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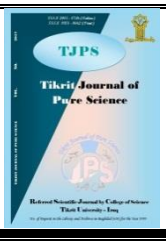

Analysis of the Synergistic Effect of Antibiotics with Copper Oxide Nanoparticles Against *Pseudomonas aeruginosa* Isolated from Different Clinical Cases

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ABSTRACT

Synergism between antibiotics and nanoparticles is one of the most important ways to combat antibiotic resistance. Copper nanoparticles were produced using a green, environmentally friendly method of mixing aqueous *Ficus Sycomorus* leaf extract with copper sulfate. The work aimed to study the synergistic effect of six antibiotics with copper oxide nanoparticles prepared in a green approach against bacteria *Pseudomonas aeruginosa* isolated from patient. A total of 110 specimens were collected from patients with burn, wound and urine infections. in Samarra General Hospital, Tikrit Teaching Hospital, Tikrit Military Hospital, and Baghdad Medical City of both genders from July to September 2023. A bacteriological examination was conducted to select bacterial pathogens, with a particular focus on *Pseudomonas aeruginosa*. From a total 110 cases, 50 (45.5%) gave positive cultures for *Pseudomonas aeruginosa*. CuO-NPs was characterized using different analytical techniques, Scanning Electron Microscope (SEM). showed that the CuO-NPs have a spherical shape, its average size is 29.2 nm. Energy-dispersive X-ray (EDX) results confirmed the presence of copper and oxygen in the composition. The synergistic effect of six antibiotics with CuO-NPs was studied. By evaluating the minimum inhibitory concentration of *P. aeruginosa*. The results show the highest inhibition against bacterial isolates was due to the synergistic effect of CuO-NPs with Impenem, Ciprofloxacin, Ceftazidime, Polymyxin B, Amikacin, and Cefepime respectively, especially on isolate (15) with zones of inhibition/mm were 19.33 ±0.33, 18.33 ±0.33, 16.33 ±0.33, 15.33 ±0.33, 14.67 ±0.33, and 12.7±0.58, respectively. The results indicated that the synergistic effect gave clear zones of inhibition against all isolates compared with the inhibition effect of nano-copper oxide and antibiotics.

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دراسة التأثير التآزري للمضادات الحيوية مع جزيئات أوكسيد النحاس النانوية ضد بكتيريا الزائفة الزنجارية المعزولة من حالات سريرية مختلفة

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الملخص

يعد التآزر بين المضادات الحيوية والجسيمات النانوية أحد أهم الطرق لمكافحة مقاومة المضادات الحيوية. تم إنتاج جسيمات النحاس النانوية باستخدام طريقة خضراء صديقة للبيئة تتمثل في خلط المستخلص المائي لأوراق نبات التين*Ficus Sycomorus* مع كبريتات النحاس. تم العمل على دراسة التأثير التآزري لستة مضادات حيوية مع جزيئات أكسيد النحاس النانوية المحضرة بالطريقة الخضراء ضد بكتيريا الزائفة الزنجارية المعزولة من المرضى. تم جمع 110 عينات من اصابات الحروق والجروح والادرار في مستشفى سامراء العام ومستشفى تكريت التعليمي ومستشفى تكريت العسكري ومدينة الطب ببغداد من كلا الجنسين للفترة من تموز الى ايلول 2023. وتم اجراء الفحص البكتريولوجي لعزل مسببات الامراض البكتيرية، مع التركيز بشكل خاص على. بكتيريا الزائفة الزنجارية من إجمالي 110 حالة، أعطت 50 (45.5٪) نتائج إيجابية لبكتيريا الزائفة الزنجارية تم تشخيص CuO-NPs باستخدام تقنيات تحليلية مختلفة، استخدمت تقنية المجهر الإلكتروني الماسح (SEM). أظهر أن CuO-NPs لها شكل كروي، ويبلغ متوسط حجمها 29.2 نانومتر . وأكدت نتائج EDX وجود النحاس والأوكسجين في التركيبة. تمت دراسة التأثير التآزري لستة مضادات حيوية مع CuO-NPs. من خلال تقييم الحد الأدنى من التركيز المثبط لل*P. aeruginosa*. أظهرت النتائج أن أعلى تثبيط ضد العزلات البكتيرية كان نتيجة للتأثير التآزري لـ CuO-NPs مع اميبينيم، سيبروفلوكساسين، سيفتازيديم، بولي مكسين ب، اميكايسين ، وسيفيبيم على التوالي، خاصة على العزلة (15) حيث كانت مناطق التثبيط / ملم 19.33 ± 0.33، 18.33 ±0.33، 16.33 ±0.33، 15.33 ±0.33، 14.67 ±0.33، و12.7±0.58، على التوالي. أشارت النتائج إلى أن التأثير التآزري أعطى مناطق تثبيط واضحة ضد جميع العزلات مقارنة مع التأثير التثبيطي لأكسيد النحاس النانوي والمضادات الحيوية.

