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Eco-friendly Synthesis of Ag Nanoparticles with Detecting Antibacterial Activity on Gram-Positive Bacteria from UTI

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ABSTRACT

Urinary tract infection is a worldwide health issue caused by various Gram-positive and Gram-negative bacteria, and the misuse of antibiotics has contributed to increasing antimicrobial resistance among bacterial pathogens. In the present study, 150 urine specimens were collected between the beginning of December 2023 and the end of April 2024 from patients attending Baqubah Teaching Hospital and Al-Batoul Teaching Hospital in Diyala. The specimens were distributed among 60 males and 90 females. The isolates were identified by bacteriological and biochemical tests, which revealed two species belonging to Gram-positive bacteria, *Staphylococcus aureus* 50 (33.3%) and *Enterococcus faecalis* 10 (6.7%), whereas four species belonged to Gram-negative bacteria, *Escherichia coli* 30 (20%), *Acinetobacter baumannii* 22 (14.7%), *Klebsiella pneumoniae* 20 (13.3%) and *Pseudomonas aeruginosa* 18 (12%). The identification was then confirmed by the Vitek-2 system. Susceptibility tests were performed for all isolates against 14 antibiotics using the disk diffusion method, which indicated that more than half of the isolates were multidrug resistant (MDR). Silver nanoparticles (Ag NPs) were biosynthesized using silver nitrate (AgNO3) as a substrate with aqueous extract of Nigella sativa seeds. The formation of Ag NPs was evidenced by a color change from light yellow to dark brown. The synthesized nanoparticles were characterized using Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), and Transmission Electron Microscopy (TEM). AFM and SEM analyses indicated that nanoparticle sizes ranged from 25 to 60 nm, while XRD revealed an average crystallite size of 20 nm. The antibacterial activity of Ag NPs showed notable effects against bacterial isolates, with the highest inhibitory diameter observed at 200 mg/ml reaching 25 mm. The MIC of Nigella sativa ranged from 3.125 to 12.5 ug/ml, whereas that of Ag nanoparticles ranged from 1.5 to 12.5 ug/ml.

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1. INTRODUCTION

Globally, urinary tract infections are the most prevalent bacterial infections. Most often caused by uropathogenic E. coli (UPEC), infections can be mild, recurrent (rUTI), or complex (cUTI). Antibiotics are typically an easy way to treat uncomplicated UTIs, which happen to otherwise healthy people. Patients with structural or neurological disorders that affect their urinary tracts including immunosuppression, renal calculi, pregnancy, and catheters that are indwelling. UTI symptoms can be severe and incapacitating; patients frequently appear with increased urge to void, dysuria, chronic discomfort (stranguria), and urine incontinence [1]. Gram-positive bacteria, particularly *Enterococcus species*, group *B streptococci*, and *Staphylococcus* species, are responsible for 10% of UTI infections. *S. aureus* and coagulase-negative staphylococci (CoNS), previously considered uncommon causative agents of ascending urinary tract infections in outpatients, may hold greater significance in hospitalized, immunocompromised individuals.

The separating of *S. aureus* from urine may signal a more severe illness, such as endocarditis or bacteremia, when the bacteria disseminate hematogenous and ultimately infiltrate the kidneys. The research indicates that the prevalence of *S. aureus* isolation in UTIs ranges from 0.5% and 13% [2]. *Enterococcus faecalis* is the predominant species in bacteremia, endocarditis, central nervous system infections, and urinary tract infections [3]. The primary cause of increased resistance to antimicrobial drugs is the unregulated and abused use of antibacterial agents. The rising prevalence of drug-resistant diseases requires the urgent identification and isolation of novel bioactive components from medicinal plants using standardized modern analytical methods. Compounds obtained from medicinal plants could provide new, simple strategies against infectious agents [4].

A common traditional herbal medicinal plant, black seed has been used as a food additive and traditional medicine in many countries. The conventional application of black seed for wound healing expedites the recovery process, particularly for burned skin, due to its antioxidant properties. Thymoquinone in black seeds also inhibits cyclooxygenase and 5-lipoxygenase, which reduces inflammation by preventing membrane lipids from peroxiding into cells. Numerous studies suggest that thymoquinone and thymohydroquinone have inhibitory effects against bacteria [5]. Silver nanoparticles (Ag NPs) are extensively utilized across several domains due to their physical attributes, such as size, shape, morphology, and surface area, as well as their magnetic, electrical, and optical capabilities. Silver's medicinal and preservation qualities are mostly used to protect vessels against bacterial infections and to maintain the reusability of water and other liquids. Ag nanoparticles have strong antimicrobial qualities and are also excellent at decoding DNA. The synthesis of Ag NPs by Cryphonectria sp. has antibacterial efficacy against Salmonella typhi, E. coli, S. aureus, and Candida albicans [6].

2. METHOD

2.1 Collection of Samples

150 samples of urine were taken from patients of all ages and genders at Baquba Teaching Hospital and Al-Batoul Teaching Hospital in Diyala, between the beginning of December 2023 and the end of April 2024.

2.2 Culturing of Samples

A widely used procedure involves streaking 0.001 ml of urine across a culture plate filled with blood agar and MacConkey agar that supplies the nutrients needed for bacterial growth in a sterile loop. After covering, streaked plates are incubated for at least 18 h at 35°C. The existence and quantity of bacterial colonies are observed on plates [7].

2.3 Isolation and Identification of Bacteria

Bacteria were isolated and identified using morphological characteristics of the colonies, microscopic examination of bacterial cells, and biochemical tests. In order to verify identification, 10 bacterial isolates were chosen using the Vitek-2 method [8].

2.4 Collection of Plant Samples

Seeds of *Nigella sativa* were procured from a local market and identified by Prof. Dr. Khazal D. Wadi, from the College of Sciences, University of Diyala.

