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Synthesis, Characterization, and Antibacterial Activity of Silver Nanoparticles with Expired Minocycline as Reducing Agent: Proposed as a Recycling Technique

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Abstract

In this research, expired minocycline, a broad-spectrum tetracycline antibiotic, was employed as both a reducing and stabilizing agent for the eco-friendly synthesis of silver nanoparticles. This sustainable method avoids the use of toxic chemical reducing agents, providing a greener alternative for nanoparticle production. The synthesized silver nanoparticles were analyzed using several characterization techniques, such as UV-Vis's spectroscopy, scanning electron microscopy, transmission electron microscopy, and Edex analysis, to assess their structural, morphological, and optical properties. When measuring their antibacterial activity, silver nanoparticles showed clear activity against several harmful bacteria. The findings underscore the effectiveness of minocycline-assisted synthesis in creating bioactive nanoparticles with promising applications in biomedicine.

Keywords: Antibacterial activity, biomedical applications, characterization, expired minocycline, green synthesis, pathogenic bacteria, silver nanoparticles, tetracycline antibiotic

INTRODUCTION

Nanoparticles

The real power of nanoparticles comes down to their incredibly small size – just 1 to 100 nanometers. Being this tiny gives them unique physical and chemical properties that larger particles simply don't have. Why? Mainly because their small size means they have a much larger surface area relative to their volume. This minute scale has opened up huge possibilities across all sorts of fields, from medicine and industry to agriculture. Take medicine, for example. Nanoparticles are now widely used for targeted drug delivery, sending medication straight to specific cells in the body. They also help with medical imaging and diagnostics. One big plus here is that these approaches can really cut down on treatment side effects [1–3]. In the energy sector, nanoparticles are making their way into more advanced solar panels and better batteries [4]. On the environmental side, they're helping to purify water and tackle pollution [5]. And in electronics, they are probably used most extensively – helping make devices smaller, smarter, and more efficient (3). But for all their potential, there's still some worry about how safe they are. Their possible toxicity means we need more research to make sure we're using them safely [6]. There are different ways to make nanoparticles – physical, chemical, or biological methods – but these days, "green" synthesis is getting more attention as the eco-friendlier option [7]. To really understand what we're working with, scientists use various analysis techniques, with electron microscopy and spectroscopy being two of the most important for figuring out these particles' properties [8].

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Despite their potential, regulatory frameworks for nanoparticle use remain underdeveloped, highlighting the need for standardized guidelines [9]. Overall, nanoparticles represent a transformative technology with immense potential, provided their risks are adequately managed [10].

Silver Nanoparticles

Silver Nanoparticles (AgNPs) are highly versatile nanomaterials that can be synthesized using various techniques, each offering distinct advantages based on particle size, shape, and application suitability. The most common approach is chemical reduction, where silver ions (Ag^+) are reduced using agents, like sodium borohydride or citrate, often stabilized by surfactants or polymers [11]. Green synthesis uses plant extracts or microorganisms to create nanoparticles. These biological materials serve as reducing agents, transforming regular elements into their nano-sized forms. Essentially, they remove electrons from the elements, reducing them to a zero-valent state. A common way this happens is through plant extracts rich in phenolic compounds, which can effectively pull electrons away. Silver is one of the most well-known elements processed this way.

Physical methods, such as laser ablation and evaporation-condensation, yield high-purity AgNPs free from chemical residues [12], while electrochemical synthesis offers precise control over particle size by reducing silver ions at an electrode surface [13]. Microwave-assisted synthesis ensures rapid and uniform heating, accelerating nanoparticle nucleation and growth [14]. Similarly, sol-gel techniques involve the transition of a colloidal sol into a gel, resulting in AgNPs with a high surface area [15].

Biological synthesis using fungi, bacteria, and algae relies on natural biomolecules for AgNP formation, with significant potential in biomedical applications [16]. Photochemical methods utilize light to drive silver ion reduction in the presence of photosensitizers, ensuring controlled particle formation [17]. Sonochemical synthesis employs ultrasound to create localized high temperatures and pressures, facilitating the reduction process [18]. The polyol method, where silver salts are reduced in a polyol solvent, like ethylene glycol, produces well-dispersed nanoparticles [19].

Advanced strategies, such as microemulsion techniques, enable precise control over AgNP size and morphology [20]. Shape-controlled synthesis and photochemical routes further enhance the tunability of nanoparticles for specialized applications [21, 22]. Given their unique properties, AgNPs find extensive use in antimicrobial, medical, and therapeutic fields [23, 24]. The wide range of synthesis methods underscores the adaptability of AgNPs for diverse scientific and industrial applications.

Expired Drugs as Reducing Agents

The utilization of pharmaceutical compounds as reducing agents for nanomaterial synthesis has attracted considerable interest due to their dual role in facilitating nanoparticle formation while also imparting biocompatibility and therapeutic functionality. Various drugs, including doxorubicin, curcumin, and ascorbic acid, have been employed for metal ion reduction, leveraging their intrinsic redox properties. For instance, doxorubicin, a chemotherapeutic agent, has been used in the synthesis of gold nanoparticles, resulting in a hybrid system with both therapeutic and diagnostic potential [25]. Similarly, curcumin, a natural polyphenol, has been utilized to produce curcumin-capped silver nanoparticles, enhancing their antimicrobial and anticancer efficacy [26]. Ascorbic acid, a well-known antioxidant, has demonstrated its ability to synthesize various metal nanoparticles, including silver and gold, by offering mild reduction and size control [27].