Introduction

The development of new antibacterials is one of the top priorities flagged by the World Health Organization and the scientific community [1]. A recent meta-analysis revealed that antibiotic resistance was responsible for 4.95 million fatalities in 2019. This study emphasized the immediate necessity to take action in order to combat bacterial illnesses that are resistant to antibiotics [2]. Wound infection is a form of persistent infection that can result in serious outcomes, such as the need for limb amputation and, if left untreated, death. Between 15-27% of cases involving bacterial wound infection result in gangrene, necessitating limb amputation. This highlights the inefficiency of present tactics for managing wound infections and emphasizes the need for new approaches [3].

Currently, in the realm of standard antibiotics, the concept of combinatory synergism is seen as a highly promising method for treating bacterial infections and preventing resistance. The utilization of a variety of medications enables the reduction of drug dosage, resulting in a decrease in the occurrence of adverse effects when compared to the usage of a single therapy[4]. Nevertheless, antibiotics are delivered in a manner that affects the entire body, and the use of many antibiotics together might result in pharmacokinetic profiles that are difficult to anticipate. Applying combinations of (Nanoparticles) topically would overcome the limitations associated with using antibiotics systemically[5]. An effective method to overcome this problem is to enhance the potency of antibiotics by combining them synergistically with other antibiotics or with natural phytochemicals, such as thymoquinone, a benzoquinone [6], or pterostilbene, a polyphenol [7], which have therapeutic qualities. The concurrent use of numerous drugs enhances the susceptibility of pathogens to antimicrobial agents, mitigates the toxicity associated with high

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doses of individual agents, and diminishes the likelihood of resistance development by targeting multiple bacterial sites. In recent times, metal oxide nanoparticles, specifically copper [8], have been employed to enhance the effectiveness of antibiotics. CuO NPs exhibit biocompatibility and efficacy against infections. Various techniques can be employed to synthesize CuO NPs, including microwave irradiation [9], sonochemical irradiation [10], and others. One of the various techniques for creating copper nanocubes is through green synthesis, which involves using water instead of harmful solvents [11]. Bacteria [12], fungi [13], algae [14], or plants [15] can be employed as environmentally friendly approaches for synthesizing CuO NPs. *Ficus sycomorus*, is one of the plants that has proven its effectiveness as antibacterial substances because it contains many vital and biologically active molecules such as sugars and flavonoids, tannins, and alkaloids[16]. Therefore, the utilization of synergistic nanoparticles is highly encouraging. Our hypothesis suggests that when applied jointly, copper oxide nanoparticles (CuO-NPs) exhibit enhanced antibacterial effectiveness due to their distinct mechanisms of action on bacteria. Additionally, copper increases the antibacterial efficacy of antibiotics, making them the most effective metal-based antibacterials currently known[17]. Furthermore, copper is classified as a microelement and plays a favorable role in wound healing. It stimulates the movement of fibroblasts, enhances the production of collagen, and is crucial for the formation of new blood vessels. CuO-NPs also supports the process of wound healing and aids in the regeneration of bones. Therefore, the combination of CuO-NPs could exhibit a superior antibacterial action, enhance the process of wound healing, and serve as a very advantageous treatment for infected wounds[18].

The aim of the study is to isolate and diagnose bacteria from different clinical samples, evaluate the minimum inhibitory concentration of CuO-NPs and six antibiotics against *P. aeruginosa* bacteria isolated from patients. The synergistic effect of CuO-NPs with the antibiotics used was also studied.

