increased and significantly increased again when the biofilm concentration reached 40% over the course of the two separate 24-48-hour incubation periods. It was also found that the use of concentrations and the decrease in biofilm production differed significantly, as evidenced by the p-value of 0.0012 and the correlation coefficient between concentration and antibiotic.

Keywords: Garlic extract; *Pseudomonas aeruginosa*; Virulence factors; Correlation coefficient

1. Introduction

Fresh or crushed garlic produces both sulfur-containing and sulfur-free compounds. Compounds that contain sulfur, such as: allene, ajoene, diallyl polysulfide, phencyclidine, allyl cysteine. [1,2]. Allicin is the most effective component in garlic as it has antibacterial and antifungal properties. [3]. Its effect is stronger when the garlic is cut into very small pieces or mashed [4,5]. The smaller the cut or the more crushed it is, the greater the health benefits of garlic is full of secondary plant compounds that help eliminate viruses, bacteria, and fungi. [6]. In addition, it contains B vitamins that are important for nerve health and vitamin C, which works to strengthen immunity, Lysine is an organosulfur compound with the formula C6H10OS2, which is found naturally in garlic. [7,8]. It exists under standard conditions as a colorless liquid. chemically, it is a thioester of sulfenic acid, also known as allyl thiosulfate, garlic has many benefits. It is a natural antibiotic, a stomach disinfectant, and a killer of bacteria and viruses [9,10]. Therefore, it is recommended to include garlic in meals on a regular basis, garlic is a natural treatment for pneumonia in adults, as it contains antibacterial and antiviral substances. It also helps lower body temperature, as well as remove phlegm from the chest and lungs.[4] Garlic is especially beneficial for patients with cystic fibrosis, a genetic disorder that increases the risk of pneumonia [11,12]. Garlic is widely used in the treatment of urinary tract infections, especially cystitis. Not only does it have antimicrobial properties, but it is also effective against antibiotic-resistant strains of bacteria that cause bladder infections or urinary tract infections in general. Garlic extract has shown bactericidal effects against *P. aeruginosa*. Minimum bactericidal concentration (MBC) values for garlic extract were observed at 1 g/ml for Streptococcus pyogenes and 0.5 g/ml for *Pseudomonas aeruginosa* [13]. Synergistic effects with antibiotics such as ciprofloxacin enhance bacterial clearance [14]. In a mouse model of urinary tract infection, oral garlic treatment reduced renal bacterial counts and protected kidney

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tissue from damage by reducing QS-mediated virulence factors. Allicin disrupts biofilm formation and directly inhibits the secretion of virulence factors. DADS disrupts QS genes and inhibits elastase, lectins (LecA/LecB), and pyocyanin [15]. It targets RNAIII in Staphylococcus aureus and sRNAs in P. aeruginosa*, which expands the spectrum of its antiviral effect [16]. Therefore, our study aimed to determine the effect of raw garlic extract *on Pseudomonas* bacteria and their secreted enzymes.

2. Material and methods

2.1. Preparation of garlic extract

Garlic extract is usually prepared by crushing or squeezing garlic cloves and then extracting the active compounds using solvents or other methods. There are several ways to prepare garlic extract, including steam distillation, Soxhlet extraction, maceration, and ultrasonic extraction [17].

2.2. Methods for investigating the viability of isolated bacteria upon formation Biofilm

Obtaining the bacterial isolation from the educational hospital in Diyala, according to the experts in the laboratory, Loopful transplanting of bacteria was performed in ml medium glass tubes and cultured at C°37 for 82 hours. After the lap time had passed, the medium was withdrawn from the tubes and grafted multiple times with broth soy Trypticase containing 0% glucose sugar in PBS buffer (pH = 7.3), phosphate buffer saline. He had already attended and departed. Allow to thoroughly dry at room temperature before staining with Crystal% 1.0 violet. The surplus color was drained, bathed with distilled water, and then left [18].

3. Statistical Analysis

The results were statistically analyzed using the student's t-test, followed by Fisher's test ($P < 0.05$ for significance) to compare the plant extract to the standards, and the least significant difference was determined.

4. Results and discussion

4.1. Effect of garlic extract on protease activity of *P. aeruginosa*

The impact of varying concentrations of plant extract on the protease enzyme activity in *P. aeruginosa* bacteria is depicted in Figure 1. There were three different plant extract concentrations used 10, 20, 30, and 40%. The enzyme's activity was assessed twice over the course of two separate 24-48-hour incubation periods. During these incubation periods, the enzyme's effectiveness significantly decreased as the concentration of the plant extract increased and significantly reached 40% at the final concentration. The usage of concentrations and the decline in the enzyme's efficacy were also found to differ significantly, as evidenced by the p value of 0.0023 and the correlation coefficient between the treatments.