This drug-mediated approach simplifies nanoparticle synthesis by eliminating the need for additional stabilizers, thereby reducing potential toxicity [24]. Moreover, drug-functionalized nanoparticles exhibit enhanced biocompatibility and targeted delivery, making them highly suitable for biomedical applications such as cancer therapy, drug delivery, and imaging [28]. The ability to integrate multiple therapeutic functionalities into a single nanomaterial further amplifies its efficacy [29].

Additionally, biological methods utilizing plant extracts and microorganisms offer eco-friendly and biocompatible alternatives for nanoparticle synthesis [30]. However, careful optimization of drug selection and synthesis conditions is crucial to ensure controlled nanoparticle formation and stability [31]. Overall, drug-assisted synthesis presents a promising green approach for the development of multifunctional nanomaterials with significant potential in nanomedicine.

While several studies [32] have reported the synthesis of minocycline-assisted silver nanoparticles, the scope and objectives of the present work differ substantially from previously published reports. For instance, a recent study investigated minocycline-modified silver nanoparticles (mino/AgNPs) for their antioxidant and antidiabetic potential in an in vivo alloxan-induced diabetic mouse model**,** emphasizing biomedical and therapeutic applications. In that study, minocycline was used as a reducing and stabilizing agent, and the nanoparticles were extensively evaluated using UV-Vis spectroscopy, FT-IR, XRD, and TEM, followed by antioxidant assays and biological evaluations related to glucose regulation, lipid profile, and histopathological recovery of vital organs.

In contrast, the present study focuses on the eco-friendly valorization of expired minocycline as a dual reducing and stabilizing agent for silver nanoparticle synthesis, highlighting a sustainable waste-to-resource approach. Unlike the previous work, which employed pharmacologically active minocycline and targeted systemic antidiabetic effects, this study emphasizes green synthesis, physicochemical characterization, and antibacterial activity of the synthesized AgNPs against pathogenic bacteria. Moreover, additional characterization techniques, such as SEM and EDX analysis, were employed to confirm morphology and elemental composition, providing complementary structural insights.

Importantly, the antibacterial performance observed in this study demonstrates that expired minocycline-derived AgNPs retain strong bioactivity, supporting their potential application in antimicrobial and environmental contexts rather than systemic therapeutic use. Therefore, while both studies utilize minocycline-mediated silver nanoparticle synthesis, they differ markedly in material source (expired vs. active drug), experimental design, biological targets, and intended applications**,** underscoring the novelty and relevance of the present work in sustainable nanomaterial development.

ECO-FRIENDLY APPROACHES FOR NANOMATERIAL SYNTHESIS

Sustainable methods for nanomaterial synthesis have garnered significant interest due to their minimal environmental impact, cost-effectiveness, and alignment with green chemistry principles. Unlike chemical or physical methods – which often involve environmentally risky chemicals or energy-intensive processes – green technology takes a different path. It harnesses extracts from a wide range of plants to produce nanoparticles, positioning it as the most eco-friendly approach available. Beyond plants, it can also make use of microorganisms and biopolymers to achieve the same goal.

Plant extracts, rich in bioactive compounds, like polyphenols, flavonoids, and terpenoids, have been extensively utilized for synthesizing metal nanoparticles, including silver, gold, and zinc oxide, providing an environmentally friendly alternative to conventional methods [33]. Similarly, microorganisms, such as bacteria, fungi, and algae, employ enzymatic processes to facilitate nanoparticle formation, ensuring biocompatibility and controlled morphology [34]. Additionally, biopolymers, like chitosan and cellulose, enhance nanoparticle stability and regulate growth, further improving synthesis efficiency [27].

Advanced techniques, such as microwave- and ultrasound-assisted synthesis, contribute to the eco-friendliness of these methods by reducing reaction time and energy consumption [14]. These green approaches not only produce biocompatible nanomaterials suitable for biomedical applications but also reduce environmental pollution and waste generation [24]. Their scalability and simplicity make them highly attractive for industrial applications, particularly in medicine, energy, and environmental remediation [35].

Despite their advantages, challenges, such as achieving precise control over particle size, shape, and reproducibility, remain requiring further refinement and optimization [31]. Nonetheless, green synthesis methods offer a sustainable and efficient alternative for producing functional nanomaterials, supporting the shift toward environmentally responsible technologies.

CHARACTERIZATION OF NANOMATERIALS

Characterizing nanomaterials is essential for understanding their structural, chemical, and functional properties, which directly impact their performance across various applications. Multiple analytical techniques are employed to assess key attributes such as size, morphology, crystallinity, surface chemistry, and optical behavior.

The techniques used to analyze nanoparticles vary depending on their intended application. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are used to determine nanoparticle size and also define their shape and fine surface structure [8]. Meanwhile, X-ray diffraction (XRD) determines the stability and purity of the material by identifying the particles' crystalline structure [36]. On the other hand, particle size distribution can be measured using dynamic light scattering (DLS). As for evaluating surface charge, this is done through zeta potential analysis, which helps in analyzing the behavior and assessing the stability of the colloidal material [37]. Fourier-transform infrared spectroscopy (FTIR) and Raman spectroscopy help identify functional groups and molecular interactions on nanoparticle surfaces, offering valuable chemical characterization [38]. UV-Vis spectroscopy is widely used to analyze optical properties, including surface plasmon resonance in metallic nanoparticles like gold and silver [39]. X-ray photoelectron spectroscopy (XPS) provides elemental composition and chemical state information, enabling precise surface analysis [40].