2.5 Preparation of Nigella sativa Seeds

The Nigella sativa seeds were transported to the laboratory, purified with distilled water, and dried at oven at 30°C for four days. They were then crushed with an electric grinder to produce a fine powder and stored in a plastic bag until required [9].

2.6 Preparation of Aqueous Extract of Nigella sativa Seeds

The aqueous extract was made by dissolving 50g of seed powder in 100 ml of hot water using Whatman No. 1 filter paper. Five filter sheets were employed to isolate the requisite filtrates from the solid residues after 24 h at room temperature and 150 rpm of agitation. Crude extracts were obtained by pre-concentrating all filtrates with a rotary evaporator operating at reduced pressure and a temperature range of 40–60°C. The extract was preserved at 4°C until utilized [10].

2.7 Antibiotic Susceptibility Test of Isolated Bacteria

An antibiotic susceptibility test was performed on two types of gram-positive pathogenic bacteria, assessing sensitivity against fourteen distinct groups of antibiotics for each bacterium, as reported by CLSI [11]. This was conducted to identify the sensitivity of isolates or their resistance to antibiotics prevalent in healthcare institutions, selected for their common application in treating bacterial infections.

2.8 Determination of the Antimicrobial Activity of Nigella sativa

Burgaz *et al*. [12] produced suspensions of bacterial isolates, which were inserted in sterile brain heart infusion tubes and cultured for 18 to 24 h at 37°C. To the suspension, which was compared to the standard MacFarland solution (1.5×10^8 CFU/ml), 100 ul of the aqueous extract of *N. sativa* seeds was added to each well previously created in the culture media using a cork borer. The efficacy of each concentration was assessed by measuring the diameter of the inhibitory zone surrounding each well.

2.9 Biosynthesis of Ag NPs

To synthesize silver nanoparticles (Ag NPs), 2.5 g of silver nitrate (AgNO3) was dissolved in 50 ml of deionized water utilizing a magnetic stirrer at 800 rpm at ambient temperature. Subsequently, 100 ml of Nigella sativa plant extract was included in the precursor solution. Following a duration of 72 h, 0.5 M sodium hydroxide (NaOH) was introduced to the aforementioned solution, resulting in a color change to brown. The precipitate is isolated using centrifugation and subsequently washed with water and ethanol five times. The drying procedure was conducted utilizing an oven at 40°C [13].

2.10 Characterization of Silver Nanoparticles

Fourier Transform Infrared Spectroscopy (FTIR) from Shimadzu (Germany) was employed to characterize Ag nanoparticles and identify specific functional groups inside the Ag nanoparticles [14]. Scanning electron microscopy (SEM) was employed to determine the shape, dimensions, and size distribution of green synthesized silver nanoparticles [15]. The UV-Vis spectrophotometer is an effective, straightforward, and sensitive technique for analyzing silver nanoparticles, with the reduction of pure Ag+ ions evaluated through the measurement of the UV-Vis spectrum of the reaction medium [16]. An investigation of X-ray diffraction (XRD) was conducted utilizing a Japanese Shimadzu XRD 6000 apparatus, which aids in the structural characterization of nanoparticles, including crystallite size and crystal structures [17]. Atomic Force Microscopy (AFM) was employed to ascertain the dimensions, surface morphology, and granularity volume of the Ag nanoparticles [18]. Transmission electron microscopy (TEM) employs an electron beam to engage with ultrathin samples, yielding the most accurate and high-resolution imaging data regarding the size, shape, morphology, aggregation state, and distribution of nanoparticles at nanoscale resolution [19].

2.11 Antibacterial Activity of Ag NPs

260 mg of nanoparticle powder was dissolved in 2.6 ml of distilled deionized water to prepare a stock solution of silver nanoparticles, further concentrated to 100 mg/ml. The solution was heated to 45°C in a water bath, and a vortex was employed to guarantee the complete dissolving of the powder. Five distinct concentrations (200, 100, 50, 25, and 12.5 mg/ml) were prepared from stock. A comparison was conducted using the streak method on a McFarland tube (1.5 x 10^8 CFU/ml). The bacteria were cultured on Muller-Hinton agar, and six wells (5 mm) were formed in the plate using a sterile cork borer. Five varied concentrations of Ag NPs, each comprising 100 ul, were introduced into the wells, while the sixth well served as a control with the addition of 100 ul ddH2O. The plates were subsequently incubated at 37°C for 24 h [20].

3. RESULTS AND DISCUSSION

3.1. Distribution of Specimens According to Gender

A total of 150 specimens from all age groups and both genders were collected from a clinical source (urine), comprising 60 (40%) male specimens and 90 (60%) female ones. The findings of the present study concur with those of Czajkowski *et al*. [21], who indicated that urinary tract infections (UTIs) are among the most prevalent infections impacting women at various life stages. Due to the anatomical structure of the female lower urinary tract and its proximity to the reproductive organs, women are far more susceptible to urinary tract infections than men.

3.2. Distribution of Specimens According to the Bacterial Species

Following the isolation and identification of bacteria through bacteriological and biochemical analyses, as well as confirmation via VITEC compact 2 systems, the results presented in Table 1 indicate that two species of Gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus faecalis*, were isolated from urine samples 50 (83.33%) and 10 (16.67%), respectively. This finding is consistent with the study by Pirkani *et al*. [22].