Materials and methods

Study design and Samples collection

One hundred ten swab specimens were collected from burn , wounds, and infections patients in Samarra General Hospital. Tikrit Teaching Hospital, Tikrit Military Hospital, and Baghdad Medical City of both genders from July to September 2023.

Microbiological culture

This study involved cultivation of clinical samples on Blood agar, cultivating clinical specimens of Blood Agar, MacConkey's Agar, and cetrimide agar. The identification of *Pseudomonas aeruginosa* was conducted based on various criteria, including colony characteristics such as grape-like odor, pigment production, motility, oxidase positivity, Gram staining (specifically gram-negative bacilli), non-fermentative nature, indole test, ability to hemolyze blood agar, urease activity, vegas-Proskauer activity, growth at temperatures of 42/4°C, and positivity for citrate utilization. In the VITEK 2 COMPACT system, the confirmation process is conducted.

Preparation of CuO-NPs from *Ficus sycomorus* Leaves Extract

Plant material was collected in accordance with national and international guidelines. The *F. sycomorus* Leaves samples were placed in a paper bag and then taken to the laboratory within a period of 48 hours. The gathered samples were washed with distilled water and left to dry in the shade for a duration of 14 days. The desiccated samples were dissected into small fragments using aseptic scissors and subjected to heating in a water bath with a concentration of 10% weight-to-volume at a temperature of 60 °C for a duration of 20 minutes using a heating mantle. The solution was cooled to ambient temperature, subjected to vacuum filtration to get the concentration of the aqueous extract (which had a brown colour), and then kept at a temperature of 4 °C for future use[20]. Afterward, 10 ml of extract was mixed with 90 ml solution of $CuSO_4$ (1 mM). The solution was maintained under conditions of darkness during the mixing process. The development of a change color from black to brown indicated the synthesis of CuO-NPs. Following the completion of the reaction, the CuO-NPs obtained were subjected to centrifugation at a speed of 6000 revolutions per minute for 10 minutes using Centrifuge. The CuO-NPs that had formed were rinsed multiple times with direct distilled water and then dried overnight at a temperature of 50 °C in a laboratory oven [19].

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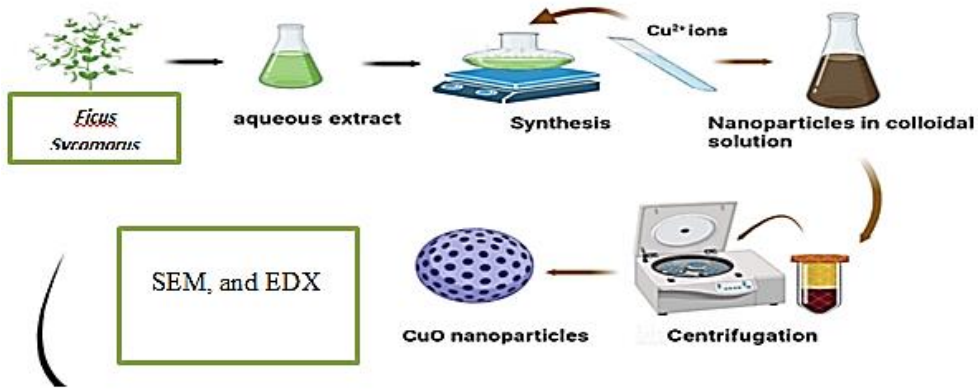

Fig. 1: Shows the green synthesis of CuO NPs using aqueous extract of *F. sycomorus* leaves [20]

Characterization of CuO-NPs

1. Scanning Electron Microscope (SEM) analysis

SEM technique was used to evaluate the size and morphological characterization of CuO-NPs that were synthesized using green methods, the sample was performed by dispersion of the samplein aluminum foil at 5.0 kV operating voltage using JSM-7500F and images were recorded at different magnifications [21].

2. Energy-dispersive X-ray (EDX) analysis

EDX analysis was used to determine the element present in samples. EDX was performed by using Hitachi S-3400 NSEM instrument equipped with Thermo EDX for which the synthesized CuO-NPs were dehydrated and coated on carbon film [22].

