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Evaluation of the antibacterial effect of ciprofloxacin coated with silver nanoparticles (AgNPs) on *Pseudomonas aeruginosa* isolated from wounds

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen commonly associated with burn wound infections and known for its resistance to multiple antibiotics. In this study, ciprofloxacin was combined with biosynthesized silver nanoparticles (AgNPs) derived from *Thymus capitatus* leaf extract to evaluate their antibacterial efficacy against *Ps. aeruginosa*. Clinical isolates were obtained from burn patients and identified using morphological and biochemical methods. Silver nanoparticles were synthesized via green synthesis, characterized by X-Ray Diffraction) XRD), Fourier Transform Infrared Spectroscopy) FTIR), Energy Dispersive X-ray Spectroscopy) EDX), Scanning Electron Microscopy) SEM), and Transmission Electron Microscopy) TEM) analyses, and confirmed to be predominantly spherical. The antibacterial activity was evaluated using the agar diffusion method. Results demonstrated that AgNPs alone exhibited a strong inhibitory effect, with a maximum inhibition zone of 18.67 ± 0.58 mm at 64 µg/mL, whereas ciprofloxacin alone showed an inhibition zone of 14.00 ± 1.00 mm. The combination of ciprofloxacin with silver nanoparticles) AgNPs)slightly enhanced the antibacterial activity (15.33±0.58 mm), indicating a synergistic effect. These findings suggest that silver nanoparticles) AgNPs) have significant potential as an adjuvant to conventional antibiotics for combating multidrug-resistant *Psedomonas aeruginosa* in wound infections.

Introduction

Bacterial infections remain a plague to modern healthcare systems, particularly with the ominous rise of antimicrobial resistance. One such preeminent opportunistic pathogen is *Pseudomonas aeruginosa*, which has widespread notoriety accorded by the World Health Organization for its propensity to develop resistance and cause severe infections in immunocompromised individuals. Common reservoirs of *Ps. aeruginosa* are moist surfaces such as sinks, respiratory devices, and catheters, which allow it to be transferred to vulnerable patients who have open wounds, burns, or those who receive invasive procedures [1].

The pathogen's virulence is due to a complex arsenal that includes biofilm formation, secretion systems (e.g., Type III), tissue-degrading enzymes, quorum sensing, and the complex

iron acquisition via siderophores. These mechanisms enhance its adaptability and foster chronic, hard-to-treat infections [2].

Nanotechnology has been proposed as a promising strategy against these problems. Nanoparticles, and more specifically silver nanoparticles (AgNPs), exhibit broad-spectrum antimicrobial activity through mechanisms that involve cell wall disruption, DNA interaction, and induction of reactive oxygen species (ROS) [3]. More importantly, AgNPs have also demonstrated synergistic effect when combined with conventional antibiotics, with enhanced efficacy compared to that of individual agents [4]. Ciprofloxacin, a fluoroquinolone antibiotic with wide application against Gram-negative bacteria, including *Ps. aeruginosa*, works by inhibiting DNA gyrase and topoisomerase IV. Its efficacy has, however, decreased due to resistance via mutations or efflux mechanisms [5].

It has been revealed in recent work that enhanced antibacterial efficacy of AgNPs as a co-therapy with ciprofloxacin. Silver nanoparticles biosynthesized using the metabolites of Kocuria flava, for instance, decreased the MIC of ciprofloxacin against sharp multidrug-resistant *Ps. aeruginosa*, exhibiting a clear synergism [6]. In another study, AgNPs alone were shown to be of high activity against MDR and XDR isolates of *Ps. aeruginosa*, preventing biofilm formation, motility, and production of virulence factors such as swarming, protease, gelatinase, and pyocyanin, and changing expression of important virulence genes [7].

Apart from silver, other nanoparticle systems have also been found useful in supplementing antibiotic delivery and overcoming biofilm defense, pH-responsive, charge-reversing smart nanoparticles penetrated *Ps. aeruginosa* biofilms more effectively, enabling higher delivery of antibiotics like tobramycin and causing a significant decrease in bacteria [8]. Apart from that, the novel nanocomposite made of ciprofloxacin-derived carbon dots and silver nanoparticles displayed intense antibacterial and antibiofilm activity with the potential to decrease up to 5-log colony-forming units and induce massive biofilm removal through positive electrostatic interaction [9]. Aim of the study A comparison was made between the effect of the antibiotic ciprofloxacin coated with silver nanoparticles and the antibiotic alone on the activity of *Pseudomonas aeruginosa* isolates obtained from clinical wound samples.

