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Green Synthesis of Ag Nanoparticles Using *Musa Acuminata* Fruit Peels and Determination of Its Inhibitory Effect on Pathogenic Bacteria

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ABSTRACT

Background: Creation of biocompatible compounds using nanotechnology as anticancer agent, on other hand, were highly effective against MCF-7 breast cancer cells and had reasons of cell death, making it one of the novel approaches in field of cancer therapy. **Aim:** Impact of Ag nanoparticles using *Musa acuminata* on pathogenic bacteria.

Methods: 125 samples were collected with various infections. All necessary examinations for preliminary diagnosing of isolated bacteria were carried out and confirmatory diagnosis of these isolates by the Vitek- 2 system. Biosynthesis of silver nanoparticles was conducted via green methods by using *Musa acuminata* alcoholic extracts, and properties of silver nanoparticles were defined by spectrophotometer.

Results: The largest Ag NPs 75 nm for alcoholic *Musa acuminata* extract and FTIR analysis showed presence of flavonoids and tannins in abundance in *Musa acuminata* fruits, using an agar diffusion assay, silver nanoparticles biosynthesized from *Musa acuminata* isolates demonstrated antimicrobial efficacy against *E. coli*, *S. epidermidis*, *K. pneumoniae*, *S. aureus* and *P. aeruginosa*. At 12,5 mg/ml Ag NPs, it was shown that growth of these bacteria was suppressed. Concentrations of nanoparticles (12,5, 25, 50, 100 and 200 mg/ml) exhibited greatest inhibitory effects on *S. aureus* (20 mm), *E. coli* (18 mm), *K. pneumonia* (20 mm), and *P. aeruginosa* (15 mm) and *S. epidermidis* (24 mm).

Conclusion: *Klebsiella pneumonia* was the most common infection in diabetic foot. Ag NPs could be used as potent nanomedicines that are ideal substitutes for crude extract in treatment of diabetes mellitus.

Keyword: *Musa acuminata*, Bacterial, Diabetic Infection, Ag nanoparticales.

INTRODUCTION

Antimicrobial resistance has become alarming in recent years and poses a serious global public health threat according to the World Health Organization (WHO). The prevalence of multidrug resistant (MDR) strains has created significant difficulties in the treatment of burn infections. Multidrug-resistant bacterial infections are becoming a serious problem around the world necessitating an immediate search for alternatives. The emergence of MDR isolates has complicated antibiotic efficacy even further reducing therapeutic options in health services as a result increasing medical costs, morbidity and mortality rates [(1)]. The high efficacy and therapeutic index of nanotechnology against microbes makes it a promising therapeutic approach [(2)].

Nanoparticle have the potential to become a vital viable therapeutic option for treating drug-resistant infections. They are viewed as potential replacements and supplements to existing antimicrobials [(3)].

Silver nanoparticles (Ag NPs) have become a popular class of nanomaterials for various commercial and medical uses. One of the unique methods in the field of cancer therapy is the development of biocompatible molecules employing nanotechnology as an anticancer agent on the other hand were particularly efficient against MCF-7 breast cancer cells and have diverse causes of cell death [(4)].

MATERIALS AND METHODS

Collection of *Musa acuminata* samples: *Musa acuminata* fruit used in this research was collected from Baaquba market in Diyala. An appropriate amount of banana peel drying under sunlight irradiation for a certain period. Then, cutting it into very small pieces. For preparing plant extract, the banana peel is washed with ethanol then 25 g of peel pieces are added to a glass flask containing 300 ml of the ethanol under a magnetic stirrer for 180 min and temperature heat for 80 ^{0}C. After that a yellow extract was obtained and the precipitation was separated by filter paper. The plant extract is saved in the refrigerator [(5)].

Collection of specimens

Several 125 specimens were obtained from Baaquba Teaching Hospital in Diyala province from October 2021 to February 2022 from people suffering from various illnesses. The sources of samples included diabetic foot ulcers. These samples underwent tests like catalase and oxidase as well as culturing in ready-made media like Blood agar, Brain Heart Infusion broth (BHIB), MacConkey agar, Mannitol Salt agar, Muller-Hinton agar, Nutrient agar, and Nutrient broth. By autoclaving, the culture media were made sterile. After being prepared and kept at 4°C until they were used, they were incubated for 24 hours at 37°C to ensure their sterility. By adding fibrinogen plasma, the media can also detect coagulase action. [(6)].

Antibiotic Discs

Antibiotic discs that were used in this study to test the antimicrobial sensitivity by the disc diffusion method were listed in table (1) according to (Clinical and Institute, 2021).

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Table (1): The study's antibiotic disk

Antibiotic	Symbol	Concentration0 µg	company0	country0
Amikacin	AK	30	Mast	UK
Aztreonam	ATM	30	Mast	UK
Cefepime	CPM	30	Mast	UK
Chloramphenicol	C	30	Mast	UK
Ciprofloxacin	CIP	5	Mast	UK
Clarithromycin	CLA	15	Mast	UK
Clindamycin	CD	2	Mast	UK
Gentamicin	G.M.	10	Mast	UK
Imipenem	IMI	10	Mast	UK
Levofloxacin	LEV	5	Mast	UK
Meropenem	MEM	10	Mast	UK
Nitrofuration	NIT	300	Mast	UK
Ofloxacin	OFX	5	Mast	UK
Rifampicin	RIF	5	Mast	UK
Streptomycin	S	10	Mast	UK
Tetracycline	T	30	Mast	UK
Trimethoprim	TM	5	Mast	UK
Trimethoprim, Sulfamethoxazole	STX	1.25/23.75	Mast	UK
Vancomycin	VA	30	Mast	UK

Biosynthesis of Ag NPs from extract *Musa acuminata*

Three grams of silver nitrate were used in the biosynthesis of Ag NPs, and they were dissolved in 40 ml of ethanol with a magnetic stirrer for 15 min. After that, 80 ml of plant extract were added, and the mixture was stirred for an additional hour. The solution turns red after a while, and after 24 hours, we get a black or grey precipitation that is repeatedly washed and rinsed with ethanol and distilled water. then 4 hours of drying at 60 °C [7, 8].

