

Green synthesis of zinc oxide nanoparticles and its antibacterial Activity on *Pseudomonas aeruginosa*

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ABSTRACT

Background: Zinc oxide (ZnO) nanoparticles have wide-ranging applications and can be synthesized using environmentally friendly green synthesis methods as an alternative to conventional approaches. *Pseudomonas aeruginosa* (*P. aeruginosa*) is an extremely perilous bacterium known for its multidrug-resistant (MDR) nature, posing a significant threat to hospitalized patients and those with compromised immune systems. The bacterium's ability to withstand multiple antibiotics, combined with its capacity to form biofilms, contributes to its high rates of morbidity and mortality. The intrinsic resistance of *P. aeruginosa*, along with its ability to form biofilms, further complicates treatment and exacerbates the severity of infections, particularly in susceptible patient populations. **Objective:** The main objective of this study was to utilize extracts from *Ocimum basilicum* leaves in a green synthesis approach to produce zinc oxide nanoparticles. Additionally, the research aimed to assess the antibacterial effectiveness of these synthesized nanoparticles against *P. aeruginosa*. **Materials and Methods:** In this study, ZnO nanoparticles were synthesized using the leaf extract of *O. basilicum* plants under various parameters. The biosynthesis of zinc oxide nanoparticles was verified using a UV-visible spectrophotometer and characterized using Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), and Fourier Transform Infrared Spectroscopy (FTIR). The synthesized ZnO nanoparticles exhibited significant antibacterial efficacy. Specifically, they demonstrated antimicrobial activity against *P. aeruginosa* pathogens. Different concentrations of both *O. basilicum* extract and synthesized ZnO nanoparticles were tested against *P. aeruginosa* and observed antibacterial activity. **Results:** indicated that the ZnO NPs synthesized from *O. basilicum* exhibited stronger antibacterial activity compared to the plant extract alone. The most effective concentrations were found to be 1.0, 1.5, and 3 mg/ml of prepared ZnO nanoparticles. Increasing the concentration of ZnO nanoparticles resulted in enhanced inhibition of bacterial growth in *P. aeruginosa*. **Conclusion:** The green synthesis of ZnO NPs using plant extracts has demonstrated significant antibacterial activity against *P.aeruginosa*. The high concentration of the ZnO NPs resulted in larger inhibition zones at higher concentrations. These findings underscore the possibility of green-synthesized ZnO NPs as an antimicrobial agent for *P. aeruginosa*. The environmentally friendly and cost-effective nature of the green synthesis method further enhances its appeal for future applications in antibacterial treatments.

Keywords: Antibacterial, spectrophotometer, zinc oxide nanoparticles, *Ocimum basilicum*.

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1- Introduction

Nanoscience focuses on the synthesis and utilization of materials at the nanoscale level. Zinc oxide nanoparticles at the nanoscale have garnered significant focus in recent research because of their broad range of potential applications. These applications include their potential as antibacterial and antifungal agents, their ability to aid in wound healing processes, their antioxidant properties, and their optical characteristics (1,2,3). Nanotechnology in agriculture offers significant benefits, including improved food quality and safety, reduced farming inputs, and enhanced nutrient absorption from the soil. These advantages make nanotechnology a crucial field of research and development (4,5). In cropping systems, nanotechnology has specific applications such as nanofertilizers and nanopesticides for enhancing nutrient levels and crop productivity. Nanoparticles are synthesized using chemical and green methods. The synthesis of nanoparticles must therefore be environmentally friendly and cost-effective (2,6). To overcome the challenges associated with chemical production methods of nanoparticles, green synthesis methods utilizing bacteria, fungi, algae and plants have been introduced. These methods offer a more environmentally friendly approach, reducing the need for large doses of chemicals and extreme production conditions (7). Inorganic nanostructures are currently synthesized using a variety of microorganisms and plant extracts. The synthesis of shape- and size-controlled nanocrystals is an important step for every practical application since nanoparticles differ in size, orientation, and physical characteristics (8,9). The use of nanotechnology to synthesize zinc oxide nanoparticles (ZnO NPs) has generated significant interest due to their potential antibacterial efficacy. Microorganisms typically fall within the nanometer to micrometer size range, making ZnO NPs particularly attractive for antimicrobial applications. The enhanced specific surface area of ZnO NPs resulting from their reduced particle size contributes to their antimicrobial properties. Additionally, ZnO NPs are considered a safe biomaterial and exhibit photocatalytic effects on both biological and chemical types (10).

