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Effect of Tropolone And Some Plants Extracts on Elastase Production From *P. Aeruginosa* Isolates

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Abstract:
P.aeruginosa is a significant opportunistic pathogen known for its production of elastase, an enzyme that contributes to tissue damage and virulence. The increasing prevalence of antibiotic resistance necessitates the exploration of alternative antimicrobial agents, including natural compounds.To evaluate the antibacterial activity of tropolone and various plant extracts (garlic, onion, moringa, and leek) against elastase-producing isolates of *P. aeruginosa* (P79). isolates were collected and evaluated for their production of the elastase enzyme. Antibacterial activity was assessed using the disc diffusion method, with varying concentrations of tropolone and plant extracts. Minimum Inhibitory Concentration (MIC) values were determined to quantify the effectiveness of each compound against the bacterial isolates.All tested compounds exhibited varying degrees of antibacterial activity. Tropolone was the most effective, completely inhibiting bacterial growth at all concentrations tested (15.6 µg/mL). Garlic extract showed significant inhibition at higher concentrations (500 µg/mL and 62.5 µg/mL), while onion extract also demonstrated activity at elevated concentrations (1000 µg/mL and 31.2 µg/mL). Moringa extract inhibited growth at the highest concentration (500 µg/mL), whereas leek extract exhibited limited antibacterial activity across all concentrations.These findings support the exploration of natural compounds as alternatives to conventional antibiotics in the treatment of infections caused by this pathogen.

Keywords: Tropolone*;* Elastase enzyme*; P. aeruginosa; Inhibitory effect of Tropolone ;plant extracts.*

Introduction

P. aeruginosa is a versatile opportunistic pathogen known for its ability to produce a variety of virulence factors, including elastase. The pathogenesis of *P. aeruginosa* infections involves a series of steps, including attachment, colonization, local invasion, and the potential for systemic disease [1]. These infections are diverse, affecting individuals with conditions such as cystic fibrosis (CF), chronic obstructive pulmonary diseases, and

immunodeficiency [2]. It exhibits tolerance to extreme environmental conditions, including high salt and dye concentrations, various antibiotics, and even distilled water. Also, it can survive in the presence of some disinfectants [3].

Elastase enzyme, primarily produced by the strain's elastolytic activity, is a proteolytic enzyme that breaks down elastin and plays a significant role in various physiological and pathological processes, including tissue remodeling and inflammation. It is produced by some bacterial species including *P. aeruginosa* and plays a significant role in the pathogenicity of *P. aeruginosa*, particularly in chronic infections such as those seen in cystic fibrosis and burn patients [4]. Elastase facilitates tissue invasion and degradation by hydrolyzing elastin, a key protein in connective tissues, which leads to the destruction of host tissues and the evasion of the immune response [5]. Excessive elastase activity is implicated in conditions such as emphysema, chronic obstructive pulmonary disease (COPD), and skin aging. Therefore, the search for effective elastase inhibitors is crucial in developing therapeutic strategies. The production of elastase is regulated by various environmental factors, including the availability of nutrients and the presence of host tissues [6]. Additionally, the expression of elastase is often linked to the formation of biofilms, a common lifestyle of *P. aeruginosa* in chronic infections, where the enzyme contributes to the breakdown of the extracellular matrix, allowing for further colonization and persistence within the host [7]. Understanding the mechanisms behind elastase production and its role in *P. aeruginosa* infections is crucial for developing targeted therapies to combat this resilient pathogen.

Tropolone is an organic compound with a unique bicyclic structure belonging to the phenols group known for its antimicrobial properties. It has garnered interest in the scientific community for its diverse biological activities, particularly its potential as an antielastase agent. It exhibits its antielastase activity through competitive inhibition of the enzyme responsible for elastase production in P. aeruginosa. It binds to the active site of elastase, thereby preventing the substrate (elastin) from being hydrolyzed. The inhibitory effect could be beneficial in controlling the growth and spread of the bacteria in the body as shown in Figure (1). It has been suggested that tropolone inhibits specific enzymes involved in essential cellular processes, such as cell wall synthesis, protein synthesis, or DNA replication. By targeting these vital enzymatic activities, tropolone disrupts bacterial growth and impairs the normal functioning of bacterial cells [8]. This inhibition helps

maintain the structural integrity of elastin-rich tissues and may mitigate the effects of diseases associated with elastase overactivity.

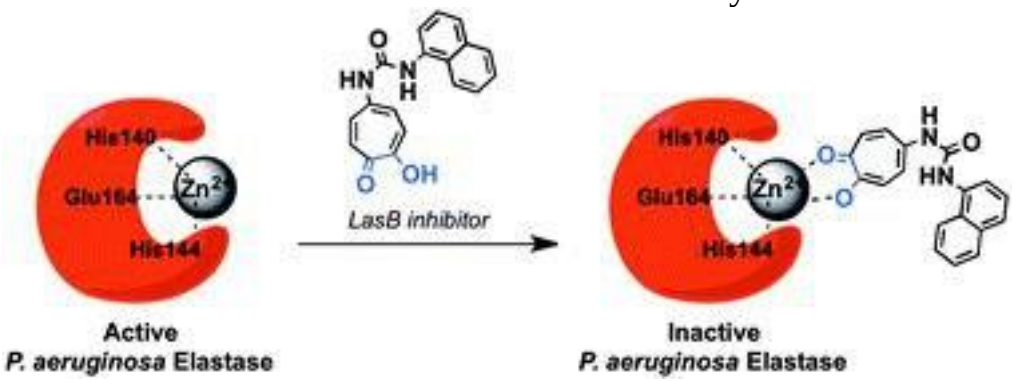

Figure 1. The inhibitory effect of Tropolone on elastase bacterial enzyme [1].

Several studies have explored the antielastase properties of tropolone. For instance, a study by [9] demonstrated that tropolone effectively inhibited porcine pancreatic elastase in vitro, showing a dose-dependent relationship. The study highlighted the potential of tropolone as a lead compound for developing new elastase inhibitors. investigated the effects of tropolone on human neutrophil elastase, finding that it significantly reduced elastase activity and subsequently decreased the degradation of extracellular matrix components in lung tissues. This suggests that tropolone may have therapeutic implications for respiratory diseases characterized by excessive elastase activity [10]

Research into the effects of herbal extracts on elastase enzyme activity in bacteria, particularly in pathogens like *P. aeruginosa*, has gained attention due to the potential of these natural compounds to serve as alternative therapeutic agents. Various studies have demonstrated that certain herbal extracts possess significant elastase inhibitory properties, suggesting their role in mitigating tissue damage caused by elastase-producing bacteria. For instance, a study by [11] investigated the elastase inhibitory effects of extracts from *Curcuma longa* (turmeric) and *Zingiber officinale* (ginger). The researchers found that both extracts significantly reduced elastase activity in vitro, indicating their potential to protect against elastin degradation and associated tissue damage. The active compounds, curcumin from turmeric and gingerol from ginger, were identified as the primary contributors to this inhibitory effect, showcasing their promise in developing natural elastase inhibitors. Another significant finding was reported by [12], who explored the effects of *Ginkgo biloba* extract on elastase production in P. aeruginosa.