Evaluation of the inhibitory efficacy of biosynthesised copper oxide nanoparticles against *P. aeruginosa*

The inhibitory activity of biosynthesised copper oxide nanoparticles from fig leaf extract was studied against *P. aeruginosa*. Velsankar *et al* [23] used different concentrations of 100, 200, 300, 400 and 500 µg/ml of CuO nanoparticles. The bacterial suspension was spread on the surface of Muller Hinton plates and then etched with 5 etches per plate, after which 50 µl of each concentration of CuO nanoparticles was transferred.

Study of the synergy between biosynthesised copper oxide nanoparticles and antibiotics

The inhibitory efficacy of the synergy between biosynthesised copper oxide nanoparticles and the antibiotics used in the experiment was studied against *P. aeruginosa* using three concentrations of 300, 400 and 500 µg/ml, the diffusion method in pits and on Muller Hinton medium and for each concentration three replicates with a control sample without any treatment.

Statistical Analysis

The results were reported as Mean±SD. The researchers employed the t-test methodology to ascertain the existence of statistical disparities, with a p-value below 0.05 being deemed statistically significant. All biological experiments in this study were conducted three times.

Results and Discussion

Isolation and Identification of *P. aeruginosa*

All 110 clinical specimens were collected from patients with burn, wounds and urine infections both sexes patients in Samarra General Hospital, Tikrit Teaching Hospital, Tikrit Military Hospital, and Baghdad Medical City of both genders from July to September 2023. 50 (45.4%) isolates belonging to the *P. aeruginosa* bacteria were diagnosed as show in (Fig. 2), and the rest were neglected as they were diagnosed based on cultural and microscopic characteristics. Microscopy and biochemical tests. Isolates were grown on culture media for primary isolation, MacConkey agar, Nutrient agar, Blood agar, and then on selective Cetrimide agar.

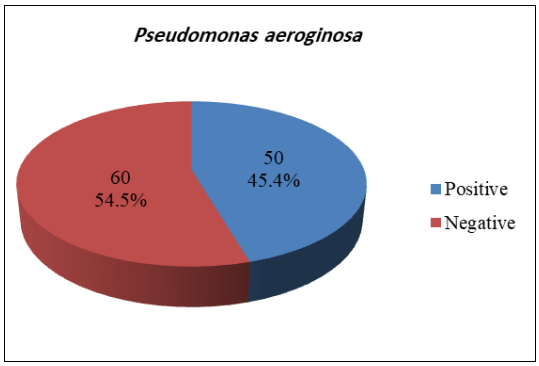

Fig. 2: Percentage of *P. aeruginosa* isolates From patient specimens study

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Characterization of CuO-NPs

The characteristics of CuO NPs are shown in Figure 3. According to the SEM images in Figure 3(C), CuO-NPs that were produced through the environmentally friendly manufacture by employing *F. sycomorus* leaf extract had a spherical shape. and the dimensions were found to be limited between 18.00 - 52.00 nm and its average size is 29.2 nm, these results agree with [24]. EDX show the element compostion in nano-materials, The synthesis of CuO-NPs using *F. sycomorus* leaf extract exhibits peaks associated with Cu and O[25].