Pseudomonas aeruginosa is a Gram-negative bacterium that is known to cause severe infections in healthy humans, particularly in individuals with weakened immune systems or compromised barriers (11). This opportunistic pathogen is responsible for a wide range of infections, including pneumonia, urinary tract infections, and sepsis. One of the key virulence factors of *P. aeruginosa* is the ability to form biofilms, in which bacterial communities are attached to surfaces and are resistant to antibiotics and the immune system of the host (12). Bacterial pathogenicity is influenced by several factors, including lipopolysaccharides, flagella, type IV pili, type III secretion systems, proteases, alginate, exotoxin A, quorum sensing (QS), biofilm formation, type VI secretion systems, and airborne oxidants. Bacteria colonize, evade the host immune system, damage tissues, and establish infections based on these factors (13).

The main objective of this study was to utilize extracts from *O. basilicum* leaves in a green synthesis approach to produce zinc oxide nanoparticles—additionally, the research aimed to assess the antibacterial effectiveness of these synthesized nanoparticles against *Pseudomonas aeruginosa*.

2- Materials and Methods

Plant Sample Collection

For this study, the selected experimental material was *O. basilicum*, a significant medicinal plant. The plant material was obtained from markets in Baghdad during March 2023, and its classification was confirmed by the National Herbaceous/General Authority for Agricultural Research.

Plant Aqueous Extracts preparation

Traditional extraction techniques were used to prepare the plant extracts. In order to remove surface impurities, fresh and healthy plants were collected, cleaned thoroughly, and washed. To remove all moisture, the plants were air-dried for 30 minutes. The dried plant material was then fragmented into small

pieces and boiled in a water bath with 50 ml of distilled water at 50 °C for approximately 30 minutes. The extracts were filtered using Whatman No. 1 Filter paper and stored in a freezer at 4 °C for future use (14).

Green Synthesis of ZnO Nanoparticles

In the preparation of zinc oxide nanoparticles from the *O. basilicum* extract, a method by (15) was adopted with some modifications; a water extract of 50 ml was heated on a hot plate at a temperature of 40 – 45 °C. 5 grams of zinc acetate were melted using deionized water and then added to the plant extract and heated, the mixture was then subjected to a centrifuge to separate the precipitate from the filtrate. The resulting paste from the precipitate was collected in a glass petri dish and dried at 300 °C for two hours. After drying, the product was skimmed using a spatula to obtain a white powder, which was then appropriately packaged for further characterization and treatment.

Scanning electron microscope (SEM)

A SEM scanner was used to analyze the particles in the prepared samples. Five microliters of ready-made solutions were placed on a gold and carbon buckle electronic microscope holder for examination and left to dry at room temperature. Different magnifying forces were used to analyze the samples (16)

Atomic force microscopy (AFM)

The size, roughness, surface, granularity volume distribution, and topography of the prepared ZnO nanoparticles samples were characterized using an Atomic Force Spectroscopy system. A thin film of the sample was created on a glass slide by depositing 100 µl of the sample and allowing it to dry at room temperature. This prepared sample was then ready for testing and analysis using the AFM system (17).

Fourier transforms infrared spectroscopy (FTIR) analysis.

A volume of 100 µl from the prepared ZnO solution was subjected to ultrasonic shaking and then tested in its liquid form at room temperature. The spectral scanning was conducted within the range of 200 to 4000 cm⁻¹(18).

UV-Visible spectrophotometer (UV-VIS spectroscopy)

An optical spectrophotometer can easily detect the creation of ZnO nanoparticles by analyzing the color of nanoparticles in the range of (200-800 nm) (19).