The study revealed that Ginkgo biloba extract not only inhibited elastase activity but also reduced biofilm formation, which is often linked to increased virulence in bacterial infections. This dual action suggests that Ginkgo biloba could be beneficial in managing chronic infections where elastase plays a role in tissue destruction. Additionally, research by [13] examined the effects of various herbal extracts, including those from *Ocimum sanctum* (holy basil) and *Allium sativum* (garlic), on elastase activity. The findings indicated that both extracts exhibited significant elastase inhibitory effects, with garlic extract showing the most potent activity. The study highlighted the potential of these herbal remedies to complement conventional treatments in managing elastase-related pathologies. This study suggests that tropolones, with their metal-chelating properties, are being explored as potential inhibitors of *P. aeruginosa* elastase, to develop new strategies to combat infections caused by this bacterium.

Materials And Methods

Collection of samples

A total of 88 clinical isolates of *P. aeruginosa* were obtained between September and December 2023. The samples were sourced from outpatients and hospitalized individuals visiting Medical City, Alsedar City, and the Specialized Sensitivity Center in Alsedar, Iraq. All bacterial isolates included in this study were collected from various clinical specimens, including blood, respiratory tract infections (RTI) aspirates, sputum, urine, ear swabs, cerebrospinal fluid (CSF), burn swabs, and wound samples.

Identification of Pseudomonas aeruginosa:

Morphological Characteristics

The phenotypic characteristics of the bacterial isolates were studied by growing them on culture media such as MacConkey agar and Cetrimide agar,Nutrient agar,King A agar and incubating them at a temperature of 37 °C for 18 to 24 h, and then observing the shape of the colony, its texture, color, and edges, and the type of decomposition and its production of pigmen[14]

Microscopical Examination

Microscopical examination of the cells of bacterial isolates was carried out by stained using a gram stain and examined under a light microscope to observe the shape of the bacterial cells, their color[15]

Biochemical Testsand arrangement

An oxidase and catalase test was performed to test the ability of the isolates to produce the enzymes oxidase and catalase[16]

Antibiotic Susceptibility Test

Susceptibility test was performed for isolates using Kirby-bauer disk diffusion method [17]. The tested antibiotics were meropenem (MEM; 10 µg), amikacin (AK; 10 µg), gentamicen (CN; 10 µg), piperacillin (PRL; 100 µg), tetracycline (TE; 30 µg), colistin (CT; 10 µg), ceftazidime (CAZ; 30 µg), ciprofloxacin (CIP; 10 µg), cefepime (FEB; 10 µg) and ceftriaxone (CTX; 30 µg). The clear zone's diameter was measured, and the Clinical and Laboratory Standards Institute (CLSI) 2023 was used to evaluate the observations [18].

Plants extract preparation

Aqueous extracts

The preparation of garlic, leeks, Moringa, and onion leaf extracts involved sourcing dried leaves from local markets in Baghdad, Iraq, followed by their transport to the laboratory. The leaves were ground into a fine powder and stored in tightly sealed flasks. For the preparation of aqueous leaf extracts, 1 gram of the powdered leaf material was combined with 10 mL of distilled water. The mixture was shaken at 37°C for 4 hours and subsequently left to stand overnight at room temperature. The solution was then strained using gauze and further filtered through Whatman filter paper to obtain a clear filtrate. This filtrate was dried in a glass petri dish at 37°C in an incubator to yield a crude leaf extract. The resultant extract was weighed and stored in a sealed container at 5°C until further use [19].

Ethanolic extract

The methodology involved obtaining dried leaves of garlic, leek, Moringa, and onion plants, which were ground into a fine powder. A total of 50 grams of the powdered material was placed into tightly sealed flasks, and 500 mL of 95% ethanol was added to each flask. The mixtures were agitated at a speed of 120 revolutions per minute for 30 minutes, after which they were left at room temperature for 72 hours to facilitate precipitation. Following this, the mixtures were filtered to remove solid residues, and the resulting filtrates were concentrated using a rotary evaporator. The concentrated extracts were then dried at room temperature to obtain the ethanolic extracts of the leaves [20].

Screening of elastase production from *p. aeruginosa* isolates

Qualitative assay

The streaking method was employed to identify elastase-producing isolates using an elastin agar medium containing 1% elastin from ligamentum nuchae and 2.5% nutrient agar. Each isolate was cultured individually at 37°C for 24 hours. Elastase-producing isolates were identified by the presence of clear zones surrounding the colonies, indicating elastin degradation [21].

Quantitative assay

Each isolate was cultured in nutrient broth containing 0.2% elastin (from ligamentum nuchae) at 37°C for 24 hours in a shaking incubator at 200 rpm. Following incubation, the cells were removed by centrifugation at 5000 g for 10 minutes, and the resulting supernatant was sterilized using a 0.22 µm Millipore filter. To assess elastase activity, 1 mL of the sterilized supernatant was incubated with 20 µg of elastin Congo red (ECR) for 30 minutes at room temperature in a shaking incubator set at 200 rpm. The reaction was terminated by adding 0.1 M NaOH. Soluble Congo red dye released into the supernatant was separated by centrifugation at 10000 g for 10 minutes at room temperature. The absorbance of the soluble Congo red was measured at A495. Fresh nutrient broth (NB) medium served as the control [22].

Determining the optimal conditions for elastase production by the chosen isolate.

The optimal conditions for elastase production were found based on the study by [23].

pH effect

The culture medium (nutrient broth + 0.2% elastin) was modified to several pH levels (4, 7, 8, 10) to investigate their impact on elastase synthesis. The medium was infected with 1% of *P. aeruginosa* suspension containing

0.5x108 colony-forming units per millilitre (cfu/ml) and cultured in a shaking incubator at 37°C for 24 hours with controlled agitation at 200 rpm. Following the incubation time, the culture was centrifuged at 6000 rpm for 10 minutes. The supernatant was then sterilised by filtering through a 0.22mm Millipore filter before being collected for assessing elastase production.