Materials and methods

Collection and Identification of Clinical Samples

Clinical samples were collected from patients with infected surgical wounds attending private clinics using sterile cotton swabs. The samples were cultured on various media, including nutrient agar, blood agar, MacConkey agar, and Cetrimide agar, to observe colony growth characteristics such as shape, color, and hemolysis patterns. Identification of bacterial isolates was performed through microscopic examination and a series of biochemical tests. These included the indole test for indole production, methyl red test to assess sugar fermentation and acid production, Voges-Proskauer test for acetoin detection, citrate utilization test to evaluate citrate consumption and sodium carbonate formation, urease test to detect urea hydrolysis, oxidase test for cytochrome production, catalase test, and sugar fermentation assays [10].

Preparation of Plant Extract

Approximately 20 g of *Thymus capitatus* leaves were thoroughly washed four times with deionized water to remove impurities and then air-dried at room temperature. The dried leaves were ground into a fine powder and mixed with 100 ml of deionized water. The mixture was heated at 60ºC and boiled for 20 minutes. After boiling, the extract was cooled to room temperature, filtered, and 75 ml of the clear yellowish extract was collected and stored at 4ºC for further use [11].

Synthesis of Silver Nanoparticles (AgNPs)

A 4 mM silver nitrate ($AgNO_3$) solution was prepared by dissolving 0.067 g of $AgNO_3$ in 100 ml of deionized water. The solution was kept in the dark to prevent photo-degradation. Then, 5 ml of the prepared *Thymus capitatus* leaf extract was added to 45 ml of the silver nitrate solution. The mixture was heated on a hot plate with continuous stirring using a magnetic stirrer for one hour. The color of the solution changed from transparent yellow to dark brown, indicating the formation of silver nanoparticles. The resulting AgNPs solution was centrifuged to precipitate the nanoparticles, and the supernatant was discarded. The pellet was dried in an electric oven at 40ºC until completely dry, yielding a fine silver nanopowder [12].

Antibiotic loading on silver nanoparticles

A 0.02 g of silver nanoparticles were dissolved in 100 mL distilled water using a magnetic stirrer rod at 1000 (rpm) for 30 min, 0.2 g of antifreeze powder was dissolved in 100 mL distilled water using a magnetic stirrer for 15 min, then mixed 25 ml of each of the two solutions by magnetic stirrer for 45 minutes, after homogenization the mixture was placed in the ultrasonic device for 45 minutes, to obtain the smallest possible volume. The solution was filtered using a 0.22 µm Whatman filter paper [13].

Characterization of Prepared Silver Nanoparticles

The synthesized silver nanoparticles (AgNPs) were characterized using energy-dispersive X-ray spectroscopy (EDX) to determine their elemental composition. Additionally, transmission electron microscopy (TEM) was employed to evaluate the morphology and size distribution of the nanoparticles.

Antibacterial Activity Assessment

The inhibitory effects of silver nanoparticles alone, ciprofloxacin antibiotic alone, and ciprofloxacin coated with silver nanoparticles against *Pseudomonas aeruginosa* were evaluated using the agar well diffusion method. Wells of 6 mm diameter were drilled into Mueller-Hinton agar plates using a cork borer to ensure uniform size and prevent overlap of inhibition zones. A bacterial suspension of 0.1 ml was evenly spread over the agar surface. Subsequently, 60 µl of each test solution—AgNPs at concentrations of 64, 32, 16, 8, and 4 µg/ml, ciprofloxacin alone, and ciprofloxacin coated with AgNPs—was added to the wells. Plates were refrigerated for one hour to allow diffusion of the test substances, then incubated at 37°C for 24 hours. The diameters of inhibition zones were measured in millimeters using a ruler [14].

Statistical Analysis

Data obtained from inhibition zone measurements were statistically analyzed using analysis of variance (ANOVA) based on a completely randomized design (CRD). Means were compared using Tukey's multiple comparison test at a significance level of $p \leq 0.05$ [15].

