



Effect of Bio-Zinc Nanoparticles synthesized from *Lawsonia inermis* as Antibacterial, Antibiofilm On Multi Resistance *Staphylococcus aureus*



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Abstract

Background: Plant extract -mediated green synthesis has gained important attention due to its friendly of environmental nature and cost is low. Also, it may be used as an alternative method treatment of antibiotics resistance in bacteria. **Objective:** The current study aimed to biosynthesize zinc nanoparticles from an aqueous extract of *Lawsonia inermis* and determine their effectiveness on bacteria and biofilms. **Methodology:** This was a cross-sectional study that was conducted in the Department of Biology, College of Science, University of Tikrit, Tikrit, Iraq, from May 2025 to December 2025. A sterile swabs used to collect samples from wounds of diabetic foot ulcers of Fifty patients swabs cultured on Mannitol salt agar and Gram-staining, and a series of biochemical tests and VITEK-2 technique A susceptibility test was conducted for seven antibiotics while Biofilm formation detect by Congo red and Microtiter plate Methods, the bio-synthesis of Zinc nanoparticles characterized by devices include UV-Vis spectroscopy, scanning electron microscopy (SEM), FTIR spectroscopy. The inhibition effect of concentrations (25.0%, 50.0%, 75.0%, and 100.0%) of nanoparticles against the bacterial isolates was detected also detect effect ant-biofilm of these nanoparticles through SubMIC concentrations (25.0%, 50.0%, 75.0%. **Results:** *Staphylococcus aureus* isolates appeared resistance to most seven antibiotics except one isolate was sensitive to Meropenem and Levofloxacin, Zinc nanoparticles green synthesized using Hena aqueous extract showed high absorbance at 372 nm, with a spherical shape and sizes ranging from (19.17 -45.33) nm, FTIR analysis indicated a range of at (677.95 to 3416.85) cm^{-1} , The antibacterial activity was appeared zone inhibition to *Staphylococcus aureus* between (15-17) mm also results showed bacterial colony black color on Congo red agar and value numbers (0.445-0.621) by Microtiter method before treat with Zinc nanoparticles while after treat bacteria with Zinc nanoparticles lack black colony and value numbers decrees. **Conclusions:** Biosynthesized zinc nanoparticles showed activity as antibacterial effect synthesis from aqueous extract of *Lawsonia inermis*. Therefore, it may suggest their potential as a novel therapeutic approach as an antibacterial agent. [Bangladesh Journal of Infectious Diseases, June 2026;13(1):331-340]

Keywords: *Staphylococcus aureus*; Zinc nanoparticles; *Lawsonia inermis*

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Introduction

The increasing emergence of multidrug-resistant bacterial pathogens, usually resulting from the indiscriminate use of antibiotics, is causing

infectious diseases. This has directly impacted public health and the economy due to the rise in healthcare expenditures for treating patients with these diseases¹. Therefore, there is a pressing need

to find alternatives to antibiotics to replace those ineffective against resistant bacteria, such as *Staph. aureus*, a major clinically significant lead to different infectious diseases². From minor skin infections include cellulitis and abscesses and to more threat- infections such as endocarditis, sepsis, osteomyelitis, pneumonia, syndrome also UTIs³. It related to antibiotic resistance and production of biofilms represent a serious public health and complicated the treatment of infections caused by these bacteria⁴. In context, nanotechnology has emerged as a promising approach to address challenge of antibiotic resistance⁵.

Nanoparticles are three-dimensional particles ranging in size from 1 to 100 nanometers⁶, several types of nanoparticles including Fe₃O₄, TiO₂, CeO₂, and ZnO₂ have significant importance due to their bioavailability and low cost⁷. These particles can be synthesized from various environmentally friendly sources, including bacteria, fungi, algae, and plant components⁸ zinc nanoparticles synthesized from plant extracts exhibit antibacterial activity compared to conventional treatments⁹. Therefore, the study aimed to determine effect of biosynthesized zinc nanoparticles on *Staphylococcus aureus* growth and biofilm production.

Methodology

Study Design: This was a cross-sectional study which was conducted in the Department of biology, College of Science, University of Tikrit, Tikrit, Iraq from May 2025 to December 2025. A sterile swabs used to collect samples from diabetic foot ulcers of Fifty patients (34) male and (16) female arrived to Tikrit Teaching Hospital in Tikrit City from the period of May 2025 to December 2025 then swabs samples transfer to Microbiology Laboratory of Tikrit Teaching Hospital, Iraq and cultured on Mannitol salt agar and Gram-staining, and a series of biochemical tests include catalase and coagulase test also identified by using VITEK-2 technique (BioMérieux, France). Then detect antibiotics sensitivity test of *staph. aureus* and Biofilm formation.

Antibiotic Resistance Test: The Kirby-Bauer method detected the resistance of bacterial isolates to seven antibiotics (Manufacture Company, Bioanalyse, Turkey), following the methodology^{10,11} (Table 1). Bacterial suspension was adjusted to match the McFarland 0.5 standard; it was spread on Mueller-Hinton agar (Oxweed, England) using a sterile swab. The inoculated plates were left for five minutes, then antibiotic discs were transferred to the

plate surface using sterile forceps. Then petri plates were incubated in an incubator for 24 hours at 37°C. The results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

Qualitative method of Biofilm production: Congo Red Agar method used to detect Biofilm production like qualitative method¹² bacterial suspension (1×10⁸) inoculated on plates by sterile lobe, then plates incubated for 24 hours at 37°C, the result appeared colony Black, indicate production biofilms while colony red color negative result.