Atomic force microscopy (AFM) allows for topographical mapping and mechanical property evaluation at the nanoscale [41]. Moreover, Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) are employed to assess thermal stability and phase transitions [42].

These diverse characterization techniques offer a comprehensive understanding of nanomaterials, facilitating their optimization for applications in medicine, electronics, energy, and environmental science. Their integration ensures precise material design, enhancing functionality in emerging technologies.

Medical Applications of Nanosilver

Nanosilver has gained significant attention in medicine due to its exceptional physicochemical properties and strong antimicrobial activity Studies have proven that silver nanoparticles have diverse medical applications due to their high efficacy against a wide range of bacteria and viruses, and they are also used in infection control and the protection of medical equipment [23]. Silver nanoparticle-based wound dressings accelerate healing by reducing bacterial load, minimizing inflammation, and promoting tissue regeneration [43].

In dentistry, nanosilver is incorporated into dental composites and implants to prevent biofilm formation and secondary infections, enhancing oral health outcomes [44]. Its integration into drug delivery systems improves the therapeutic potential of antibiotics and anticancer agents by enabling targeted delivery and controlled release [24]. Additionally, nanosilver has shown promising anticancer properties, inducing apoptosis in cancer cells through reactive oxygen species (ROS) generation and mitochondrial dysfunction [45].

To mitigate hospital-acquired infections, nanosilver is applied in the coatings of catheters and surgical instruments, reducing microbial colonization and enhancing sterility [46]. Functionalized silver nanoparticles, particularly those modified with vitamin C and its derivatives, exhibit enhanced stability, biocompatibility, and antibacterial efficacy against pathogens, like *Escherichia coli* and *Staphylococcus aureus*, making them valuable for antimicrobial therapies and medical device coatings.

Despite its numerous benefits, concerns regarding nanosilver's potential toxicity and long-term health effects necessitate careful evaluation and regulatory oversight [47]. Ongoing research focuses on optimizing nanosilver formulations to enhance therapeutic benefits while minimizing risks, ensuring safe and effective applications in nanomedicine. Those functionalized with vitamin C and its derivatives show effective antibacterial properties against *Escherichia coli* and *Staphylococcus aureus*.

These modified nanoparticles enhance stability, biocompatibility, and antimicrobial efficacy, making them promising candidates for medical applications such as wound healing, coatings for medical devices, and antimicrobial therapies. Their novel functionalization improves their potential use in nanomedicine by reducing toxicity while maintaining high antibacterial activity [48].

AIMS

This study is based on selecting an environmentally friendly method for synthesizing silver nanoparticles by using expired minocycline as a reducing agent. Subsequently, the products are analyzed using various techniques to determine their shape, size, and elemental composition. Additionally, the effectiveness of the silver nanoparticles as antibacterial agents against a number of bacterial species is studied.

Objectives

1. Using expired minocycline as a reducing agent to prepare silver nanoparticles as an environmentally friendly green method.
2. Several techniques were employed to confirm the formation of the desired nanoparticles. Specifically, Ultraviolet-Visible (UV-Vis) spectroscopy, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Energy Dispersive X-ray (EDX) analysis were used.
3. To prepare a stable stock solution of minocycline-mediated AgNPs for further biological applications.
4. To evaluate and compare the antibacterial efficacy of:
   - Pure silver nitrate,
   - Expired minocycline alone, and
   - Minocycline-mediated AgNPs against multidrug-resistant clinical bacterial isolates.
5. To conduct a comparison between the effect of the synthesized silver (nanoparticles) and the selected antibiotics on bacteria. The purpose of this approach is to study the potential of selecting silver nanoparticles as antimicrobial agents. To assess the potential reuse of expired pharmaceuticals in nanomaterial synthesis as part of sustainable and environmentally friendly pharmaceutical waste management.

RESEARCH METHODOLOGY

Preparation of Silver Nitrate Nanoparticles

Silver nitrate ($AgNO_3$) (purchased from Sigma-Aldrich solution is prepared freshly in the lab. The selected drug (Minocycline Expired date July 2024 with reducing properties was dissolved in distilled water with continuous stirring until the drug was completely dissolved. The drug solution is added gradually dropwise using a pipette or dropper to silver nitrate until the gradual color changes from colorless to yellow or brown, indicating the reduction of silver ions to silver nanoparticles. To ensure the reaction goes to completion, stir the mixture at room temperature for 30-60 minutes. After the drug solution is added, continue stirring for an additional 30 minutes at room temperature to ensure thorough reduction and stabilization of the nanoparticles. Once the reaction is complete (indicated by color change), the silver nanoparticles will be suspended in the solution. This solution can be stored for future use or used immediately for further applications [49].

Characterization of Nanoparticles

Uv-vis Spectroscopy

Characterization was performed using different techniques including UV-Vis spectrophotometer Scan – LAMBDA 365 Perkins Elmers wavelength scan range to 300–700 nm. Identifying the wavelength of maximum absorbance (λ max), which indicates the SPR peak.