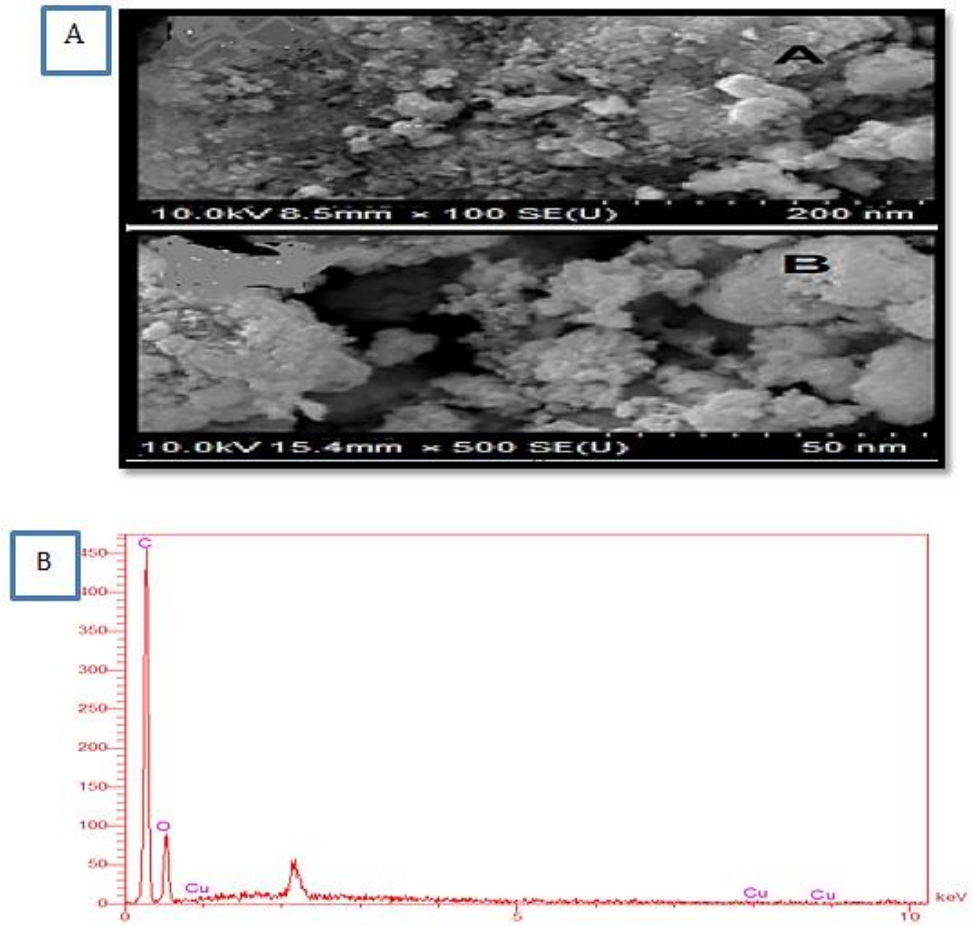

Fig. 3: Characterization of CuO-NPs by (A) SEM image and (B) EDX of green synthesized of CuO-NPs:

Determination of antibiotic activity against *P. aeruginosa*

Investigating the sensitivity of isolates to antibiotics is essential for proper antibiotic therapy. The susceptibility of the isolated bacteria to different classes of antibiotics was assessed, and the results are presented in Table 1. The inhibitory effectiveness of different antibiotics against *P. aeruginosa* bacteria at different concentrations of 300, 400, and 500 μg/ml, using the method of diffusion in holes was assessed. The inhibitory effectiveness of antibiotics was observed by measuring the inhibitory diameters that were determined around the etching areas on Muller Hinton medium. The results of the current study are consistent with the study conducted by Alaallah *et al.* The results of the study indicated an increase in bacterial inhibition with increasing dose of nanoparticles[22].

The results of the current study showed that six antibiotics had a significant effect on inhibition, as the inhibitory effectiveness is directly proportional to the increase in concentration, the results showed that the concentration of 500 mg/ml was the most effective against all isolates with all antibiotics, and Ciprofloxacin gave the highest rate of inhibition, (20.7±1.15), followed by Amikacin (20.6±0.58). The lowest rate of inhibition at the concentration of 500 μg/ml was Cefepime (16.6±0.58)[25]. These results are consistent with the study of [27], as they showed that common antibiotics differ in the degree of

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inhibition depending on the type of antibiotic and the target location, in addition to the concentration. The study of [28]also confirmed that different antibiotics work in a specific way towards the bacterial cell. Some of them are It works to destroy the cell wall, some of which work to cause a defect in the permeability of the cell membrane, and some of them lead to the denaturation of the proteins that make up the cell wall or the proteins that make up the amino acids or peptides responsible for the formation of the cell membrane. For this reason, differences have appeared in the rates of bacterial inhibition, because bacteria may resist A certain antibiotic in certain proportions, while you cannot resist another antibiotic in the same proportion.

Table 1: Antibiotic sensitivity profiles of bacteria *Pseudomonas aeruginosa* at 300,400 and 500 µg/ml