Bacterial samples collection

P. aeruginosa samples were obtained from a previous study (20). (Unpublished data).

Antibacterial Activity

The antibacterial activity of the synthesized ZnO nanoparticles was evaluated using the agar well diffusion method, following the protocol described by (21). The concentrations of 1.0, 1.5, and 3.0 mg/ml of the synthesized ZnO nanoparticles were utilized. A culture of *P. aeruginosa* was diluted to a concentration of 1.5×10^8 CFU/ml in Mueller-Hinton broth. The bacterial suspension was then spread evenly over the surface of the Mueller-Hinton agar medium using a sterile cotton swab, and the plates were allowed to dry. Wells with a diameter of 8 mm were created on the agar plates, and each well was filled with 100 µL of the prepared ZnO nanoparticles at concentrations of 1.0, 1.5, and 3.0 mg/ml. The plates were incubated at 37°C for 18-24 hours. Following incubation, the diameter of the inhibition zone surrounding each well was measured in millimeters.

The MIC (minimum inhibitory concentration) of the Plant Extract and prepared ZnO NPs against *P. aeruginosa* was determined through a series of steps. *P. aeruginosa* colonies were suspended in sterile saline, and a microtiter plate was prepared with Mueller-Hinton broth. The Plant Extract and ZnO NPs were added to specific wells in the plate, followed by gentle mixing and serial dilutions. Bacteria were added to the wells, and the plate was incubated. After incubation, the plate was scanned at 630 nm using an ELISA

Reader, and the inhibitory concentration, pre-concentration, and post-concentration were cultured on agar to assess bacterial growth. The MIC was identified as the lowest concentration that inhibited bacterial growth.

3- Results

The SEM image provided in Figure (1) shows the synthesized nano zinc oxide particles using basil extract. The particles appear spherical with an average diameter of around 300 nanometers. While some particles aggregate into larger clusters, individual particles can still be observed. The SEM image indicates the successful role of basil extract as a reducing agent in the synthesis, resulting in uniform sized and shaped ZnO nanoparticles. This confirms the success of the basil extract-mediated synthesis of ZnO nanoparticles, demonstrating excellent dispersion and consistency in their characteristics. (22,23).

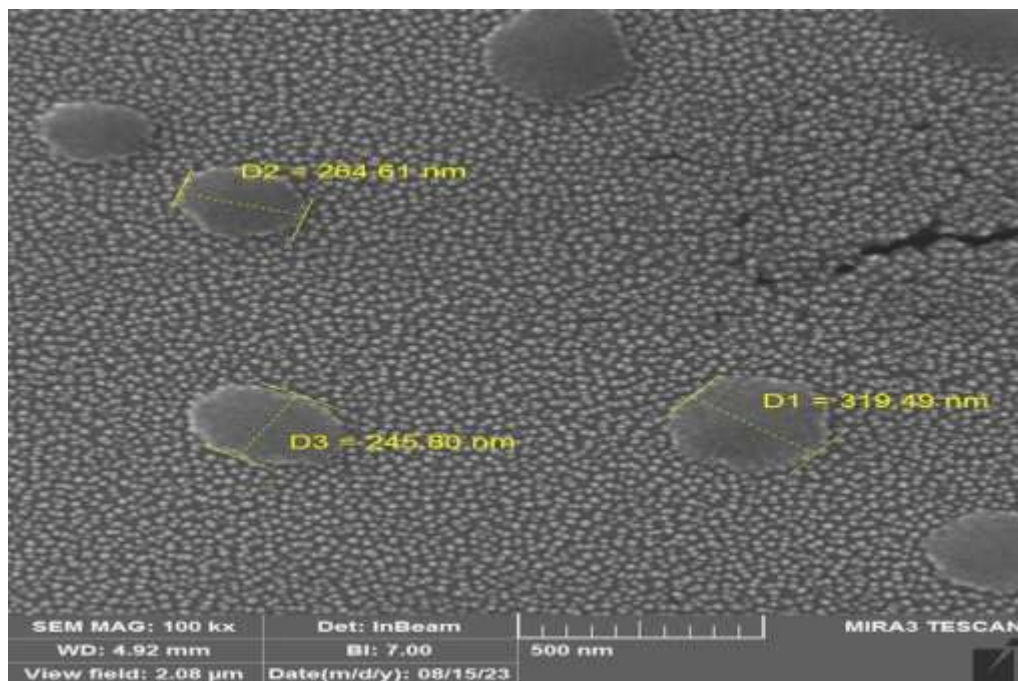
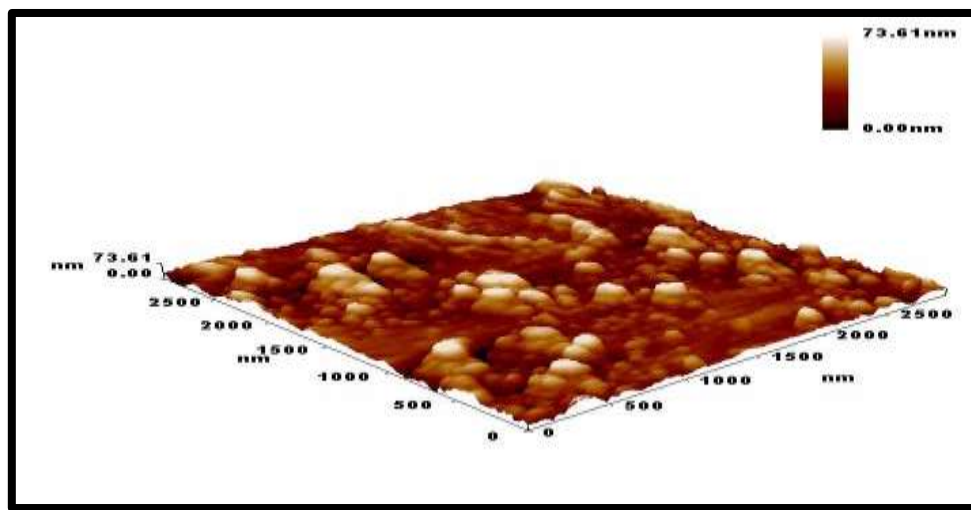