Quantitative method of Biofilm production: Microtiter plate assay used according to a study¹³, added 200 µL/well of the prepared bacterial suspension (1×10⁸) to A sterile 96-hole microtiter plates used to detect ability of bacteria produce biofilm. After incubation at 37°C for 48 hours then washed well used by sterile phosphate-buffered saline (PBS) gently after that plates a dry it in oven at 60°C for 60 minutes the well of plate staining by added 200 µL of 0.5% (w/v) crystal violet solution and let it 15 minutes, remove stain through rinsed off with sterile distilled water. And dried plate a 37°C incubator for 30 minutes. Finally, add 200 µl of 96.0% ethanol to each well, and then detect biofilm biomass by measuring optical density at the appropriate wavelength

Green synthesis of Zinc nanoparticles: The solution extract of plant and zinc with synthesis method was according to the studies with some modifications^{14,15}.

Preparation of Lawsonia inermis plant extract and Zinc Solution: The plant extract of henna (Buy them from local markets (was prepared by weighing 10 grams of henna powder and heating it in 100 ml of deionized distilled water placed in a 250 ml volumetric flask and heating it for 5 minutes in a water bath. Then the extract was filtered using Whitman filter paper, while, a zinc nitrate solution was prepared at a concentration of 0.1 M (Zn (NO₃)₂·6H₂O) by dissolving it at 60°C (prepared by Merck, Germany) and stored in an opaque bottle at 4°C B-Synthesis Zinc nanoparticles Zinc nanoparticles were manufactured by gradually adding 10 ml of aqueous henna extract to 90 ml of zinc nitrate solution at 70°C with continuous stirring through a magnetic stirrer until a color change occurred, which lasted 30 minutes.

Characterization of Zinc Nanoparticles: Zinc nanoparticles generated with *Lawsonia inermis* extracts were analyzed through used UV-vis spectroscopy for detect nanoparticles (Shimadzu, Tokyo, Japan), SEM (Gallenkamp, England), FTIR

spectroscopy (Shimadzu, Germany), and characterized according to protocols¹⁶. Screening of Zinc Nanoparticles as Antibacterial agent. The antibacterial activity of zinc nanoparticles was determined using the well diffusion method against *Staphylococcus aureus*, according to studies by¹⁷ bacterial suspension, stabilized with McFarland's solution 1×10^8 , was spread on Mueller-Hinton agar using a sterile cotton swab. The plates were left to stand for 15 minutes, then wells (6 mm in diameter) were prepared using a sterile cork drill, one hundred μL of zinc nanoparticles (25.0%, 50.0%, 75.0%, and 100.0%) was added (one concentration each well). Additionally, 100 μL of sterile distilled water was added to one well as a negative control. The Petri dishes incubated at 37°C for 24 hours After incubation, the diameters of the inhibition zones that formed around those wells were recorded.

Antibiofilm Effect of Zinc Nanoparticles: Effect Zinc nanoparticles on biofilm of *Staphylococcus aureus* detect through:

Qualitative Method: The Congo red agar used to detect effect zinc nanoparticles on biofilm formation through Added 120 μL of bacterial suspension (1×10^8) to 80 μL zinc nanoparticles concentrations (25.0%, 50.0%, 75.0%) in test tubes sterilized (tube each concentration), Then tubes incubated at 37°C for 24 hours, after that 5 microliter for each tube transfer by micropipette to Congo red plates incubated at 37°C for 24 hours¹⁸.

Quantitative Method: Microtiter plate method was used to determined zinc nanoparticles effect on production biofilms of *Staphylococcus aureus* according to¹⁹ with some modifications, 100 μL of bacterial suspension (1×10^8) added to each well of plate, after that added 100 μL of concentrations

zinc nanoparticles SubMic (25.0%, 50.0%, 75.0%) then incubated at 37°C for 24 hours after incubated time bacterial solution removed, and well washed twice by used phosphate buffer solution and dried it then added for each well 200 μL of 1.0% stain crystal violet solution. escape stain, added 200 μL of absolute ethanol, calculated OD through used an ELISA reader at 630nm, the medium Brain heart infusion broths used as a negative control.

Statistical Analysis: The study results do not require statistical software; only percentages were used in the reported results of the study

Ethical Consideration: Ethical Consideration was conducted according to the Declaration of Helsinki with formal Ethics approval from the Biology Department, College of Science, Tikrit University, Salah al-Din, Iraq (Approval No. 0422; on 23 April 2025). Also, written informed consent was obtained from all author participants.

Results

Bacterial Isolates of *Staphylococcus aureus*: Only nine bacterial isolates of *Staphylococcus aureus* 18.0% of total fifty swabs samples taken from patients appeared results while others not

Antibiotic Resistance Profile: Four bacterial isolates of *Staphylococcus aureus* in this study showed resistance to seven types antibiotics while other isolates five isolates showed sensitivity to antibiotics in the study (Table 1).

The results of the zinc biosynthesis include a color change to yellow and characterization of zinc nanoparticle formation through ultraviolet spectroscopy examination, which showed an absorption peak at 372 nm (Figure IA&B).

Table. 1- Antibiotic effect (inhibition zone in mm) on *Staphylococcus aureus*

Bacteria	Antibiotics						
	MEM	E	LEV	CFM	AK	SXT	P
<i>Staph.aureus 1</i>	S	RS	S	RS	RS	RS	RS
<i>Staph.aureus 2</i>	RS	RS	RS	RS	RS	RS	RS
<i>Staph.aureus 3</i>	RS	RS	RS	RS	RS	RS	RS
<i>Staph.aureus 4</i>	RS	RS	RS	RS	RS	RS	RS
<i>Staph.aureus 5</i>	S	RS	S	S	S	RS	S
<i>Staph.aureus 6</i>	S	RS	S	S	S	S	S
<i>Staph.aureus 7</i>	RS	S	S	S	S	RS	RS
<i>Staph.aureus 8</i>	S	RS	S	S	S	RS	RS
<i>Staph.aureus 9</i>	S	RS	S	RS	S	S	S

S=Sensetive, RS=Resistance, MEM=Meropenem, E=Erythromycin, LEV= Levofloxacin, CFM=Cefixime, AK= Amikacin, SXT= Trimethoprim-sulfamethoxazole, P= Penicillin.

