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The Antimicrobial Activity of Silver Nanoparticles Synthesized by *Pseudomonas aeruginosa* Against UTI Pathogenic Microorganisms

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Abstract

Nowadays, there is an increase in interest in creating and researching silver nanoparticles (AgNPs) because of their numerous applications in many fields, especially medical fields such as burn, wound healing, dental and bone implants, antibacterial, viral, fungal, and arthropodal activities. *Pseudomonas aeruginosa* was isolated from burn patient identification tests by using various methods (cultural characteristics, biochemical tests, and the vitek2 system) and used to produce silver nanoparticles according to biological methods. Physical and morphological characterization was used to identify silver nanoparticles, and analysis using Fourier transform infrared spectroscopy showed the presence of 15 bands. Atomic force microscopy had an average diameter of 46.15 nm. Field emission scanning electron microscopy shows particles are aggregated into spherical to hexagonal shapes; the X-ray diffraction method results in peaks of the organic compounds in the extract that reduce the amount of silver ions and stabilize; and UV-visible spectroscopy analysis reveals a peak with maximum absorbance at 454 nm. The results showed that silver nanoparticles have antimicrobial activity against UTI pathogenic microorganisms.

Keywords: *P. aeruginosa*, characterization silver nanoparticles, antimicrobial activity, fourier transform infrared spectroscopy.

1. Introduction

Microbes evolved a number of strategies during molecular evolution to maintain genomic flexibility, and this genomic adaptability creates an adequate environment for growth and survival when exposed to harsh environments [1, 2]. Researchers have linked the causes of drug resistance to the overuse of antibiotics in the treatment of infectious diseases, which can lead to other side effects [3-5]. Professor Norio Taniguchi of Tokyo Science University coined the term nanotechnology in 1974 to describe the production of substances at the nanometer level [6, 7]. Silver has at least one dimension between 1 and 100 nm [8, 9]. Pharmaceutical, cosmetic, engineering, medical, and other industries can all use silver in various ways. In general, nanomaterials are smaller and have a lot more surface area than their bulk counterparts [10, 11]. As human fluid bodies contain high levels of sulfide and chloride ions, they defend against silver toxicity by forming insoluble salts containing silver ions. For this reason, silver has relatively low

toxicity in humans [12]. Silver nanoparticles can enter bacteria's cell walls, alter cell membrane composition, and produce reactive oxygen species, interfere with deoxyribonucleic acid replication, and increase cell membrane permeability by releasing silver ions [13]. Bacteria are also more sensitive to silver nanoparticles because the negative charge of the lipopolysaccharides promotes the adhesion of positively charged silver ions [14].

2. Materials and methods

2.1. Samples collection

All the samples (burn patients, UTI) were clinically isolated from Iraqi patients' hospitals. All swabs of the samples were inoculated on agar media like Macconkey, Cetrimide, Sabouraud dextrose, and Mannitol salt) and incubated aerobically at 37 °C for 24 hours.

2.2. Identification of pathogenic microorganisms

The initial isolate diagnosis was carried out based on the colonies' observed morphological characteristics, such as shape, color, texture, and edges. Cetrimide agar is used as a selective medium for *Pseudomonas aeruginosa* in addition to biochemical tests such as (oxidase, urease, catalase, and IMViC, and for microorganisms from UTI infection, Macconkey, Mannitol salt, and Sabouraud dextrose agar are used in addition to the vitek2 system.

2.3. Synthesis of silver nanoparticles (AgNPs)

After isolation and identification of P. aeruginosa, the inoculum was prepared by culturing a colony from an agar plate with a loop and aseptically transferred into a 100-ml brine heart infusion broth medium dissolved in deionized water. We incubated the medium for 2 days at 180 rpm at 37 °C to prepare a suspension of the *P. aeruginosa* bacterium. Next, we centrifuged the supernatant at 8000 rpm for 10 minutes. To get rid of any solids, we put the supernatants through germ-free membranes with 0.2-m pores. We did this before using them as AgNP catalysts. Four gm of $AgNO_3$ were added to this suspension and incubated in a dark environment at 37°C under agitation at 120 rpm for 24 h. The color of the reaction mixture changed to brown, which indicates the formation of silver nanoparticles. We then centrifuged the mixture at 8000 rpm for 10 minutes. After centrifuging for 5 minutes at 8000 rpm to remove the supernatant, we washed the precipitate with deionized water and placed it in a glass Petri dish for evaporation, resulting in powdered AgNPs after drying, as per [15].