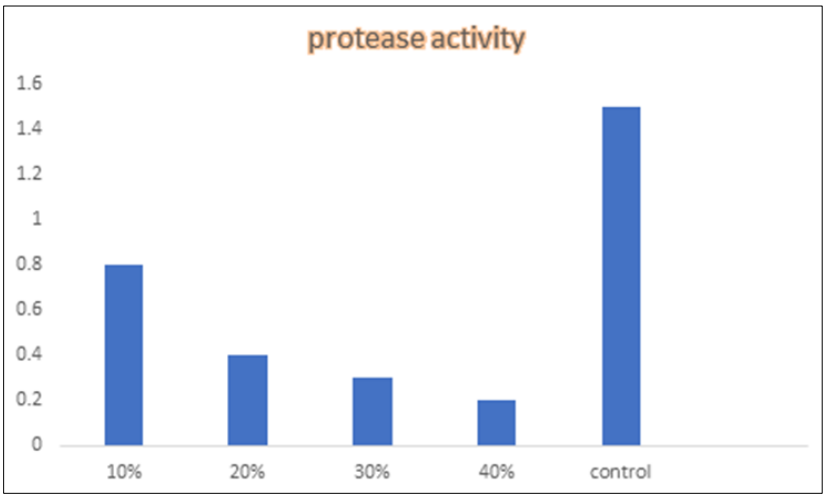

Figure 1 Effect of garlic extract on **P**rotease activity of *P. aeruginosa*(LSD5%=1.239)

The impact of varying concentrations of plant extract on the protease enzyme activity in *Pseudomonas aeruginosa* bacteria is depicted in Figure 2. The plant extract was employed in the following concentrations: 10, 20, 30, and 40%. The biofilm production was measured over the course of two separate 24-48-hour incubation periods. The biofilm production significantly decreased as the plant extract concentration increased and significantly increased again when the biofilm concentration reached 40% over the course of the two separate 24-48-hour incubation periods. The usage of concentrations and the decline in biofilm generation were also found to differ significantly, as evidenced by the p-value of 0.0012 and the correlation coefficient between the concentration and the control representing the antibiotic

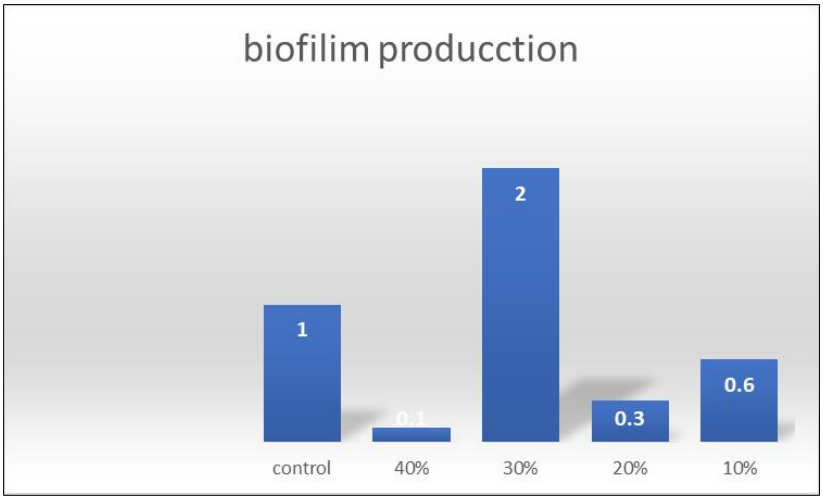

Figure 2 Effect of garlic extract on biofilm production *of p. aeruginosa* (LSD5%=0.092)

4.2. Effect of garlic extract on hemolysin activity of *P. aeruginosa*

The effect of varying plant extract concentrations on the hemolysin's enzyme activity in *P. aeruginosa* bacteria is depicted in Figure 3. There were three different plant extract concentrations used: 10, 20, 30, and 40%. The enzyme's activity was measured over the course of two separate incubation periods of 24 and 48 hours. During these times, the enzyme's effectiveness significantly decreased as the concentration of the plant extract increased, and it finally reached a significant 40% during the final two incubation periods. Additionally, as the p value was 0.0011 and the correlation coefficient between the concentrations used and the decline in the enzyme's efficacy was seen, it was noticed that there are noteworthy distinctions.