Zinc nanoparticles characterization

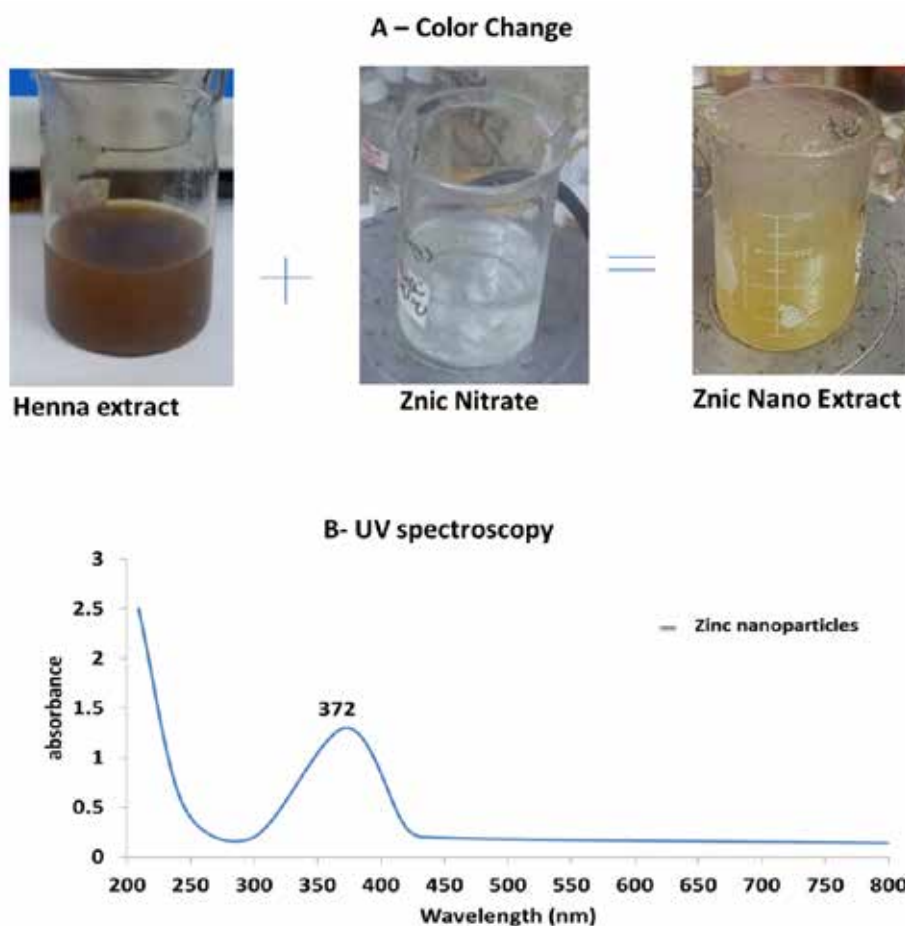


Figure I: A) Zinc nanoparticles color, B) UV-Spectroscopy Spectrum Peak Of Synthesized Bio-Zinc Nanoparticles

The size of zinc nanoparticles was detected by using scanning electron microscope examination,

showing a spherical shape and a size range from 19.17 to 45.33 nm (Figure II).

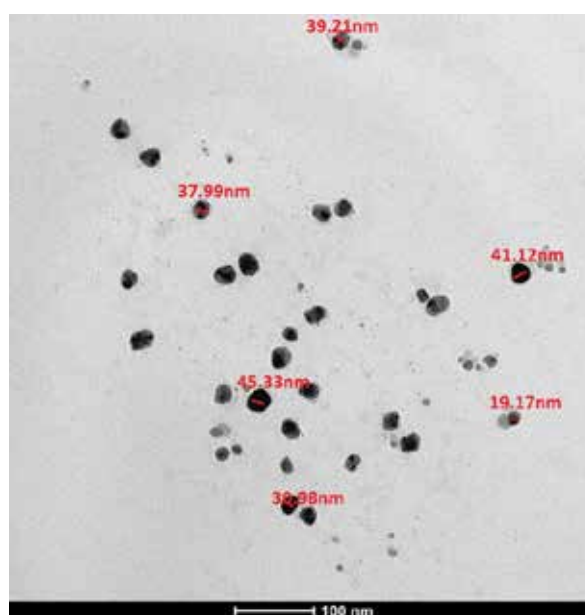


Figure II: Scanning Electron Microscopy image of zinc nanoparticles

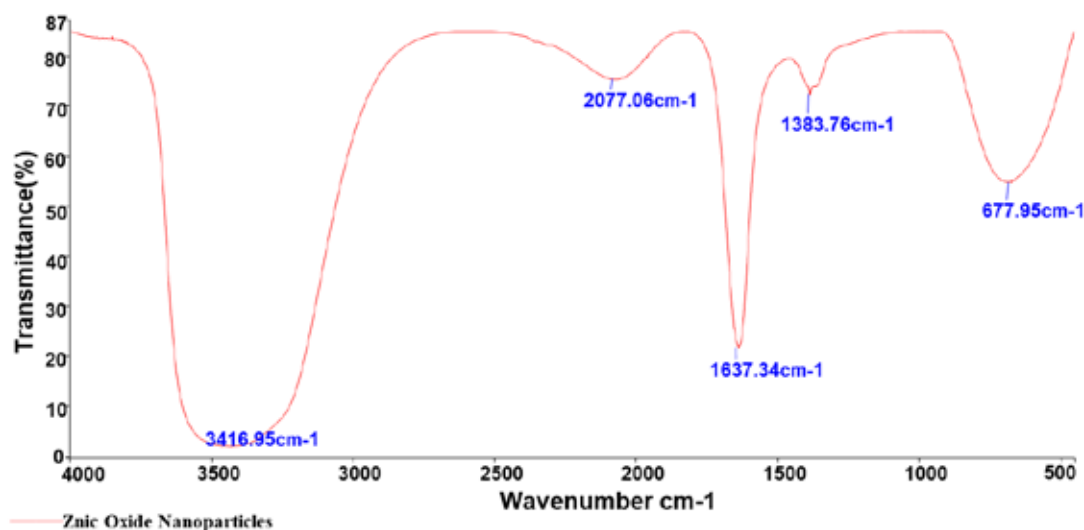


Figure III: FTIR analysis of Zinc nanoparticles

Table 2: Antibacterial effect (inhibition zone in mm) of synthesized Zinc nanoparticles