Distilled water was used as solvent after the formation of the final solution, the mixture was filtered using a Büchner funnel to separate the solid precipitate, which consists of silver nanoparticles. The precipitate is collected and weighed, yielding 1.1 g of silver nanoparticles. To prepare a stock solution, dissolve the 1.1 g of silver nanoparticle solid in 100 mL of dimethyl sulfoxide H_2O. To ensure homogeneity, gently mix the solution using a vortex mixer or an ultrasonic bath. This step ensures that the nanoparticles are uniformly dispersed throughout the solution, making the stock solution consistent for subsequent experimental use.

SEM-TEM and Edex Analysis

The SEM, TEM, and EDX Analysis were performed at Alexandria University.

A scanning electron microscope (SEM) (JEOL JSM, Japan) was used to examine all prepared nanoparticles, it was primarily used to study the surface topography and texture of solid materials. Image-J software (Laboratory for Optical and Computational Instrumentation, University of Wisconsin) was used to measure the diameter of each nanoparticle sample, and an average value was calculated from about 30 measurements that were randomly selected for each sample.

EDX (FEI Quanta FEG 450) (Thermo Fisher Scientific, Waltham, MA, USA) was utilized to gather qualitative information on constituents in the product of Ag and Au nanoparticles.

To investigate the internal structure of materials, such as crystal structure, defects, grain boundaries, and biological cells TEM, was performed on the acceleration voltage was 20.00 Kv, magnification x 600, process time T2, live time 30.00 s, real-time 30.04 s, and count rate was 286.00 CPS The elemental composition of the sample was determined using Energy Dispersive X-ray Spectroscopy (EDX).

Experimental Procedure for Evaluating the Antibacterial Activity of Silver Nanoparticles

Materials Required

96-well microtiter plate, Mueller Hinton broth, Antibiotic solution (0.1 mM), silver nitrate (0.1 mM), and drug nanoparticles (62.5 µg/ml) in 10% DMSO, Micropipettes with sterile tips, Bacterial inoculum, sterile normal saline 0.9% and 10% DMSO.

Tested Microorganisms

Testing their sensitivity against bacteria to silver nitrate, Minocin Drug, and Minocin silver nanoparticles (MagNPs), comparing them, and seeing the effect of the Minocin in the form of silver nanoparticles and choosing bacterial (gram positive and gram negative). Hundred clinical isolates belonged to seven different genera: *(1) Staphylococcus aureus, (2) Escherichia coli, (3) Pseudomonas aeruginosa, (4) Staphylococcus epidermidis, (5) Acinetobacter baumanii, (6) Streptococcus pneumoniae and (7) Klebsiella pneumonia.* These were isolated from patients attending different departments Al-jalaa hospital for wounds and accidents in Benghazi, east of Libya, July 2024.

Evaluation of the Antibacterial Activity of Silver Nitrate, Minocycline, and Silver Nanoparticles

Primary antibacterial screening of silver nitrate, minocycline, and AgNP's was performed by the disc diffusion assay. All tested standard clinical bacterial strains were separately swabbed with one hundred microliters of their respective suspensions. This process was carried out on the surface of Muller Hinton Agar (MHA). The inoculum was then left to dry for 5 minutes. The particles to be tested were individually loaded with 20 microliters onto 6 mm diameter paper discs. Their concentrations were 0.1 mM $AgNO_3$, MagNP's (5 mM), and (1.62 mM) Minocycline. They were placed on the surface of Muller Hinton Agar. The solvent DMSO was used as a control for the solvent system. The bacterial culture plates were incubated for 72 hours at a temperature of 37°C. The inhibition zones were measured in millimeters. The experiment was also repeated twice [50]. All determinations were conducted in duplicates, and all inhibition zone results were calculated as mean ± standard deviation (SD).

RESULTS

Note the peak absorbance value. A sharp, distinct peak indicates well-dispersed nanoparticles, while a broader peak may suggest aggregation or polydispersity [51]. The formation of silver nanoparticles was first observed through visual inspection. Upon adding the minocycline solution to silver nitrate, the solution underwent a distinct color change. Initially, the solution was colorless, but within 10 minutes of the reaction, it changed to a pale yellow, which progressively intensified to dark brown. This color transformation indicates the formation of silver nanoparticles, attributed to surface plasmon resonance (Figure1), which causes the brownish tint characteristic of colloidal silver solutions [52]. Stability throughout the inspection period, no significant precipitation was observed and remained free from aggregation.

Figure 1. $AgNO_3$ solution before and after adding drug solution.

UV-Spectrophotometer Results

The following table presents the UV-Vis spectrophotometer results, highlighting the absorbance.

The UV-Vis spectrophotometric analysis was performed to monitor the absorbance of the sample at various wavelengths. This technique helps in determining the optical properties and confirming the formation of Minocyclin silver nanoparticles The resulting curve provides insight into the interaction of light with the sample, particularly at specific wavelengths that correspond to characteristic absorption peaks. By observing the wavelength at which maximum absorbance occurs, we can infer the successful synthesis and stability of the minocycline silver nanoparticles in solution.

The synthesized nanoparticles were identified through UV-Vis spectrophotometry, revealing a distinct surface plasmon resonance (SPR) peak at 400–450 nm, confirming successful reduction of silver ions and nanoparticle formation based on peak intensity, sharpness, size distribution, and concentration in the solution (Figure 2).

