Enhanced antibacterial activity of green synthesized Ag NPs against Gram-negative bacteria by *Nigella sativa* isolated from UTI

Shahad khalid Hassan

University of Diyala, College of Science, Department of Biology

scibioms2204@uodiyala.edu.iq **(**corresponding author)

Abbas Yaseen Hasan

University of Diyala, College of Science, Department of Biology

abbasyaseen@uodiyala.edu.iq

Received: 26 December 2024 \ Revised: 17 January 2025 \ Accepted: 25 January 2025

ABSTRACT

Background: The improper use of antibiotics results in significant bacterial resistance to numerous antibiotics, particularly in prevalent bacterial species such as *E. coli, S. aureus, E. faecalis, K. pneumoniae, A. baumannii,* and *P. aeruginosa*. This is a challenge in the management of urinary tract infections induced by certain bacterial species. Consequently, it stimulates scientists to search for an alternative antibacterial agent with fewer adverse effects and lower bacterial resistance. Nanotechnology recently represented alternative therapeutic methods by eco-friendly synthesis of nanoparticles which showed a significant result on pathogenic bacteria in many studies including the current study.

Objective: This study aims to indicate the antibacterial activity of biosynthesized Ag NPs by *Nigella sativa* aqueous extract against antibiotic resistant bacteria.

Materials and methods: 130 specimens of different age groups for both genders from clinical source (urine) were collected, between the beginning of December 2023 and end of April 2024 from patients of Baqubah Education Hospital and Al-Batoul Teaching Hospital in Diyala, after cultured in the cultures media, the total clinical isolates were 125 isolates of a different genus of bacteria.

Results: The result showed that four species belong to the Gram negative bacteria, *Escherichia coli, Acinetobacter baumannii, Klebsiella pneumoniae,and Pseudomonas aeruginosa,* which isolated from urine 42 (33.6%),33 (26.4%), 27 (21.6%),and 23 (18.4%),respectively. Atomic Force Microscopy (AFM) was used to determine the average size and shape of the nanoparticles, which came out to be 54 nm. The Ag NPs have a smooth surface texture and a spherical shape, according to scanning electron microscopy (SEM). The wavelength range was evaluated using UV–visible spectroscopy (UV-Vis), which investigate a noticeable peak at 420 nm. Fourier Transform Infrared Spectroscopy (FTIR) shows that the reduction and capping processes are made possible by several functional groups found in biomolecules. Ag NPs at different concentrations (12.5, 25, 50, 100, and 200 mg/ml) were evaluated against isolates that were multiple drug-resistant (MDR). According to the results, at a dose of 200 mg/ml, the highest inhibition zone diameters against *K. pneumoniae, A. baumannii, E. coli, and P. aeruginosa* were (25, 23, 22, and 21) mm, respectively. On the other hand, the same isolates showed the smallest zones at 12.5 mg/ml to be (15, 12, 14, and 0) mm, respectively.

Conclusion: The research shown that The *Nigella sativa* aqueous extract displays a potent action against Gram-negative bacteria, however the antibacterial efficacy of silver nanoparticles (Ag NPs) surpassed that of *Nigella sativa* seed extracts.

Keywords- UTI, Ag NPs, Biosynthesis, Nanoparticles, MDR.

INTRODUCTION

Urinary tract infection (UTI) is a major global health concern caused by several Gram-positive and Gram-negative bacteria, including *E. coli, P. mirabilis, E. faecalis, K. pneumoniae, and S. saprophyticus*, along with certain fungi [1]. UTI encompasses a variety of infectious illnesses affecting the urinary tract, from the urethra to the kidneys. Reports indicate that (50-60) % of women may encounter at least one UTI throughout their lifetime, rendering it one of the most widespread ailments. An inflammatory response is generally initiated by bacteria that inhabit the urethra or periurethral area and subsequently invade the bladder [2]. Antimicrobial resistance (AMR) in UTIs poses an ongoing challenge and global concern, prompting

persistent awareness campaigns on the rise of resistant microbes due to the improper therapeutic application of antimicrobial medicines, exacerbating the AMR issue [3].

Black seed, a widely recognized traditional herbal medicinal plant, has been utilized as both a food additive and a form of traditional medicine in numerous nations [4]. 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 inhibits cyclooxygenase and 5-lipoxygenase, hence diminishing inflammation by inhibiting the peroxidation of membrane lipids within cells. Multiple investigations indicate that thymoquinone and thymohydroquinone exhibit antibacterial inhibitory effects[5]. Due to their distinctive physical and chemical properties, morphology, dispersion, size, shape, and elevated surface area, silver nanoparticles (Ag NPs) are extensively utilised across several sectors, including food, medicine, healthcare, and industry [6].

Ag NPs are among the most extensively researched particles at the moment. In the field of biomedicine, Ag NPs are becoming increasingly powerful because of their use as antibacterial agents, coatings for medical equipment, and carriers for chemotherapy medications. Effective antibacterial qualities are demonstrated by Ag NPs against methicillin-resistant strains of bacteria as well as Gram-positive and Gram-negative bacteria. Additionally, Ag NPs have anti-biofilm properties and function in conjunction with other antibiotic classes, such as B-lactams, macrolides, and lincosamide [7].

Materials and methods

Collection of specimens: Between December 2023 and April 2024, 130 of urine specimens collected from patients of all ages and genders at Baquba Education Hospital and Al-Batoul Teaching Hospital in Diyala.

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 [8].

Isolation and identification of bacteria: Bacteria were isolated and identified using morphological characteristics of the colonies, microscopic examination of bacterial cells, and biochemical tests. IMViC test was used to perform biochemical identification. For each isolated sample, sterile slants of indole, methyl red, VogesProskauer, and citrate were stabbed with ($1.5x\ 10^8$) CFU/ml fresh bacterial inoculum and then incubated for 24–48 h. at 37°C. In order to verify identification, 10 bacterial isolates were chosen using the Vitek-2 method [9].

Collection of plant samples: Seeds of Nigella sativa were obtained from a local market and subsequently verified by Prof. Dr. Khazal D. Wadi from the College of Sciences at the University of Diyala.

***Nigella sativa* seeds preparation:** After being transported to the lab, the Nigella sativa seeds were cleaned with distilled water and dried for four days at 30 °C by using oven. After being further ground into a fine powder using an electric grinder, the ingredients were put away in a plastic bag until they were needed [10].

Preparation of aqueous extract of *Nigella sativa* seeds: Using Whatman No. 1 filter paper, 50g of seed powder was dissolved in 100ml of hot water to create the aqueous extract. Five filter sheets were employed to isolate the requisite filtrates from the solid residues after 24 h. at room temperature and 150 rpm 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 [11]

Determination of the antimicrobial activity of *Nigella sativa:*

According to Burgaz *et al*.[12] produced suspensions of bacterial isolates, which were inserted in sterile brain heart infusion tubes and cultured at 37 °C for 18 to 24 hours. In contrast to (1.5×10^8) CFU/ml, the conventional MacFarland solution, 100 µl of the aqueous extract of *Nigella sativa* seeds was added to each well formed in the culture media using a cork borer. The effectiveness of each concentration was evaluated by measuring the diameter of the inhibitory zone around each well.

Biosynthesis of Ag NPs: A magnetic stirrer was used to dissolve 2.5 g of silver nitrate (AgNO3) in 50 ml of deionized water at 800 rpm at room temperature in order to create silver nanoparticles (Ag NPs). Subsequently, 100 ml of Nigella sativa plant extract was included into the precursor solution. After 72 h., 0.5 M sodium hydroxide (NaOH) was introduced to the aforementioned solution, resulting in a brown coloration. The precipitate is isolated using

centrifugation and subsequently rinsed with water and ethanol five times. The drying procedure was conducted via an oven at 40°C [13].