2.4. Characterization of silver nanoparticles using different analysis methods

Size, shape, composition, crystal structure, and other factors all affect the characteristics of silver nanoparticles

2.4.1. Atomic force microscopy (AFM)

The department of chemical science at AL-Nahrain University measured the granularity accumulation distribution, roughness, and grain size of the AgNPs nanostructures using atomic force microscopy, as stated in [16].

2.4.2 UV-Visible spectroscopic analysis

A UV-visible spectrophotometer (Optizen Pop) was used to scan the silver nanoparticle powder (200–700 nm) at UV, visible, and near-infrared wavelengths after it had been dissolved in deionized water [17].

2.4.3 Fourier transforms infrared spectroscopy (FTIR) analysis

The specimen was ground by pressing the samples into KBr granules in a 1:90 ratio for the FT-IR spectrum and pressed into disks under vacuum using a Spectra Lab Pelletiser, which was then scanned by using an FTIR spectrophotometer (FT/IR-4100; Shimadzu-Japan) with a resolution of

4 cm1 between 4000 and 500 cm1. We analyzed shifts in peak maxima in different regions of the spectra [18].

2.4.4 X-Ray Diffraction Method Analysis (XRD)

The specimen was formed into 1 cm-diameter and 1-2 mm-thick discs and scanned with an X-ray diffractometer lab XRD-600 (Shimadzu-Japan) at a wavelength of 1.54056 A^0, a step size of 0.02, and a scanning rate of 2^0/min X-ray beam at room temperature. We measured the intensity of scattering as a function of the scattering angle [19].

2.4.5 Field emission scanning electron microscopy (FE-SEM)

Dehydrate samples were dried on a slide and then coated with gold at 50 nm in a PE-5000 sputter coater. At a detector angle of 00, a Scanning Electron Microscope- S-4160 (Japan) was used to view the specimen. Fields were photographed randomly, mounted on well-contrast negative films, and projected using a slide projector [20].

2.5 Antimicrobial activity of silver nanoparticles by well-diffusion assay (WDA)

AgNPs were used to examine their anti-microbial activity against seven human clinical isolate pathogens (*Staphylococcus Haemolyticus, Staphylococcus aureus, Proteus mirabilis, Escherichia coli*, *Enterococcus faecalis*, *Acinetobacter baumannii,* and *Candida albicans*). The impact of silver nanoparticles was investigated using the agar-well diffusion method. Müller-Hinton agar medium was sterilized, cooled, and then poured into sterilized Petri dishes and set aside at room temperature to solidify. The bacterial loads of overnight cultures were maintained at 1.5×10^8 CFU/mL according to McFarland standards. We used sterile cotton swabs to transfer and spread the test microorganism onto the agar medium, followed by creating 5 wells in the agar using a sterile gel puncture with a diameter of 4 mm. After that, various concentrations (8, 16, 32, 64, 128, 256, 512, and 1024 µg/ml) of AgNPs were added to the wells and then incubated for 24 hours at 37 °C. An ultrasonic cleaner device dissolved the AgNPs in deionized, sterile water. We measured the inhibition zones in millimeters after incubation to determine the activity of silver nanoparticles.

3. Results and Discussion

3.1 Isolation and Identification of *Pseudomonas aeruginosa* and UTI pathogens

We conducted the preliminary identification of *P. aeruginosa* on MacConkey agar, where it manifests as a pale colony (lactose non-fermenter). On nutrient agar, the isolate demonstrated growth at 42 °C and emitted a sweaty grape odor. **Figure 1** shows that *P. aeruginosa* is different from other Pseudomonas species because it can grow at high temperatures, make the color pyocyanin, and grow on cetrimide agar, which is a selective or differential medium with 0.03% cetrimide to stop microorganism growth. These characteristic colonies resembled those observed by [21], which formed spherical mucoid smooth colonies on nutrient agar without fermenting lactose. It also released a sweaty grape odor.