SEM Analysis Results

Scanning electron microscopy (SEM) analysis was employed to assess the morphology and surface structure of the produced minocycline-silver nanoparticles.

The SEM images (Figure 3) indicate the successful synthesis of primarily spherical nanoparticles, with some irregular shapes due to agglomeration. These characteristics suggest that the synthesis process was successful.

TEM Analysis Results

Transmission Electron Microscopy (TEM) is an essential tool for examining the structural and morphological characteristics of nanoparticles at a nanometer scale. It provides high-resolution images that allow for the assessment of particle size, shape, and distribution, as well as insights into their crystallinity and potential agglomeration.

The results of Transmission Electron Microscopy (TEM) (Figure 4) showed that the silver nanoparticles were within the desired size range. It was revealed that their size ranged between 27.83 and 62.45 nanometers. These figures confirm a wide-size distribution, which enables them to perform better in various applications.

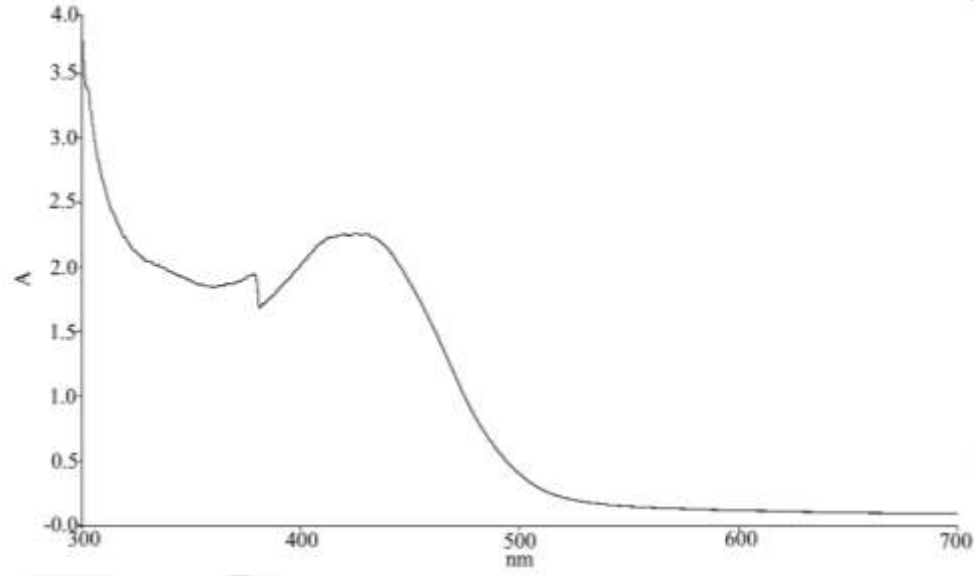

Figure 2. UV-visible spectrum of minocycline-modified silver nanoparticles.

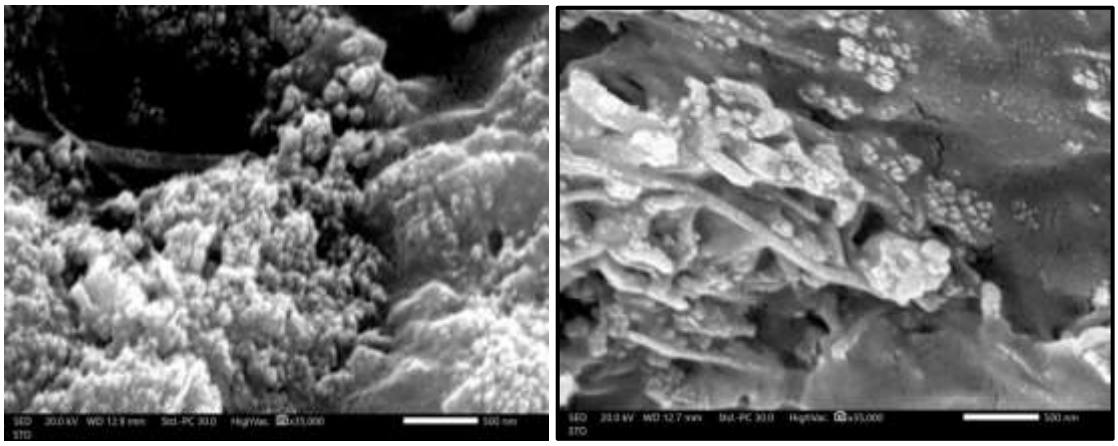

Figure 3. SEM analysis of silver nanoparticles.