Characterization of Ag NPs: The characterization and the identification of specific functional groups inside the Ag NPs were performed using Fourier Transform Infrared Spectroscopy (FTIR) from Shimadzu (Germany)[14]. Scanning electron microscopy (SEM) was utilized to ascertain the morphology, dimensions, and size distribution of [15]. The UV-Vis spectrophotometer is an efficient, direct, and sensitive method for analyzing silver nanoparticles, and the reduction of pure Ag^+ ions was assessed by measuring the UV-Visible spectrum of the reaction medium [16]. The size, surface texture, and granular volume of the Ag nanoparticles were assessed using Atomic Force Microscopy (AFM)[17].

Antibacterial activity of Ag NPs: 260 mg of nanoparticle powder were dissolved in 2.6 ml of distilled deionized water to prepare a stock solution of silver nanoparticles, subsequently 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 the stock solution. After conducting a comparison examination using the streak method on a McFarland tube (1.5×10^8) CFU/ml, six wells 5 mm were made on the plate using a sterile cork borer, and the bacteria were cultivated on Muller-Hinton agar. Five distinct concentrations of Ag NPs at100 µg/ml were administered to the wells, while the sixth well functioned as a control with the inclusion of 100 µg/ml ddH2O. After that, the plates were incubated for 24 h. at 37°C [18].

Results

Distribution of specimens according to gender

130 specimens of different age groups for both genders from clinical source (urine) were collected, 59 (45.4%) of specimens were males while 71(54.6%) were females as shown in table (1).

Table (1): number of isolates according to gender

Sources	Specimens No. (%)	Gender	
		Male No. (%)	Female No. (%)
Urine	130 (100%)	59(45.4%)	71(54.6%)

Distribution of specimens according to the bacterial species

Following the bacteriological examination, biochemical tests and VITEC compact 2 systems was done for confirmation, the result showed that four species belong to the Gram negative bacteria, *Escherichia coli, Acinetobacter baumannii , Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, which isolated from urine 42 (33.6%), 33 (26.4%), 27 (21.6%), and 23 (18.4%) respectively, table (2).

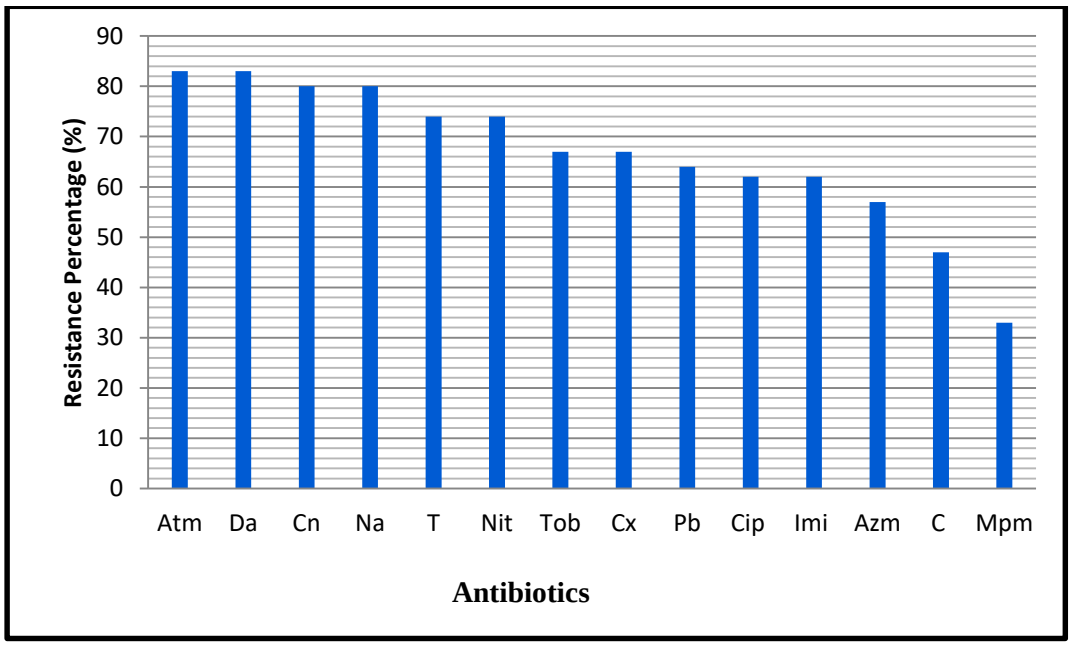
Table (2): the number and percentage of the isolates for each bacteria

Bacteria	**No. (%) of urine plates**
E.coli	42 (33.6%)
A. baumannii	33 (26.4%)
K.pneumonia	27 (21.6%)
P. aeruginos	23 (18.4%)
Total	125 (100%)

Antibacterial Susceptibility test

An antibiotic susceptibility test was performed on four species of pathogenic bacteria, assessing sensitivity against 14 distinct families of antibiotics for each bacterium, as published by CLSI [18].To assess the sensitivity of isolates or their resistance to antibiotics prevalent in healthcare settings, these drugs were selected due to their common application in treating bacterial illnesses.

Antibiotic Susceptibility of Bacteria



Escherichia coli

The results shown in figure (1) indicated that the all *E.coli* isolates (42) showed resistance which include: Aztreonam 35 (83%), Clindamycin 35 (83%), Gentamicin 34 (80%), Nalidixic acid 34 (80%), Tetracycline 31(74%), Nitrofurantoin 31 (74%), Tobramycin 28 (67%), Cefoxitin 28 (67%), Polymyxin B 27 (64%), Ciprofloxacin 26 (62%), Imipenem 26 (62%), Azithromycin 24 (57%), Chloramphenicol 20 (47%), and

Meropenem 14(33%).

Fig (1): The percentages of antibiotic resistance of *E.coli*

Cefoxitin (CX), Tetracycline (T), Gentamicin (CN), Clindamycin (Da), Polymyxin B (PB), Ciprofloxacin (CIP), Meropenem (MPM) , Imipenem (IMI), Aztronam (ATM), Nalidixic acid (NA), Nitrofurantoin (Nit), Tobramycin (TOB), Azithromycin (AZM), Chloramphenicol (C).

Acinetobacter baumannii

According to the current study's findings, which are displayed in figure (2), all 33 isolates of *A. baumannii* exhibited numerous antibiotic resistance traits, including: Piperacillin 28 (85%), Trimethoprim 28 (85%), Ampicillin 25 (76%), Ceftazidime 24 (73%),Cefotaxime 24 (73%),Clindamycin 23 (70%), Polymyxin B 23 (70%), Ciprofloxacin 23 (70%), Levofloxacin 22 (67%), Gentamicin 22 (67%), Amikacin 20 (60%), Doxycycline 17 (52%), Imipenem 12 (36%), and Meropenem 12 (36%).