Bacteria	Zinc nano-Concentrations			
	25%	50%	75%	100%
<i>Staph. aureus 1</i>	9	12	14	17
<i>Staph. aureus 2</i>	10	13	15	16
<i>Staph. aureus 3</i>	10	12	15	17
<i>Staph. aureus 4</i>	9	10	13	15

While FTIR analysis detects bands at 677.95, 1383.76, 1637.34, 2077.06, and 3416.85 cm^{-1} (Figure III).

Screening of Zinc Nanoparticles as an Antibacterial Agent: All concentrations of zinc nanoparticles showed inhibitory activity against multidrug-resistant *Staphylococcus aureus* and increase zone inhibition according to increase concentration the range zone inhibition (15 to 17) mm (Table 2, Figure IV).

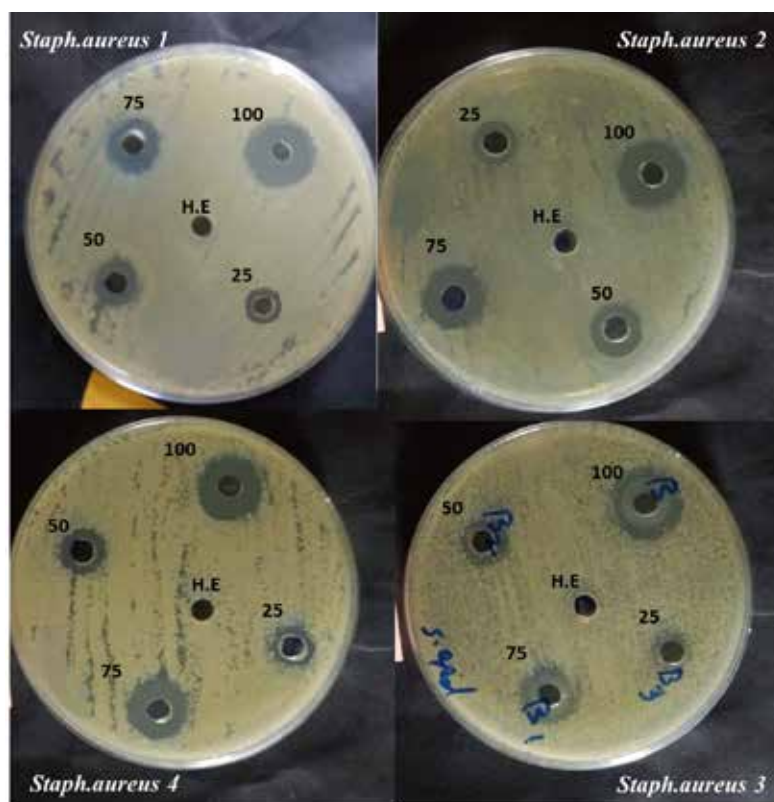


Figure IV: Antibacterial Effect (inhibition zone in mm) of Zinc nanoparticles concentrations (100,75,50,25) %, H. E.= Henna extract (*Lawsonia inermis*) to *Staphylococcus aureus* bacteria

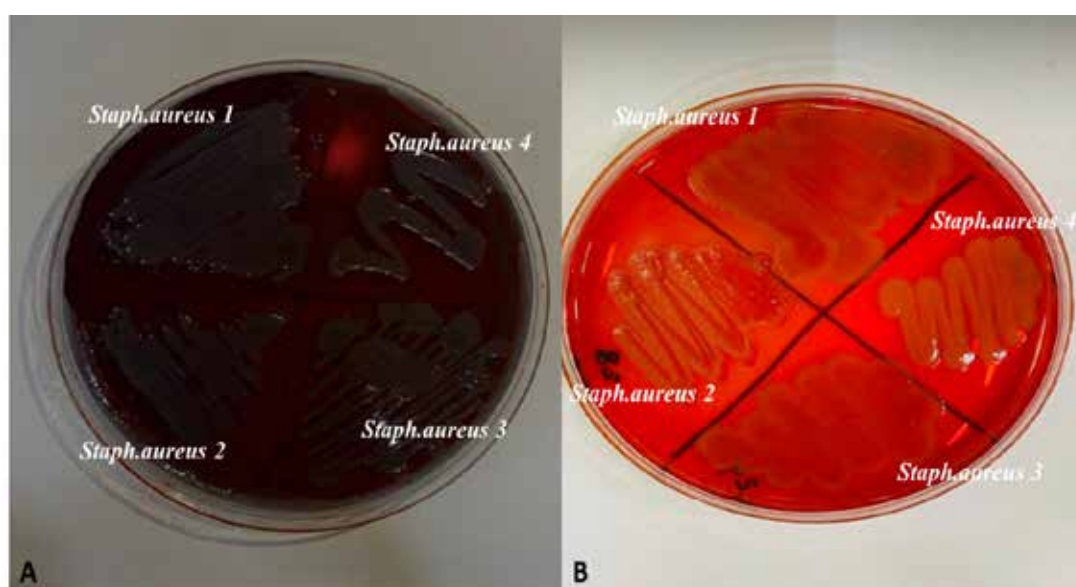


Figure V: Antibiofilm Effect of Zinc nanoparticles on Staphylococcus aureus bacteria (A) After treatment (B) Before treatment Staphylococcus aureus with Zinc nanoparticles

Table 3: value formation biofilm before treat with Zinc nanoparticles and after treatment

NO.	Bacterial Isolates	Before treatment		After treatment	
		OD at 630nm	Biofilms	OD at 630nm	Biofilms
1	<i>Staph. aureus 1</i>	0.445	Strong	0.067	Weak
2	<i>Staph. aureus 2</i>	0.512	Strong	0.102	Weak
3	<i>Staph. aureus 3</i>	0.460	Strong	0.090	Weak
4	<i>Staph. aureus 4</i>	0.621	Strong	0.112	Weak

Screening bacterial Biofilm and antibiofilm of Zinc Nanoparticles

Congo Red Agar Method: Total nine isolates four isolates produce strong to biofilm while others weak, *Staphylococcus aureus* four isolates showed color black colony on plate Congo red agar while showed red color after bacteria treat with SubMic (25.0%, 50.0%, 75.0%) zinc Nanoparticles (Figure V).