Table1. Number of isolates for gram-positive bacteria

Bacteria	No. (%) of urine isolates
Staphylococcus aureus	50 (83.33%)
Enterococcus faecalis	10 (16.67%)
Total	60 (100%)

3.3. Antibiotic Susceptibility Test of Bacterial Isolates

The results of the current study shown in Figure 1 indicated that from the total 50 isolates of S. aureus showed resistance to Oxacillin 43(86%), Cefoxitin 38(76%), Penicillin 35(71%), Chloramphenicol 35(70.5%), and Nitrofurantoin 34(69%), Doxycycline 33(66%), Vancomycin 33(65.5%), Gentamicin 32(63%), Ciprofloxacin 31(61%), Trimethoprim 30(59.5%), Levofloxacin 28(56%), Rifampicin 27(55%), Azithromycin 25(50%) and Erythromycin 25(50%).

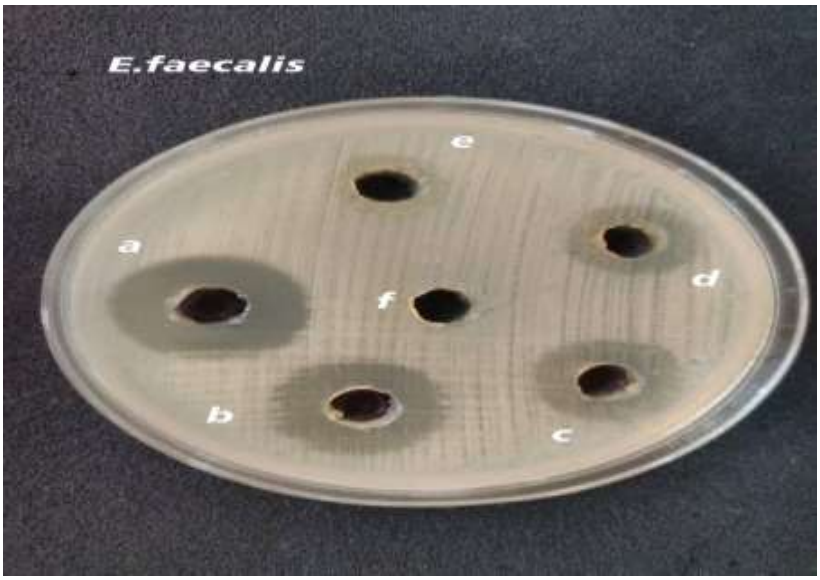


Figure 1. Percentages of antibiotic resistance of S. aureus [Oxacillin (OX), Cefoxitin (CX), Penicillin (P), Chloramphenicol (C), Nitrofurantoin (NIT), Doxycycline (DOX), Vancomycin (VA), Gentamicin (CN), Ciprofloxacin (CIP), Trimethoprim (TR), Levofloxacin (LEV), Rifampicin (RA), Azithromycin (AZM), Erythromycin (E)]

The present study showed high resistance to Oxacillin, Cefoxitin and Penicillin; these results were close to a study done in Iraq by Naqid *et al*. [23]. In other studies, in Iraq, Hassan Beg *et al*. [24] mentioned the resistance of *S. aureus* isolates to Nitrofurantoin 70% and to Vancomycin was 64.9% which agreed with the results of the current study. The resistance to Erythromycin was 50% which agreed with Salman et al. [25]. *S. aureus* has thrived in humans due to its adaptability and potential to gain antibiotic resistance, rendering it a significant pathogen across various environments [26]. Antibiotic resistance in *S. aureus* is associated with the plasmid-mediated production of beta-lactamases and other mechanisms [27]. The primary causes of antibiotic-resistant bacteria are genetic alterations and subsequent selection mechanisms induced by antibiotics [23]. The results of the current study shown in Figure 2 indicated that from the total 10 isolates of Enterococcus faecalis showed resistance to Ampicillin 7(70%), Rifampicin 7(70%), Doxycycline 6(60%), Vancomycin 6(60%), Chloramphenicol 6(60%), Clindamycin 6(60%), Norfloxacin 5(50%), Nitrofurantoin 5(50%), Erythromycin 5(50%), Levofloxacin 4(40%), Trimethoprim 4(40%), Penicillin 4(40%), Ciprofloxacin 3(35%) and Fosfomycin 3(30%).

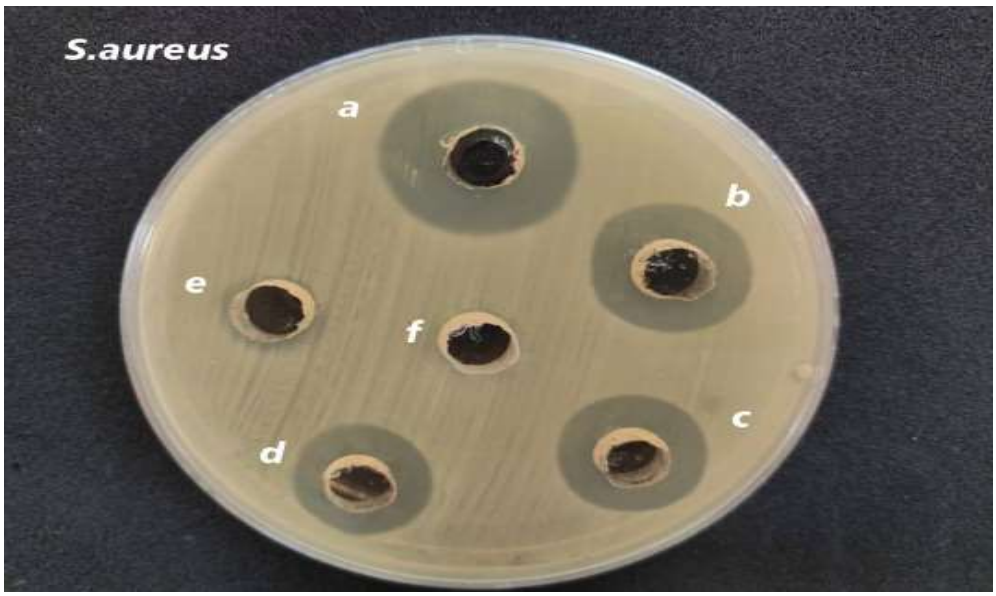


Figure 2. Percentages of antibiotic resistance of E. faecalis [Ampicillin (AMP), Rifampicin (RA), Doxycycline (DOX), Vancomycin (VAN), Chloramphenicol (C), Clindamycin (DA), Norfloxacin (NOR), Nitrofurantoin (NIT), Erythromycin (E), Levofloxacin (LEV), Trimethoprim (TR), Penicillin (P), Ciprofloxacin (CIP), Fosfomycin (FOS)]