Impact of Temperature

A culture medium consisting of nutritional broth and 0.2% elastin was inoculated with a 1% *P. aeruginosa* suspension containing 0.5x10^8 cfu/ml and incubated at temperatures of 28°C, 37°C, and 42°C for 24 hours. The sample was centrifuged at 6000 rpm for 10 minutes, and the resulting supernatant was sterilized by filtering through a 0.22mm Millipore filter before being used to measure elastase production.

Antibacterial activity of tropolone and plant extracts on the growth of elastase-producing isolates

Under aseptic conditions, sterile 96-well microtiter plates were prepared and used. Mueller Hinton broth was used to serially dilute 100 µL of different concentrations of the test materials. The assay involved placing 100 microliters of sterile Mueller-Hinton broth in all wells and adding 100 microliters of the stock extract in well 1. The stock extract was then diluted from well to well, except for well 12 which did not contain any extract. A tube containing the same number of bacteria as the McFarland standard of the activated bacteria was prepared and 0.1 ml to 10 ml from the McFarland tube was transferred to a sterile Mueller-Hinton tube. From this tube, 100 microliters were added to all wells except well 11. The plate was incubated at 37°C for 24 hours. After incubation, 10 µL of resazurin reagent solution was added to each well, and the color change of the resazurin reagent was evaluated. Positive results were noted when the color changed from purple to pink or remained purple, indicating a negative result. The MIC value, is the smallest concentration of the test material at which color change occurred.

Effect of tropolone and some plant extracts on elastase production from *P. aeruginosa*

The effect of tropolone and plant extracts (onion, garlic, moringa, and leek extracts) on elastase production by *P. aeruginosa* (P79) was investigated. After determining the minimal inhibitory concentration (MIC) of tropolone and the plant extracts, they were added to tryptic soy agar broth cultures adjusted to match the MIC and inoculated with *P. aeruginosa* (P79). The cultures were then incubated in a shaking incubator at 37°C for 24 hours. Subsequently, the cells were removed by centrifugation at 5000g for 10

minutes and sterilized with a 0.22 µm paper filter. Following this, one milliliter of the sterilized supernatant was incubated with 20 µg of elastin Congo red (ECR) for 30 minutes in a shaking incubator at 200 rpm and room temperature. To stop the reaction, 0.1 M NaOH was added. The insoluble ECR was then removed by centrifugation at 6000 rpm for 10 minutes at room temperature, and the soluble Congo red dye released into the supernatant was measured at A495 on an ELISA reader and spectrophotometry.

RESULTS AND DISCUSSION

Collection and re- identification of *P.aeruginosa* Isolates

In this study, a total of 88 from 120 clinical *P.aeruginosa* isolates were gathered from patients suffering from various types of infections. These isolates were sourced from different hospitals located in Baghdad city and were collected over a period spanning from September to December 2023. The isolates were derived from a variety of clinical sources, including in Table (1):

Table (1): Number of isolates according to their collection sources.

clinical source	NO. of isolation	Percentage
Blood	8	9.09%
Aspiration Of RTI	2	2.27%
Bronchial Wash	2	2.27%
Sputum	18	20.45%
Urine	16	18.18%
Ear Swab	12	13.64%
C.S.F	4	4.55%
Burn Swab	6	6.82%
Wound swab	20	22.73%
Total	88	100%

The results of the table show that the most common clinical sources of isolation are wound, sputum, urine, and ear swab respectively. The high number of wound infections in the table may be due to the fact that the data was collected from a medical city, Al-sader city, and Ibn- Albalady Hospital . These facilities are likely to see a high number of patients with complex and difficult-to-treat infections. Additionally, the high number of wound infections may be due to the fact that the data was collected during the winter months, when wound infections are more common

Characteristics of culture

The identification of P. aeruginosa, a notorious opportunistic pathogen, has been extensively studied due to its clinical significance and resistance to multiple antibiotics. Previous research has consistently highlighted the effectiveness of selective media in promoting the growth and identification of this bacterium. MacConkey agar, for instance, has been widely utilized for the differentiation of lactose fermenters from non-fermenters. As noted in the literature, *P. aeruginosa* is characterized as a non-lactose fermenter, resulting in pale yellow, smooth colonies on MacConkey agar, which aligns with the

findings presented in Figure (2) [24]. This characteristic is pivotal for initial screening in clinical microbiology laboratories.

Further studies have demonstrated that the metabolic byproducts of P. aeruginosa, particularly pyocyanin, play a crucial role in its identification. The production of this blue-green pigment on Nutrient agar not only serves as a phenotypic marker but also signifies the bacterium's pathogenic potential [25]. The presence of pyocyanin has been linked to the organism's virulence factors, enhancing its ability to establish infections in immunocompromised hosts.

Cetrimide agar, a selective differential medium, has been specifically designed to isolate *P. aeruginosa* from mixed microbial populations. Research indicates that Cetrimide agar facilitates the growth of *P. aeruginosa* through the incorporation of its pigments, pyoverdine and pyocyanin, which further aid in the visual identification of the organism [26]. The dual pigmentation system not only enhances the visibility of *P. aeruginosa* colonies but also provides a means of differentiating them from other non-fermenting Gram-negative bacteria. Moreover, King A agar has been recognized for its specificity in isolating and identifying *P. aeruginosa* based on pigment production. The distinctive blue-green coloration imparted by pyocyanin on this medium serves as a reliable indicator of the organism's presence [27]. This characteristic has been corroborated by multiple studies, reinforcing the notion that culture media play a vital role in the accurate identification of bacterial pathogens in clinical settings. Microscopic examination showed that *P. aeruginosa* appear as gram-negative small rods in a single cells or in pairs Examine the ability of isolates to produce oxidase and catalase enzymes was positive for all isolates.

