RESEARCH PAPER

Biosynthesis of Ag/ZnO Nanocomposite Using *Ziziphus Jujuba* Leaves Extract for Enhanced Synergistic Antibacterial Activity

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ARTICLE INFO

Article History:
Received 05 September 2025
Accepted 27 October 2025
Published 01 January 2026

Keywords:
Antibacterial
Biosynthesis
Nanocomposite
Silver/zinc oxide
Synergistic effect
Ziziphus jujuba

ABSTRACT

Over the past ten years, there has been a clear trend toward finding eco-friendly, long-lasting ways to make nanomaterials, especially composites with better properties. This study successfully synthesized silver/zinc oxide nanocomposite (Ag/ZnONC) utilizing an aqueous extract of Ziziphus jujuba leaves as a reducing agent and green filler, adhering to an eco-friendly approach. Spectroscopic and microscopic techniques were employed to characterize the biosynthesized nanocomposite. Fourier transform infrared spectroscopy (FTIR) elucidated the role of flavonoid compounds in Ziziphus jujuba extract during reduction and stabilization. Images from a field emission scanning electron microscope (FESEM) confirmed that mixing silver nanoparticles with zinc oxide nanoparticles made a hybrid and homogeneous structure. X-ray diffraction (XRD) spectroscopy also showed that the silver/zinc oxide nanocomposite was made correctly. This was shown by X-ray diffraction patterns that showed clear peaks for both materials. The wide diffraction peaks confirmed that the composite had a nanostructure. The Debye-Scherer equation says that the particle size of the composite component is about (45–55) nm. The synthesized nanocomposite consistently demonstrated significant efficacy against Enterococcus faecalis (Gram-positive) and Klebsiella pneumoniae (Gram-negative) bacteria, exhibiting a minimum inhibitory concentration (MIC) of 1.25% for both bacterial strains examined. The high effectiveness was due to a synergistic effect between the composite parts, which worked together in a number of complementary ways, such as producing reactive oxygen species (ROS), damaging the cell membrane in two ways, and interfering with cellular processes in the bacteria at the same time. The current study highlights the potential of Ziziphus jujuba leaves as an effective agent in the green synthesis of knowledgeable nanocomposites. Thus, offers the biosynthesized nanocomposite as a promising choice for biomedical applications throw the fight against multi-resistant microbes, supporting efforts to find green alternatives to traditional chemical methods.

How to cite this article
Shuker K., Fadhil H., Mohammed Z., Naser J. Biosynthesis of Ag/ZnO Nanocomposite Using Ziziphus jujuba Leaves Extract for Enhanced Synergistic Antibacterial Activity. J Nanostruct, 2026; 16(1):508-516. DOI: 10.22052/JNS.2026.01.046

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INTRODUCTION

In the last two decades, nanomaterials have become a cornerstone of technological and medical progress, due to their unique properties that are fundamentally different from their micro-sized counterparts. These properties, which include a huge surface area, high surface activity, and outstanding electromagnetic and optical capabilities, have enabled them to have pioneering applications in diverse fields such as medicine; such as targeted diagnostics and drug delivery [1], energy; solar cells and batteries [2], environment; water treatment and photocatalysis [3] and the field of antimicrobial coatings [4].

Among the nanomaterials group, silver nanoparticles (AgNPs) and zinc oxide nanoparticles (ZnONPs) stand out as some of the most studied and applied materials due to their broad antimicrobial activity. The antibacterial activity of silver nanoparticles is ascribed to their ability to release silver ions (Ag^{+}) that interact with the components of bacterial cellular, which leads to inhibition of cellular respiration, damage to the membrane of plasma, interference with of the gene expression, and the formation of reactive oxygen species (ROS), it causes oxidative stress and cell death [5]. Similarly, ZnONPs show strong antibacterial action, particularly when exposed to ultraviolet radiation, as they produce large amounts of ROS, and the release of zinc ions (Zn^{2+}) contributes to damage the bacterial cell membranes [6].

Despite the effectiveness of each individual material, the current trend in nanobiology is focus on nancomposites designing. These composites combine two or more nanomaterials not only to enhance individual properties but also to produce a synergistic effect that enhances overall performance. In this context, silver/zinc oxide nanocomposite (Ag/ZnONC) is a promising material. Research indicate that combining silver and zinc at the nanoscale can significantly enhance ROS generation, provide multiple mechanisms of bacterial cells damage, reduce the developing probability of bacterial resistance and increase the broad stability and efficiency of material [7].