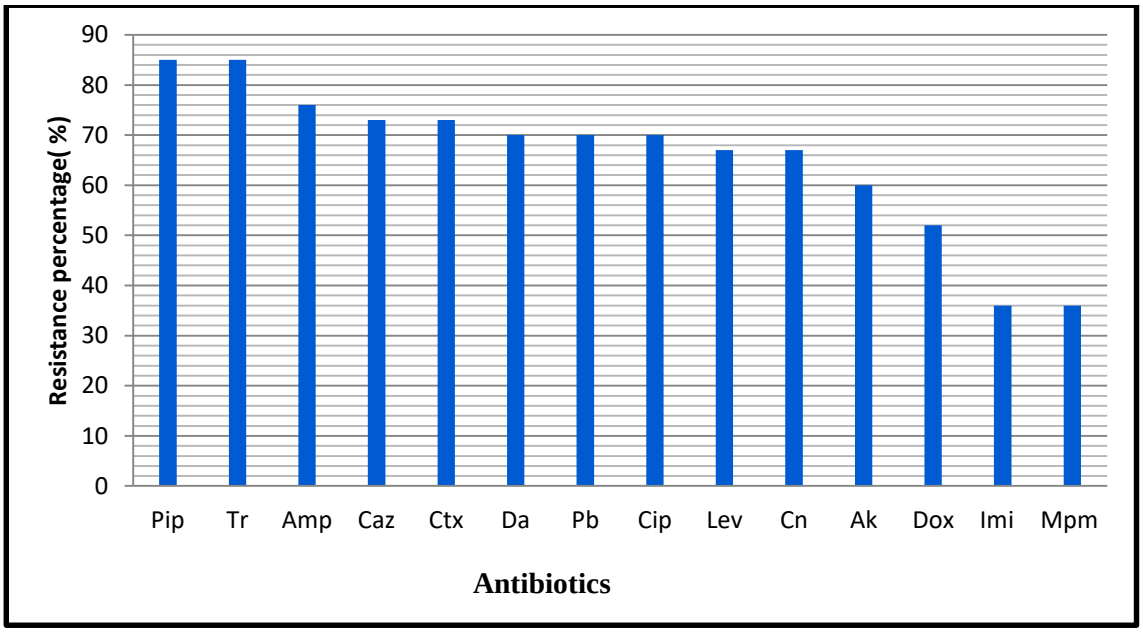


Figure (2): the percentages of antibiotic resistance of *A. baumannii*

Cefotaxim (CTX), Trimethoprim (TR), Piperacillin (PIP), Gentamicin (CN), Ceftazidime (CAZ), Clindamycin (Da), Polymyxin B (PB), Doxycycline (Dox), Ciprofloxacin (CIP), Amikacin (AK), Levofloxacin (LEV) , Meropenem (MPM) , Ampicillin (AMP), Imipenem (IMI).

Klebsiella Pneumoniae

The results of the antibiotics sensitivity of all *K. pneumoniae* (27) isolates showed variable resistance as shown in Figure (3) which include: Nitrofurantoin 22(81%), Clindamycin 22 (81%), Aztreonam 20 (74%), Ciprofloxacin 20 (74%), Azithromycin 19 (70%), Gentamicin 19 (70%), Imipenem 19 (70%), Polymyxin B 18 (66%), Nalidixic acid 18 (66%), Tetracycline 16 (59%), Tobramycin 14 (51%), Cefoxitin 14 (51%), Chloramphenicol 11 (40%), and Meropenem 8 (29%).

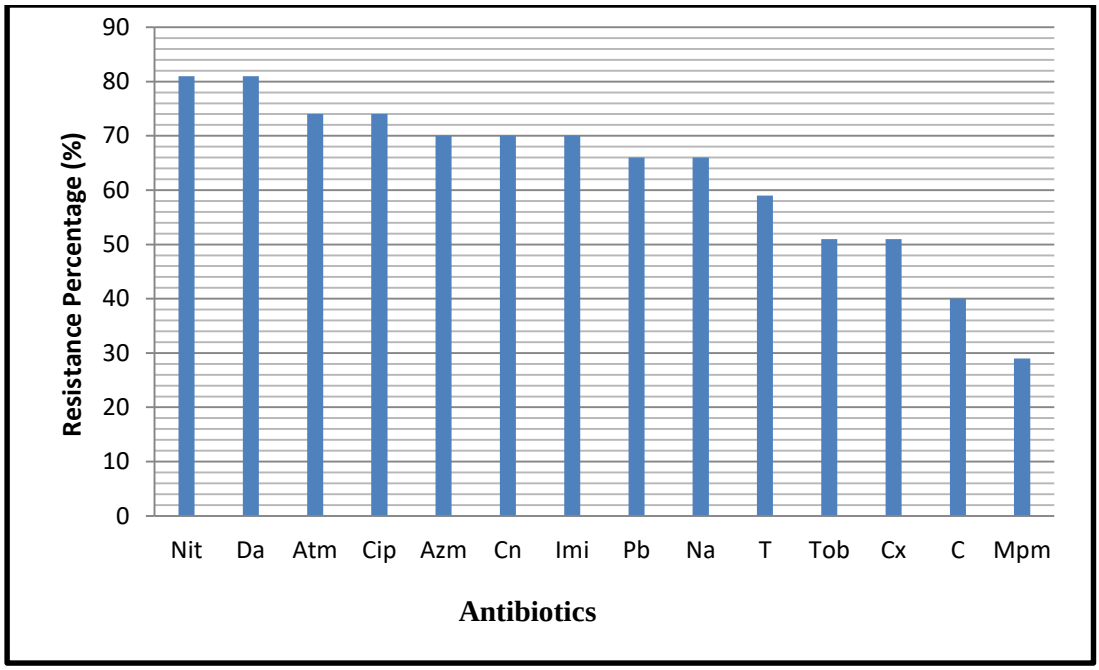


Figure (3): the percentages of antibiotic resistance of *K.pneumoniae*

Nitrofurantoin (NIT), Clindamycin (DA), Aztreonam (ATM), Ciprofloxacin (CIP), Azithromycin (AZM), Gentamicin (CN), Imipenem (IMI), Polymyxin B (PB), Nalidixic acid (NA), Tetracycline (T), Tobramycin (TOB), Cefoxitin (CX), Chloramphenicol (C), Meropenem (MPM)

Pseudomonas aeruginosa

The results of the current study shown in the figure (4) indicated that from the total 23 isolates of *P. aeruginosa* showed resistance to Aztreonam 20 (87%), Trimethoprim 19(83%), Piperacillin 18 (78%), Gentamicin 18 (78%), Ceftazidime 17 (74%), Tobramycin 15 (65%), Polymyxin B 15 (65%), Chloramphenicol 15 (65%), Ciprofloxacin 14 (61%), Amikacin 14 (61%), Levofloxacin 13 (57%), Norfloxacin 10 (43%), Meropenem 10 (43%), and Imipenem 10 (43%).