The highest rate of resistance to antibiotics was documented against Ampicillin 70% and Rifampicin 70%; these results agree with a study done by Alduhaidhawi *et al*. [28]. Resistance of bacteria to vancomycin and Norfloxacin in the present study were 60% and 50% respectively, which agree with Gilmore et al. [29] in the case of vancomycin but differ from the current results in the case of Norfloxacin, who showed the rate of resistance was 95%. Among multidrug-resistant enterococci, three perilous resistance mechanisms are proliferating: vancomycin-resistant enterococcus (VRE), Glycopeptide-resistant enterococcus (GRE), and Linezolid-resistant enterococcus (LRE) [30]. The administration of Glycopeptides and Linezolid in empirical therapy may augment enterococcal resistance to these antibiotics, resulting in the proliferation of super-resistant germs that are untreatable [31].

3.4. Multidrug Resistance Patterns of Bacteria

The results of the current study showed that all the clinical isolates of gram positive isolates were multiple drug-resistant MDR. The number of S. aureus and E. faecalis MDR were 38(76%) and 8(80%) respectively, and the number of extensively drug-resistant (XDR) isolates were 12(24%) and 2(20%) respectively, as shown in Table 2.

Table2. Antibiotic resistant patterns of gram positive bacteria

Bacteria	MDR No. (%)	XDR No. (%)
Staphylococcus aureus	38 (76%)	12 (24%)
Enterococcus faecalis	8 (80%)	2 (20%)

Antibiotic resistance constitutes a significant dilemma within contemporary healthcare, posing a grave threat to public health. Initially, antibiotic-resistant bacteria were confined to the hospital setting [32]. Antimicrobial resistance is the ability of a bacterium to withstand the effects of various antimicrobials. When resistance develops to several medications, it is referred to as multidrug resistance (MDR). Microbes exhibit many resistance mechanisms, including inherent resistance to specific antimicrobials, genetic mutations, and acquired resistance from other species [33]-[34].

3.5. Antibacterial Activity of the Aqueous Extract of Nigella sativa Seeds

The results of the current study in Table 3 showed that the aqueous extract of *Nigella sativa* seeds has a notable effect on the Gram-positive bacteria. The aqueous extract of Nigella sativa was more effective against isolates of *S. aureus*, and the inhibition diameter ranged from 20 mm at concentration 200 mg/ml, while the inhibition diameter of *E. faecalis* was 19 mm at concentration 200 mg/ml. Whereas, the aqueous extract had the lowest inhibition diameter (0, 12) mm respectively at a concentration 12.5 mg/ml.

The results of the aqueous extracts of Nigella sativa were close to Gawron *et al*. [35], which showed the aqueous extracts of *Nigella sativa*, had a high effect against gram-positive *S. aureus* with the inhibition diameter reaching 19 mm at concentration (200) mg/ml. The present findings are consistent with those of Shafodino *et al*. [36] who indicated that Nigella sativa extract exhibits antibacterial properties against pathogenic bacterial strains (*E. coli, P. aeruginosa, S. aureus, and B. subtilis*). *Nigella sativa* seeds contain alkaloids, proteins, saponins, as well as fixed and volatile essential oils. In particular, a significant amount of the biological activity of the seed extracts is caused by thymoquinone (TQ), the primary component of the essential oil [37]. As show Figure2.

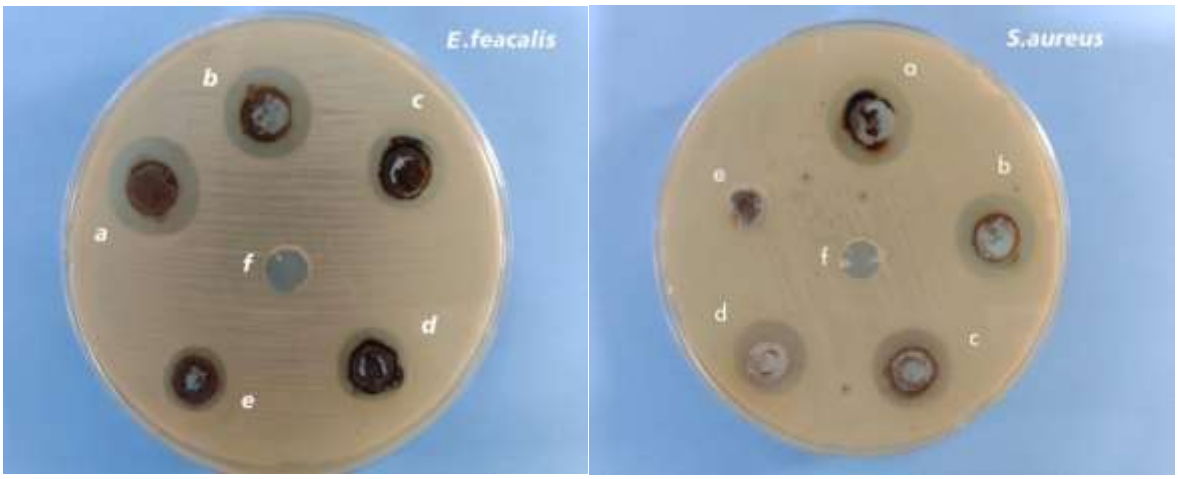


Figure3. Inhibition zone of N. sativa on Bacterial isolates (a=200, b=100, c=50, d=25, e=12.5, f=Control)