Results and Discussion

Clinical bacterial swabs were collected from burn infection patients attending private clinics in Samarra. Following morphological and biochemical testing, a total of 20 bacterial isolates were obtained. Pseudomonas aeruginosa was identified based on its phenotypic characteristics. On MacConkey agar, colonies appeared pale due to the bacterium's inability to ferment lactose "[16]". On blood agar, the colonies exhibited beta-hemolysis, indicating production of the hemolysin enzyme [17]. Additionally, growth on cetrimide agar showed colonies with characteristic pigmentation: greenish-yellow (pyoverdine) or greenish-blue (pyocyanin), both of which fluoresce under ultraviolet light and are water-soluble [18].

Biosynthesis of Silver Nanoparticles (AgNPs)

The aqueous leaf extract of *Thymus capitatus* was utilized as a reducing and stabilizing agent for the biosynthesis of silver nanoparticles. The formation of AgNPs was confirmed by the appearance of a precipitate at the bottom of the reaction mixture, indicating successful nanoparticle synthesis. The biosynthetic approach offers advantages such as low cost, environmental safety, ease of operation, and reduced toxicity.

Characterization of AgNPs

Fourier Transform Infrared Spectroscopy (FTIR)

The AgNPs samples were examined using FTIR spectroscopy to identify the presence of capping agents as well as the effective stability of the synthesized metallic nanoparticles (NPs). The FTIR analysis of the silver nanoparticles (AgNPs) showed characteristic transmittance peaks at 3435.56, 2354.57, 2080.76, 1958.82, 1635.80, 1455.50, 1046.57, and 667.44 cm^{-1}, as shown in Figure 1.

The peak at 3435.56 cm^{-1} was assigned to the hydroxyl (–OH) group, as part of the O–H stretching that forms during the oxidation process when moisture is absorbed onto the highly reactive surface of the silver nanoparticles, this is in agreement with previously reported findings, Muzamil *et al*. [19].

FTIR testing is considered one of the precise laboratory techniques used to identify chemical elements in the compounds under study. The identity of chemical compounds depends on how the chemical bonds within the compounds absorb infrared radiation, as each compound has its unique absorption spectrum [20].

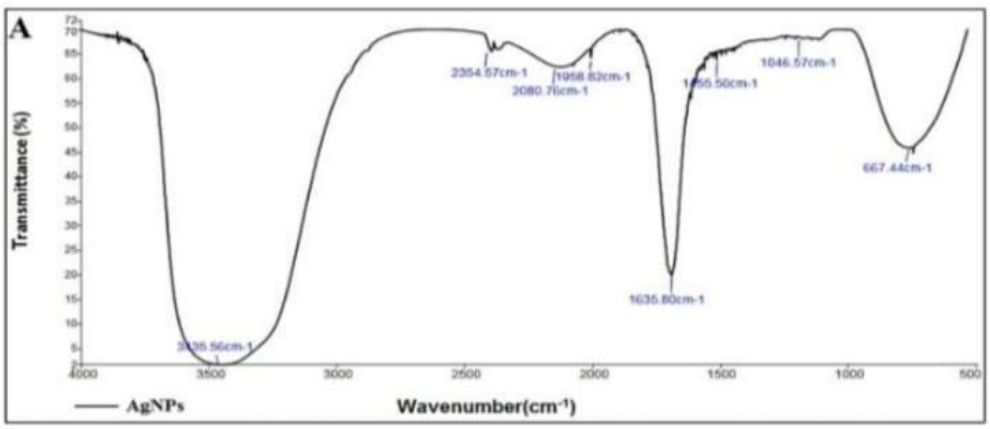


Fig 1: Fourier Transform Infrared Spectroscopy (FTIR) Analysis of AgNPs Compound

Field Emission Scanning Electron Microscopy (FE-SEM)

The size, surface morphology, and distribution uniformity of the nanoparticles (NPs) were evaluated using Field Emission Scanning Electron Microscopy (FE-SEM). This technique allows for both qualitative and quantitative data acquisition, in addition to providing detailed information regarding the morphology and size of the nanoparticles [21].

The FE-SEM images of the green-synthesized silver nanoparticles (AgNPs) confirmed the formation of highly dense nanostructures, as shown in Figure 2. The images revealed that the AgNPs were spherical and well-dispersed in the solution without any noticeable aggregation. Figure 2 also showed that most of the NPs had smooth surfaces, with diameters ranging from 32.08 nm to 43.28 nm. These results were consistent with the XRD analysis. The slight variations in particle size could be attributed to the fact that SEM images generally reflect the metallic core of the nanoparticles.