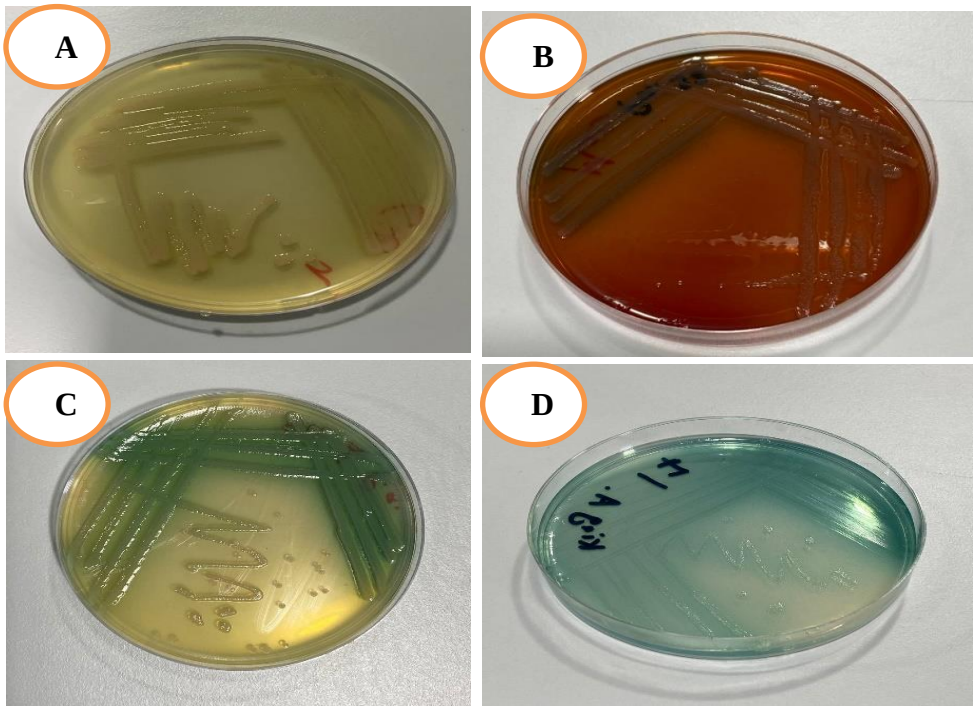

Figure 2. *P. aeruginosa* isolates cultured on media A: Nutrient agar B:MacConkey agar C: Cetrimide agar D: king A agar

Antibiotic Susceptibility Test

The Figure (3) showed the results of antibiotic susceptibility testing for a panel of 13 antibiotics on a set of isolates. The antibiotics tested were Ciprofloxacin, Gentamicin, Ceftazidime, Meropenem, Cefepime, Amikacin, Piperacillin, Colistin, and Ticarcillin.

The resistance patterns of *P. aeruginosa* to various antibiotics were extensively studied across different research papers. The Kirpy- Bauer method was utilized to determine the susceptibility of isolates to 13 antibiotics, revealing high resistance rates, with Ceftazidime, Doxycycline, and Tetracycline showing 100% resistance, followed by Cefepime (90%), Piperacillin (50%), Amikacin (45%), Gentamicin (40%), Ticarcillin (30%), Tobramycin (25%), Ciprofloxacin, Meropenem, and Colistin (20%), and Aztreonam (15%).

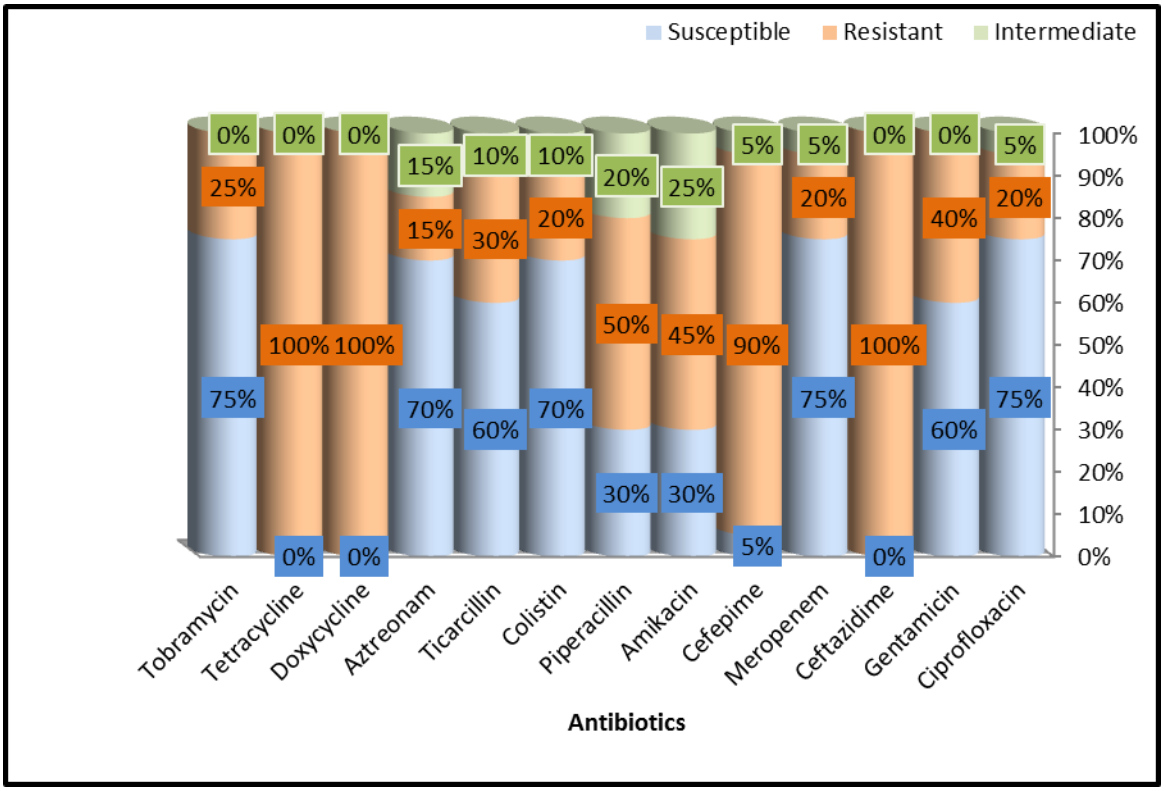

Figure (3): Antibiotic susceptibility test for *P. aeruginosa*

Screening of elastase production from *P. aeruginosa* isolates

Qualitative assay

The production of elastase by bacterial isolates, particularly those from clinical environments, has garnered significant attention due to its implications for pathogenicity and tissue damage[28]. In this study, the

induction of elastase production in bacterial isolates through cultivation in a nutritional broth containing 1% elastin powder at 37 °C for 24 hours was successful, as evidenced by the formation of clear halos around the colonies on nutritional elastin agar. This methodology is consistent with established techniques for evaluating elastase activity, which have been effectively employed in previous research.

The current findings, which indicate that 81 out of 88 clinical isolates (92%) were elastase productive, align with the literature highlighting the prevalence of elastase among pathogenic bacteria. For instance, [29] reported that 70% of clinical isolates from cystic fibrosis (CF) patients produced detectable levels of LasB, a well-characterized elastase. This suggests that elastase production is a common trait among CF isolates, likely contributing to the chronic lung infections characteristic of this condition. The remaining 30% of isolates in their study were found to be inactive, indicating variability in elastase production that may be influenced by factors such as genetic background or environmental conditions.

Moreover, the study conducted by [30] on 96 Brazilian clinical isolates provides further evidence of the widespread occurrence of elastase production, reporting a striking 100% prevalence among the isolates examined. This finding underscores the potential role of elastase as a virulence factor in various bacterial pathogens, particularly in contexts where tissue degradation and immune evasion are critical for infection persistence.