Figure (1): Scanning Electron Microscopy (SEM) of biosynthesized zinc nanoparticles.

Synthesized ZnO NPs were subjected to Atomic Force Microscopy (AFM) which is a powerful technique for analyzing the surface morphology, nanoscale particle size, and surface roughness of the zinc nanoparticles synthesized from plant extraction. Figure (2) presents three-dimensional atomic force microscopy images of ZnO nanoparticles. The synthesized zinc oxide nanoparticles exhibit an average size distribution ranging from 18 nm to 30 nm across different percentages, showcasing a smooth surface (24,25).



Fourier Transform Infrared (FTIR) spectroscopy was employed to examine the synthesized zinc oxide (ZnO) nanoparticles and the basil leaf extract. This technique aims to identify the functional groups present in compounds and determine their molecular structures (26).

The FTIR spectrum displays absorption peaks corresponding to the vibrational modes of these functional groups, enabling the identification of distinctive functional signatures, such as OH, C=O, NH, CH, and others, thus confirming the presence of specific chemical bonds. The FTIR spectra of the synthesized ZnO samples were examined within the wavelength range of 4000–500 cm^{-1} , as shown in Figure (3 B), using the aforementioned method. The absorption peak at (3280 cm^{-1}) is attributed to the O–H bond stretching, while the peak at (2182 cm^{-1}) corresponds to the C–C bond. The absorption band around (1636 cm^{-1}) corresponds to the stretching of Alkenyl C=C. Strong absorption peaks observed in the range of 500–600 cm^{-1} are indicative of ZnO nanoparticles, specifically denoting the stretching frequency of Zn–O bonds. This observation confirms the presence of a metal-oxide compound (24). The peak at (2182 cm^{-1}) is due to the C–C bond. In the context of comparing the results, the FTIR spectra of the basil leaf extract were examined within the wavelength range of (4000 to 500 cm^{-1}), as shown in Figure (3 A). FTIR analysis revealed distinctive peaks in the spectrum. Peaks at 3366 cm^{-1} and (3276 cm^{-1}) indicate the presence of hydroxyl groups.