Table 3. Effect of aqueous extract of Nigella sativa seeds on bacterial growth (average inhibition zone diameter, mm)

Type of isolates	Con. 200 mg/ml	Con. 100 mg/ml	Con. 50 mg/ml	Con. 25 mg/ml	Con. 12.5 mg/ml
S. aureus	20mm	18mm	16mm	15mm	0mm
E. faecalis	19mm	17mm	15mm	14mm	12mm

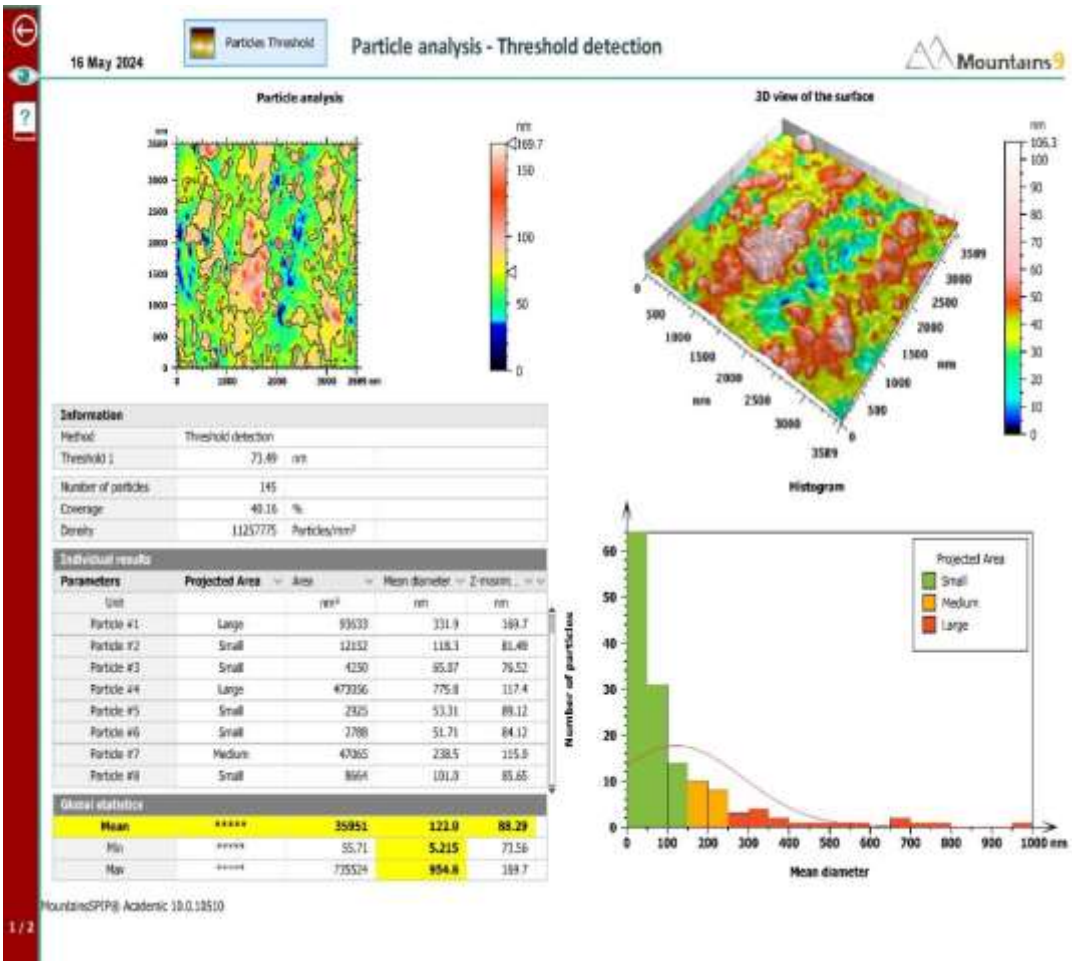
3.6. Characterization of Silver Nanoparticles

Atomic Force Microscopy (AFM)

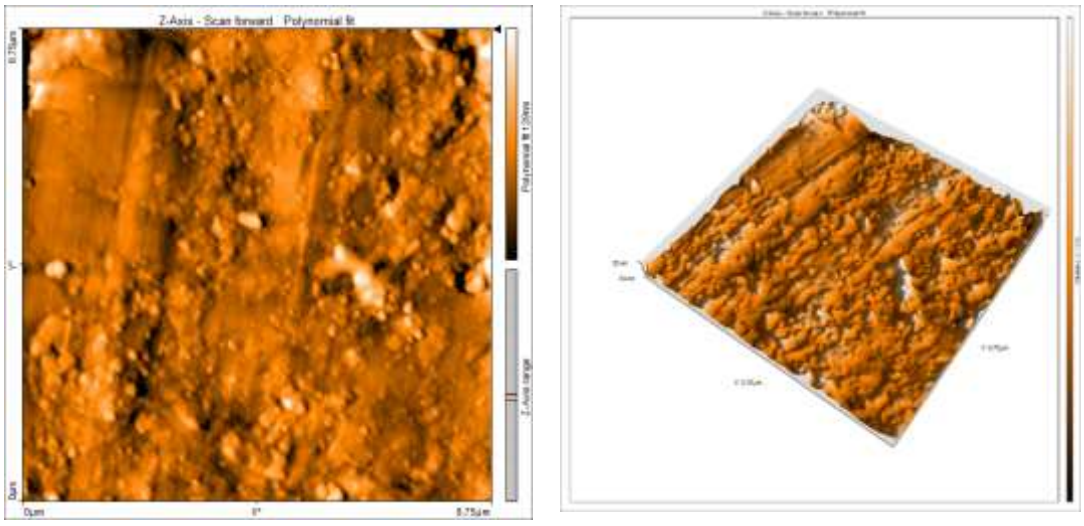
Atomic force microscopy (AFM) was used to validate the surface shape of the biosynthesized silver nanoparticles by using *N. sativa* aqueous extract; the two dimensions and three dimensions were determined. The results showed there were differences in the silver nanoparticles' phenotypic properties. The average diameters of Ag NPs biosynthesized by N. sativa were 14.12 nm and the calculated size of nanoparticles ranged between (23.30 - 54.41) nm (Table 4, Figure 4). These results demonstrated that the synthesized particles were ultrafine particles that have a diameter of less than 100 nm, proving that N. sativa extract was efficient for synthesizing smaller NPs; these results were in agreement with Chand et al. [38] and Gulbagca *et al*. [39].