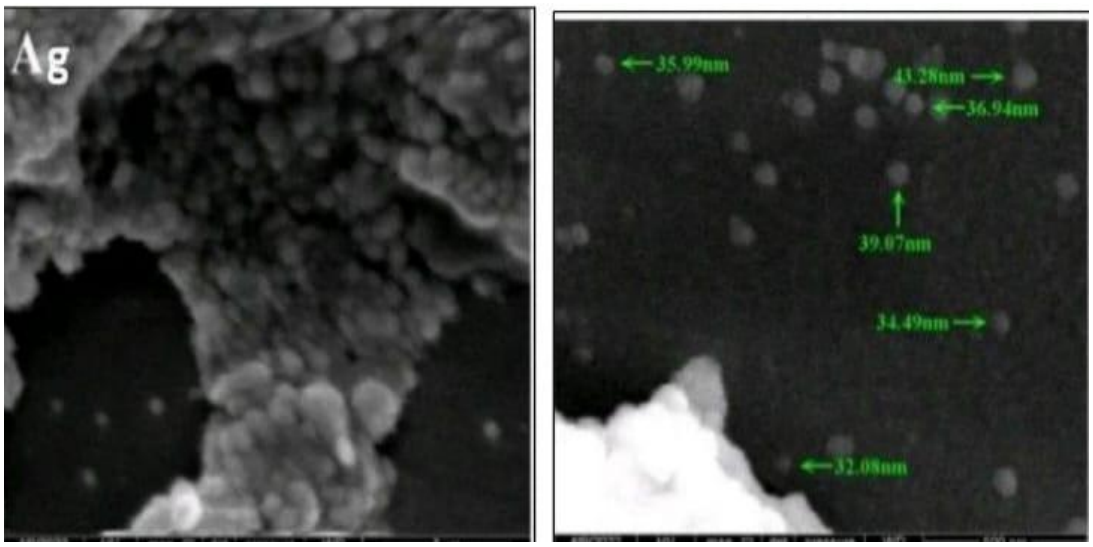


Fig 2: Field Emission Scanning Electron Microscopy (FE-SEM) Image of AgNPs

X-ray Diffraction (XRD) Analysis

The XRD pattern of the synthesized AgNPs showed four prominent peaks at 2θ values of 32.12º, 38.04º, 46.21º, and 64.18º, which correspond to the crystallographic planes (101), (111), (200), and (220), respectively (Figure 3).

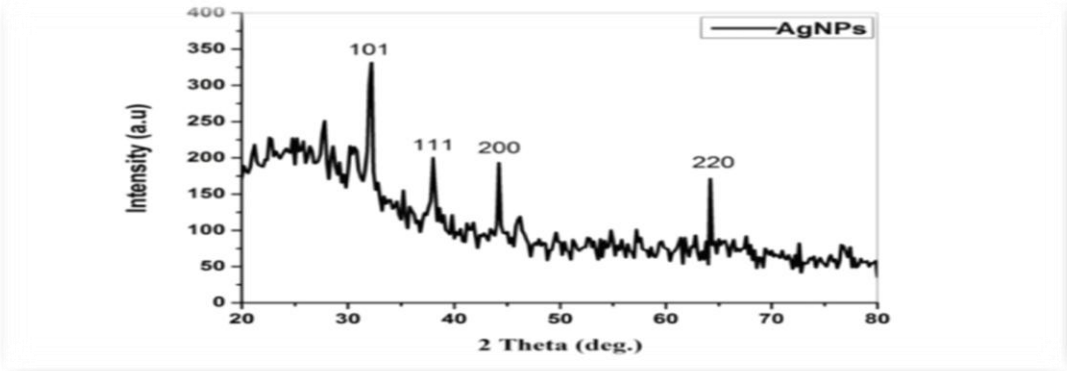
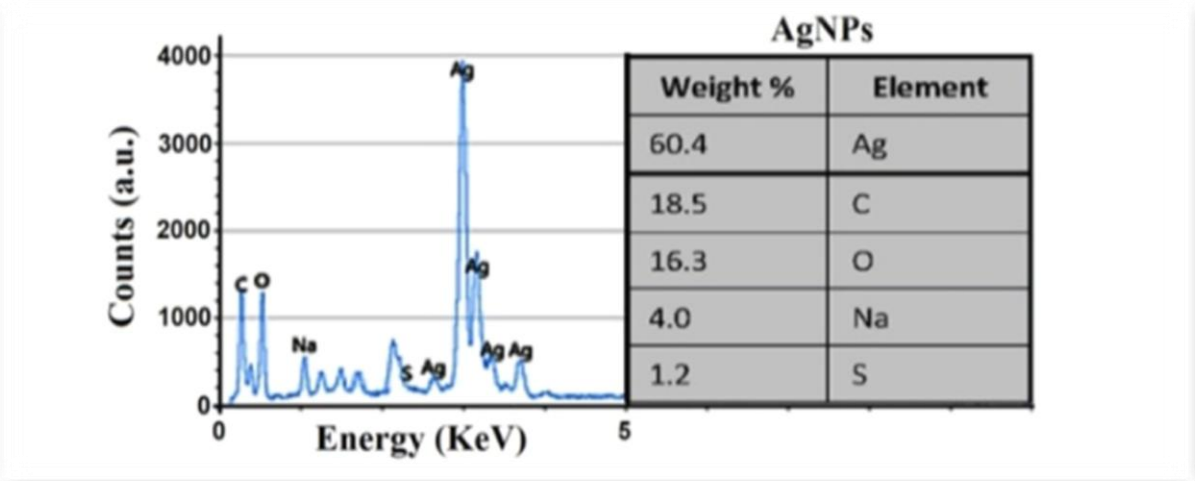