Additionally, peaks at (2904 cm^{-1}) correspond to the stretching vibration of C–H bonds. A weaker peak observed at (2103 cm^{-1}) may be attributed to the C=N stretching of R–N=C= groups. The sharp peak at (1636 cm^{-1}) signifies N–H stretching. In the lower frequency range, the peak at (602 cm^{-1}) is associated with N–H bending. The appearance of a peak at (1277 cm^{-1}) suggests the existence of C–O vibrations, potentially originating from polyols like hydroxy flavonoids (27,28).

Another notable peak at (1058 cm^{-1}) could be linked to secondary alcohols. In terms of comparing the functional groups, the ZnO nanoparticles display distinct absorption features related to their composition, while the basil leaf extract exhibits characteristic peaks corresponding to its unique molecular components, It's worth noting that the functional groups present in the extract contribute electrons, which have the potential to facilitate the reduction of zinc ions (Zn^{2+} to Zn^{+1}) and ultimately lead to the formation of zinc nanoparticles (ZnO) as in the studies (29).

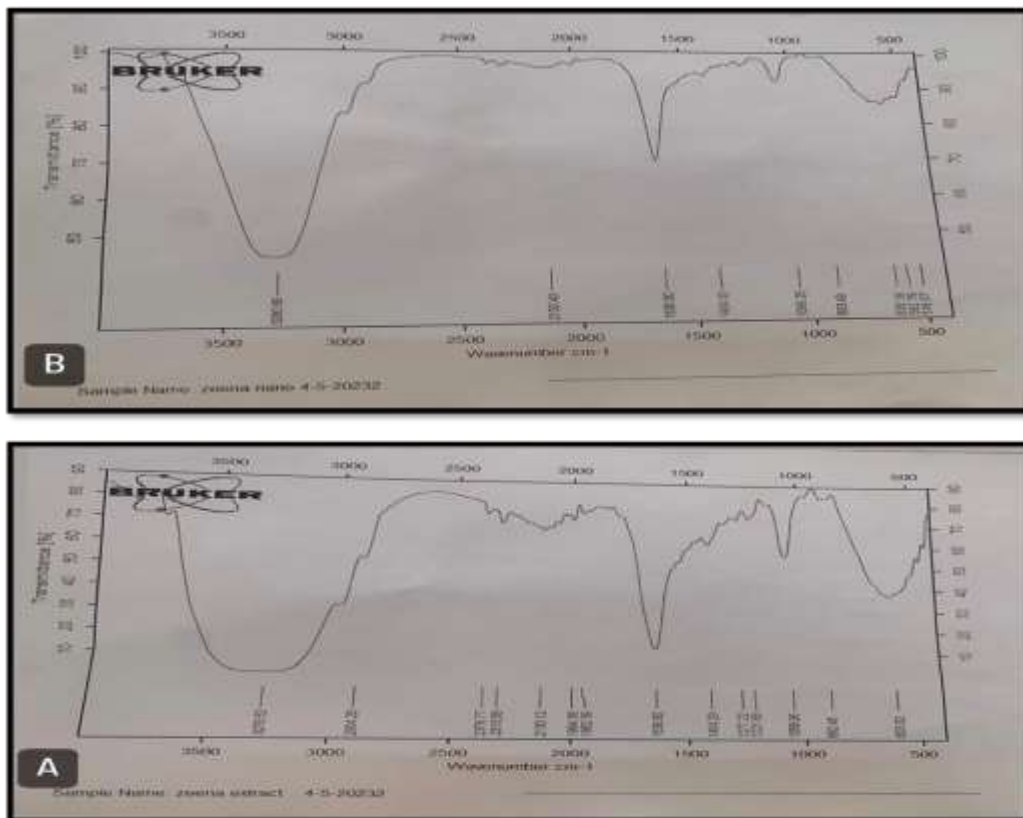


Figure (3): FTIR spectrum of A) plant extract, and B) biosynthesized ZnO nanoparticles.

The optical properties of the synthesized ZnO nanoparticles were investigated using a UV/visible spectrophotometer in the wavelength range of 200-1200 nm. The UV-Vis spectrum of the ZnO nanoparticles, as depicted in Figure (4), displayed a prominent absorption peak at 290 nm. This observation is consistent with previous studies that have reported absorption bands between 290 and 300 nm for ZnO nanoparticles synthesized through photosynthesis (30). The broad absorption band in the UV-Vis spectra suggests a heterogeneous size and shape distribution of the ZnO nanoparticles, indicating the presence of a diverse population of nanoparticles. This highlights the importance of UV-visible analysis in characterizing nanomaterials (31).