Antibiotic sensitivity at 500/ concentration of Inhibition Zone (mm)						
Isolates	Polymyxin B	Amikacin	Cefepime	Impenem	Ceftazidime	Ciprofloxacin
1	17.3±0.58	20.6±0.58	16.6±0.58	19±1	19±1	20.7±1.15
2	19.7±1.53	18.6±0.58	14.6±0.58	18.7±1.15	19±1	18.3±0.58
3	23. ±2.65	17.7±0.58	14.3 ±0.58	18 ±1	15.7 ±0.58	20.3 ±1.53
4	19.3±0.58	20±1	17.3±2.08	19.7±1.15	19±1	20.7±1.15
5	18±1	16.7±0.58	15.7±0.58	16	19±1	19.3±1.15
Antibiotic sensitivity at 400/ concentration of Inhibition Zone (mm)						
1	16.7±0.58	17.3±1.15	15.3±0.58	16.3±1.53	16±1	18.7±1.15
2	17.7 ±0.58	15.3±0.58	13.6 ±0.58	16±1	16 ±1.73	15 ±1
3	20.3±2.89	16±1	14.3±0.58	15.3±1.53	14.7±0.58	17±1
4	R	16.3±0.58	14.3±0.58	17±1	17.3±1.15	18±1
5	16±1	16±1	13.7±0.58	16±1	16.3±1.53	16.3±1.53
Antibiotic sensitivity at 300/ concentration of Inhibition Zone (mm)						
1	15.3 ±0.58	14.7±2.08	13.3 ±0.58	15±1	14.7±0.58	15±1
2	R	14.7±2.08	13±1	15.3 ±0.58	16±1	14.7 ±0.58
3	16±3.46	13.3±0.58	11.7±0.58	15	14.7±0.58	15.3±0.58
4	R	14±1	13±1	15.3±0.58	15.3±1.53	16±1
5	15.3±0.58	12±1	12.7±0.58	14±1	14.7±0.58	14.7±0.58

2.4. Determination of Antibacterial Activity of CuO-NPs against *P. aeruginosa*

Table 2 displays the bactericidal activity of CuO-NPs at different concentrations ranging from 300 µg/mL to 500 µg/mL, with the activity increasing as the concentration increases. The highest level of bactericidal activity, measuring (19.3±1.53, 21.7±1.53, 19±1, 16.6±1.15, and 18±1.73) mm, was recorded when using a concentration of 500 µg/mL against *P. aeruginosa*. To assess the antibacterial properties of the *F. sycomorus* extract, it was employed as a control. The results showed that it exhibited bactericidal activity with an inhibition zone of (13.1±2.51,18.7±1.8 3, 15±1.5, 13.3 ±1.19, and 14±1.99) mm against *P. aeruginosa*. 5 isolates were selected from the rest of the isolates because they were highly sensitive to antibiotics. CuO -NPs have a strong bacterial *P. aeruginosa* inhibition. This is due to the fact that smaller molecules have a larger surface area to interact with microorganisms. Copper ions have the potential to disrupt the bacterial cell wall or cell membrane, which gives them the ability to inhibit the growth and development of bacteria. Upon entering the cell, they will engage in interactions with molecules that are rich in phosphorus and sulphur, such as DNA, due to their affinity for these molecules [29]. It is possible that these occurrences may set off a chain of redox reactions, which will result in the production of reactive oxygen species, the disruption of bacterial membranes, and the occurrence of oxidative stress and cell death.

Table 2: Antibacterial activity of CuO NPs (diameter of inhibition zone in mm).

Concentration of CuO-NPs (ppm)			
Isolates	**500**	**400**	**300**
P2	19.3±1.53	16.7±0.58	14.3±2.08
P3	21.7±1.53	19±1	17.7±0.58
P7	19±1	16.7±0.58	15.7±0.58
P8	16.6±1.15	14.7±0.58	12.3±0.58
P15	18±1.73	16.3±1.53	14.3±0.58
Concentration of fruit extract at (500 µg/ml)			
P2	13.1±2.51		
P3	18.7±1.8 3		
P7	15±1.5		
P8	13.3 ±1.19		
P15	14±1.99		

2.5 Synergistic Effect of Antibiotics with CuO-NPs

The synergistic effect between antibiotics and CuONPs at levels significantly inhibited bacterial