The ability of elastase-producing bacteria to degrade elastin not only facilitates tissue invasion but also plays a role in the modulation of host immune responses[31]. The clear halo formation observed in this study indicates the enzymatic breakdown of elastin surrounding the bacterial colonies, which is a hallmark of elastase activity. This enzymatic property may contribute to the pathogenicity of these isolates, particularly in patients with compromised immune systems or chronic infections.

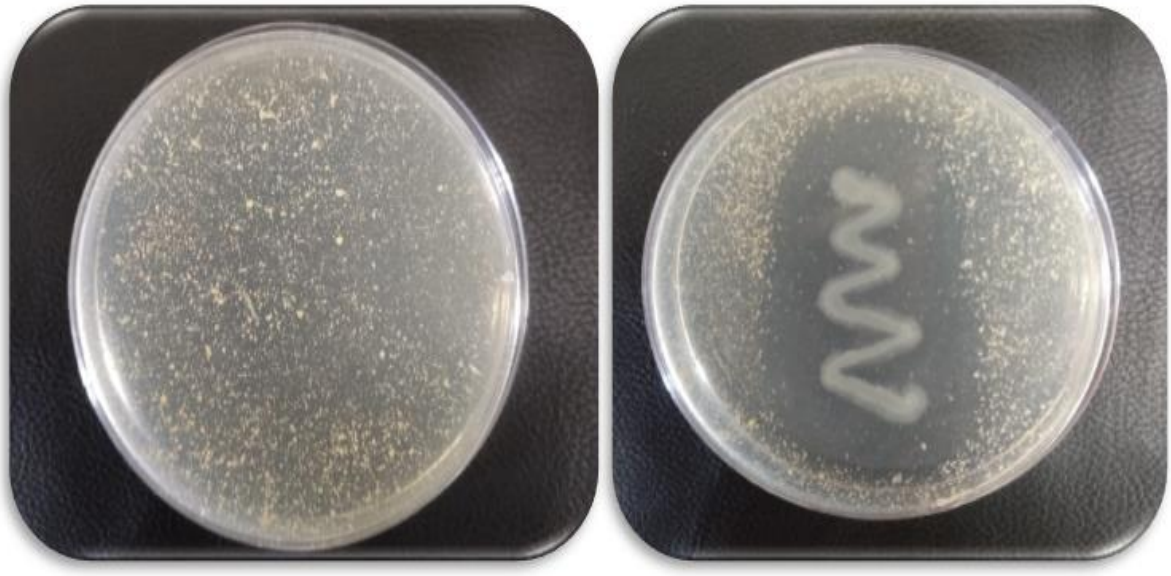

Figure (4):A: Negative control elastin nutrient agar without growth B: Elastase-producing *P. aeruginosa* (P17) showing a clear halo around its growth positive for elastase production

Quantitative assay

The elastase production assessment in bacterial isolates is crucial for understanding their pathogenic potential, particularly in the context of infections associated with tissue damage. In this study, the quantitative measurement of elastolytic activity was performed using a spectrophotometric method, specifically by detecting the release of Congo red dye from elastin degradation at an absorbance of 495 nm (A495). This approach not only corroborates the qualitative findings of elastase production but also provides a more precise quantification of enzymatic activity, which is essential for comparing the elastolytic capabilities of different isolates.

Previous research has demonstrated the utility of spectrophotometric methods for quantifying elastase activity. For instance, a study by [32] utilized similar methodologies to evaluate the elastolytic activity of various bacterial strains, emphasizing the reliability of spectrophotometry in enzyme activity assays. The results presented in this study, particularly the high elastolytic activity of the *P. aeruginosa* isolate (P79), align with findings from other studies that have identified *P. aeruginosa* as a significant producer of elastase. This bacterium is well-known for its role in chronic infections, especially in immunocompromised patients, where its elastase production contributes to tissue destruction and immune evasion [33].

The identification of *P. aeruginosa* (P79) as the isolate with the highest elastolytic activity opens avenues for further investigation into its pathogenic mechanisms and potential therapeutic interventions. Following the quantification of elastase activity, the isolate was subjected to antibiotic susceptibility testing. This step is critical, as it provides insights into the potential treatment options available for infections caused by elastase-producing bacteria. Previous studies have highlighted the importance of understanding the antibiotic resistance profiles of P. aeruginosa, given its notorious ability to develop resistance to multiple classes of antibiotics [34].
Furthermore, the subsequent investigation into the anti-elastase activity of tropolone and plant leaf extracts using the highly elastolytic isolate *P. aeruginosa* (P79) is particularly noteworthy. The exploration of natural compounds as potential inhibitors of elastase activity is gaining traction, as these compounds may offer alternative therapeutic strategies against elastase-producing pathogens. Research by [35] has shown that certain plant extracts possess significant anti-elastase properties, suggesting their potential use in managing infections associated with elastase-producing bacteria.

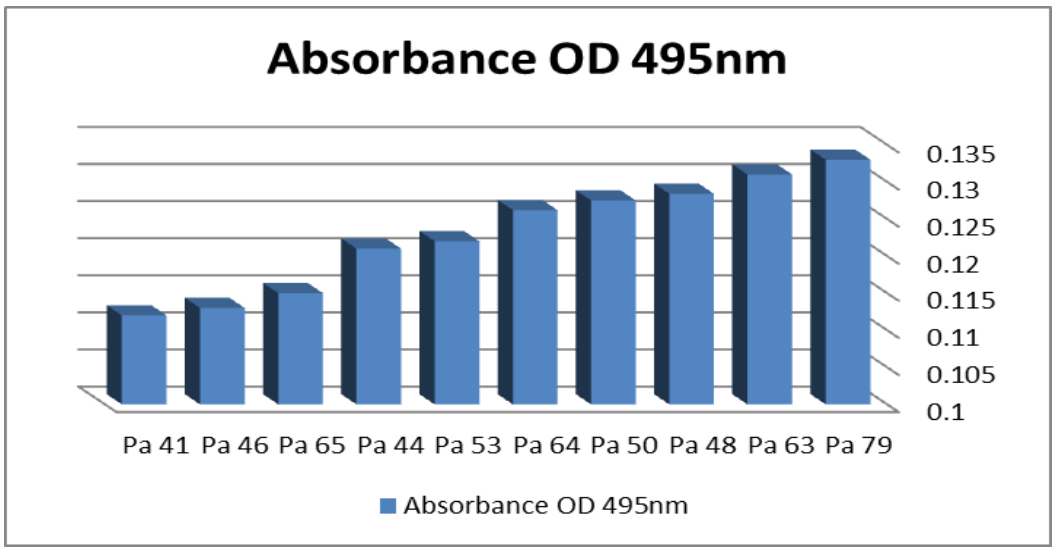

Figure (5): Spectrophotometer reading at (A495)

Optimizing conditions for elastase production

Optimizing the conditions for elastase production by *P. aeruginosa* (P79) is essential for enhancing our understanding of its enzymatic activity and potential applications in biotechnology and medicine. In this study, we assessed the effects of pH and temperature on elastase production, revealing critical insights into the optimal conditions for enzyme synthesis.