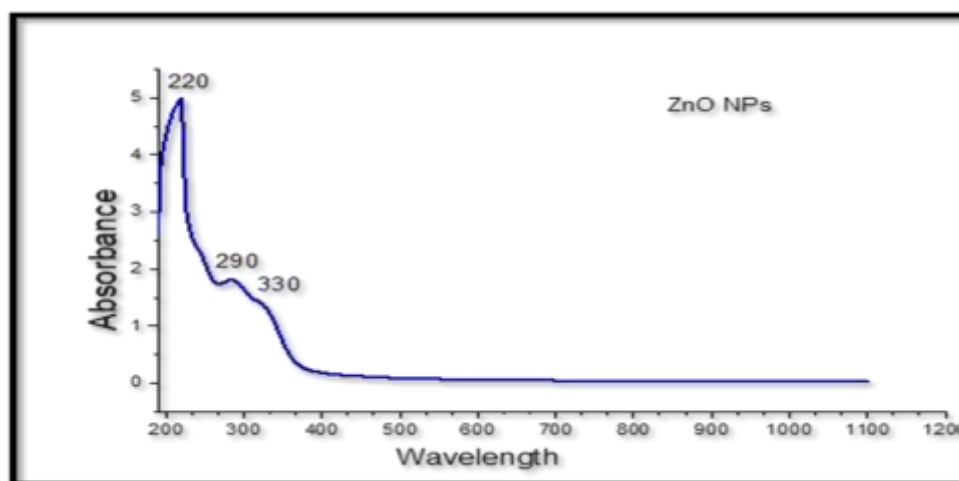


Figure (4): Ultraviolet-visible (UV-Vis) spectrum of biosynthesized ZnO nanoparticles

Zinc oxide nanoparticles (ZnO-NPs) are gaining popularity in medical applications due to their biocompatibility, low toxicity, and affordability. The study assessed the efficacy of plant-mediated green synthesis of ZnO-NPs in inhibiting pathogenic microbes using the agar well diffusion method. Three concentrations (1.0, 1.5, and 3.0 mg/ml) of both the plant extract and the prepared ZnO NPs were tested against three different *P. aeruginosa* isolates. The inhibitory effect was determined by measuring the diameter of the inhibition zones formed around the wells containing the plant extract and ZnO NPs. The results indicated that the ZnO NPs synthesized from *O. basilicum* exhibited stronger antibacterial activity compared to the plant extract alone. The inhibition zones formed by the ZnO NPs had varying diameters, as presented in Table (1).

Table (1): Zone of Inhibition produced by *Ocimum basilicum* extract and ZnO NPs against three *Pseudomonas aeruginosa* isolates.

Antibacterial Name	Concentrations (mg/ml)	Diameter of Inhibition Zone (mm)		
		Isolate 1	Isolate 2	Isolate 3
ZnO NPs	1.0	40	25	28
	1.5	42	29	30
	3.0	45	35	30
Plant Extract	1.0	13	12	14
	1.5	15	14	17
	3.0	16	15	18

Minimum inhibitory concentrations (MICs) refer to the lowest effective doses of antimicrobial substances that prevent the visible growth of microorganisms after an overnight incubation period. Alexander Fleming pioneered the concept of MIC by using broth turbidity to quantify the antibacterial activity of drugs. In the late 1980s, the Clinical and Laboratory Standards Association established standardized methods and criteria for determining MICs in clinical settings to evaluate the bacteriostatic effects of antibiotics. In the context of this study, both the *O. basilicum* plant extract and the green synthesized ZnO nanoparticles (NPs) demonstrated antibacterial activity against *P. aeruginosa* isolates. MIC values were determined by testing a range of concentrations (from 0.75 mg/ml to 1.5 µg/ml) of the plant extract and ZnO NPs in nutrient broth

culture media. The green synthesized ZnO NPs exhibited a minimum inhibitory concentration of 0.048 mg/ml against *P. aeruginosa* isolates, while the plant extract showed a minimum inhibitory concentration of 0.37 mg/ml.