Microtiter Plate Method: The biofilm formation of *Staph. Aureus* before treating with zinc nanoparticles at concentrations according to absorbance value between 0.621 to 445 AU compare with absorbance values which decrease after treating *Staphylococcus aureus* isolates with SubMic (25.0%, 50.0%, 75.0%) concentration zinc nanoparticles appeared rang (0.112 to 0.067) AV (Table 3).

Discussion

Diabetic foot ulcers are a major complication of diabetes. If left untreated, these ulcers and wounds can lead to more serious complications, including osteomyelitis, gangrene, and amputation. Surgery and

antibiotic therapy are used to control these infections, especially bacterial ones. Males were found to have a higher incidence of these infections compared to females; this may be attributed to the role males play in outdoor activities that can lead to work-related injuries and ulcer development. Similarly, the majority of participants with diabetic foot ulcer infections were between 50 and 60 years old, consistent with a study²⁰. The infections are caused by several types of bacteria, including *Staphylococcus aureus*, which is the predominant Gram-positive strain, consistent with the observation²¹ Gram-positive bacteria 41 (32.0%). Among the isolated bacteria, the most predominant bacteria were *Staphylococcus aureus* 25.19% (32/127).

The resistance of four isolates of *Staphylococcus aureus* to antibiotics was very high, reaching 100% resistance to all antibiotics except for one bacterial isolate, which was sensitive to meropenem and levofloxacin. This is similar to the results of a study²², which observed that *Staphylococcus aureus* bacteria isolated from urine samples exhibited high levels of resistance to cefotaxime 100.0% and levofloxacin 53.0% while meropenem and ampicillin 76.0%. Also, resistance to cefixime (48.6%), amikacin (52.9%),

meropenem (44.3%), and levofloxacin (64.3%). Congdon et al²³ also observed 100.0% resistance to ampicillin, cefoxitin (78.6%), and Amikacin (73.8%), while meropenem showed resistance (19.0%).

Bacteria have different mechanism to Resistance antibiotic include Resistance to β -lactam antibiotics as penicillin is associated with ability to inhibit synthesis bacterial cell wall. All beta-lactam antibiotics act on PBPs (penicillin-binding proteins). Also, Modifications of PBPs one of resistance mechanisms of β -lactam antibiotics. Bacteria reduce affinity to PBPs and increase expression of β -lactamase led to hydrolysis of β -lactam ring of antibiotics²⁴ While aminoglycoside antibiotics such amikacin resistance bacteria due to produce aminoglycoside -modifying enzymes led to hydrolyze susceptible bonds like esters and amides in aminoglycosides groups²⁵.

Fluoroquinolone antibiotics resistance as levofloxacin antibiotic mediated mutations in DNA gyrase enzyme and topoisomerase genes²⁶, another mechanism include Modification of metabolic pathways as antibiotic Trimethoprim-sulfamethoxazole through absence or alteration of para-aminobenzoic acid, was a precursor to nucleic acid and synthesis folic acid, allows bacteria to overcome inhibitory effects of antibiotic targeting these pathways²⁷.

Zinc Nanoparticles Synthesis: Color change is the first step in the formation of zinc nanoparticles. A pale-yellow color was observed, a result consistent with that obtained by an author¹⁴ who observed a color change in henna extract after adding zinc nitrate. Similarly²⁸, also found a color change from dark brown to pale yellow in henna after adding zinc nitrate. It is known that the plant is rich in active compounds, including flavonoids, saponins, methylxanthines, and phenolic acids, which act as reducing and capping agents and play a role in nanoparticle synthesis²⁹. The reduction of the metal ion also occurs through a mixture of bioactive substances in the plant extract, including proteins, enzymes, and polysaccharides²⁸.

Confirming the formation of nanoparticles requires tests that determine the optical properties of zinc nanoparticles using UV spectroscopy. An absorption band of 372 nm was obtained, similar to that of¹⁵ (395 nm), which indicates the synthesis of zinc nanoparticles, suggesting the formation of zinc nanoparticles from henna extract³⁰. Exhibited a prominent absorption band at approximately 385 nm, a characteristic feature of zinc oxide nanoparticles. This band is used to describe zinc nanoparticles. The absorption range exhibited by zinc nanoparticles is influenced by surface plasmon resonance (SPR)³¹.

Furthermore, plant chemical compounds contributed to the reduction of zinc ions and acted as stabilizing agents³². The sizes of the zinc nanoparticles were determined to be in the range of (19.17 to 45.33) nm, which is similar to what¹⁵ found, where zinc nanoparticles ranged in size from 5 to 50 nm³³. Observed zinc nanoparticle sizes of 40 to 70 nm, which is similar to our results.

The important functional groups involved in the reduction and stabilization of zinc nanoparticles were identified using FTIR; the results of the current study are similar to those obtained by³⁴ who observed peaks at 538.39 cm^{-1} , 1,637.54 cm^{-1} , and 3,415.04 cm^{-1} . They are also similar to those found by¹⁴, where the band 1618.92 cm^{-1} indicates C=C in the aromatic ring, while 1,344.98 cm^{-1} indicates C-N extension of amide-I protein compounds. The band at 3422 cm^{-1} could indicate O-H hydroxyl groups associated with polyphenols of the plant extract, which contribute to the biosynthesis of zinc nanoparticles, while the band at 664 to 676 cm^{-1} indicates the aromatic ring³⁵.