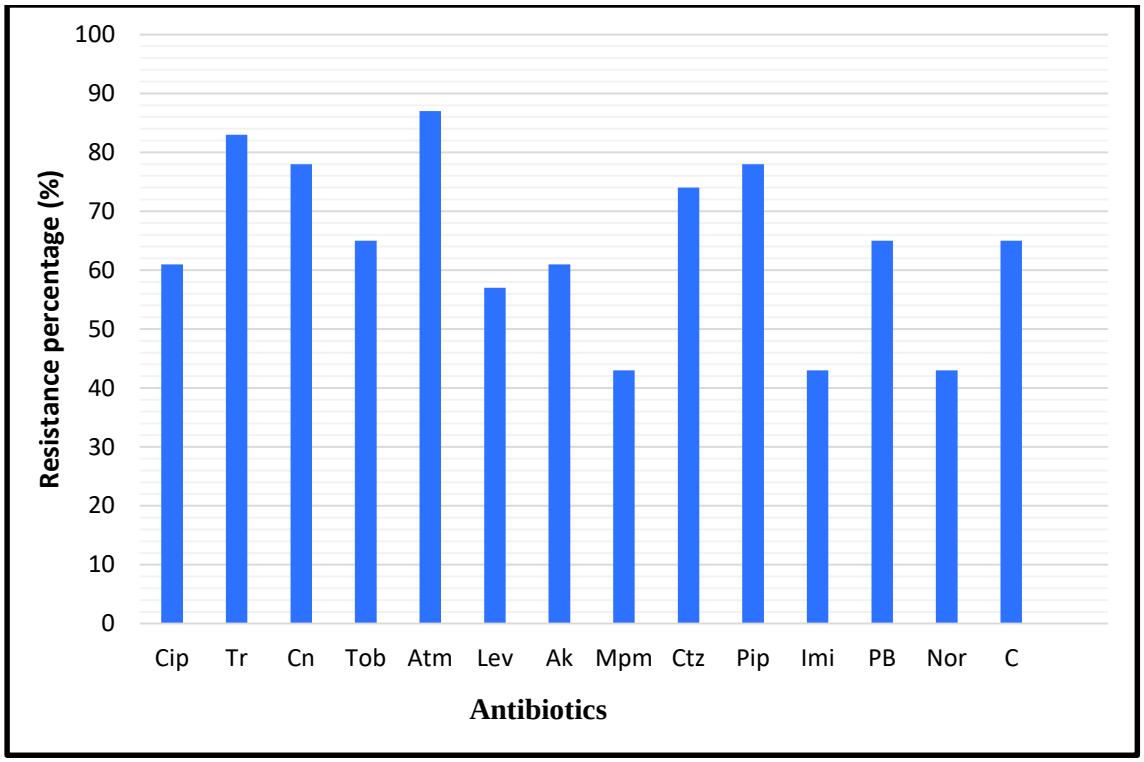


Figure (4): The percentages of antibiotic resistance of *P.aeruginosa*

Aztreonam (ATM), Trimethoprim (TR), Piperacillin (PIP) , Gentamicin (CN), Ceftazidime (CAZ), Tobramycin (TOB), Polymyxin B (PB), Chloramphenicol (C), Ciprofloxacin (CIP), Amikacin (AK), Levofloxacin (LEV) , Norfloxacin (NOR), Meropenem (MPM) , Imipenem (IMI)

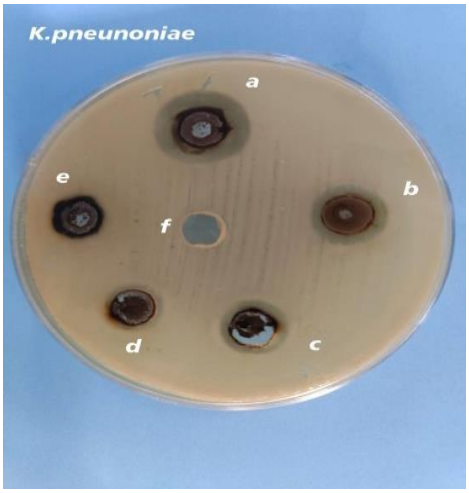
Antibacterial activity of the aqueous extract of *Nigella sativa* seeds

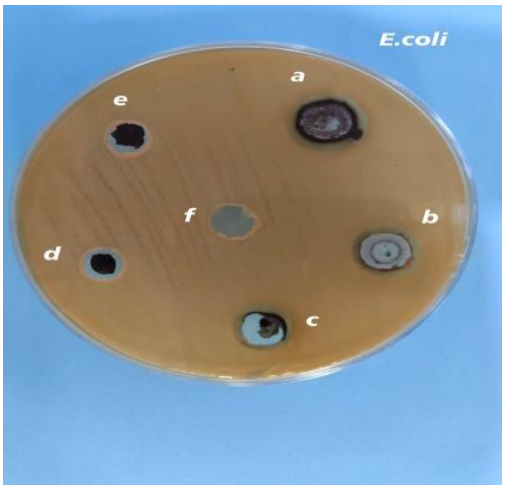
The results of current study showed that the aqueous extract of *Nigella sativa* seeds have a noticeable effects on Gram negative bacteria.

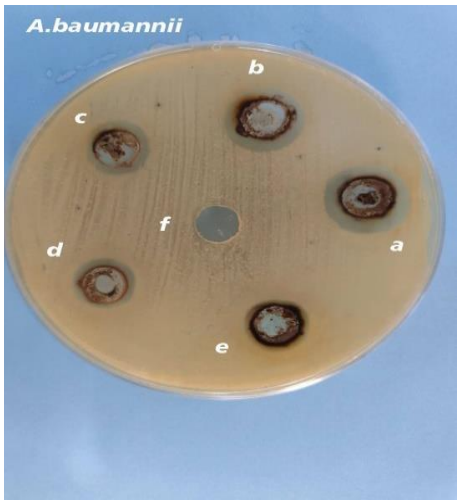
Table (3) and figure (5) shows the aqueous extract of *N. sativa* was more effective against isolates of *K. Pneumoniae*, and the inhibition diameter was19 mm at concentration 200 mg/ml, while the inhibition diameter was (18, 15, 18) mm respectively against each of *P. aeruginosa*, *E. coli* and *A. baumannii*, whereas, the aqueous extract recorded the lowest activity against same isolates at concentration 12.5 mg/ml.

Table (3): the effect of aqueous extract of *Nigella sativa* seeds on bacterial growth in different concentration

Bacterial of isolate	Average of inhibition zone diameter(mm)				
	12.5mg/ml	25mg/ml	50mg/ml	100mg/ml	200mg/ml
P. aeruginosa	0mm	12mm	13mm	15mm	18mm
K. pneumoniae	0mm	11mm	13mm	15mm	19mm
E. coli	0mm	0mm	11mm	13mm	15mm
A. baumannii	12mm	13mm	14mm	16mm	18mm







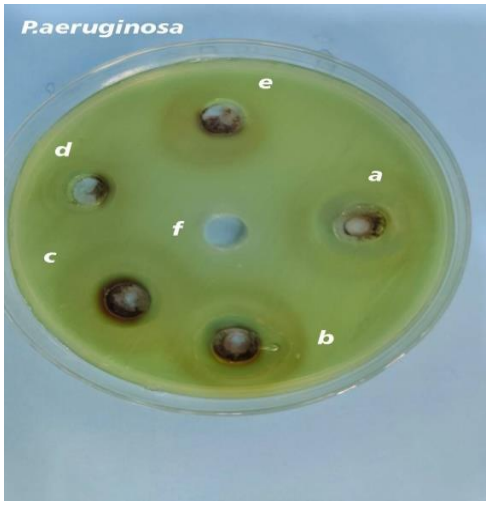


Figure (5): the inhibition zone of *N. sativa* on Bacterial isolates (a=200, b=100, c=50, d=25, e=12.5, f= Control)