Impact of pH levels

Our findings indicate that the highest elastase production occurred at pH 8, with an optical density (OD) reading of 0.26. This observation is consistent with previous research that identified pH 8 as optimal for elastase production in *P. aeruginosa* [36]. The decrease in elastase activity at pH levels of 10 and 4, as well as at pH 7, highlights the sensitivity of elastase production to hydrogen ion concentration (Figure 6). This aligns with the work of. [36], which demonstrated that elastase production tends to decline when pH deviates from the optimum range. The pH environment significantly influences microbial growth and enzyme production, as it affects the ionization of substrates and the overall metabolic activity of the organism.

Interestingly, while our findings support the optimal pH of 8, they contrast with other studies that reported pH 7.5 as the peak for elastase production. This discrepancy may arise from variations in experimental conditions, such as the growth medium or strain differences, underscoring the need for further investigation into the specific environmental factors that influence elastase synthesis. It was observed that elastase production decreased when the pH value was either below or above the optimum level.

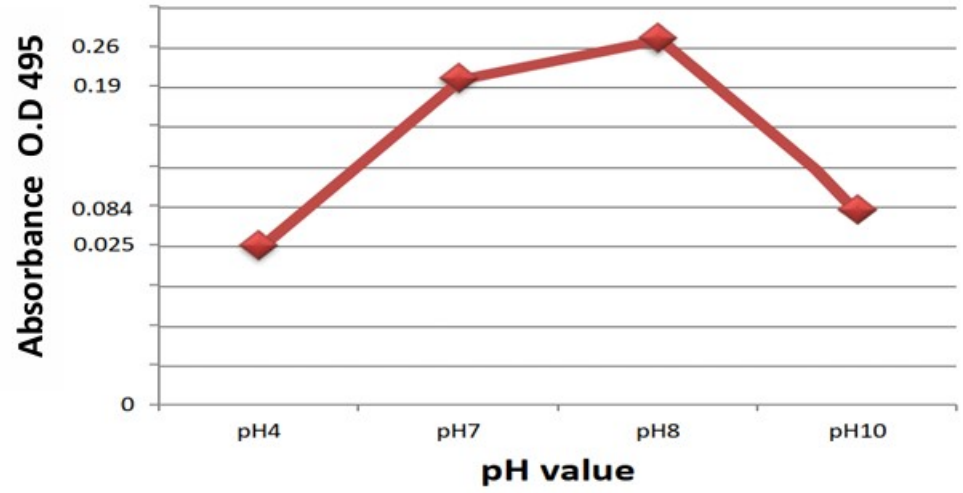

Figure 6. The impact of pH on the production of elastase by *P. aeruginosa* strain(P79).

The impact of temperature

Regarding temperature, our results indicated that the maximum elastase production occurred at 37°C, with an OD of 0.231. This finding corroborates previous studies that have consistently identified 37°C as the optimal temperature for elastase production in *P. aeruginosa* [37]. The decline in

elastase activity at temperatures of 28°C and 42°C suggests that lower and higher temperatures adversely affect enzyme synthesis, likely due to the impact on cellular metabolism and enzyme stability (Figure 7).

However, our results differ from those reported by [38], who found that the optimal elastase production occurred at 30°C, yielding a maximum of 652 U/ml. This variation may be attributed to differences in experimental design, such as the duration of incubation and the specific strain of *P. aeruginosa* used. Temperature is a critical factor in regulating enzymatic activity, as it can influence reaction kinetics and enzyme conformation [39]. The ability to manipulate temperature for enhanced enzyme production has significant implications for industrial applications, where optimized conditions can lead to increased yields.

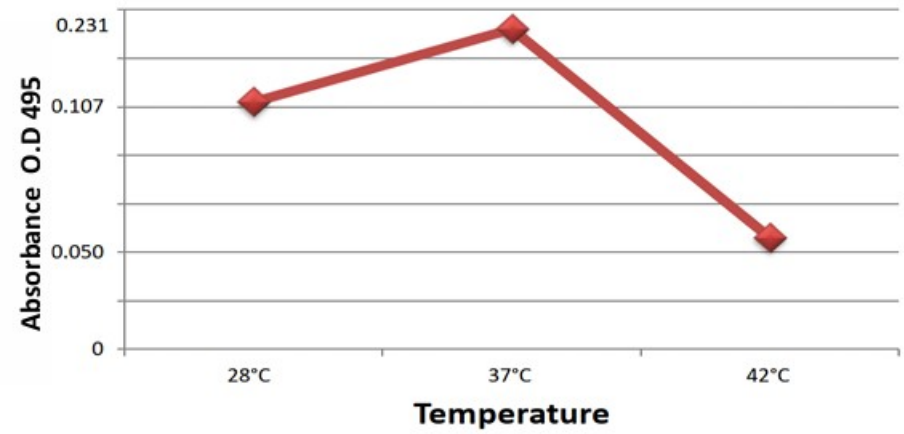

Figure 7. The impact of Temperature on the production of elastase by *P. aeruginosa* strain(P79).

Antibacterial activity of tropolone and plant extracts on the growth of elastase-producing isolates

The exploration of antibacterial agents against elastase-producing isolates of *P. aeruginosa* is critical for addressing the challenges posed by this opportunistic pathogen, particularly in clinical settings where it is associated with chronic infections. In this study, we investigated the antibacterial activity of tropolone and various plant extracts, including onion, garlic, moringa, and leek, on the growth of *P. aeruginosa* (P79), specifically focusing on their effects on elastase production.

Our findings indicate that all tested samples of tropolone exhibited antibacterial activity against the elastase-producing isolates. Notably, tropolone (A) was identified as the most effective agent, completely inhibiting bacterial growth at all concentrations tested. This aligns with

previous research that has highlighted the antimicrobial properties of tropolone, a naturally occurring compound known for its ability to disrupt bacterial cell membranes and inhibit enzymatic activity [40]. The complete inhibition of *P. aeruginosa* growth by tropolone suggests its potential as a therapeutic agent in managing infections caused by elastase-producing strains.