Table 4. Particle (grain) diameter size of Ag NPs

Parameter	Value
Total Particles numbers	100
Average diameter	14.12 nm
10% Diameter	5.10 nm
50% Diameter	13.22 nm
90% Diameter	22.11 nm



(A) The range sizes of biosynthesized Ag NPs



(B) **(C)**

Figure4. (A) Range sizes of biosynthesized Ag NPs. (B) Topography of two-dimensional Ag NPs. (C) Topography of three-dimensional Ag NPs.

Scanning Electron Microscopy (SEM) Analysis

The Scanning Electron Microscope (SEM) is another method employed to ascertain the dimensions, morphology, and distribution of green produced silver nanoparticles. Figure 5 shows the particles were spherical with 25-60 nm of smooth surface area. The present study succeeded in achieving good results in establishing narrow range silver nanoparticles sizes, which is in agreement with Zare-Bidaki *et al*. [40] and Palanisamy *et al*. [41] who produced silver nanoparticles sized 27-65 nm.

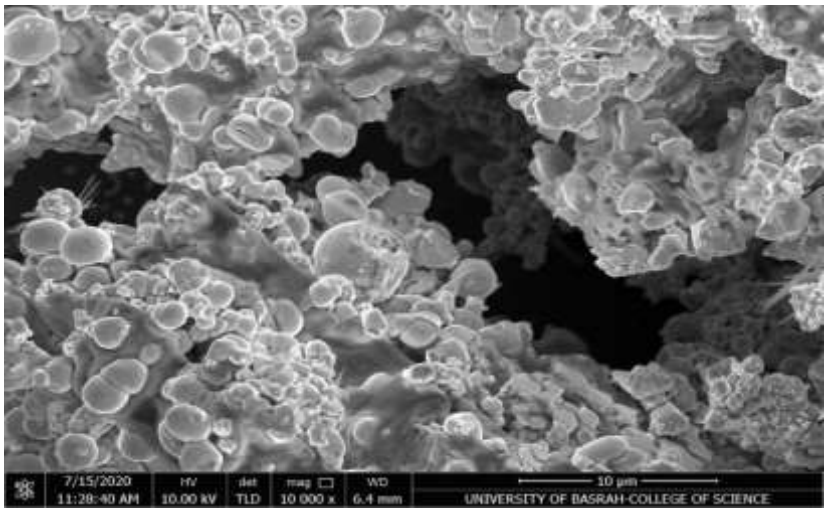


Figure5. SEM image of Ag Nanoparticles

X-ray Diffraction (XRD)

The bio-synthesized Ag nanoparticles were examined using X-ray diffraction to determine crystallinity and average particle size. Figure 6 illustrates the significant peaks associated with the diffraction levels recorded at 2theta angles of 35.1° (110), 38.1° (111), 68° (113), and 78.4° (004), which align well with the Joint Committee on Powder Diffraction Standards (JCPDS) card No. 4-783. The mean particle size (D) of the produced nanoparticles was determined using the Debye-Scherrer equation [42, 40]: D = 0.9lambda / beta*cos(theta), where lambda represents the wavelength of X-ray sources (CuKalpha lines - 0.1541 nm), beta denotes the full width at half maximum (FWHM) in radians, and theta signifies Bragg's diffraction angle. The computed value of D was determined to be 40 nm, corroborating the findings of Daoudi et al. [43].