4- Discussion

In this study, an environmentally friendly approach was employed to synthesize zinc oxide nanoparticles. Basil leaf extract was utilized as a natural reducing and capping agent, making the process biocompatible, cost-effective, and safe. It is worth noting that the green synthesis of zinc oxide nanoparticles is considered much safer and more eco-friendly in differentiation from chemical synthesis methods (33). The formation mechanism of ZnO NPs can be described as the bioreduction process of zinc ions facilitated by different plant metabolites or phytochemical components present in the plant extract. These components include terpenoids, tannins, polyphenols, sugars, proteins, alkaloids, phenolic acids, flavonoids, glycosides, and more. Upon interaction with these compounds, complexation of zinc ions occurs, followed by hydrolysis reactions that result in the formation of zinc hydroxide. The presence of hydroxyl groups in the polyphenols aids in these hydrolysis reactions. Ultimately, the zinc hydroxide undergoes further transformation to yield zinc oxide nanoparticles. Furthermore, these phytochemicals not only facilitate the bioreduction of zinc ions but also serve as capping agents or stabilizers for the nanoparticles. They play a crucial role in controlling the growth of the nanoparticles and preventing their aggregation. Consequently, the synthesis of zinc oxide using basil leaves led to the formation of a light brown precipitate as the end product. The final step in the process involved calcination, which is a decomposition reaction, resulting in the production of pale-yellow zinc oxide nanoparticles in the form of a powder (34).

Synthesized ZnO NPs were subjected to Atomic Force Microscopy (AFM) which is a powerful technique for analyzing the surface morphology, nanoscale particle size, and surface roughness of the zinc nanoparticles synthesized from plant extraction (26). Fourier Transform Infrared (FTIR) spectroscopy was employed to examine the synthesized zinc oxide (ZnO) nanoparticles and the basil leaf extract. This technique aims to identify the functional groups present in compounds and determine their molecular structures (28). The optical properties of the synthesized ZnO nanoparticles were investigated using a UV/visible spectrophotometer in the wavelength range of 200-1200 nm. The UV-Vis spectrum of the ZnO nanoparticles displayed a prominent absorption peak at 290 nm. This observation is consistent with previous studies that have reported absorption bands between 290 and 300 nm for ZnO nanoparticles synthesized through photosynthesis (31). The broad absorption band in the UV-Vis spectra suggests a heterogeneous size and shape distribution of the ZnO nanoparticles, indicating the presence of a diverse population of nanoparticles. This highlights the importance of UV-visible analysis in characterizing nanomaterials (34).

Zinc oxide nanoparticles (ZnO-NPs) are gaining popularity in medical applications due to their biocompatibility, low toxicity, and affordability. They have shown potential as antifungal, antibacterial, anticancer, antidiabetic, antioxidant, anti-insect, and anti-inflammatory agents. Additionally, they can be utilized for drug delivery and bioimaging (35).

Recent studies have shown that green-synthesized ZnO nanoparticles (ZnO-NPs) exhibit enhanced antibacterial activity compared to plant extracts alone. For instance, (21) found that ZnO-NPs synthesized with *O. basilicum* leaf extract exhibited effective antibacterial activity against various pathogenic bacteria, including *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *P. aeruginosa*. The study revealed that a concentration of 50 µl resulted in the maximum zone of inhibition, with *E. coli* exhibiting the largest inhibition zone (16 mm), followed by *S. aureus* (14 mm), *S. typhi*, and *B. subtilis* (13 mm). The superior antibacterial activity of green-synthesized ZnO-NPs can be attributed to their small size, larger surface area, and higher reactivity compared to bulk ZnO particles. These characteristics facilitate enhanced interaction between the nanoparticles and the bacterial cell membrane, resulting in increased antibacterial

efficacy. Moreover, the eco-friendly and cost-effective nature of the green synthesis method makes it a promising alternative to traditional chemical methods (36).