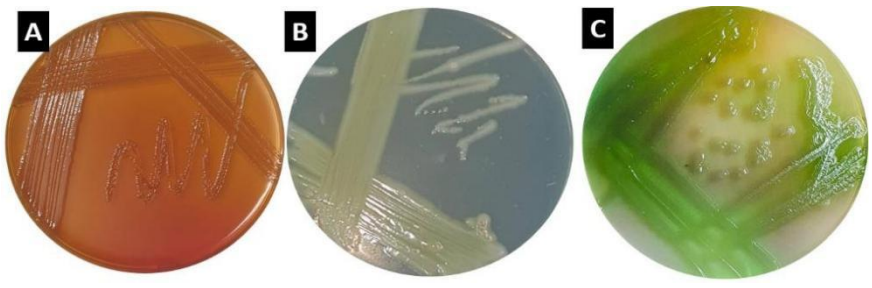

Figure 1. Culture media of *p. aeruginosa* on (A) MacConkey agar (B) cetrimide agar (C) nutrient agar

The biochemical tests in **Table 1** and **Figure 2** showed that the isolates were positive for oxidase, urease, and catalase. The IMViC tests showed that they were negative for indole, methyl red (MR), and voges-proskauer (VP), and they could use citrate as their only carbon source, which is similar to what was said by [22]. Macconkey, Mannitol Salt, Sabouraud Dextrose Agar, and the vitek2 system identified UTI pathogens.

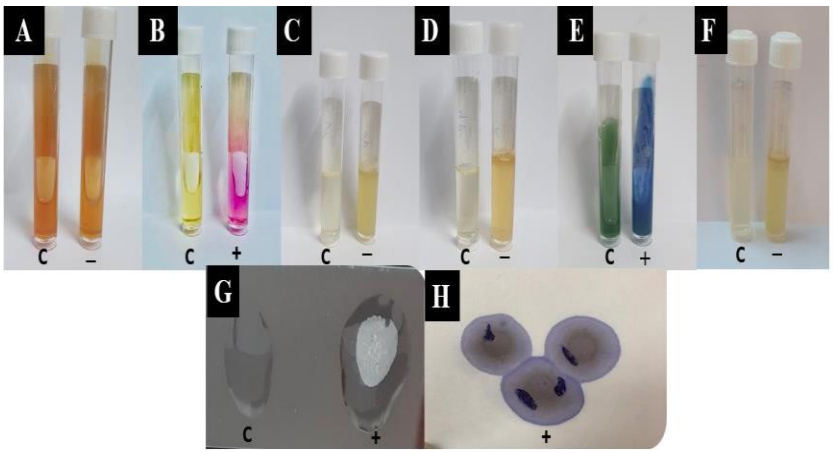

Figure 2. Biochemical tests of *p. aeruginosa* (A) Triple Sugar Iron (B)Urease test (C) Voges- Proskauer (D) Methyl red test (E) Citrate test (F) Indole test (G) Catalase test (F) Oxidase test.

Table 1. Results of biochemical test for *P. aeruginosa* isolates.

Biochemical tests	Result
Oxidase production	+
Triple Sugar Iron	–
Catalase production	+
Indole production	–
Voges- Proskauer	–
Methyl red	–
Citrate utilization	+
Urease production	+
Lactose fermentation	–

3.2 Characterization of silver

3.2.1 Nanoparticles Atomic force microscopy (AFM) analysis

Results from this research demonstrated that the biosynthesized AgNPs by *P. aeruginosa* had an average diameter (46.15) nm, as shown in **Table 2** and **Figure 3**. According to [23], we calculated the average particle diameter in nanoscale size, and the average size range of the synthesized silver nanoparticles falls between 5 and 45 nm. In contrast, the average particle size is 68.13 nm, according to [24].