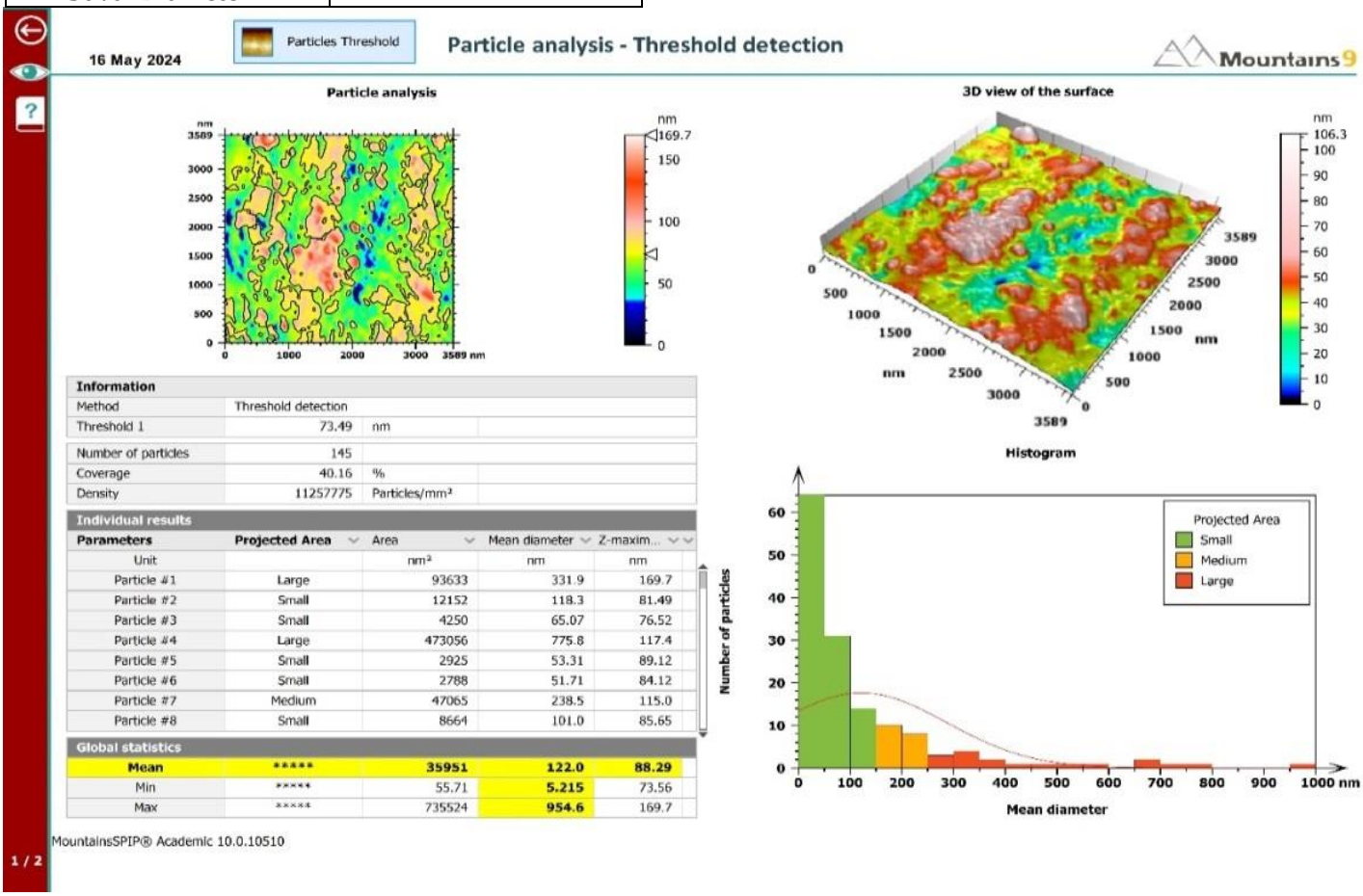
Characterization of Ag NPs nanoparticles

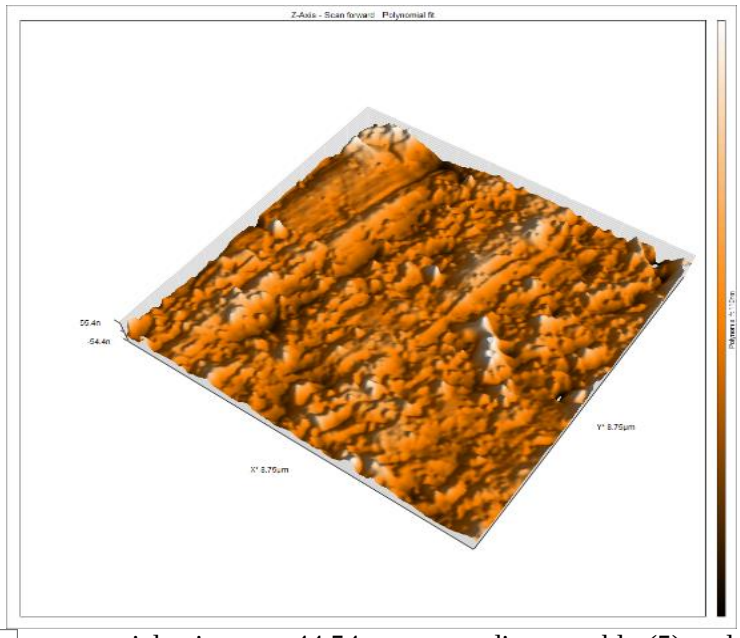
Using N. sativa aqueous extract, AFM was utilized to confirm the surface form of the biosynthesized silver NP, the two dimensions and three dimensions were determined image with AFM. The results showed that the average diameters of Ag NPs biosynthesized by *N. sativa* was 14.12 nm and the calculated size of nanoparticle was ranged between (23.30 – 54.41) nm table (4), figure (6)**.**

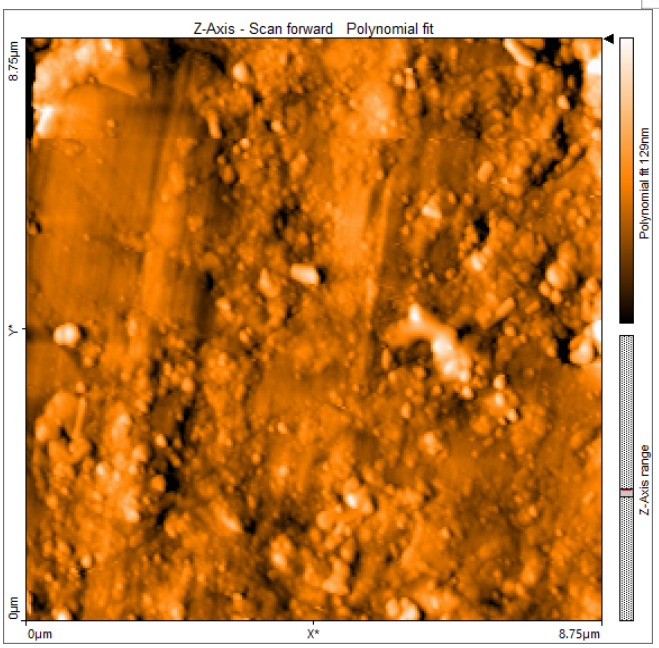
Table (4): Particle (grian) diameter size of Ag NPs

Total Particles numbers	**100**
Average diameter	14.12 nm
10% Diameter	5.10 nm
50% Diameter	13.22 nm
50% Diameter	22.11 nm

A







B C

Figure (6): A- the range sizes of biosynthesized Ag nanoparticles.
B- Topography of two-dimensional silver nanoparticles.
C- Topography of three-dimensional Ag nanoparticles.

To determine the size, form, and distribution of green synthesized silver nanoparticles, the Scanning Electron Microscope (SEM) was utilized. The average particle size was 44.54 nm, according to table (5) and image (6).

Table (5): particle size (nm) of Ag NPs biosynthesized using *N. sativa* extract

Particles Size (nm)	Particles Size (nm)	Particles Size (nm)
30.12	40.39	47.65
31.22	41.20	48.98
32.14	42.36	49.50
33.5	43.33	50.26
33.7	44.61	51.70
36.23	45.22	52.55
38.30	46.72	60.20

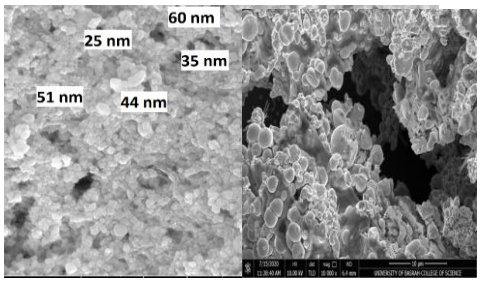


Figure (7): SEM image of Ag Nanoparticles

The absorption characters' spectra have important properties of the Ag NPs and the UV-Visible spectra had proved to be very useful for Ag NPs analysis and were a good method for characterizing Ag NPs formation and production. The synthesis of Ag NPs from the aqueous extract of N. sativa was further stimulated by ultraviolet-visible spectroscopy (UV/VIS) in the 300–600 nm range. The absorbance peak was recorded at 420 nm in figure (8).