The common chemical and physical methods of synthesis these nanomaterials often involve the utilization of toxic and expensive chemicals, consume high energy, and may release harmful byproducts to the environment. As a result, green or biosynthesis using plant extracts has emerged as a sustainable and eco-friendly alternative. Secondary component that found in plant, such as phenols, flavonoids, alkaloids and terpenes act as natural reducing agents and stabilizers in synthesizing process of nanoparticles [8]. In this study, Ziziphus jujuba leaves extract a plant known for its richness in phenolic compounds and volatile oils with antioxidant activity has been used, making it a perfect base for the green synthesis of nanocomposite [9].

Numerous investigations were carried out on the biosynthesis of individual nanomaterials. Continuously, Almatroudi synthesized AgNPs by use the extract of green tea and tested their antibacterial activity [10]. Therefore, Elumalai et al. produced ZnONPs utilizing the neem leaf extract [11]. Previous studies on the chemical synthesis of Ag/ZnO nanocomposites also showed improved antibacterial activity compared to individual materials [7].

The present research novelty offers a comprehensive of an integrated green strategy for the synthesis of silver/zinc oxide nanocomposite (Ag/ZnONC). Three interconnected supports this work; first, the utilization of a single-step, simultaneous synthesizing approach, rather than standard methods that depend on synthesis the two nanomaterials separately and then mixing them. This provides the creation of a homogeneous nanocomposite with an effective hybrid structure. Second, the use of Ziziphus jujuba leaves extract for the first time -to the researchers' knowledge- in the synthesis of this specific nanocomposite. This offers a new source to the scientific literature of plant to explore the biological activity of this plant's compounds in the composite production. Third, an extensive evaluation of the synergistic effect created between the components of composite, establishing the hypothesis that the synthesized hybrid structure utilizing this green technique will has significantly enhanced antibacterial activity in comparison to individual silver or zinc oxide nanoparticles.

This work aims to synthesis silver/zinc oxide nanocomposite (Ag/ZnONC) in an environmentally friendly approach using extract of Ziziphus jujuba leaves and characterization it by several techniques such as; Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and X-ray diffraction (XRD) spectroscopy. The research also intended to evaluate the antibacterial activity of the synergistic compound against selected bacteria, Enterococcus faecalis (Gram-positive) and Klebsiella pneumoniae (Gram-negative), to verify the hypothesis of a synergistic effect between the two substances in the compound, thus opening up potential applications for it as an active ingredient in antimicrobial and antibacterial agents.

MATERIALS AND METHODS

Materials

Chemical Materials

Silver nitrate ($AgNO_3$), purity ≥ 99.8%, zinc nitrate ($Zn(NO_3)_2.6H_2O$), purity ≥ 99.0%, and sodium hydroxide (NaOH), purity ≥ 97%, were used. All the above materials were supplied by Sigma-Aldrich.

Culture Media

In this study, Muller-Hinton Agar and Nutrient Broth bacterial culture media prepared by Himedia were used.

Instruments

Shimadzu IRSpirit Fourier Transform Infrared Spectrometer (FTIR), Shimadzu-6100 X-ray Diffraction Spectrometer (XRD), TESCAN VEGA3 Scanning Electron Microscope (SEM), Memmert Incubator, Grant Water Bath, Hettich EBA 20 Centrifuge.

Methods

Preparation of Plant Extract

Ziziphus jujuba leaves were collected from Baghdad Governorate, Iraq. The leaves were washed, dried in the shade, and then ground into a fine powder. To prepare the aqueous extract, 1 g of the powder was added to 100 mL of distilled water and heated in a water bath at 70 °C for 30 min. The solution was filtered using Whatman No. 1 filter paper and stored in the refrigerator for later use.

Synthesis of Silver/Zinc Oxide Nanocomposite (Ag/ZnONC)

10 mL of plant extract was added to a mixture containing 45 mL of silver nitrate and zinc nitrate solution (1:1 molar ratio). The pH was adjusted to 11 using sodium hydroxide. The mixture was heated in a water bath at 80 °C for 45 min. The resulting precipitate was subsequent washed with a warm 50 % v/v water-ethanol solution to remove any remaining salts. The produced nanopowder was then dried in oven at 80 °C.