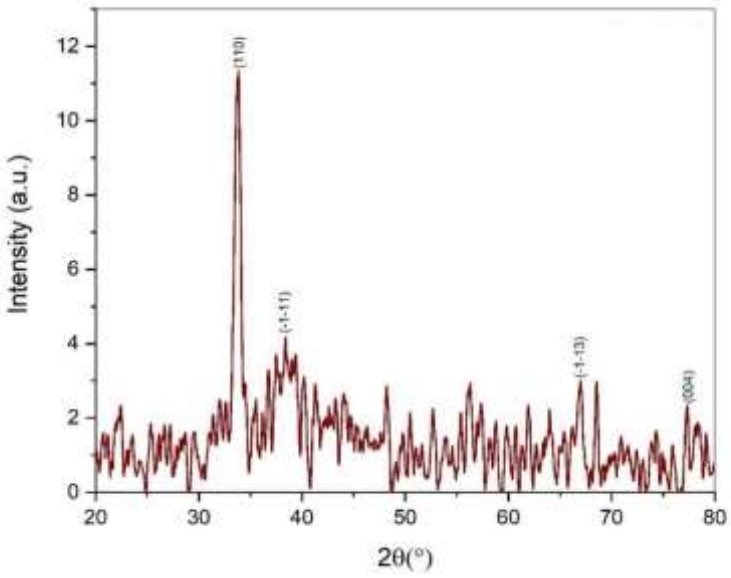


Figure6. XRD spectra of Ag NPs

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was performed to assess the possible functional classes of biomolecules implicated in the reduction of silver ions and the stabilization of biosynthesized Ag NPs produced by N. sativa aqueous extract. The band intensities of the test sample were examined as depicted in Figure 7. The significant peak at 3278 cm-1 corresponds to the N-H stretching vibration of amines, while the peaks of alkanes and alkynes at 2921 cm-1 are characteristic of methyl groups or -CH. The detected signal at 2850 cm-1 corresponds to the -OH stretching vibration of flavonoid, polyphenol, and alcohol functional groups within carboxylic compounds. The significant peaks at 1740 and 1637 cm-1 correspond to the alkene stretching vibration C=C, while the peak at 1040 cm-1 is associated with the aliphatic amine stretching vibration C-N. Alkenes correspond to the peaks at 767 and 717 cm-1. This outcome closely aligned with that of Gavamukulya et al. [44].

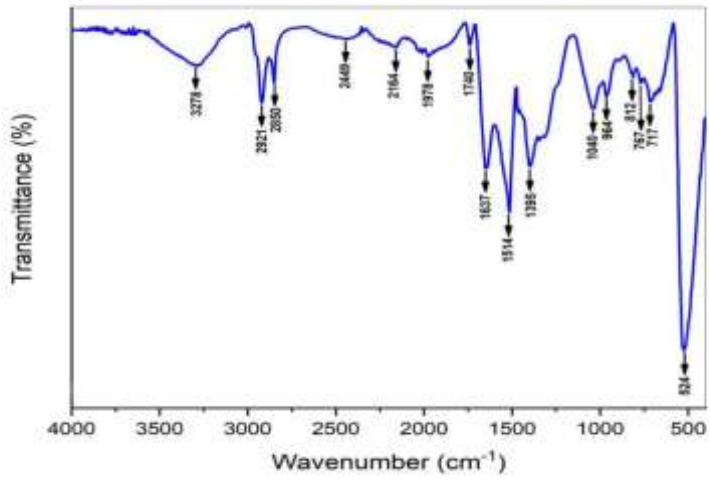


Figure7. FTIR spectra of functional groups of the Ag NPs synthesized from N. sativa

Transmission Electron Microscope (TEM)

The morphology and size of biosynthesized Ag NPs were observed by TEM which showed that they were quasi-spheres in shape with few agglomerations. Ag NPs aggregation verifies that the produced particles are capped, as was previously mentioned in the section on chemical interactions in FTIR. The size of NPs ranged from 5-33 nm (Figure 8). The elemental composition analysis which provides qualitative and quantitative details about elements was done by EDX (Figure 9), and showed a strong signal from metallic Ag NPs at 3 keV due to their surface plasmon resonance, in addition to signals of silicon which may have acted as capping organic agent attached to the surface of Ag NPs.

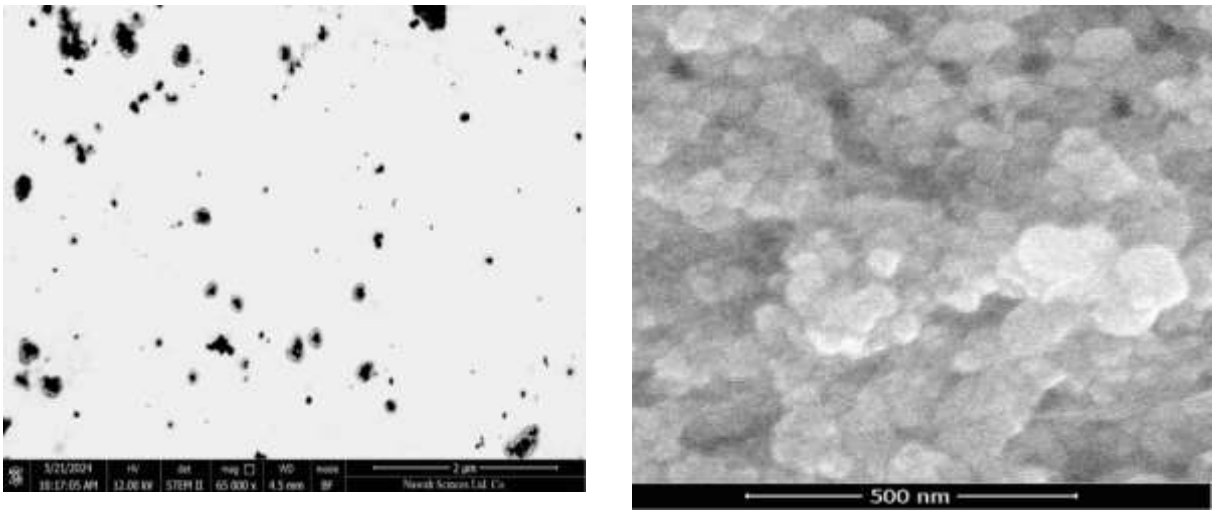


Figure8. (A) Silver nanoparticles (B) TEM image of N. sativa with silver nanoparticles