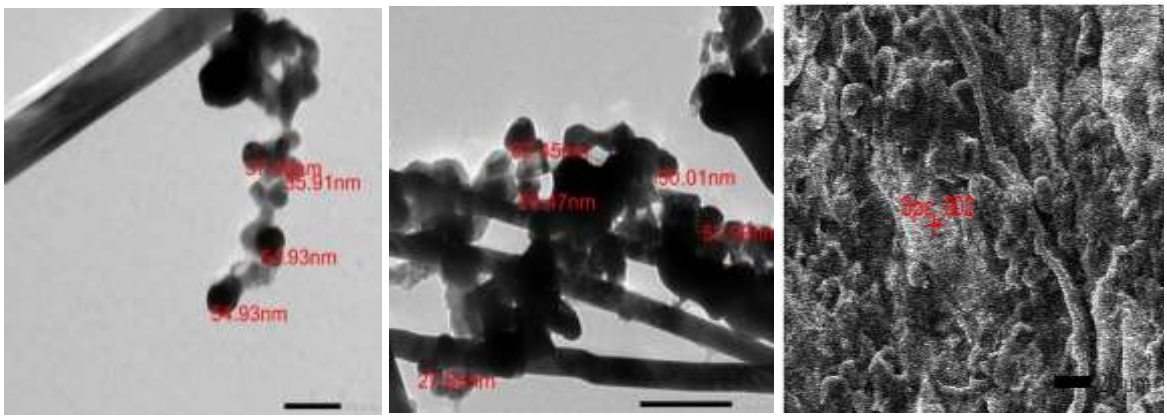

Figure 4. TEM analysis of silver nanoparticles.

EDX Analysis Results

Energy Dispersive X-ray (EDX) analysis is a key technique used to determine the elemental composition of a sample. In the context of nanoparticle synthesis, EDX.

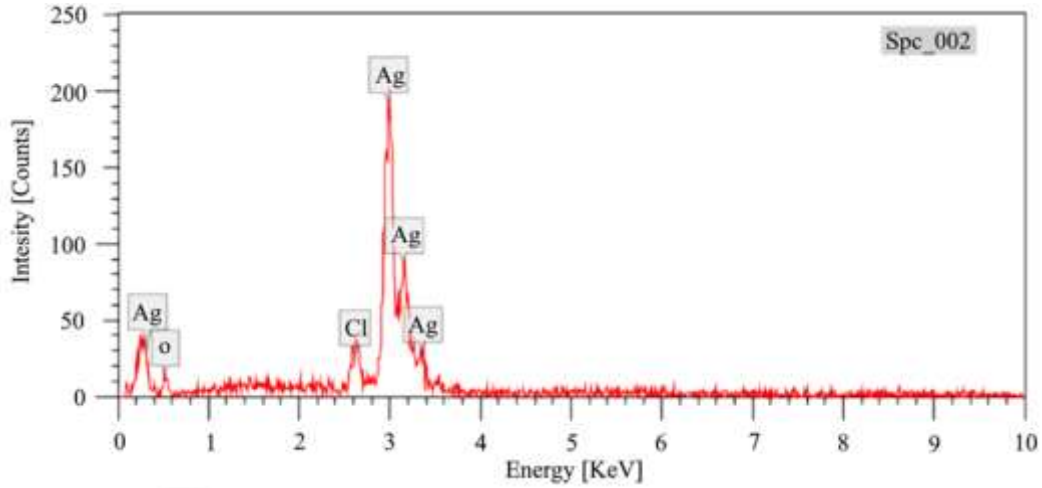

Figure 5. EDX analysis of silver nanoparticles.

This technique helps us determine the distribution of elements within the sample and confirms the success of the reaction and the synthesis process .By analyzing the X-rays emitted from the sample after being exposed to an electron beam, we can identify the elements present and assess their relative concentrations (Figure 5).

The Energy Dispersive X-ray (EDX) analysis was conducted (Table 1) to confirm the presence of silver.

Table 1. Displayed X-ray (EDX0 results).

Display name	**Standard data**	**Quantification method**	**Result type**
Spc_002	Standardless	ZAF	Metal
Element	*Line*	*Mass%*	*Atom%*
O	K	11.75 ± 1.25	45.20 ± 4.82
Cl	K	3.80 ± 0.38	6.60 ± 0.66
Ag	L	84.45 ± 2.27	48.20 ± 1.29
Total		100.00	100.00
Spc_002			Fitting ratio 0.3277

Nanoparticles in the synthesized sample. The EDX spectrum reveals prominent peaks corresponding to silver (Ag), with the most significant peak appearing at 3 keV, characteristic of the L-line of silver.

Quantitative analysis further supports this, with silver making up 84.45% of the mass and 48.20% of the atomic content. These values strongly indicate that silver is the dominant material present. Minor peaks for oxygen (O) and chlorine (Cl) were also detected, likely due to surface oxidation or residuals from the chemical reagents used in the synthesis process.

Results of Antibacterial Evaluation of Silver Nanoparticles

A comparison of the results, as shown in (Figures 7, 8 and Tables 2, 3), indicates that the silver nanoparticles have the strongest effect on the studied bacteria, whether gram-positive or gram-negative, compared to silver nitrate and minocycline alone. Furthermore, the results of the silver nanoparticles were better than those of the antibiotics used against these bacteria. Together, these mechanisms suggest that the synthesized AgNPs hold great potential as an effective.