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activity after 24 h of incubation at 37°C. Measurement of the inhibition zone for *P. aeruginosa* isolates showed a significant decrease for all isolates. Figure 4: Shows the zones of inhibition of synergistic effect of CuO-NPs with the antibiotics used in this study. The results indicate the highest inhibition against bacterial isolates was due to the synergistic effect of CuO-NPs with Impenem, Ciprofloxacin, Ceftazidime, Polymyxin B, Amikacin, and Cefepime respectively, especially on isolate (P15) with zones of inhibition/mm were 19.33 ±0.33, 18.33 ±0.33, 16.33 ±0.33, 15.33 ±0.33, 14.67 ±0.33, and 12.7±0.58, respectively. The results are agreement with the work conducted by 30], which found that the inhibition zones decreased as the concentration of CuO-NPs with antibiotics increased. The increase in the inhibition diameter ratios may be due to an increase in the ability of the antibiotics after the synergistic process to oxidize the phosphorylated lipids of the wall of the *P. aeruginosa* bacteria, but in varying proportions depending on the concentration of the antibiotic under study. It may also be due to the physiological difference between the composition of the wall or the nucleic acids between the isolates of the bacteria under study. These results are consistent with the study by [31], which confirmed that when an antibiotic binds to one of the cellular receptors, it leads to abnormal growth of the cell wall or a defect in the vicinity of the cell wall. Some species work to stop the reactions related to cell wall formation, and that nanoparticles give a higher efficiency for the antibiotic. This leads to the inhibition of the formation of Peptidoglycan, the main component of the cell wall. For example, the glycopeptide group binds to the D-alanyl ends of the Peptidoglycan fifth nascent peptide chain, and this will prevent the growth or elongation of Peptidoglycan. The results of this study are consistent with the study [31], where they showed that polymyxin B is an antibiotic used primarily to treat Gram-negative bacterial infections. The ability of this antibiotic increases through synergy with other compounds, especially nanoparticles, which makes the bacterial cell membrane more permeable, resulting in water absorption, which leads to the death of the bacterial cells.

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Table 3: Sensitivity of isolates of *P. aeruginosa* to the synergy between the antibiotic and CuO-NPs at 300,400,and 500 µg/ml

Isolates	Polymyxin B	Amikacin	Cefepime	Impenem	Ceftazidime	Ciprofloxacin
Sensitivity of isolates of *P. aeruginosa* to the synergy between the antibiotic and CuO-NPs at 500/ Concentration of diameter Inhibition Zone (mm)						
P2	19.67 ±0.33 AB a	22.67 ±0.33 A a	16.6±0.58	26.00 ±1.00 A a	17.33 ±0.33 C a	18.33 ±0.33 C a
P3	22.33 ±0.33 ABC a	20.33 ±0.33 C a	14.6±0.58	26.33 ±1.76 A a	18.67 ±0.33 BC a	20.67 ±0.33 B a
P7	22.00 ±0.57 BC a	21.00 ±0.57 BC a	14.3 ±0.58	23.67 ±0.88 A a	17.66 ±0.88 C a	18.67 ±0.33 C a
P8	21.33 ±0.33 C a	22.00 ±0.57 AB a	17.3±2.08	27.33 ±1.20 A a	23.00 ±0.57 A a	18.67 ±0.33 C a
P15	23.67 ±0.33 A a	21.00 ±0.57 BC a	15.7±0.58	27.67 ±1.56 A a	20.33 ±0.33 B a	23.33 ±0.33 A a
Sensitivity of isolates of *P. aeruginosa* to the synergy between the antibiotic and CuO-NPs at 400/ Concentration of diameter Inhibition Zone (mm)						
P2	18.67 ±0.33 A b	21.33 ±0.88 A a	15.3±0.58	20.67 ±0.67 A b	16.33 ±0.33 C a	17.67 ±0.88 BC a
P3	18.67 ±0.33 A b	18.00 ±1.00 B b	13.6 ±0.58	20.00 ±1.00 A b	17.66 ±0.33 B a0	18.33 ±0.33 B b
P7	17.66 ±0.33 AB b	14.67 ±0.33 C b	14.3±0.58	20.67 ±1.20 A ab	16.33 ±0.33 C ab	16.67 ±0.33 C b
P8	17.00 ±0.57 C b	20.33 ±0.33 A b	14.3±0.58	22.00 ±1.15 A b	19.67 ±033 A b	17.33 ±0.33 BC b
P15	17.00 ±0.57 BC b	19.67 ±0.33 AB a	13.7±0.58	23.33 ±1.45 A b	16.67 ±0.33 BC b	20.67 ±0.33 A b
Sensitivity of isolates of *P. aeruginosa* to the synergy between the antibiotic and CuO-NPs at 300/ Concentration of diameter Inhibition Zone (mm)						
P2	15.67 ±0.33 A c	16.67 ±0.88 A b	13.3 ±0.58	17.67 ±0.88 A c	14.67 ±0.33 B b	15.33 ±0.33 BC b
P3	15.67 ±0.33 A c	16.67 ±0.33 A b	13±1	18.00 ±0.57 A b	14.67 ±0.33 B b	15.67 ±0.33 B c
P7	14.67 ±0.33 A c	13.00 ±0.57 B b	11.7±0.58	18.00 ±1.15 A b	14.67 ±0.33 B b	14.33 ±0.33 C c
P8	15.00 ±0.57 A b	17.67 ±0.33 A c	13±1	18.33 ±0.33 A c	15.33 ±0.33 A c	14.67 ±0.33 BC c
P15	15.33 ±0.33 A c	14.67 ±0.33 B b	12.7±0.58	19.33 ±0.33 A b	16.33 ±0.33 B c	18.33 ±0.33 A c