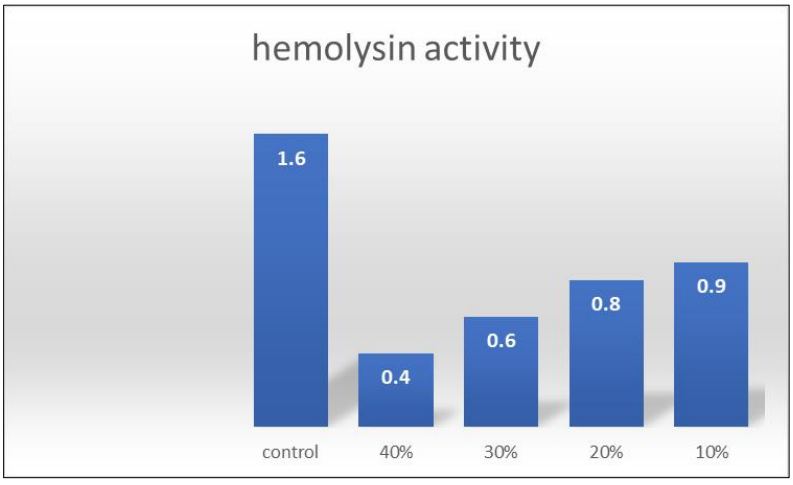

Figure 3 Effect of garlic extract on hemolysin activity of *P. aeruginosa* (LSD5%=0.037)

Research has shown that garlic extract has a remarkable ability to reduce the viability of *P. aeruginosa*, a dangerous opportunistic bacterium that threatens public health. This effect manifests through multiple mechanisms, most notably inhibiting the bacterial quorum sensing system, a vital system through which it controls the production of virulence factors. This inhibition leads to a reduction in the secretion of these factors, thereby weakening the bacteria's ability to

cause infection [19,20]. The effect of garlic extract is not limited to inhibiting quorum sensing alone; it also reduces the production of several important virulence factors, such as elastase and pyocyanin, reducing the bacteria's ability to destroy tissue and cause inflammation. Garlic extracts also contributes to increasing the fragility of bacterial biofilms and preventing their formation [20], reducing their resistance to antibiotics and thus enhancing the effectiveness of these drugs, especially when used in conjunction with antibiotics such as cefotaxime and ceftriaxone. Allicin, a compound extracted from garlic, plays an important role in reducing virulence factors, such as elastase, pyocyanin, pyoverdine, and rhamnolipids. Additionally, diallyl disulfide, a compound found in garlic oil, regulates genes responsible for quorum sensing systems in bacteria, enhancing its inhibitory effect on the virulence of these bacteria [21,22].

Garlic oil has been shown to inhibit various virulence factors and quorum-sensing pathways in *Pseudomonas aeruginosa*, an important opportunistic pathogen. The main components responsible for these effects include diallyl disulfide (DADS) and allicin [23]. Garlic oil disrupts the quorum-sensing (QS) systems of P. aeruginosa, particularly the las, rhl, and pqs pathways [24]. This inhibition results in decreased expression of virulence genes and reduced production of signaling molecules such as C12-HSL [25]. Treatment with garlic oil or its components results in decreased production of elastase and pyocyanin, reduced biofilm formation, impaired swarming motility, and decreased levels of pyoverdine and rhamnolipid [26]. Garlic oil increases the permeability of the outer membrane of P. aeruginosa, which may enhance its susceptibility to antibacterial compounds. Treatment with garlic oil reduces the activity of the efflux pump, which may increase the effectiveness of antibiotics against multidrug-resistant strains [27]. Garlic oil shows promise in enhancing the efficacy of antibiotics, especially when combined with drugs such as colistin. The ability of garlic oil to inhibit biofilm formation suggests its potential use in the prevention of chronic P. aeruginosa infection [28]. By suppressing various virulence factors, garlic oil can help reduce the pathogenicity of P. aeruginosa infection. Treatment with garlic oil enhances the clearance of P. aeruginosa bacteria from human blood, which may aid the host's innate immune response [29].

Overall, these multiple effects of garlic extract reduce the ability of *P. aeruginosa* to cause infection, opening new avenues for the development of effective treatments to combat this dangerous bacterium.

5. Conclusion

Garlic extract exhibited a notable ability to diminish the survival of *P. aeruginosa* in the current investigation. This effect appears to be mediated through the disruption of quorum sensing mechanisms, consequently leading to a reduction in the production of key virulence determinants, including elastase, pyocyanin, and biofilm development. The outcomes of this research imply that garlic-derived compounds hold promise for creating novel therapeutic interventions against this challenging bacterium. Ultimately, this could benefit public health by providing alternative approaches to manage *P. aeruginosa* infections and potentially alleviate the growing issue of antibiotic resistance.

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