Garlic extract (B) also demonstrated significant antibacterial efficacy, slightly surpassing the effectiveness of other plant extracts. This observation is consistent with the body of literature that recognizes garlic (Allium sativum) as a potent antimicrobial agent, primarily attributed to its active compound, allicin, which has been shown to inhibit the growth of various pathogenic bacteria, including *P. aeruginosa* [41]. The Minimum Inhibitory Concentration (MIC) and Sub-MIC values for both tropolone and garlic extracts, as presented in Table 2, provide important insights into the concentrations required to inhibit bacterial growth effectively.

While the other plant extracts (onion, moringa, and leek) were also tested, their effectiveness in inhibiting elastase production was comparatively lower. This may be due to the varying compositions of phytochemicals present in these extracts, which may not exert the same level of antibacterial activity as tropolone and garlic. Previous studies have shown that the antimicrobial efficacy of plant extracts can vary significantly based on factors such as extraction method, concentration, and the specific phytochemicals present [42].

The implications of these findings are noteworthy, particularly in the context of developing alternative therapeutic strategies against *P. aeruginosa* infections. The ability of tropolone and garlic extract to inhibit elastase production not only suggests a potential dual action against bacterial growth and virulence factors but also highlights the importance of exploring natural compounds as viable alternatives to traditional antibiotics, especially in an era of increasing antibiotic resistance.

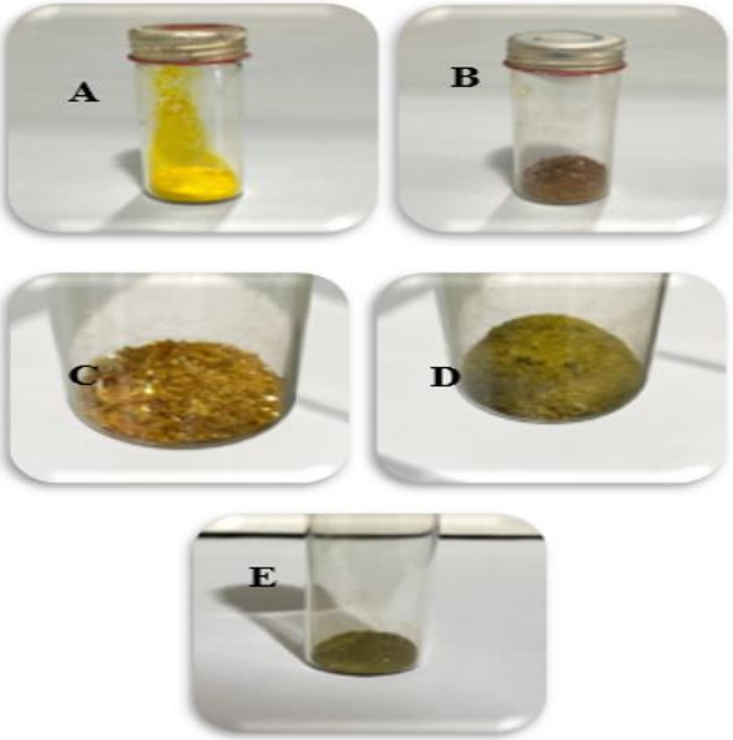

Figure 8. A: tropolone B: garlic leaves extract C: onion leaves extract D: moringa leaves extract E: leeks leaves extract

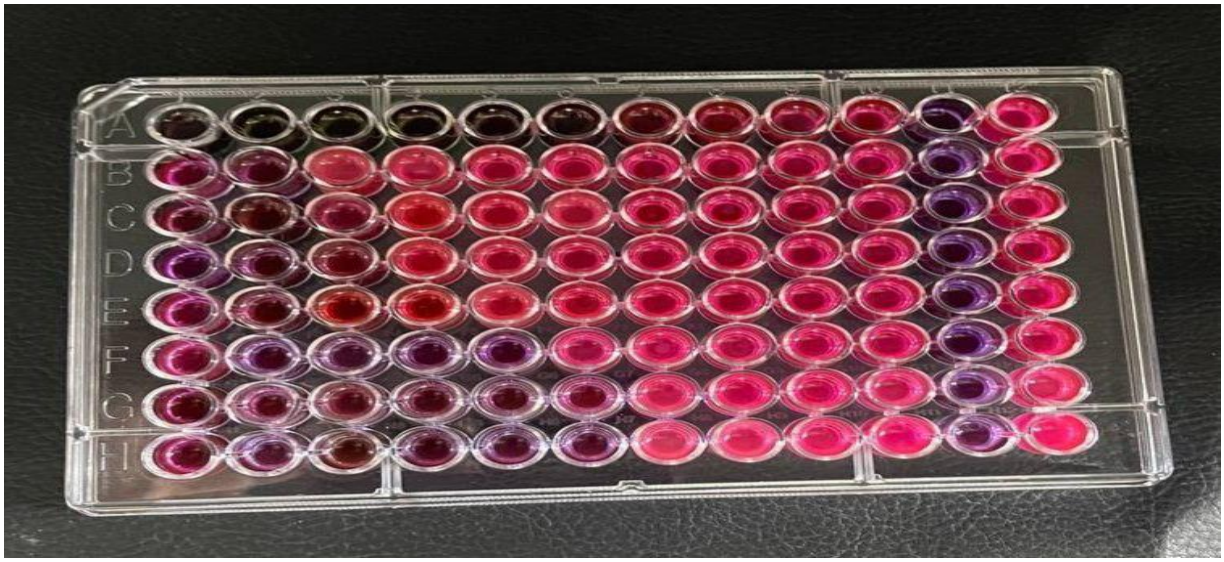

Figure (9): Antibacterial activity against growth of *P. aeruginosa* (A): Tropolone (B): Garlic aqueous extract (C): Onion aqueous extract (D): Leek aqueous extract (E): Moringa aqueous extract (F): Garlic alcoholic extract (G): Onion alcoholic extract (H): Leek alcoholic extract . No. 11: negative control No. 12:positive control.