Fig 3: X-ray diffraction (XRD) of AgNPs

Energy Dispersive X-ray Analysis (EDX)

The EDX analysis revealed that silver (Ag) constituted 60.4% by weight of the total elements present in the synthesized nanoparticles. Minor amounts of carbon (C), oxygen (O), sulfur (S), and sodium (Na) were also detected, which are likely residues from the chemical reagents and plant extract used during the biosynthesis process. The elemental composition confirms the successful synthesis of silver nanoparticles. Additionally, statistical analysis demonstrated significant differences in the inhibitory effects across all tested concentrations of AgNPs, as illustrated in Figure 4.



Weight %	Element
60.4	Ag
18.5	C
16.3	O
4.0	Na
1.2	S

Fig 4: Energy **dispersive X-ray analysis EDX**

Transmission Electron Microscopy (TEM) Analysis

TEM analysis was conducted at a magnification of 77,500x to investigate the morphology and size distribution of the synthesized silver nanoparticles. The micrographs revealed that the majority of the nanoparticles exhibited a predominantly spherical shape with varying sizes, as shown in Figure 5.

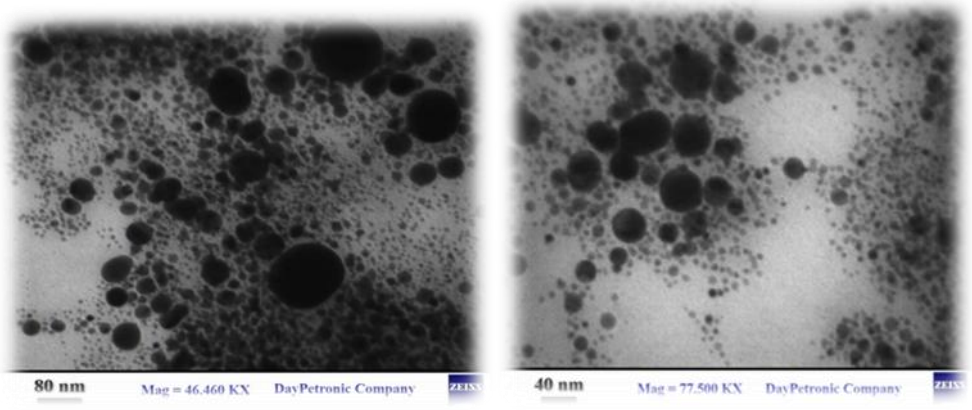


Fig 5: AgNPs under TEM electron microscopy

Determination of Antibacterial Effectiveness of AgNPs, Ciprofloxacin, and Ciprofloxacin-Coated Silver Nanoparticles

The antibacterial activity against *Pseudomonas aeruginosa* was assessed using the agar diffusion method with five concentrations of silver nanoparticles (64, 32, 16, 8, and 4 µg/ml).