Evaluation of Antimicrobial Activity

The antibacterial activity of the synthesized nanocomposite (Ag/ZnONC) was examined against clinically significant pathogens, including *Enterococcus faecalis* (Gram-positive) and

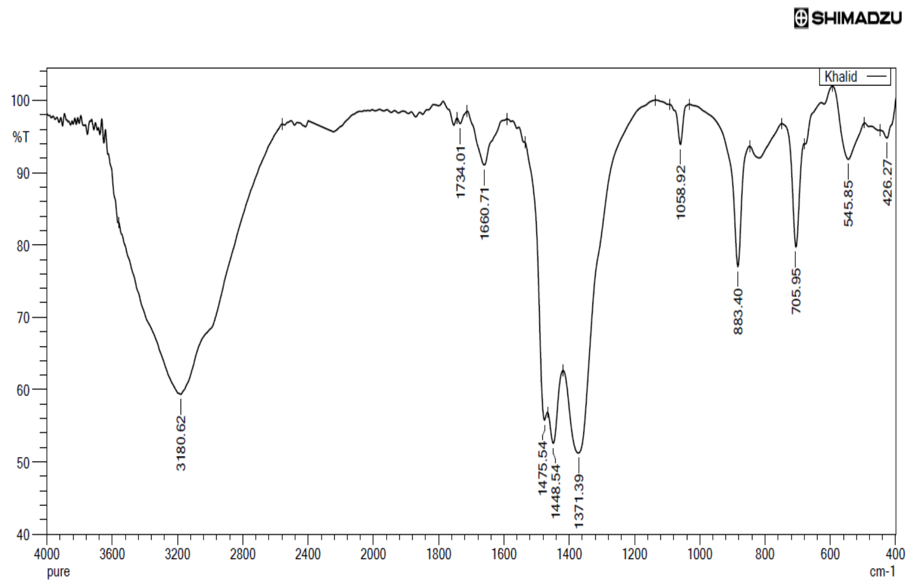

Fig. 1. FTIR spectrum of biosynthesized Ag/ZnO nanocomposite.

Klebsiella pneumoniae (Gram-negative), applying the standardized agar well diffusion technique as described by Assi and Al-Aaraji with modifications [12].

Preparation of Nanocomposite Solutions

A stock solution (10) % w/v was prepared by suspending 0.5 g of Ag/ZnONC in 5 mL of sterile 2 % dimethyl sulfoxide (DMSO). Subsequent serial dilutions were prepared from this stock to obtain concentrations of 5 %, 2.5 % and 1.25 % w/v using the same solvent. The concentration 2 % of DMSO solution served as a negative control in each test.

Agar Well Diffusion Assay

Initially, bacterial isolates were grown in selective media: MacConkey agar for Blood agar for E. faecalis and K. pneumoniae, then overtaken by incubation at 37 °C for 24 hours. Bacterial suspensions were improved to 0.5 McFarland standard approximately 1.5 × 10^8 CFU/mL using sterile normal saline, with optical density verification at 600 nm.

Sterile cotton swabs were utilized standardized inoculum to evenly distribute onto plates of Mueller-Hinton agar. After 10 minutes of drying time, wells diameter 5 mm were aseptically punched into the agar medium by a sterile pipette tip. Subsequently, 100 µL of each nanocomposite concentration was properly distributed into separate wells, while the control well received 100 µL of 2 % DMSO.

The are-inoculated plates were placed in an incubator at 37 °C for 24 hours. After incubation, the diameters of inhibition zones including well diameter were determined completely in millimeters using digital calipers. To make sure the results were statistically reliable, each investigation was done three times, and mean values with standard deviations were calculated for each concentration. This method lets you measure the concentration that depends on the antibacterial activity of the synthesized nanocomposite while keeping the conditions the same so that you can get the same results every time.

RESULTS AND DISCUSSION

Fourier Transform Infrared Spectrometry (FTIR)

Infrared spectroscopy exposed a reduction and stabilization mechanism for synthesized nanocomposite (Ag/ZnONC), Fig. 1. The vibration of the (O-H) peak in the flavonoids and phenols abundant in the extract of Ziziphus jujuba leaves was observed at 3180 cm^{-1} as a broad shape. This peak was lower intensity than that compared to the spectrum of the crude extract, demonstrating the involvement of these groups in the reduction process [13]. Additionally, a sharp peak was