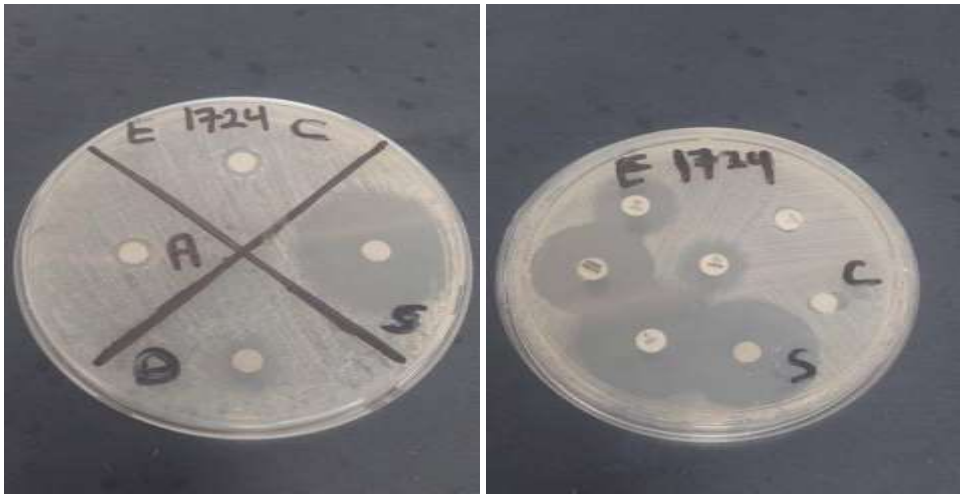

Figure 6. Zone of inhibition discs.

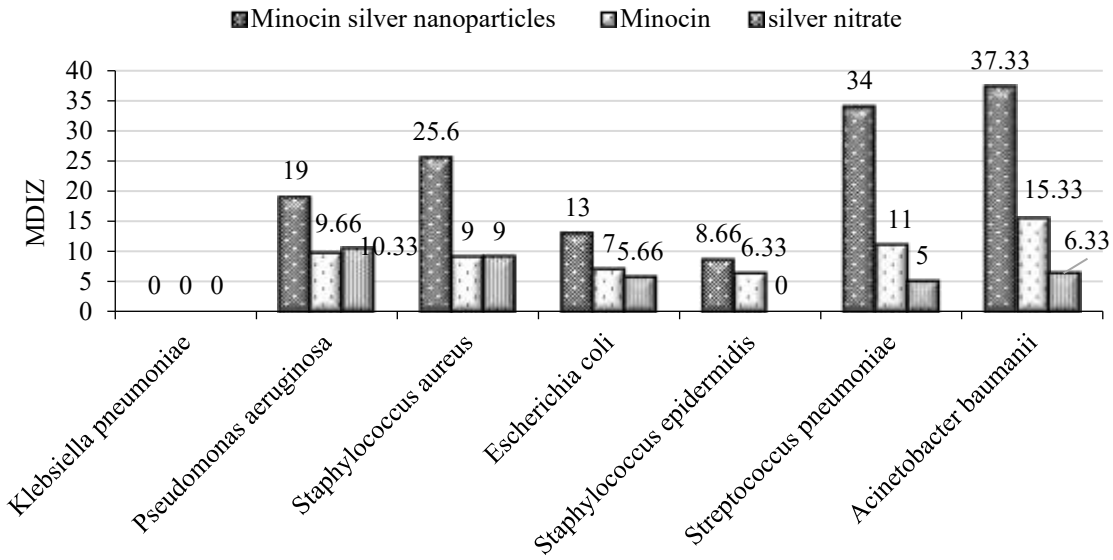

Figure 7. Means of inhibition zones diameter (MDIZ) in (mm) of silver nitrate, Minocin, and Minocinm silver nanoparticles (MSNPs) against clinical isolates.

The biological performance of silver nanoparticles is strongly governed by their physicochemical characteristics, including particle size, morphology, surface chemistry, and elemental composition. In the present study, comprehensive structural characterization using UV-Vis spectroscopy, SEM, TEM, and EDX analysis provide critical insights into the observed antibacterial activity of the synthesized AgNPs.

The appearance of a characteristic surface plasmon resonance band in the UV-Vis spectrum confirms the formation of metallic silver nanoparticles and indicates good colloidal stability, which is essential for sustained interaction with bacterial cell membranes. SEM and TEM analyses revealed predominantly nanoscale particles with relatively uniform morphology, a feature that is known to enhance antibacterial efficacy by increasing the surface area available for contact with microbial cells. Smaller and well-dispersed nanoparticles can more efficiently adhere to bacterial cell walls, penetrate membrane structures, and promote the generation of reactive oxygen species, ultimately leading to cell membrane disruption and bacterial death. Furthermore, EDX analysis confirmed the presence of elemental silver with minimal impurities, supporting the conclusion that the antibacterial activity arises primarily from silver-mediated mechanisms rather than residual organic contaminants.