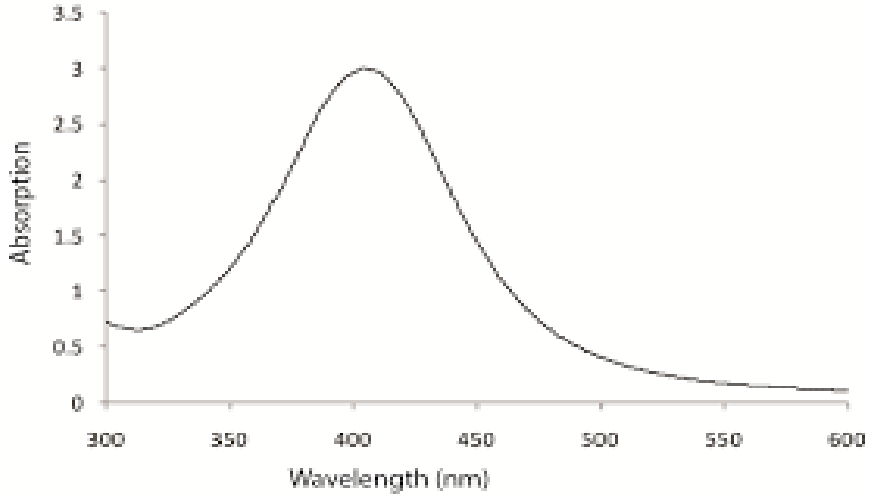


Figure (8): UV-Vis spectrum of synthesized silver NPs

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 analyzed as depicted in the figure (9).

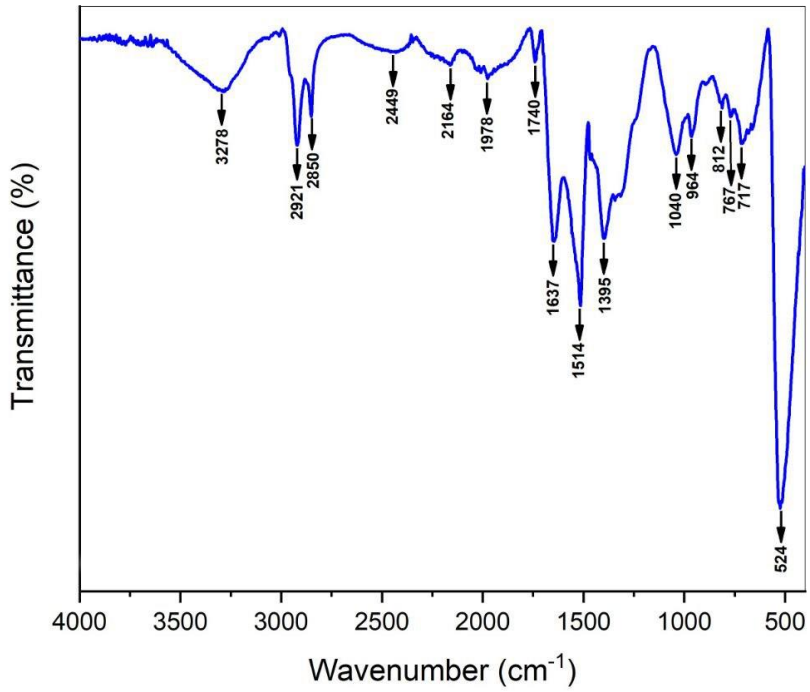


Figure (9): FTIR spectra of functional groups of the Ag NPs synthesized from *N. Sativa*

Antibacterial activity of Ag NPs against pathogenic bacteria

Ag NP showed a remarkably antibacterial effect toward Gram-negative bacteria (multidrug-resistance) as shown in Table (6), Figure (10) .The Ag NPs showed the highest diameter of inhibition zone at concentration 200 mg/ml against *P.aeruginoas, K.pneumoniae, E.coli* and *A. baumannii* reaching (21, 25, 22, 23) mm respectively, while the Ag NPs recorded at12.5 mg/ml concentration lowest diameter of inhibition zone toward the same isolates (0, 15, 14, 12) mm respectively.

Table (6): the effect of Ag NPs on bacterial growth

Type of isolate	Average of inhibition zone diameter (mm)				
	concentration 12.5mg/ml	concentration 25mg/ml	concentration 50mg/ml	concentration 100mg/ml	concentration 200mg/ml
P. aeruginosa	**0mm**	**12mm**	**15mm**	**18mm**	**21mm**
K. pneumoniae	**15mm**	**17mm**	**19mm**	**21mm**	**25mm**
E. coli	**14mm**	**15mm**	**17mm**	**19mm**	**22mm**

A. baumannii	**12mm**	**14mm**	**16mm**	**21mm**	**23mm**

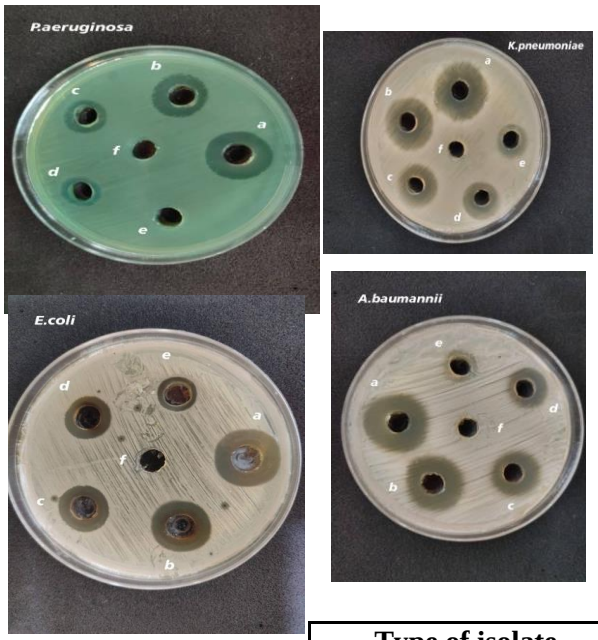


Figure (10): the inhibition zone of Ag NPs on Bacteria isolates (a=200, b=100, c=50, d=25, e=12.5, f= Control)

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

The results appeared in table (7), serial dilutions of Ag NPs (100, 50, 25, 12.5, 6.25, 3.125, 1.5, and 0.78) µg/ml were prepared. The MIC of N. *sativa* ranged (3.125-12.5) µg/ml and for Ag NPs (1.5-12.5) µg/ml

Table (7): Minimum Inhibition Concentration (MIC) of aqueous *N. sativa* and Ag NPs against pathogenic bacteria

Type of isolate	Aqueous *N.sativa*	Ag Nps
P. aeruginosa	**3.125**	**3.125**
K. pneumoniae	**12.5**	**12.5**
E. coli	**6.125**	**1.5**
A. baumannii	**3.125**	**1.5**

Discussion

Distribution of isolates varied according to gender with the higher rate of UTI in women, while distribution according to bacterial species the numbers varied as *E. coli* 42 (33.6%). *A. baumannii* 33(26.4%), *K. pneumoniae* 27(21.6%) and *Pseudomonas aeruginosa* 23(18.4%). this result agreed with Pirkani *et al*. [19].

The bacterial isolates of the current study showed a high variable pattern in the antibacterial susceptibility test for different fourteen antibiotics. As *P. aeruginosa* showed a high resistance to Aztreonam , Trimethoprim , Piperacillin , Gentamicin and Ceftazidime, these results agree with Hasan *et al*. [20]who showed that *P.aeruginosa* isolates revealed highly resistance against the same antibiotics.When comparing the present result with Roy *et al*. [21] found the

resistance of *A. baumannii* isolates against Piperacillin , Trimethoprim , Ampicillin and Ceftazidime were 85%, 86% , 77%, and 71%

Naqid *et al*. [22] reported that resistance to Clindamycin, Gentamicin and Nalidixic acid were 88%, 90% and 81% respectively, these result were close to the present results. *K.pneumoniae* isolates possessed a high resistance to Nitrofurantoin, Clindamycin, Aztreonam and Ciprofloxacin which agreed with Ahmed *et al*. [23].