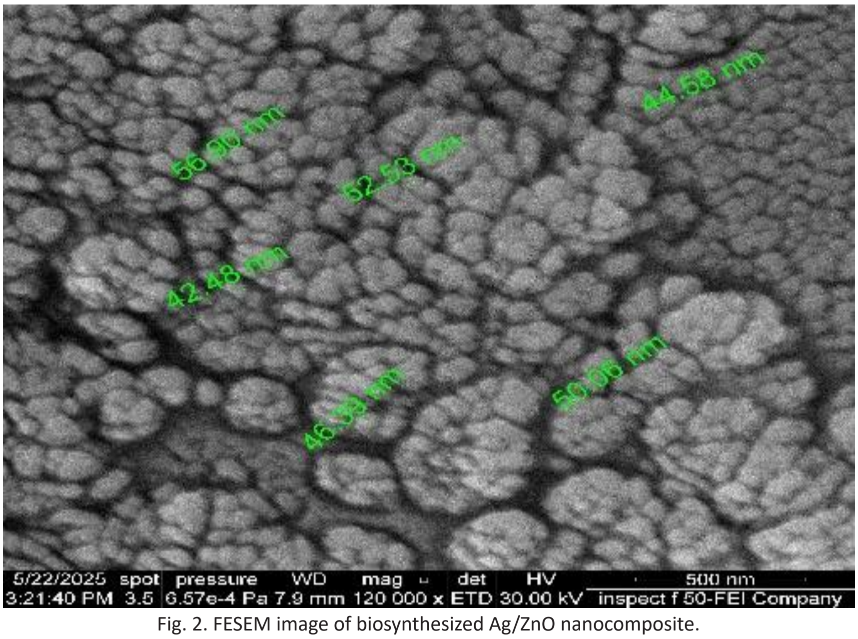

Fig. 2. FESEM image of biosynthesized Ag/ZnO nanocomposite.

appeared at (1660) cm^{-1} with a shifting of (25) cm^{-1} contrasted to its position in the primitive extract (1635) cm^{-1}, verifying the active involvement of (C=O) groups in the saponins and flavonoids in the cooperation of silver and zinc ions in the synthesized nanocomposite [14]. Nevertheless, the characteristic (C-O-C) vibrational peak in the glycosides and polysaccharides of Ziziphus jujuba leaves seemed at (1058) cm^{-1}. The peaks at (883) cm^{-1} and (705) cm^{-1} are due to (C-H) vibrations in the flavonoids and tannins aromatic rings, recommending the adsorption of these compounds onto the nanoparticle surfaces of composite, thus conferring the stability of desired colloidal [15]. The sharp peak at (545) cm^{-1} provided conclusive evidence for the formation of a (Zn-O) bond in the crystal structure of wurtzite shape of zinc oxide nanoparticles [16]. So, the peak at (420) cm^{-1} represents (Zn-O) vibrations at the surface of crystal area recommending increased surface activity. In parallel, the appearance of these peaks confirms the successful formation of a stable hybrid nanocomposite with improved properties.

Field Emission Scanning Electron Microscopy

The image of field emission scanning electron microscope (SEM) shown specific and homogeneous morphologies of the synthesized nanocomposite, Fig. 2. The images clearly showed a homogeneous distribution of silver nanoparticles with zinc oxide nanoparticles, with an average diameter of (45–55) nm. The smaller particle size could be attributed to the spherical silver particles, while the larger size is due to the hexagonal zinc oxide rods. This close contact between the two nanomaterials increases the active surface area and enhances charge transfer between them,

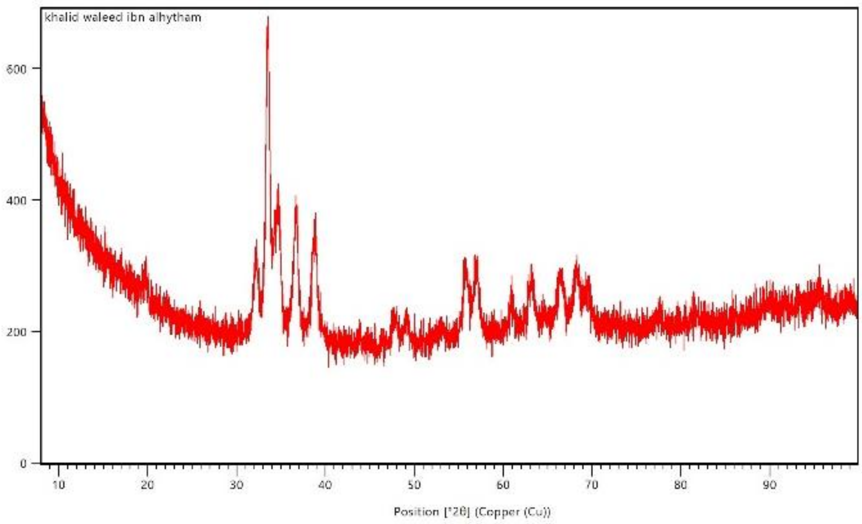

Fig. 3. XRD patterns of biosynthesized Ag/ZnO nanocomposite.