The active compounds that plant extracts typically possess play a role in the reduction, stabilization, and capping of zinc nanoparticles. These include phenols, amines, alkenes, alcohols, and carboxylic acids³⁶.

Antibacterial effect of zinc nanoparticles: The inhibitory effect of zinc nanoparticles against *Staphylococcus aureus* bacteria showed an increase with increasing nanoparticle concentration; similar to a study³⁷ it was observed, zone inhibition (15 to 26 mm for *S. aureus* at 40 $\mu\text{g mL}^{-1}$). Also, 38 found that the inhibitory diameter of zinc nanoparticles against *Staphylococcus aureus* was about 27 mm; 39 also noted the role of zinc nanoparticles in inhibiting *Staphylococcus aureus*.

Zinc oxide nanoparticles exhibit antibacterial activity, particularly against Gram-positive bacteria. Their increased antibacterial efficacy may be attributed to their ability to produce reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), which can readily penetrate the bacterial cell membrane, leading to cell death⁴⁰. The increased ROS production, resulting from the large surface area-to-volume ratio of zinc nanoparticles, further enhances their antibacterial activity⁴¹. In the presence of nanoparticles, zinc ions are released directly and bind to the cell membrane. This process leads to damage to genetic material and the breakdown of proteins, as well as the respiratory chain and enzyme synthesis. By interfering with the thiol group of enzymes, it leads to bacterial death^{42,43}.

Screening Bacterial Biofilm and Antibiofilm of Zinc Nanoparticles: *Staphylococcus aureus* is a bacterium

that poses a threat to human health due to its increasing resistance to antibiotics and its ability to produce biofilms. However, this biofilm production was reduced after treatment with zinc nanoparticles using both microtiter plate and Congo red agar methods. These results are similar to those found in a study³⁹ who observed that biofilm production by *Staphylococcus aureus* was reduced by 91.0% after treatment with zinc nanoparticles using the microtiter plate. This also aligns with the findings of another study⁴⁴, which observed that 99.0% of *Staphylococcus aureus* isolates produced biofilms before treatment with zinc nanoparticles but lost this ability after microtiter plate treatment. Similarly, *Staphylococcus aureus* was found among the biofilm-producing isolates using both Congo red and microtiter plate methods. These findings are consistent with those of Mendes et al⁴⁵, who found that the biofilm produced by *Staphylococcus aureus* was reduced after treatment with zinc nanoparticles using the microtiter method. Zinc nanoparticles influence the formation of the exopolysaccharide layer, which plays a role in bacterial adhesion to host cells and biofilm development.

Zinc nanoparticles can penetrate the bacterial cell wall and kill the bacteria⁴⁶. Metallic nanoparticles play a crucial role in controlling biofilm production due to their physicochemical properties⁴⁷. The anti-biofilm effect of metallic nanoparticles can be achieved by penetrating the bacterial cell wall and interfering with the extracellular polymer matrix of the biofilm⁴⁸. They can also disrupt the quorum sensing mechanism⁴⁹. The activity of reactive oxygen species (ROS) on these nanoparticles is known to induce bacterial membrane damage. Oxidative stress resulting from ROS leads to the production of free radicals, including hydroxyl radicals, hydrogen peroxide radicals, and superoxide radicals, which damage bacterial DNA and protein structures⁵⁰.

Conclusion

Biosynthesized zinc nanoparticles showed activity as antibacterial effect synthesis from aqueous extract of *Lawsonia inermis*. Therefore, it may suggest their potential as a novel therapeutic approach as an antibacterial agent.

Acknowledgements

None.

Conflict of interest:

The authors declare that there is no conflict of interest regarding the publication of this study.

Financial Disclosure

None.

Authors' contributions

All Authors in this study conceived and designed the study, conducted data collection and laboratory work, analyzed data, interpreted the findings, and drafted the manuscript.

Data Availability

Any questions regarding the availability of the study's supporting data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate

Ethical approval was granted by the Research Ethics Committee of the Biology Department, College of Science, Tikrit University, Salah al-Din, Iraq (Approval No. 0422; 23 April 2025). The study was conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants.

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References

- da Cunha KF, Albernaz DTF, Garcia MDO, Allend SO, Hartwig DD. Silver nanoparticles (AgNps) in the control of *Staphylococcus* spp. Lett Appl Microbiol. 2023;76(1):ovac032.
- Ezeh CK, Eze CN, Dibua ME, Emencheta SC. A meta-analysis on the prevalence of resistance of *Staphylococcus aureus* to different antibiotics in Nigeria. Antimicrobial Resistance & Infection Control. 2023 Apr 25;12(1):40.
- Linz MS, Mattappallil A, Finkel D, Parker D. Clinical impact of *Staphylococcus aureus* skin and soft tissue infections. Antibiotics (Basel) 2023; 12: 557.
- Tălăpan D, Sandu AM, Rafila A. Antimicrobial resistance of *Staphylococcus aureus* isolated between 2017 and 2022 from infections at a tertiary care hospital in Romania. Antibiotics. 2023 May 28;12(6):974.
- Hetta HF, Ramadan YN, Al-Harbi AI, Ahmed EA, Battah B, Abd Ellah NH, et al. Nanotechnology as a promising approach to combat multidrug-resistant bacteria: A comprehensive review and future perspectives. Biomedicines 2023;11:413.
- Faisal S, Jan H, Shah SA, Shah S, Khan A, Akbar MT, Rizwan M, Jan F, Wajidullah, Akhtar N, Khattak A. Green synthesis of zinc oxide (ZnO) nanoparticles using aqueous fruit extracts of *Myristica fragrans*: their characterizations and biological and