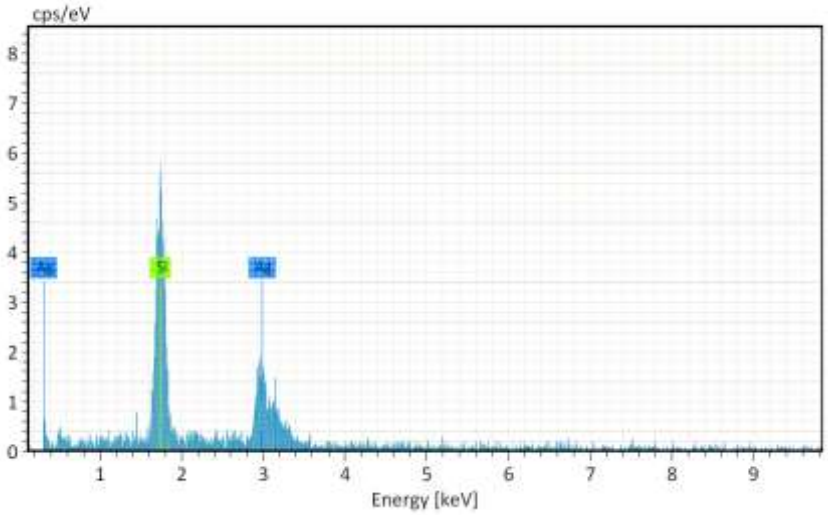


Figure9. EDX spectrum analysis of Ag NPs

3.7. Antibacterial Activity of Ag NPs against Pathogenic Bacteria

Silver nanoparticles exhibited a significant antibacterial impact against multidrug-resistant bacteria, as demonstrated in Table 5 and Figure 10. The Ag NPs exhibited a significant inhibition zone diameter at a dosage of 200 mg/ml against *S. aureus* and *E. faecalis*, measuring 25 mm and 22 mm, respectively. Conversely, at a concentration of 12.5 mg/ml, the Ag NPs had the smallest inhibition zone diameters against the same isolates, measuring 11 mm and 13 mm, respectively. These results were very close to Ezeh *et al*. [45] which showed inhibition zone diameter 18 mm at 100 mg/ml concentration against *S. aureus* and Elnosary *et al*. [46] showed that the inhibition zone was 25 mm.

Table5. Effect of Ag NPs on bacterial growth (average inhibition zone diameter, mm)

Type of isolate	Con. 200 mg/ml	Con. 100 mg/ml	Con. 50 mg/ml	Con. 25 mg/ml	Con. 12.5 mg/ml
S. aureus	25mm	22mm	20mm	18mm	11mm
E. faecalis	22mm	20mm	18mm	16mm	13mm

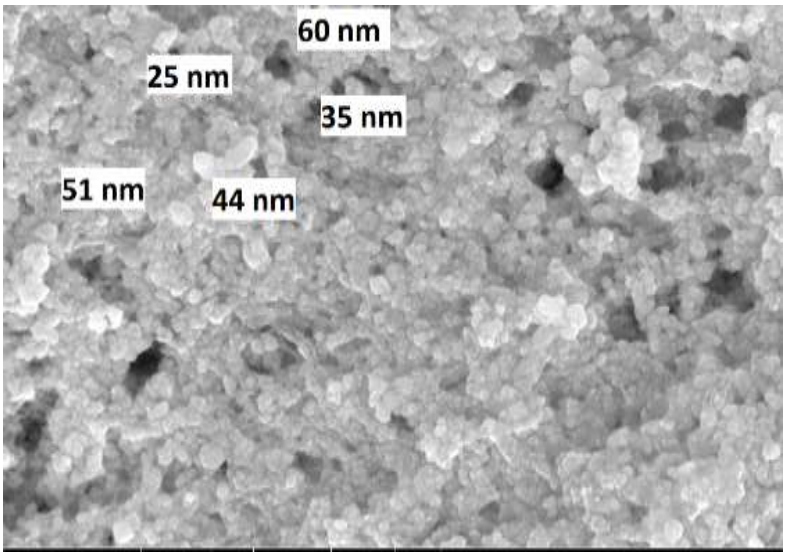


Figure10. Inhibition zone of Ag NPs on Bacterial isolates (a=200, b=100, c=50, d=25, e=12.5, f=Control)

Abd and Hasan [47] demonstrated that the inhibition zone diameter against *S. aureus* at a dose of 100 mg/ml was 22 mm, which aligns with the findings of the current study. Numerous components in N. sativa extracts, including polyphenols, flavonoids, ascorbic acid, terpenoids, and proteins, are crucial for metal ion absorption, precursor salt reduction, and the intrinsic antibacterial characteristics of capping agents [44]. Due to rising antibiotic resistance and the emergence of novel antibiotics, research has commenced on utilizing antibacterial nanoparticles as innovative medicinal devices [43].

3.8. Determination of Minimum Inhibition Concentration (MIC) of Nigella sativa and Ag NPs

The results appeared in Table 6; serial dilutions of Ag NPs (100, 50, 25, 12.5, 6.25, 3.125, 1.5, 0.78) ug/ml were prepared according to Almatroudi et al. [48]. The MIC for aqueous N. sativa was determined against clinical source isolates of *S. aureus* and *E. faecalis* which were (12.5 and 3.125) ug/ml respectively, while the MIC for Ag NPs were determined against same bacteria (3.125 and 3.125) ug/ml respectively.

Table6. Minimum Inhibition Concentration (MIC) of aqueous N. sativa and Ag NPs against pathogenic bacteria

Type of isolate	Aqueous N. sativa (ug/ml)	Ag NPs (ug/ml)
S. aureus	12.5	3.125
E. faecalis	3.125	3.125

The microbial sensitivity of metal nanoparticles varies according to the microbial species and the concentration of the metal nanoparticles [49].

4. CONCLUSION

The present study demonstrated the successful biosynthesis of silver nanoparticles using Nigella sativa seed extract through an eco-friendly method. The synthesized nanoparticles showed effective antibacterial activity against bacteria isolated from urinary tract infections, including multidrug-resistant strains. These findings suggest that biosynthesized silver nanoparticles may represent a promising alternative antimicrobial agent for controlling bacterial infections.

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