The aqueous extract of *Nigella sativa* showed a remarkable activity against the bacterial isolates, the highest activity was against *K. pneumonaie* with inhibition diameter 19mm and followed by *A. baumannii*, *P. aeruginosa*, and *E. coli* with diameter (18, 18, 15)mm at 200 mg/ml concentration, which agreed with Al Dosary *et al*. [24] who showed the extract of *Nigella sativa* possesses strong microbial inhibitory effects against most urinary pathogens that cause urinary tract infection (UTI) *K. pneumonia, A. baumannii, E. coli* and *P. aeruginosa* and the inhibition diameter were (21,23,19,19) mm respectively at 200 mg/ml, these results were agreement with the present study.

The green biosynthesis of Ag NPs from *N. sativa* seeds aqueous extract has great efficacy against pathological bacteria with multiple resistance isolated from urine samples, which exceeded the efficiency of the aqueous extract, The Ag NPs showed the highest diameter of inhibition zone at concentration 200 mg/ml against *K. pneumoniae, A. baumannii, E.coli and P. aeruginosa* was (25, 23, 22 and 21) mm respectively, while the Ag NPs recorded at concentration 12.5 mg/ml minimum diameter of the inhibitory zone against the same isolates (15,12,14 and 0) mm respectively. These results agreed with a study conducted by Abd and Hasan [25]who showed that the Ag NPs activity against *P.aeruginosa and A. baumannii* were (21, 20) mm respectively, and Ezeh *et al.* [26] who showed that the inhibition zone of *E. coli* and *K. pneumonaie* were (18, 17) mm respectively.

respectively ,which in agreement with the current study. While for *E.coli* isolates,

Ag NPs were characterized by different techniques including the Atomic force microscopy (AFM) which used to validate the surface shape of the biosynthesized silver nanoparticles by using *N. sativa* aqueous extract, the two dimensions and three dimensions was determined image with AFM. These results showed that the average diameters of Ag NPs biosynthesized by N. sativa was 14.12 nm and the calculated size of nanoparticle was ranged between (23.30 – 54.41) nm, which demonstrated that the synthesized particles were ultrafine particles that have a diameter less than 100 nm proved that N. sativa extract was efficient for synthesizing smaller NPs, these results were agreement with Chand *et al*. [26] and Gulbagça *et al*. [27].

Size, shape, and distribution of Ag NPs were determined by Scanning Electron Microscope (SEM), the present study successes to achieve good results in establishing a narrow range of silver nanoparticles sizes which in agreement with Zare-

Bidaki *et al*. [28] and Palanisamy *et al*.[29]they produced Ag nanoparticles sized 27-65 nm.

The absorption characters' spectra have important properties of the Ag NPs and the UV-Visible spectra had proved to be very useful for Ag NPs analysis and were a good method for characterizing Ag NPs formation and production. Ultraviolet-visible spectroscopy (UV/VIS) in range typically observed between 350-550 nm. Ezeh *et al* [31]. .FTIR was used to assess the probable functional classes of biomolecules implicated in the reduction of silver ions and the stabilization of biosynthesized Ag NPs derived from *N. Sativa* aqueous extract, While the peaks of alkanes and alkynes at 2921 cm^{-1} are indicative of methyl groups or –CH bonds, the notable peak at 3278 cm^{-1} is associated with the N-H stretching vibration of amines. The –OH stretching vibration of the alcohol functional groups of carboxyl, flavonoid, and polyphenol is represented by the peak seen at 2850 cm^{-1}. The aliphatic amine stretching vibration C-N is responsible for the peak at 1040 cm^{-1}, whereas the alkene stretching vibration C=C is responsible for the large peaks at 1740 and 1637 cm^{-1}. The peaks at 767 and 717 cm^{-1} are alkenes. This result aligns with the findings of Gavamukulya *et al*. [32]. In order to produce Ag NPs, functional groups such as alkanes, alkyls, alcohols, carboxylic acids, amides, alkenes, acids, and alkyl halides were used.

In MIC of *N. sativa***,** *P. aeruginosa* and *A. baumannii* showed the highest sensitivity at the concentration 3.125 µg/ml followed by *E.coli* at 6.25µg/ml followed by *K. pneumoniae* which showed less sensitivity at the concentration 12.5 µg/ml respectively. The MIC of Ag NPs indicated that *E.coli* and *A. baumannii* showed the highest sensitivity at the concentration 1.5 µg/ml followed by *P. aeruginosa* at 3.125 µg/ml followed by *K. pneumoniae* showed less sensitivity at the concentration 12.5 µg/ml respectively. *K. pneumoniae* isolates was found to be more resistant than the other isolates, which were inhibited at high concentrations of biosynthesized nanoparticles. This can be explained by the fact that the antibacterial activity of Ag NPs appears to diversify depending on the specificities of bacterial cells, which are represented by their cellular walls and appear to affect the antimicrobial effect of Ag NPs [33].

between 300 and 600 nm further provoked the synthesis of Ag NPs from the aqueous extract of *N. sativa* and the absorbance peak was reported at 420 nm, this result was agree with Elnosary *et al*.[30]noting that the Ag absorption peak was (425 nm). The pronounced maximum absorption at approximately 420 nm verifies the production of the Ag NPs, as the peak absorption for bulk Ag NPs is

Conclusion

Escherichia coli isolates were the most commonly isolates from urine infections, following by *Acinetobacter baumannii*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*. Most bacteria isolated from urine infections showed high resistance to most antibiotics, the aqueous plant extracts of *Nigella sativa* investigate antimicrobial activity against gram-negative bacteria isolated

from urine infections. The biosynthesis by aqueous extract of *Nigella sativa* showed strong effect against Gram-negative bacteria. The antimicrobial activity of Ag NPs was more efficacious than *Nigella sativa* (seeds) extracts.

References

1. Abdulkarem, A. T., Zainulabdeen, S. M. S., and Abed, S. M. (2023). Overview of urinary tract infection caused by bacteria. Muthanna Medical Journal, 10(2):227-239.

2. Al-lawati, H., Blair, B. M., and Larnard, J. (2024). Urinary Tract Infections: Core Curriculum 2024. American Journal of kidney disease, 83(1):90- 100.

3. Pothoven, R. (2023). Management of urinary tract infections in the era of antimicrobial resistance. Drug Target Insights, 17: 126-137.

4. Abdullah, S. A., Salih, T. F. M.; Hama, A. A., and Ali, S. I. (2021). The Antibacterial Property of Nigella sativa (Black seed) Oil Against Gram-positive and Gramnegative Bacteria. Kurdistan Journal of Applied Research, 6(2): 156-165.

5. Zahoor, M., Nazir, N., Iftikhar, M., Naz, S.,Zekker, I., Burlakovs, J., Uddin, F., Kamran, A.W., Kallistova, A., Pimenov, N., and Khan, F. A. (2021). A Review on Silver Nanoparticles: Classification, Various Methods of Synthesis, and Their Potential Roles in Biomedical Applications and Water Treatment. Water, *13*(16): 2216.

6. Al-Kattan, G., Abdoun, M. A., and Kareem, S. H. (2024). Antimicrobial Activity of Silver Nanoparticles on Pathogenic Bacteria. Baghdad Science Journal, 21(3): 0937-0943.

7. Amezquita-Lopez, B. A., Soto-Beltran, M.; Lee, B. G.; Yambao, J. C., and Quiñones, B. (2018). Isolation, genotyping and antimicrobial resistance of Shiga toxin-producing Escherichia coli. Journal of Microbiology, Immunology and Infection. 51(4):425-434.