At the highest concentration (64 µg/ml), AgNPs exhibited a significant inhibition zone diameter of 18.67 ± 0.58 mm. No inhibitory effect was observed at the lower concentrations of 8 and 4 µg/ml. Ciprofloxacin alone showed an inhibition zone of 14.00 ± 1.00 mm at 64 µg/ml, while ciprofloxacin coated with silver nanoparticles demonstrated a slightly enhanced inhibition zone of 15.33 ± 0.58 mm at the same concentration. Statistical analysis indicated significant differences among the treatments at a significance level of $P < 0.05$, as detailed in the accompanying Table1 and Figure 6.

The findings of this study are consistent with Kumar *et al*. [22] and Aljabali *et al*. [23] who determined the strong antibacterial activity of silver nanoparticles against Pseudomonas aeruginosa. Additionally, Ghosh *et al*. [24] and Abdallah *et al*. [25] demonstrated a significant boost in antibacterial activity when AgNPs were mixed with ciprofloxacin, with evidence of major synergism. Besides, the results of Ahmed *et al*. [26] and El-Batal *et al*. [27] are in harmony with the better effectiveness of ciprofloxacin when coated using silver nanoparticles.

Table 1: Inhibitory activity against *Pseudomonas aeruginosa*

Con. µg/ml	Diameter of Inhibition Zone (mm)		
	AgNPs	CIP	AgNPs-CIP
64	18.67±0.58 a	14.00±1.00 b	15.33±0.58 b
32	17.67±0.58 a	10.33±0.58 c	14.33±0.58 b
16	14.33±0.49 b	0.00 ± 0.00 c	0.00 ± 0.00 c
8	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c
4	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 c

*Different letters indicate a significant difference

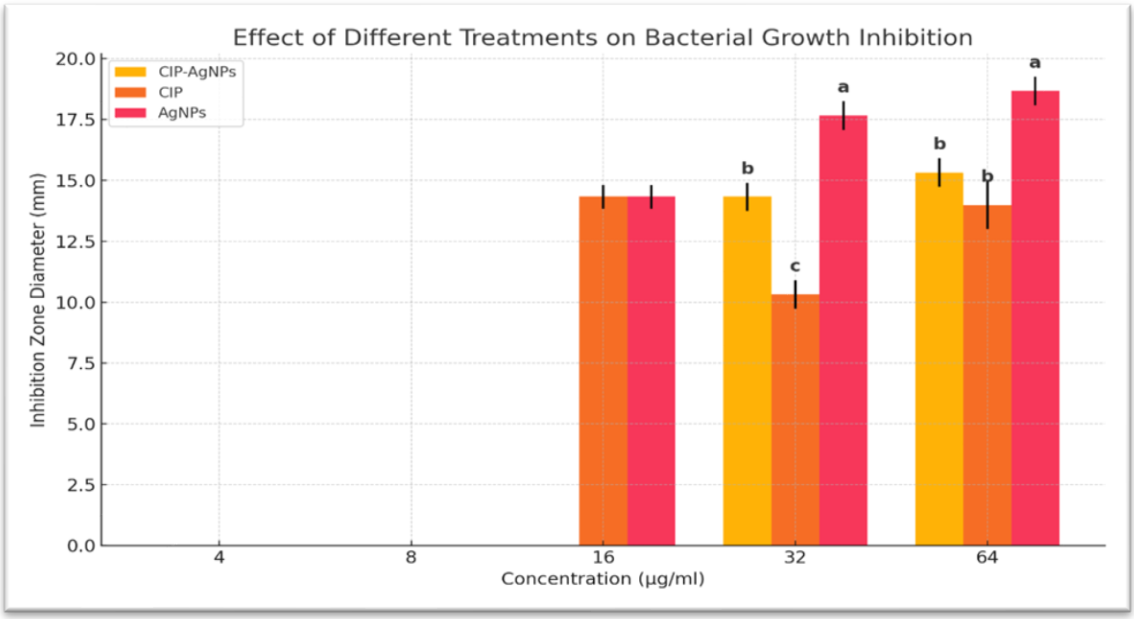


Figure 6: Inhibitory activity against *Pseudomonas aeruginosa*

The antibacterial activity of silver nanoparticles (AgNPs) can be attributed to several mechanisms, including the generation of oxidative stress and disruption of DNA replication, as well as direct bacterial cell lysis through damage to the cell membrane [17]. Silver nanoparticles may also interact with essential peptides that are critical for bacterial survival and cell division. Initially, AgNPs attach to the bacterial cell wall, subsequently penetrating the cells and inducing membrane disruption, leading to bacterial death, this underlines the potential of AgNPs as alternatives to conventional antimicrobial agents [18].