https://doi.org/10.25130/tjps.v29i4.1632

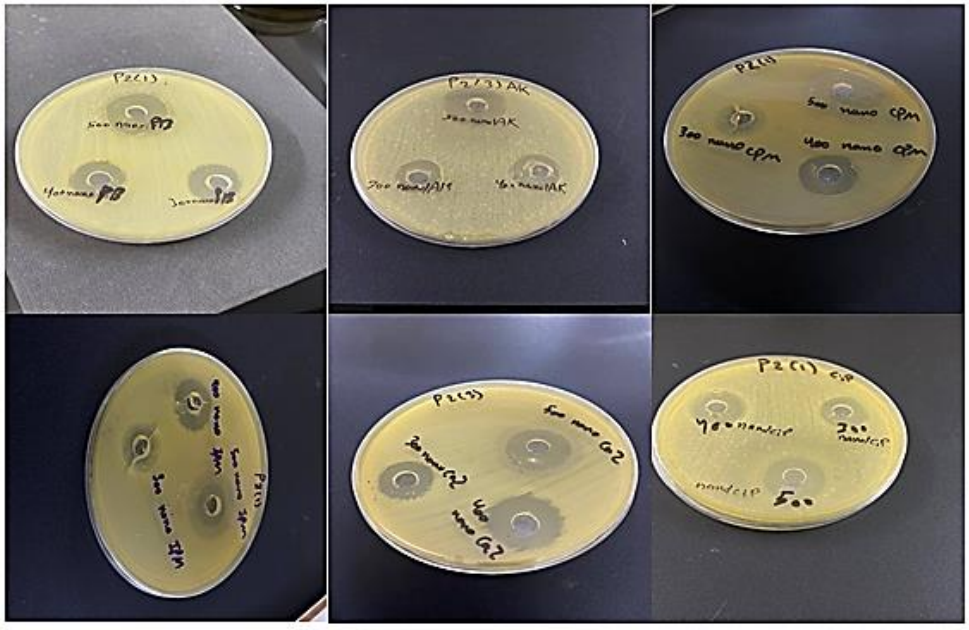

Fig. 4: Inhibition zones for the antibiotics used

Conclusion

1- The study demonstrated the possibility of isolating *Pseudomonas aeruginosa* bacteria. from people with infected wounds And burns, as well as in samples of urine from both sexes and from different age groups. The presence of this bacteria was confirmed through cultural and biochemical tests.

2- The study demonstrated the possibility of using fig leaf extract as a vital agent in the preparation of copper oxide nanoparticles, and this was confirmed through several common tests to prove the formation of nanoparticles.

3- The study showed the possibility of using biologically prepared secondary copper oxide particles as a strong inhibitory agent against *P. aeruginosa* bacteria.

4- The current study confirmed the effectiveness of the antibiotics used in the experiment against *P. aeruginosa* bacteria

5- The current study confirmed that the antibiotic Imipenem (IPM). It was the best antibiotic used in the experiment, as it gave the highest rates of inhibition compared to other antibiotics.

6- The study found that the synergism process between antibiotics used in present study, and biologically prepared copper oxide nanoparticles led to a higher efficiency of killing and inhibiting *P. aeruginosa* bacteria. With clear moral differences.

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