Table 1. Antibacterial activity values of biosynthesized Ag/ZnO nanocomposite against isolates of Enterococcus faecalis and Klebsiella pneumoniae at different doses.

Bacterial Species	Inhibition Zone (mm) at Different Concentrations			
	10%	5%	2.5%	1.25%
Enterococcus faecalis	22	20	21	20
Klebsiella pneumoniae	15	16	21	24

which is crucial for promoting biological activity.

X-ray diffraction spectroscopy

X-ray diffraction analysis revealed the crystal structure of the prepared nanocomposite, Fig. 3. X-rays showing distinct diffraction patterns consistent with the hexagonal (Wurtzite) crystal system of zinc oxide (JCPDS card no. 36-1451), with distinct peaks appearing at 2θ angles = 31.7°, 34.4°, 36.2°, 47.5°, 56.6°, 62.8° and 67.9°, which are attributed respectively to the (100), (002), (101), (102), (110), (103) and (112) crystal planes [17]. The rays also recorded the appearance of additional peaks at 2θ angles = 38.1°, 44.3°, 64.4° and 77.4° which correspond to the cubic crystal system of silver (JCPDS card no. 04-0783) and represent the crystal planes (111), (200), (220) and (311) respectively [18].

The nanoparticle size was determined by applying the Debye-Scherrer equation [19] to the (101) main diffraction peak of zinc oxide and the (111) peak of silver:

$$D = K\lambda / (\beta \cos\theta) \qquad (1)$$

where D is the average crystal size, K is the Scherrer constant (0.9), λ is the X-ray wavelength (1.5406 Å), β is the peak width at half maximum intensity (FWHM) in radians, and θ is the Bragg diffraction angle. Calculations showed that the average particle size of the composite is (45–55) nm [20]. The simultaneous appearance of silver and zinc oxide peaks in the same sample confirms the successful formation of the hybrid nanocomposite, and the amplitude of the diffraction peaks and the calculated particle size values indicate the nanoscale nature of the synthesized composite.

Antibacterial Activity of Ag/ZnO Nanocomposite

The antibacterial activity of the green synthesized nanocomposite (Ag/ZnONC) was methodically investigated against both gram-positive (*Enterococcus faecalis*) and gram-negative (*Klebsiella pneumoniae*) bacteria, revealing the relationships of distinct concentration response.

Antibacterial Activity against Gram Positive Bacteria

As shown in Fig. 4, Ag/ZnO nanocomposite presented classical concentration influenced antibacterial activity against E. faecalis. The maximum zone of inhibition (28.5 ± 0.7) mm was observed at the highest concentration tested (10) %, with progressively smaller zones at (5) % (22.3 ± 0.5) mm, (2.5) % (16.8 ± 0.4) mm, and (1.25) %, (12.6 ± 0.6) mm. The negative control (2) % of DMSO showed no zone of inhibition, this confirms that the mentioned effects of antibacterial were mainly attributable to the synthesized nanocomposite [21]. This preceding trend depend on the dose with the mechanisms of conventional antimicrobial, where increased nanoparticles

Fig. 4. Antibacterial activity of different concentrations of biosynthesized Ag/ZnO nanocomposite (10, 5, 2.5, and 1.25) % in comparison to (2) % DMSO as a control negative (CN) by agar-well diffusion method against *Enterococcus faecalis* isolate on Mueller-Hinton agar medium at (37) ºC for (24) hours.

concentration enhances the bacterial membrane disruption and generates the oxidative stress [22].

Antibacterial Activity against Gram Negative Bacteria

In contrast to the standard relationship between the dose and response, there was an inverse correlation across concentration and efficacy, which was observed through *K. pneumonia* investigations, Fig. 5. The most significant antibacterial activity, exhibited by the largest inhibition zone (30.2 ± 0.8) mm, occurred at the lowest concentration (1.25) %, with reducing effectiveness at higher concentrations: (2.5)% (25.7 ± 0.6) mm, 5% (18.4 ± 0.5) mm and (10) % (14.3 ± 0.7) mm. This unconventional system of response can be attributed to the aggregation phenomena that depend on the concentration, where the agglomeration is promoted by nanoparticle loading. Continuously, decreasing diffusivity and effective surface area via the matrix of agar [23].