Moreover, AgNPs mediate DNA damage through the ingress of Ag^+ ions between uracil and pyrimidine base pairs, resulting in the breakdown of the DNA double helix and interruption of replication processes [19]. The bactericidal effect of nanosilver also involves interference with several cellular processes, including disulfide bond formation and metabolism, which increases reactive oxygen species (ROS) production and membrane permeability, ultimately promoting bacterial death [20].

These findings align with previous studies demonstrating the synergistic effect of silver nanoparticles when combined with antibiotics against *Ps. aeruginosa*. Such synergy allows for reduced antibiotic dosages, potentially minimizing side effects and enhancing efficacy against resistant pathogenic strains [21].

Conclusions

This study demonstrated that biosynthesized silver nanoparticles (AgNPs), prepared using *Thymus capitatus* leaf extract, exhibit significant antibacterial activity against *Pseudomonas aeruginosa* isolated from wound infections. The AgNPs alone showed a strong inhibitory effect, particularly at higher concentrations. While ciprofloxacin alone was effective, its antibacterial efficacy was slightly enhanced when combined with AgNPs, indicating a synergistic interaction. The green synthesis method proved to be simple, cost-effective, and environmentally friendly. These findings highlight the potential of AgNPs as promising adjuvants to conventional antibiotics, particularly in combating multidrug-resistant (MDR) strains of *Ps. aeruginosa* in clinical settings.

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تقييم التأثير المضاد للبكتيريا للمضاد الحيوي سيبروفلوكساسين المغلف بجسيمات الفضة النانوية (AgNPs) على بكتيريا *Pseudomonas aeruginosa* المعزولة من الجروح

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معلومات البحث:
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تاريخ التعديل: 2025/07/20
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الكلمات المفتاحية:
Pseudomonas aeruginosa، سيبروفلوكساسين، الجسيمات النانوية الفضية، نبات الزعتر Thymus capitatus، مقاومة االجراثيم للمضادات.

معلومات المؤلف
الايميل:
الموبايل:

الخلاصة:
تُعد *Pseudomonas aeruginosa* من البكتيريا الانتهازية الشائعة المرتبطة بعدوى الجروح الناتجة عن الحروق، وتُعرف بمقاومتها المتعددة للمضادات الحيوية. في هذه الدراسة، تم دمج المضاد الحيوي سيبروفلوكساسين مع جسيمات الفضة النانوية (AgNPs)المُخلقة حيويًا من مستخلص أوراق نبات *Thymus capitatus* لتقييم فعاليتها المضادة للبكتيريا ضد *Ps. aeruginosa* تم الحصول على عزلات سريرية من مرضى الحروق وتشخيصها باستخدام الطرق المورفولوجية والكيميائية الحيوية. تم تحضير الجسيمات النانوية الفضية باستخدام التخليق الأخضر، وتم توصيفها باستخدام تقنيات XRD ، FTIR، EDX، SEM وTEM، حيث تبيّن أن لها شكل كروي في الغالب. تم تقييم النشاط المضاد للبكتيريا باستخدام طريقة الانتشار على وسط الأجار. أظهرت النتائج أن الجسيمات النانوية الفضية وحدها أبدت تأثيرًا مثبطًا قويًا، حيث بلغ متوسط قطر منطقة التثبيط 0.58±18.67 ملم عند تركيز 64 ميكروغرام/مل، في حين أن السيبروفلوكساسين وحده أظهر منطقة تثبيط بمقدار 1.00±14.00 ملم. أما عند دمج السيبروفلوكساسين مع الجسيمات النانوية الفضية فقد لوحظ تحسن طفيف في الفعالية المضادة للبكتيريا (0.58±15.33 ملم)، مما يشير إلى وجود تأثير تعاوني. وتشير هذه النتائج إلى أن للجسيمات النانوية الفضية إمكانات واعدة كمساعد للمضادات الحيوية التقليدية في مكافحة *Ps. aeruginosa* المقاومة للأدوية المتعددة في حالات عدوى الجروح.