These findings suggest that green synthesized ZnO NPs have a stronger inhibitory effect on *P. aeruginosa* isolates compared to the plant extract alone. Similarly, (37) demonstrated that ZnO NPs produced from pomegranate extracts exhibited a minimum inhibitory concentration of 0.83 ± 0.36 $\mu\text{g/ml}$ against *P. aeruginosa* using the 96-well titration plate technique. Another study by (38) reported MIC values of ZnO NPs synthesized using *Veronica multifida* (*V. multifida*) plant extract against *P. aeruginosa* isolates ranging from 25 to 50 $\mu\text{g/ml}$.

5- Conclusion

The utilization of plant extracts in the green synthesis of zinc oxide nanoparticles (ZnO-NPs) has exhibited significant antibacterial activity against *P. aeruginosa*. The inhibitory effect of the ZnO-NPs was found to be concentration-dependent, with higher concentrations resulting in larger zones of inhibition. These findings highlight the potential of green-synthesized ZnO-NPs as an effective antibacterial agent against *P. aeruginosa*. Moreover, the environmentally friendly and cost-effective nature of the green synthesis method further enhances its attractiveness for future applications in antibacterial treatments.

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تصنيع جسيمات أكسيد الزنك النانوية خضريا وفعاليتها المضادة للبكتريا على بكتريا الزائفة الزنجارية

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كلية التقنيات الاحيائية /جامعة النهرين- العراق

الخلاصة

خلفية البحث: تمتلك الجسيمات النانوية لأوكسيد الزنك التي تم تخليقها فعالية مضادة للبكتيريا، وتحديدًا ضد الزائفة الزنجارية وهي بكتيريا خطيرة مقاومة للأدوية وتشكل تهديدًا كبيرًا للمرضى المنومين في المستشفيات وذوي الجهاز المناعي الضعيف. **الهدف من البحث:** الهدف الرئيسي من هذه الدراسة هو تصنيع جسيمات اوكسيد الزنك النانوي خضريا باستخدام مستخلص اوراق الريحان واختبار فعاليته المضادة للبكتريا ضد بكتريا الزائفة الزنجارية **المواد وطرق العمل:** تم في هذه الدراسة تخليق جسيمات أكسيد الزنك باستخدام مستخلص أوراق نبات الريحان، وتم توصيفها وتحليلها باستخدام تقنيات مختلفة مثل مطياف الأشعة فوق البنفسجية المرئية، والمجهر الإلكتروني الماسح (SEM)، والمجهر القوى الذري (AFM)، وطيف الأشعة تحت الحمراء بالتحويل الفوري (FTIR). تمت دراسة فعالية جسيمات أكسيد الزنك المخلقة ضد بكتيريا الزائفة الزنجارية. **النتائج:** أظهرت الجسيمات التي تم تخليقها فعالية مضادة للبكتيريا، وتحديدًا ضد الزائفة الزنجارية. تم اختبار تراكيز مختلفة من مستخلص نبات الريحان وجسيمات أكسيد الزنك المخلقة، وتم ملاحظة نشاطًا مضادًا للبكتيريا. أظهرت النتائج أن التراكيز الأكثر فعالية هي 1.0 و 1.5 و 3 ملغ/مل من جسيمات أكسيد الزنك المخلقة. وزادت قوة تثبيط نمو البكتيريا في بكتريا الزائفة الزنجارية مع زيادة تركيز جسيمات أكسيد الزنك. **الاستنتاج:** تسلط نتائج هذه الدراسة الضوء على طريقة تخليق بسيطة وسريعة واقتصادية وصديقة للبيئة لجسيمات أكسيد الزنك باستخدام مستخلص أوراق نبات الريحان. وتظهر هذه الجسيمات فعالية مضادة للبكتيريا، وخاصة ضد الزائفة الزنجارية. ويؤكد التخليق الأخضر لجسيمات أكسيد الزنك باستخدام مستخلصات النباتات فعالية مضادة للبكتيريا. كما تزيد الطبيعة الصديقة للبيئة والتكلفة المنخفضة لهذه الطريقة من جاذبيتها للاستخدامات المستقبلية في علاجات مضادة للبكتيريا.

الكلمات المفتاحية: مضاد بكتيري، مقياس الطيف الضوئي، جزيئات أكسيد الزنك النانوية، نبات الريحان.