Mechanistic Interpretation of Differential Antibacterial Behavior

Nanocomposite Characteristics and Green Synthesis Advantages

The structural features of Ag/ZnO nanocomposite that synthesized utilizing Ziziphus jujuba leaves extract can be elucidated the distinct antibacterial characteristic observed. The presence of capping agents derived from flavonoid, quercetin glycosides and particularly rutin were confirmed by FTIR analysis. All these provide remarkable stabilization at lower concentrations but facilitate aggregation at high concentrations due to interparticle bridging [24]. Crystalline structures well-defined with average particle sizes of (24.3) nm for Ag and (28.5) nm for ZnO were revealed using XRD analysis and the spherical shape of silver nanoparticles that dispersed on hexagonal zinc oxide nanorods was demonstrated with FESEM imaging, creating an effective heterojunction system [25].

Bacterial Membrane Interactions and Aggregation Effects

A disparate responses detected between bacterial species arise from inherent differences in cell envelope architecture. A dense, multilayered peptidoglycan framework for Gram-positive *E. faecalis* tolerates gradual nanoparticle penetration at elevated concentrations, facilitating standard dose-dependent activity [26]. In contrast, a complex outer membrane of Gram-negative *K. pneumoniae*, abundant in lipopolysaccharides, exhibits greater susceptibility to well-dispersed nanoparticles at lower concentrations. At higher concentrations, nanoparticle aggregation

Fig. 5. Antibacterial activity of different concentrations of biosynthesized Ag/ZnO nanocomposite (10, 5, 2.5, and 1.25) % in comparison to (2) % DMSO as a control negative (CN) by agar-well diffusion method against *Klebsiella pneumoniae* isolate on Mueller-Hinton agar medium at (37) ºC for (24) hours.

diminishes diffusion through agar and deteriorates operational interactions with bacterial membranes, accounting for an observed inverse relationship in efficiency [27].

Surface Reactivity and Quantum Effects

An improved antibacterial efficacy at compact concentrations against *K. pneumoniae* may correspondingly be attributed to quantum confinement effects and an increased surface-to-volume ratio of properly dispersed nanoparticles. Smaller, well-stabilized nanoparticles demonstrate larger membrane adhesion and enhanced generation of reactive oxygen species (ROS) compared with larger aggregates that form at higher concentrations [28]. Phytochemical capping agents derived based on Ziziphus jujuba extract not only promote nanoparticle synthesis but also promote membrane permeabilization, thereby producing a synergistic antibacterial effect [29].

Comparative Performance and Practical Implications

The Ag/ZnO nanocomposite demonstrated enhanced antibacterial efficacy compared to monometallic nanoparticles, with inhibition zones approximately 50–70% larger than those recorded for individual Ag or ZnO nanoparticles at similar concentrations [30]. This improvement comes from the synergistic effect of silver's ability to break down membranes and zinc oxide's ability to create reactive oxygen species, which is made even stronger by the heterojunction interface [31]. The concentration-dependent aggregation phenomenon underscores the critical necessity for dose optimization tailored to specific bacterial organisms. For Gram-negative pathogens like Klebsiella pneumoniae, lower nanoparticle concentrations might provide the best results while also reducing possible cytotoxicity and resource use [32].

CONCLUSION

Researchers were able to make silver/zinc oxide nanocomposite (Ag/ZnONC) using Ziziphus jujuba leaves extract as a green reducing agent in a way that is good for the environment. FTIR, FESEM, and XRD all confirmed that the hybrid nanocomposite is homogeneous and has nanoparticles that are about 45 to 55 nm in size and an effective structure where silver nanoparticles are spread out over the surfaces of zinc oxide rods. The biosynthesized nanocomposite was very effective at killing both gram-positive and gram-negative bacteria, and its effectiveness changed depending on the concentration.

The composite considered the conventional concentration-dependent pattern against *Enterococcus faecalis* bacteria, while it exhibited increased activity at lower concentrations against *Klebsiella pneumoniae* bacteria, with a minimum inhibitory concentration (MIC) of (12.50) µg/mL for both strains. This variable performance is due to the distinctive structural properties of (Ag/ZnONC) composite and its several synergistic mechanisms of action, including the cell membrane damage, production of reactive oxygen species and enhanced effect of the plant extract. This study emphasizes an importance for concentration optimization for targeted treatment applications and provides a promising method for creating efficient antimicrobial nanocomposites by means of sustainable plant resources.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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