- environmental applications. ACS Omega. 2021 Mar 30;6(14):9709-22.
7. Khan AU, Khan M, Cho MH, Khan MM. Selected nanotechnologies and nanostructures for drug delivery, nanomedicine and cure. Bioprocess Biosyst Eng. 2020;43(8):1339–1357.
 8. Hamouda, R.; Elshamy, M., Using biosynthesized zinc oxide nanoparticles to alleviate the toxicity on banana parasitic-nematode, Research Square, 2021, 2021,67, 1-21.
 9. Al-Darwesh MY, Ibrahim SS, Mohammed MA. A review on plant extract mediated green synthesis of zinc oxide nanoparticles and their biomedical applications. Results in Chemistry. 2024 Jan 1;7:101368.
 10. Saleh RF, Al-Sugmiany RZ, Al-Doori MM, Al-Azzawie A. Phenotypic and Genetic Effects of Wi-Fi Waves on Some Bacterial Species Isolated from Otitis Media Infection. Tropical Journal of Natural Product Research 2020 Dec 1;4(12):1056-63.
 11. Humphries R, Bobenchik AM, Hindler JA, Schuetz AN. Overview of changes to the Clinical and Laboratory Standards Institute performance standards for antimicrobial susceptibility testing, M100. Journal of Clinical Microbiology. 2021 Nov 18;59(12):10-128
 12. Mohammed RF, Mustafa HA. Effect of Lactobacillus acidophilus bacteria and copper oxide nanoparticles on the tissues of male mice. Al-Turath Journal for Biology. 2025 Dec 31;1(4):21-47
 13. Sultan AM, Nabel Y. Tube method and Congo red agar versus tissue culture plate method for detection of biofilm production by uropathogens isolated from midstream urine: Which one could be better? African journal of clinical and experimental microbiology. 2019;20(1):60-6.
 14. Gajdacs M, Urbán E. Comparative epidemiology and resistance trends of Proteae in urinary tract infections of inpatients and outpatients: a 10-year retrospective study. Antibiotics. 2019; 8(3): 91.
 15. Al-Esawi AS, Al-Musawi RS. Inhibitory activity of zinc oxide nanoparticles (ZnONPs) synthesized using L leaf extract Lawsonia inermis. Ind. J. Eco. 2022;20(26):249-56.
 16. Yassin MT, Al-Askar AA, Maniah K, Al-Otibi FO. Green synthesis of zinc oxide nanocrystals utilizing Origanum majorana leaf extract and their synergistic patterns with colistin against multidrug-resistant bacterial strains. Crystals. 2022 Oct 25;12(11):1513.
 17. Rajaganesh R, Murugan K, Panneerselvam C, Jayashanthini S, Aziz AT, Roni M, Suresh U, Trivedi S, Rehman H, Higuchi A, Nicoletti M. Fern-synthesized silver nanocrystals: towards a new class of mosquito oviposition deterrents?. Research in Veterinary Science. 2016 Dec 1;109:40-51
 18. Saleh RF, Gaidan AM, Al-Mayah QS. Green synthesis of silver nanoparticles using aqueous leaf extract of Ocimum basilicum and investigation of their potential antibacterial activity. Tropical Journal of Natural Product Research, 2021;5(1):94-99
 19. Gupta V, Kant V, Sharma AK, Sharma M. Comparative assessment of antibacterial efficacy for cobalt nanoparticles, bulk cobalt and standard antibiotics: a concentration-dependent study. Наносистемы: физика, химия, математика. 2020;11(1):78-85.
 20. Hamzah HM, Salah RF, Maroof MN. Fusarium mangiferae as new cell factories for producing silver nanoparticles. J Microbiol Biotechnol 2018; 28(10): 1654-1663
 21. Theodora NA, Dominika V, Waturangi DE. Screening and quantification of anti-quorum sensing and antibiofilm activities of phyllosphere bacteria against biofilm-forming bacteria. BMC Research Notes. 2019 Nov 7;12(1):732
 22. Murshed M. Bacteriological profile of diabetic foot infection and its effect on limb salvation. Journal of Surgical Sciences. 2020 Nov 1;24(1):21-5.
 23. Atlaw A, Kebede HB, Abdela AA, Woldeamanuel Y. Bacterial isolates from diabetic foot ulcers and their antimicrobial resistance profile from selected hospitals in Addis Ababa, Ethiopia. Frontiers in Endocrinology. 2022 Aug 31;13:987487.
 24. Najem FM, Ali OM, Abbas FK, Jamal AA, Alzubaidi AF. Antibacterial Susceptibility Pattern of Urine Bacterial Isolates in Diyala, Iraq. Journal of Pioneering Medical Sciences. 2025 Jul 5;14:116-21.
 25. Al-Groom R, Al-Saraireh G, Saghir SA, Khan MS, Almanaseer AM, Alswalha L, Alraei W, Al-Hajjia DA, Hdaib M, Da'meh A, Burjaq SZ. Resistance profiles of Staphylococcus aureus isolates against frequently used antibiotics at private sector laboratories in Jordan. Iranian journal of microbiology. 2025 Apr;17(2):229
 26. Singh S, Numan A, Somaily HH, Gorain B, Ranjan S, Rilla K, Siddique HR, Kesharwani P. Nano-enabled strategies to combat methicillin-resistant Staphylococcus aureus. Materials Science and Engineering: C. 2021 Oct 1;129:112384
 27. Saha P, Kabir RB, Ahsan CR, Yasmin M. Multidrug resistance of Pseudomonas aeruginosa: do virulence properties impact on resistance patterns?. Frontiers in Microbiology. 2025 Feb 5;16:1508941..
 28. Aldrazi FA, Rabaan AA, Alsuliman SA, Aldrazi HA, Alabdalslam MJ, Alsadiq SA, Alhani HM, Bueid AS. ESBL expression and antibiotic resistance patterns in a hospital in Saudi Arabia: do healthcare staff have the whole picture? Journal of Infection and Public Health. 2020 May 1;13(5):759-66.
 29. Mancuso G, Midiri A, Gerace E, Biondo C. Bacterial antibiotic resistance: the most critical pathogens. Pathogens. 2021 Oct 12;10(10):1310.
 30. Shekhawat MS, Ravindran CP, Manokari M. An eco-friendly method for the synthesis of zinc oxide nanoparticles using Lawsonia inermis L. aqueous extracts. International Journal of Innovation. 2015;5(1):1.
 31. Valduga AT, Gonçalves IL, Magri E, Finzer JR. Chemistry, pharmacology and new trends in traditional functional and medicinal beverages. Food Research International. 2019 Jun 1;120:478-503.
 32. Oyewole TS, Osundiya MO, Osifeko OL, Adebajo SA, Aribidesi MO, Olaoluwa GB, Adewale OR. Green synthesis of zinc oxide nanoparticles from Morinda Lucida leaf extract for biosorption application, kinetics, and thermodynamic studies. Discover Chemistry. 2026 Dec;3(1):126
 33. Ghorbani HR, Mehr FP, Pazoki H, Rahmani BM. Synthesis of ZnO nanoparticles by precipitation method. Orient. J. Chem. 2015 Jun 1;31(2):1219-21
 34. Olowu RA, Osundiya MO, Oyewole TS, Onwordi CT, Yusuff OK, Osifeko OO, Tovide OO. Equilibrium and kinetic studies for the removal of Zn (II) and cr (VI) ions from aqueous solution using pineapple peels as an adsorbent. Eur J Appl Sci. 2022;5:34–47.
 35. Fali H, Ahmed NM, Rashid M. Green synthesis of zinc oxide nanoparticles by fresh and dry alhagi plant. Materials Today: Proceedings. 2022 Jan 1;49:3624-9.
 36. Rusli RK, Hilmi M, Mahata ME, Yuniza A, Zurmiati Z, Reski S, Hidayat C. Green synthesis of zinc oxide nanoparticles utilizing extract from Garcinia mangostana leaves: Characterization and optimization of calcination temperature. Journal of advanced veterinary and animal research. 2024 Sep 29;11(3):573.
 37. Endeshaw SB, Tufa LT, Goddati M, Lee J, Silalahi VC, Lee D, Murthy HA, Sabir FK. Croton macrostachyus leaf extract-mediated green synthesis of ZnO nanoparticles and ZnO/CuO nanocomposites for the enhanced photodegradation of methylene blue dye with the COMSOL simulation model. ACS Omega. 2023 Dec 19;9(1):559-72.
 38. Huong LM, Cong CQ, Dat NM, Hai ND, Nam NTH, An H, et al. Green synthesis of carbon-doped zinc oxide using Garcinia mangostana peel extract: Characterization, photocatalytic degradation, and hydrogen peroxide production. J Clean Prod. 2023;392:136269
 39. Abed FN, Hassan DF, Abed AN, Hasson OA. Prevalence and Distribution of Bacterial Isolates from Blood Cultures Among Patients with Fever: A Retrospective Study. Al-Turath Journal for Biology. 2026 Mar 31;2(1):110-6