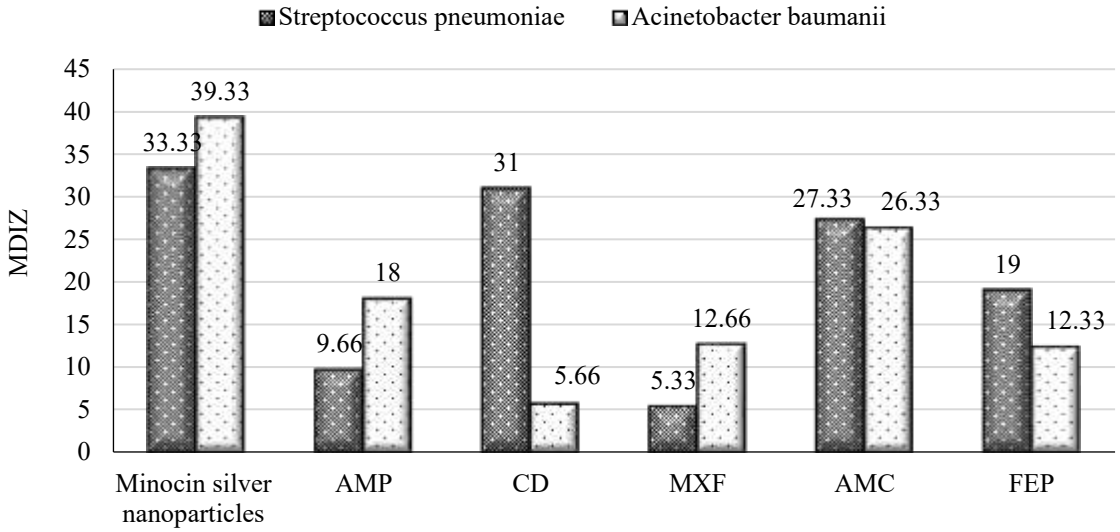

Figure 8. Susceptibility of tested clinical bacteria *Streptococcus pneumoniae* and *Acinetobacter baumanii* to Minocin silver nanoparticles and antibiotics AMP, CD, MFX, AMC, FEP reference discs.

Table 2. Means of inhibition zones diameter (MDIZ) in (mm) of silver nitrate, minocin, and minocin silver nanoparticles (MSNPs) against clinical isolates.

S. N.	Clinical isolates	Source of sample	MDIZ in (mm)		
			Silver nitrate Mean ± SD	Minocin Mean ± SD	MAgNPs Mean ± SD
1	*Klebsiella pneumoniae*	Urine	0 ± 0	0 ± 0	0± 0
2	*Pseudomonas aeruginosa*	Urine	10.33 ± 0.471	9.66 ± 0.471	19 ± 0.816
3	*Staphylococcus aureus*	Urine	9 ± 0.816	9 ± 0.816	25.6 ± 0.942
4	*Escherichia coli*	Urine	5.66 ± 0.471	7 ± 1.163	13 ± 0.816
5	*Staphylococcus epidermidis*	Urine	0 ± 0	6.33 ± 0.471	8.66 ± 0.471
6	*Streptococcus pneumoniae*	Urine	5 ± 0.816	11 ± 0.816	34 ± 0.816
7	*Acinetobacter baumanii*	Urine	6.33 ± 0.471	15.33 ± 0.471	37.33 ± 1.699

Note: MDIZ = Means of diameters of inhibition zo.
MDIZ (9–12) = partial active MDIZ (13–18) = Active MDIZ (>18 mm) = very active.

Table 3. Comparison of means inhibition zones (mm) of Minocin silver nanoparticles (MagNPs) and antibiotics references against clinical isolates

(MAgNPs) and antibiotics references	This means inhibition zones of *Streptococcus pneumoniae*	Means inhibition zones of *Acinetobacter baumanii*
(MAgNPs)	33.33 ± 1.24	39.33 ± 0.471
AMP	9.66 ± 0.471	18 ± 0.816
CD	31 ± 0.471	5.66 ± 0.471
MXF	5.33 ± 0.471	12.66 ± 0.94
AMC	27.33 ± 1.69	26.33 ± 1.52
FEP	19± 0.816	12.33 ± 0.471

Note: Minocin silver nanoparticles = (MagNPs), AMP = Ampicillin, CD = Clindamycin, MFX = Moxifloxacin, AMC = Augmentin, FEP = cefepime.

Importantly, the present work demonstrates that silver nanoparticles synthesized using expired minocycline retain pronounced antibacterial activity despite the absence of in vivo therapeutic testing. These finding highlights that the structural integrity and nanoscale features of the AgNPs, rather than the pharmacological activity of minocycline itself, play a dominant role in antimicrobial performance

(Figure 6). The use of expired minocycline further strengthens the sustainability aspect of the study, proving that drug waste can be repurposed to generate structurally well-defined and biologically effective nanomaterials.

These results suggest that structural optimization of minocycline-derived AgNPs is a key determinant of antibacterial performance, whereas therapeutic outcomes in vivo may depend on additional biological and pharmacokinetic factors.

CONCLUSION

This study employed expired minocycline – an antibiotic – as a reducing agent for synthesizing silver nanoparticles, using silver nitrate (0.01 M) as the silver ion precursor. The choice of minocycline was based on its oxidizable functional groups, which facilitate the reduction of silver ions. The combination of minocycline and silver nanoparticles exhibited potent antibacterial effects. Specifically, the silver nanoparticles act by damaging bacterial cell membranes and promoting oxidative stress, while minocycline inhibits bacterial protein synthesis. Together, these mechanisms suggest that the synthesized AgNPs hold great potential as an effective antibacterial agent, particularly in combating antibiotic-resistant strains of bacteria. The process provides a promising method for recycling expired antibiotics using green chemistry techniques.

Recommendations and Future Directions

Expanded Study on Drug-Induced Nanoparticle Synthesis by using different antibiotic drugs. It is recommended to systematically study how each antibiotic affects the size, shape, and yield of silver nanoparticles. Mechanistic Studies of Drug-Mediated Nanoparticle Formation. Investigating the role of drug-specific functional groups in nanoparticle stabilization could lead to the discovery of new synthesis methods using other pharmaceutical compounds. Additionally, time-resolved spectroscopic analysis (e.g., UV-Vis, FTIR) could provide insights into the dynamics of nanoparticle formation and the role of drugs in influencing particle size and stability.

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