Table 2. Minimum Inhibitory Concentration (MIC) for tropolone and plant leaves extract (onion, garlic, moringa, leeks extract) on the growth of elastase-producing *P. aeruginosa* (P79)

Materials	MIC	Sub – MIC
Tropolone	15.6	7.8
Garlic aqueous leaves extract	500	250
Onion aqueous leaves extract	1000	500
Leek aqueous leaves extract	250	125
Moringa aqueous leaves extract	500	250
Garlic alcoholic leaves extract	62.5	31.2
Onion alcoholic leaves extract	31.2	15.6
Leek alcoholic leaves extract	31.2	15.6
Moringa alcoholic leaves extract	62.5	31.2

Table 3. Resazurin reagent measurement method (A): Tropolone (B): Garlic aqueous extract (C): Onion aqueous extract (D): Leek aqueous extract (E): Moringa aqueous extract (F): Garlic alcoholic extract (G): Onion alcoholic extract (H): Leek alcoholic extract (I): Moringa alcoholic extract. No. 11: negative control No. 12: positive control.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1000	500	250	125	62.5	31.2	15.6	7.8	3.9	1.95	C (-)	C (+)
B	1000	500	250	125	62.5	31.2	15.6	7.8	3.9	1.95	C (-)	C (+)
C	2000	1000	500	250	125	62.5	31.2	15.6	7.8	3.9	C (-)	C (+)
D	1000	500	250	125	62.5	31.2	15.6	7.8	3.9	1.95	C (-)	C (+)
E	1000	500	250	125	62.5	31.2	15.6	7.8	3.9	1.95	C (-)	C (+)
F	1000	500	250	125	62.5	31.2	15.6	7.8	3.9	1.95	C (-)	C (+)
G	1000	500	250	125	62.5	31.2	15.6	7.8	3.9	1.95	C (-)	C (+)
H	1000	500	250	125	62.5	31.2	15.6	7.8	3.9	1.95	C (-)	C (+)
I	1000	500	250	125	62.5	31.2	15.6	7.8	3.9	1.95	C (-)	C (+)

The evaluation of antibacterial compounds against elastase-producing isolates of *P. aeruginosa* is critical for developing effective therapeutic strategies, particularly given the pathogen's role in chronic infections and its ability to produce virulence factors such as elastase. In this study, we assessed the antibacterial activity of various extracts, including tropolone, garlic, onion, moringa, and leek, against elastase-producing *P. aeruginosa*. Our results

indicate that all tested compounds exhibited varying degrees of antibacterial activity, highlighting the potential of these natural extracts as alternatives to conventional antibiotics.

Tropolone emerged as the most effective compound, achieving complete inhibition of bacterial growth at all tested concentrations (15.6 µg/mL). This finding is consistent with previous research that has documented the antimicrobial properties of tropolone, which is known to disrupt bacterial cell membranes and inhibit key metabolic processes [43]. The broad-spectrum activity of tropolone underscores its potential as a therapeutic agent against elastase-producing bacteria, particularly in the context of infections where traditional antibiotics may be ineffective.

Garlic extract also demonstrated significant antibacterial activity, particularly at higher concentrations (500 µg/mL and 62.5 µg/mL), with the alcoholic extract showing slightly greater efficacy than other forms. This aligns with the extensive literature documenting garlic's antimicrobial properties, primarily attributed to its active compound, allicin, which has been shown to inhibit the growth of a variety of pathogens, including *P. aeruginosa* [44]. The effectiveness of garlic extract at elevated concentrations suggests that it may be beneficial in formulations aimed at treating infections caused by elastase-producing bacteria.

Onion extract exhibited a similar pattern of activity, with significant inhibition observed at higher concentrations (1000 µg/mL and 31.2 µg/mL). The aqueous extract at the highest concentration demonstrated slightly greater effectiveness, reinforcing the idea that the extraction method can influence the bioactivity of plant extracts. Previous studies have shown that onion (Allium cepa) contains various sulfur compounds that contribute to its antimicrobial properties, making it a valuable candidate for further investigation.

Moringa extract also showed antibacterial activity, inhibiting growth at the highest concentration tested (500 µg/mL). Moringa oleifera is recognized for its rich nutritional profile and bioactive compounds, which have been reported to possess antimicrobial properties against various pathogens. However, the limited effectiveness of leek extract (250 µg/mL and 31.2 µg/mL) at all tested concentrations suggests that it may not be as potent as the other extracts evaluated. This variability in efficacy may be attributed to differences in the phytochemical composition of the extracts, which can significantly impact their antimicrobial activity.

Conclusion

Our study highlights the varying degrees of antibacterial activity exhibited by tropolone, garlic, onion, moringa, and leek extracts against elastase-producing isolates of P. aeruginosa. The findings support the potential use of these natural compounds as alternative therapeutic agents in managing infections associated with elastase production. Future research should focus on elucidating the mechanisms of action of these extracts and exploring their efficacy in vivo models to further assess their therapeutic potential.

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تأثير التروبولون وبعض المستخلصات النباتية على إنتاج الإيلاستاز من عزلات *Pseudomonas aeruginosa*

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مستخلص البحث:
الزائفة الزنجارية هي ممرضة انتهازية مهمة معروفة بإنتاجها للإيلاستاز، وهو إنزيم يساهم في تلف الأنسجة والضراوة. إن الانتشار المتزايد لمقاومة المضادات الحيوية يستلزم استكشاف عوامل مضادة للميكروبات بديلة، بما في ذلك المركبات الطبيعية. لتقييم النشاط المضاد للبكتيريا للتروبولون ومستخلصات نباتية مختلفة (الثوم والبصل والمورينجا والكراث) ضد عزلات *P. aeruginosa* المنتجة للإيلاستاز (P79). تم جمع عزلات *P. aeruginosa* وتقييمها لإنتاجها لإنزيم الإيلاستاز. تم تقييم النشاط المضاد للبكتيريا باستخدام طريقة انتشار القرص، مع تركيزات متفاوتة من التروبولون ومستخلصات النباتات. تم تحديد قيم الحد الأدنى للتركيز المثبط (MIC) لقياس فعالية كل مركب ضد عزلات البكتيريا. أظهرت جميع المركبات المختبرة درجات متفاوتة من النشاط المضاد للبكتيريا. كان التروبولون هو الأكثر فعالية، حيث يثبط نمو البكتيريا تمامًا في جميع التركيزات المختبرة (15.6 µg/mL). أظهر مستخلص الثوم تثبيطًا كبيرًا عند تركيزات أعلى (500 µg/L و 62.5 µg/mL)، بينما أظهر مستخلص البصل أيضًا نشاطًا عند تركيزات مرتفعة (1000 µg/mL و31.2µg/mL). كما ثبت أن مستخلص المورينجا يثبط النمو عند أعلى تركيز (500 µg/mL)، في حين أظهر مستخلص الكراث نشاطًا مضادًا للبكتيريا محدودًا في جميع التركيزات. تدعم هذه النتائج استكشاف المركبات الطبيعية كبدائل للمضادات الحيوية التقليدية في علاج الالتهابات التي يسببها هذا العامل الممرض.

الكلمات المفتاحية: تروبولون؛ إنزيم الإيلاستاز؛ الزائفة الزنجارية؛ التأثير المثبط لتروبولون؛ المستخلصات النباتية.