40. Habtemariam TH, Anjullo SH, Abebe GM. Green synthesis and antibacterial activity of zinc oxide nanoparticles using *Croton macrostachyus* Hochst. ex Delile extracts. *RSC Advances*. 2026;16(16):14492-508.
41. Rasheed ZR, Abd AN, Hassan KH. Antibacterial Activity of Zinc Oxide Nanoparticles Synthesized by Green *Eichhornia Crassipes* Extract Method. *Frontiers in Biomedical Technologies*. 2024 Oct 7;11(4):556-62.
42. Koppole K. Plant extract-mediated ecofriendly synthesis of zinc oxide nanoparticles and investigation of their photocatalytic, antibacterial, and antibiofilm properties. *Applied Physics A*. 2026 Mar;132(3):162
43. Nan J, Chu Y, Guo R, Chen P. Research on the antibacterial properties of nanoscale zinc oxide particles comprehensive review. *Frontiers in Materials*. 2024 Sep 23;11:1449614
44. Kadhim ME, Ali AM, Hanoon NM. Antibacterial, Antioxidant, and Anti-inflammatory Evaluation of a Newly Formulated Polyherbal Mouthwash. *Al-Turath Medical Journal*. 2026 Jul 16;2(1):15-26.
45. Mendes CR, Dilarri G, Forsan CF, Sapata VD, Lopes PR, de Moraes PB, Montagnolli RN, Ferreira H, Bidoia ED. Antibacterial action and target mechanisms of zinc oxide nanoparticles against bacterial pathogens. *Scientific Reports*. 2022 Feb 16;12(1):2658.
46. Jiang S, Lin K, Cai M. ZnO nanomaterials: current advancements in antibacterial mechanisms and applications. *Frontiers in Chemistry*. 2020 Jul 21;8:580
47. Hassan ZA, Al-Rubaie HM, Khalil NS. In vitro Study on the antibacterial Activity of Selected Iraqi Medicinal Plants against Multi-Drug Resistant Clinical Isolates. *Al-Turath Journal for Biology*. 2025 Dec 31;1(4):6-10
48. Bankier C, Matharu RK, Cheong YK, Ren GG, Cloutman-Green E, Ciric L. Synergistic antibacterial effects of metallic nanoparticle combinations. *Scientific Reports*. 2019 Nov 5;9(1):16074
49. Gaidan AM, Saleh RF, Hasan AY. The correlation between NOD2 and anti-Salmonella Typhi antibodies functions as a diagnostic marker for typhoid disease. *Microbial Biosystems*. 2025 Sep 1;10(3):172-177
50. Al-Bayati MH. Molecular Detection of *pilA* Gene and Antimicrobial Resistance Pattern of *Pseudomonas aeruginosa* Isolated from Burn Patients. *Al-Turath Journal for Biology*. 2026 Mar 31;2(1):104-9