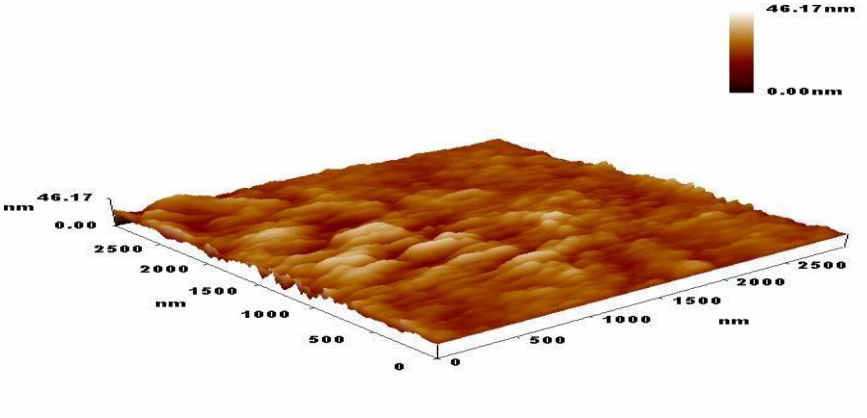

Figure 3. 3D image AFM of biosynthesized AgNPs.

Table 2. Estimation size of AgNPs biosynthesis by *P. aeruginosa* measured by AFM technique.

Sample: Sample Name	**Code:**Sample Code
Line No.:lineno	**Grain No.:**3899
Instrument:SPM	**Date:**2022-10-4

Avg. Diameter:46.15 nm	**<=10% Diameter:15.00 nm**
<=50% Diameter:40.00 nm	**<=90% Diameter:75.00 nm**

Diameter(nm) <	Volume(%)	Cumulation(%)	Diameter(nm) <	Volume(%)	Cumulation(%)	Diameter(nm) <	Volume(%)	Cumulation(%)
10.00	0.77	0.77	65.00	4.64	81.46	120.00	0.46	98.59
15.00	4.15	4.92	70.00	3.57	85.02	125.00	0.33	98.92
20.00	6.31	11.23	75.00	3.23	88.25	130.00	0.21	99.13
25.00	7.77	19.00	80.00	2.51	90.77	135.00	0.13	99.26
30.00	9.05	28.06	85.00	1.67	92.43	140.00	0.23	99.49
35.00	10.46	38.52	90.00	1.67	94.10	145.00	0.13	99.62
40.00	9.16	47.68	95.00	1.08	95.18	150.00	0.10	99.72
45.00	9.52	57.19	100.00	0.80	95.97	155.00	0.18	99.90
50.00	7.64	64.84	105.00	0.77	96.74	160.00	0.03	99.92
55.00	6.10	70.94	110.00	0.64	97.38	170.00	0.05	99.97
60.00	5.87	76.81	115.00	0.74	98.13	190.00	0.03	100.00

3.2.2 UV-Visible Spectrophotometer (UV-Vis)

Analysis of UV-vis spectroscopy of biosynthesized AgNPs illustrated in **Figure 4** revealed a peak with maximum absorbance at 454 nm. A similar study found the highest absorption peak at 429 nm [25]. AgNPs interact strongly with specific light wavelengths due to their unique optical characteristics.

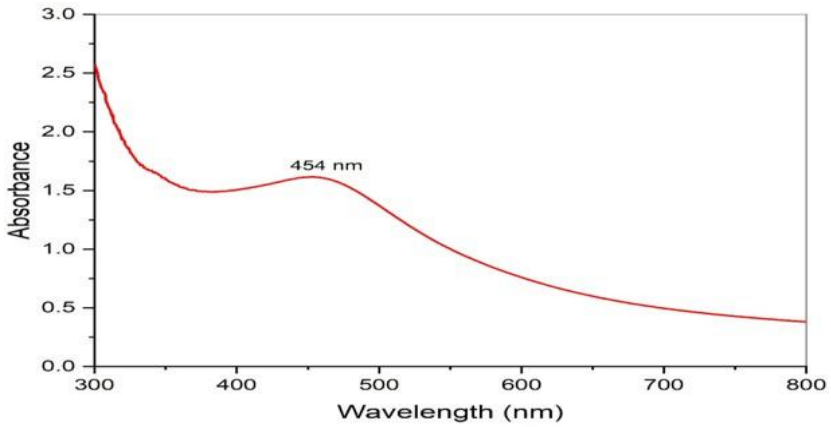

Figure 4. UV−visible absorption spectrum of AgNPs.