8. Crowley, E., Bird, P., Fisher, K., Goetz, K., Boyle, M., Benzinger, J.R. M. J., and Johnson, R. L. (2012). Evaluation of the VITEK 2 gram positive (GP) microbial identification test card: collaborative study. Journal of AOAC International, 95(5):1425-1432.

9. Bannai, J. A. A., Alrashid, I. M. and Abdul Jabbar, L. A. (2020). Effect of hot methanolic extract of Nigella sativa on the healing of infected cutaneous wounds in rabbits. Plant Archives, 20 (2): 756-760.

10. Shafodino, F. S., Lusilao, J. M., and Mwapagha, L. M. (2022). Phytochemical characterization and antimicrobial activity of *Nigella sativa* seeds, PLoS ONE 17(8): e0272457.

11. Burgaz, E., Sezener M.G., Dikbas C., Ceylan A.K., Andac M. and Çiftci A. (2021). Determination of antibacterial properties of silver nanoparticles with aqueous extracts of Brassica oleracea L. Var. acephala D.C. in cotton textiles. Journal of Elementology, 26(2): 447-462.

12. Ibrahim, N. H., Taha, G. M., Hagaggi, N. S. A. and Moghazy, M. A. (2024). Green synthesis of silver nanoparticles and its environmental sensor ability to some heavy metals. *BMC chemistry*, *18*(1): 7.

13. Pugazhendhi, A., Prabakar, D.,Jacob, J. M., Karuppusamy, I. and Saratale, R. G. (2018). Synthesis and characterization of silver nanoparticles using Gelidium amansii and its antimicrobial property against various pathogenic bacteria. Microbial pathogenesis, 114, 41-45.

14. Gamboa, S. M., Rojas, E. R., Martínez, V. V. and Baudrit, J. (2019). Synthesis and characterization of silver nanoparticles and their application as an antibacterial agent. International Journal of Biosensors & Bioelectronics, 5(5):166–173.

15. Hussain, Z., Jahangeer, M., Sarwar, A., Ullah, M., Aziz, T., Alharbi, M., Alshammari, A. and Alasmari, A. (2023). Synthesis and characterization of silver nanoparticles mediated by the *Mentha piperita* leave extract and exploration of its antimicrobial activity. Journal of the Chilean Chemical Society, 68(2), 5865-5870.

16. Nayak, S., Bhat, M. P.,Udayashankar, A. C., Lakshmeesha, T. R., Geetha, N. and Jogaiah, S. (2020). Biosynthesis and characterization of Dillenia indica-mediated silver nanoparticles and their biological activity. Applied Organometallic Chemistry, 34(4), 5567.

17. Shahid, S., Khan, S. A., Ahmad, W., Fatima, U., and Knawal, S. (2018). Size dependent bacterial

growth inhibition and antibacterial activity of Ag doped ZnO nanoparticles under different atmospheric conditions. Indian Journal of Pharmaceutical Sciences,80(1): 173- 180.

18. Clinical and laboratory standards institute (CLSI). Performance standards for antimicrobial susceptibility testing 33rd ed. CLSI supplement M100(ISBN 978-1-68440-170-3. Clinical and laboratory standards institute,USA,2023.

19. Pirkani, G. S., Awan, M. A., Abbas, F. and Din, M. (2020). Culture and PCR based detection of bacteria causing urinary tract infection in urine specimen. Pakistan Journal of Medical Sciences, 36(3): 391.

20. Hasan, S. A., Najati, A. M. and Abass, K. S. (2020). Prevalence and antibiotic resistance of "pseudomonas aeruginosa" isolated from clinical samples in Kirkuk City, Iraq. Eurasian Journal of Biosciences, *14*(1):1821-5.

21. Roy, S., Chowdhury, G., Mukhopadhyay, A. K., Dutta, S. and Basu, S. (2022). Convergence of biofilm formation and antibiotic resistance in Acinetobacter baumannii infection. *Frontiers in medicine*, *9*, 793615.

22. Naqid, I. A., Balatay, A. A., Hussein, N. R., Saeed, K. A., Ahmed, H. A. and Yousif, S. H. (2020). Antibiotic susceptibility pattern of Escherichia coli isolated from various clinical samples in Duhok City, Kurdistan Region of Iraq. International Journal of Infection, 7(3).

23. Ahmed Hasan, S., T Raheem, F., and Mohammed Abdulla, H. (2021). Phenotypic, antibiotyping, and molecular detection of Klebsiella pneumoniae isolates from clinical specimens in Kirkuk, Iraq. Archives of Razi Institute, 76(4), 1061-1067.

24. Al Dosary, R. A. A. Q. (2023). Antibacterial and Alteration of Drug Resistance Activities of Black Cumin Seed (Nigella Sativa) Extracts against Urinary Pathogens. *Journal of Public Health Sciences*, *2*(03), 148-158.

25. Abd, E. M., & Hasan, A. Y. (2023, March). Biosynthesis and characterization of silver nanoparticles by Aloe vera leaves extract and determination of its antibacterial activity. In *AIP Conference Proceedings* (Vol. 2475, No. 1). AIP Publishing.

26. Chand, K., Jiao, C., Lakhan, M. N., Shah, A. H.,Kumar, V., Fouad, D. E., and Cao, D. (2021). Green synthesis, characterization and photocatalytic activity of silver nanoparticles synthesized with Nigella Sativa seed extract. *Chemical Physics Letters*, *763*, 138218.

27. Gulbagça, F., Aygun, A., Altuner, E. E., Bekmezci, M., Gur, T., Sen, F., and Vasseghian, Y. (2022). Facile bio-fabrication of Pd-Ag bimetallic nanoparticles and its performance in catalytic and pharmaceutical applications: Hydrogen production and in-vitro antibacterial, anticancer activities, and model development. *Chemical Engineering Research and Design*, *180*, 254-264.

28. Zare-Bidaki, M., Aramjoo, H., Mizwari, Z. M., Mohammadparast-Tabas, P., Javanshir, R., and Mortazavi-Derazkola, S. (2022). Cytotoxicity, antifungal, antioxidant, antibacterial and photodegradation potential of silver nanoparticles mediated via Medicago sativa extract. *Arabian Journal of Chemistry*, *15*(6), 103842.

29. Palanisamy, C. P., Poompradub, S., Sansanaphongpricha, K., Jayaraman, S., Subramani, K., and Sonsudin, F. (2023). Biosynthesis of silver nanoparticles (AgNPs) using ethanolic extract of Nigella sativa (L.) seeds promotes wound healing via PDGF and VEGF signalling pathways activation. *Biocatalysis and Agricultural Biotechnology*, *54*, 102970.

30. Elnosary, M., Aboelmagd, H., Sofy, M. R. and Sofy, A. (2023). Antiviral and antibacterial properties of synthesis silver nanoparticles with nigella arvensis aqueous extract. *Egyptian Journal of Chemistry*, *66*(7), 209-223.

31. Ezeh, C. K., Eze, C. N., Dibua, M. E. U., Emencheta, S. C., and Ezeh, C. C. (2022). Synthesis of silver nanoparticles using Nigella sativa seed extract and its efficacy against some multidrug-resistant uropathogens. *Biomedical and Biotechnology Research Journal (BBRJ)*, *6*(3), 400-409.

32. Gavamukulya Y., El-Shemy H.A., Meroka A.M., Madivoli, E.S., Maina, E. N., Wamunyokoli, F. and Magoma, F. (2019).Advances in green nanobiotechnology: Data for synthesis and characterization of silver nanoparticles from ethanolic extracts of fruits and leaves of Annona muricata. Data Brief. 25:104194-104200.

33. Vaou, N., Stavropoulou, E., Voidarou, C.,Tsigalou, C. and Bezirtzoglou, E. (2021). Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives. *Microorganisms*, *9*(10), 2041.