3.2.3 Fourier Transform IR Analysis (FTIR)

From a Fourier transform infrared analysis of biosynthesized AgNPs **Figure 5** showed the presence of 15 bands, namely at (3847.15, 3738.61, 3671.03, 3265.99, 29181.15, 2850.37, 2355.65, 1738.32,1645.44, 1536.15,1136.31, 1076.67, 811.42, 668.51, 544.07) cm−1. The band is

around 3847.15–3738.61 cm1 due to the peptide linkages' N-H stretch vibrations or the carboxylic acid's hydroxyl group. The peaks 3671.03 and 3265.99 cm1 correspond to O-H stretching vibration, indicating the presence of alcohol and phenol. The peaks at (29181.15, 2850.37) cm1 correspond to the stretching of C-H aromatic compounds. We assigned the band at 1738.32 cm1 to represent C-C stretching (non-conjugated). Stretching of carbonyl groups (C=O) is attributed to the peak at 1645.44 cm1, whereas bands in spectrum 1536.15 and 1076.67 cm1 were assigned for N-H and C-N (amines) stretch vibrations of the proteins, respectively, and at 544.07 cm1, it corresponds to C-Cl stretching in the alkyl group. These peak results are similar to those in [26].

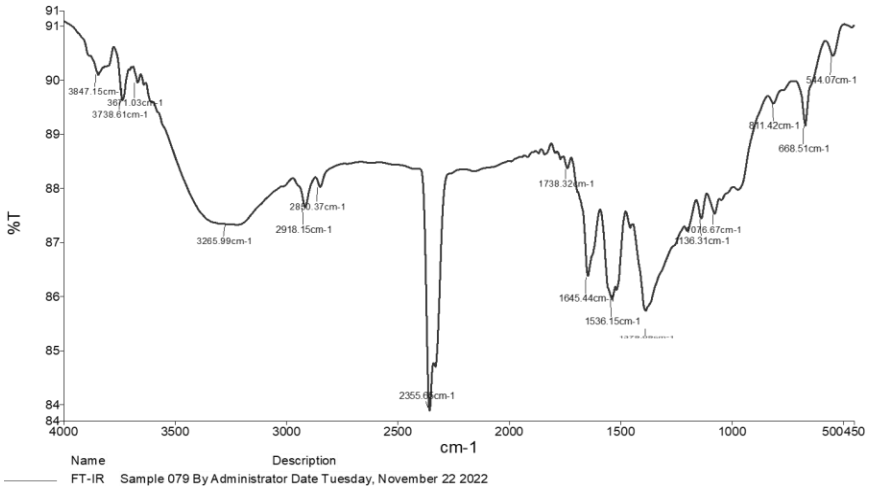

Figure 5. FTIR of AgNPs synthesized by *P. aeruginosa*.

3.2.4 Field emission scanning electron microscopy (FE-SEM)

The FE-SEM image used to investigate the morphological properties of AgNPs, as shown in **Figure 6**, with particle dimensions ranging between 19.00 and 23.72 nm, is aggregated into spherical to hexagonal shapes. It was observed that the shapes of the samples ranged from hexagonal to spherical and illustrated a lower agglomeration degree. This image is similar to [27] in that the metal particles had a spherical shape and was evenly distributed.

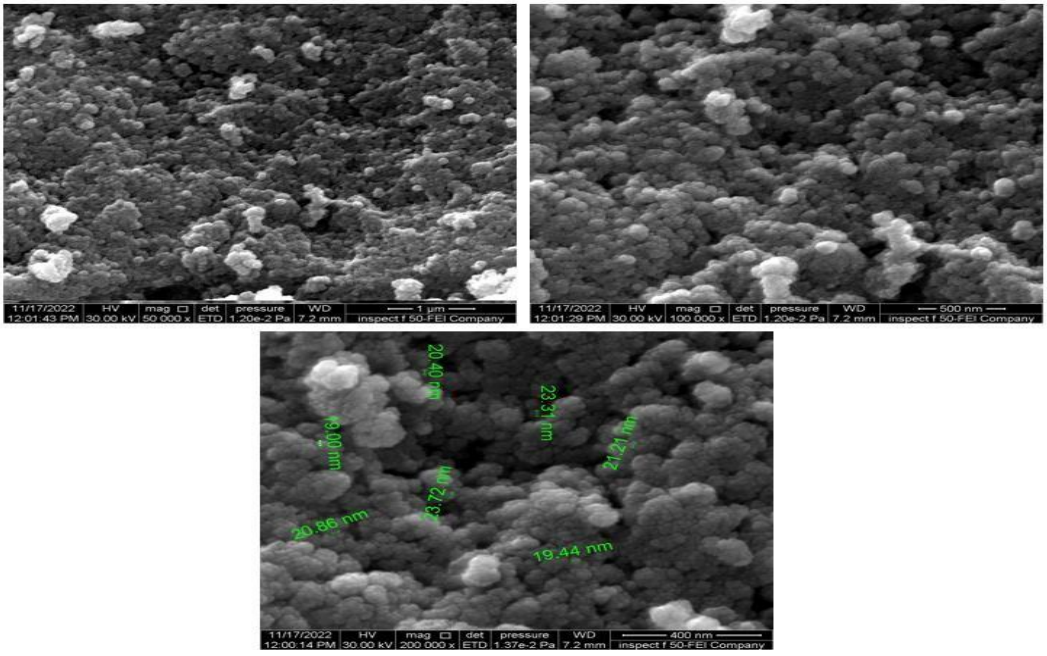

Figure 6. FE-SEM image of silver nanoparticles synthesized by *P. aeruginosa* (50000X) (100000X) (200000X).

3.2.5 X –ray Diffraction (XRD)

As shown in **Figure 7** from 20^0 to 80^0, diffracted intensities were measured, and lattice planes were observed of (100), (002), (101), (102), (110), (103), and (112), which corresponded to the 2θ values of 27.87°, 32.03°, 46.57°, 54.91°, 57.53°, 67.89°, and 77.15°, respectively. The organic compounds in the extract, which reduce the amount of silver ions and stabilize them, are responsible for these peaks, and the results align with previous research [28].

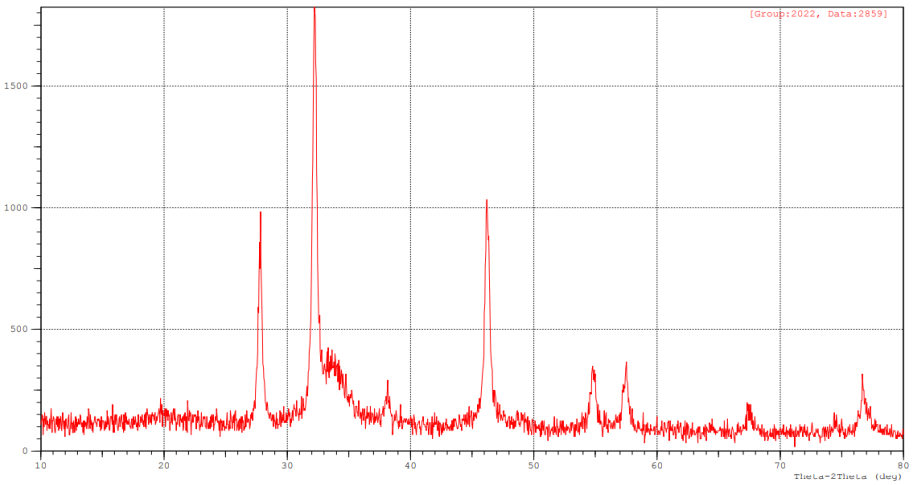

Figure 7. XRD pattern of AgNPs synthesized by *P. aeruginosa.*

3.3 The antimicrobial activity of silver nanoparticles against UTI pathogenic microorganisms

The results of silver nanoparticles against UTI isolate are shown in **Figure 8** and **Table 3.** It was found that the strongest inhibition zone for all test microorganisms was at a concentration (1024 µg/ml), which was 22 mm for *C. albicans* and *S. haemolyticus*, 18 mm for *S. aureus*, and 12 mm for *A. baumannii*, 10 mm for *E. faecalis*, and 8 mm for *P. mirabilis*. It was found that the strongest inhibition zone for all test microorganisms was at a concentration (1024 µg/ml), which was 22 mm for *C. albicans* and *S. haemolyticus*, 18 mm for *S. aureus*, and 12 mm for *A. baumannii*, 10 mm for *E. faecalis*, and 8 mm for *P. mirabilis*. These results is approximately similar as mentioned by [29, 30].

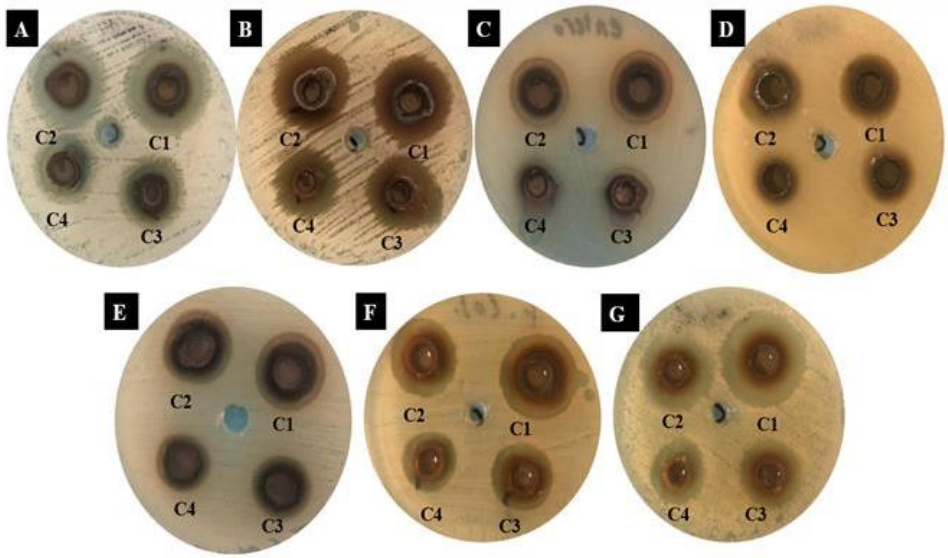

Figure 8. Effect of silver nanoparticles on UTI pathogenic microorganisms (A) *S. haemolyticus* (B) *S. aureus* (C) *E. faecalis* (D) *P. mirabilis* (E) *A. baumannii* (F) *E. coli* (G) *C. albicans*

Table 3. The effect of melanin and silver nanoparticles on UTI pathogenic microorganisms.

Type of organism	C1=1024 µg/ml	C2=512 µg/ml	C3=256 µg/ml	C4=128 µg/ml	C5=64 µg/ml	C6=32 µg/ml	C7=16 µg/ml	C8=8 µg/ml
C. albicans	22 mm	18 mm	10 mm	8 mm	8 mm	8 mm	4 mm	2 mm
S. haemolyticus	22 mm	20 mm	10 mm	8 mm	2 mm	2 mm	2 mm	2 mm
S. aureus	18 mm	18 mm	12 mm	10 mm	8 mm	8 mm	6 mm	2 mm
E. faecalis	10 mm	10 mm	6 mm	4 mm	4 mm	4 mm	0 mm	0 mm
E. coli	18 mm	14 mm	10 mm	8 mm	0 mm	0 mm	0 mm	0 mm
P. mirabilis	8 mm	8 mm	4 mm	2 mm	0 mm	0 mm	0 mm	0 mm
A. baumannii	12 mm	12 mm	6 mm	4 mm	2 mm	2 mm	2 mm	0 mm

4. Conclusions

The biosynthesis of silver nanoparticles (AgNPs) by *P. aeruginosa* is effective against UTI pathogenic microorganisms that are resistant to multiple drugs. Researchers have concluded that AgNPs possess antibacterial properties. Researchers report that the silver nanoparticles inhibit the growth of pathogenic Gram-positive and Gram-negative bacteria, as well as *C. albicans*.

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Conflict of Interest

There is no conflict of interest.

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