Characterization of Silver Nanoparticles

Using an X-ray diffractometer pattern analysis of Ag NPs was carried out (XRD). Emission Scanning Electron Microscopy was used to examine the morphology and particle size of the samples, while Fourier Transform Infrared Spectroscopy (FTIR) and a UV-Visible Spectrophotometer were used for molecular analysis. The shape topography and volume distribution of biosynthesized nanoparticles are described using atomic force microscopy. (AA-3000, Shimadzu Japan).

Antibacterial activity of Ag NPs in vitro

Prepared and poured into one petri dish was Muller-Hinton agar. All hospital-isolated bacteria (gram-positive and gram-negative) were streaked into dishes from the broth after solidifying, and then they were cultured. To ensure proper distribution of Ag NPs after the culturing, five wells were drilled into the agar but left unconnected to the plate's bottom. The well was injected with 100 µl of each Ag NPs dilution. Five concentrations were made from the stock: 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/ml. After making each concentration, the dish was sealed and placed in an incubator overnight at 37 °C to be read the following day.

Ethical approval: Ethical approvals were obtained from the Scientific Research Ethics Committee in the College of Science, Diyala University. **This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.**

RESULTS AND DISCUSSION

One hundred twenty-five clinical isolates were from different bacteria genera. The number of specimens according to their source is shown in table (2). The findings demonstrated that the isolation rate of *Escherichia coli*, *Klebsiella*, *pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis* from diabetic foot ulcers were the positive isolates with a total number of 76 divided as follows: 9 (12%), 24 (32%), 12 (16%), 21(28%), and 10 (13%) respectively.

Table (2): The number of isolates for all bacteria

Isolate	No. (%) of diabetic foot ulcers isolates
Escherichia,coli	**(%12) 9**
Klebsiella,pneumonia	**(%32) 24**
Pseudomonas,aeruginosa	**(%16) 12**
Staphylococcus aureus	**(%28) 21**
Staphylococcus epidermidis	**10 (13%)**

Characterization of Silver Nanoparticles (Ag NPs)

Utilizing X-ray diffraction analysis, the crystallinity and average particle size of the biosynthesized Ag nanoparticles were determined. Figure (1) showed the XRD pattern of Ag_2O NPs prepared by green synthesis the strongest peaks at 28.13°, 32.54°, 38.34°, 46.48°, 55.1°, 57.7°, 76.9° corresponding to the planes (110), (111), (200), (211), (220), (221), and (311), respectively. The structure of Ag_2O NPs. These peaks are in agreement with JCPDS Card No: (00-012-0793).

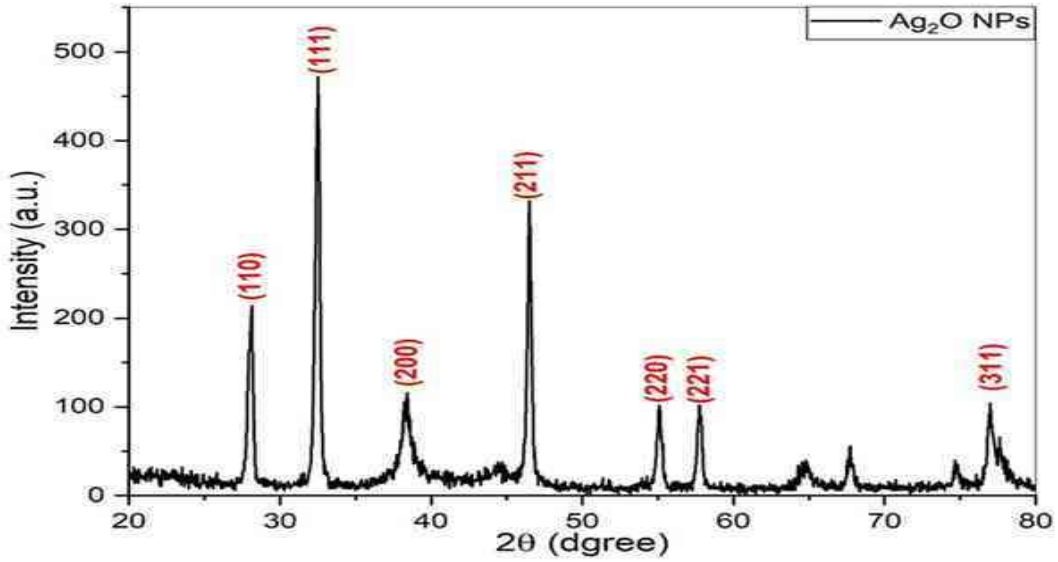

Figure (1): XRD pattern of Ag NPs prepared by green synthesis

As we can see, the peaks of the figures gave us indication for the cubic structure of Ag NPs. By using eq., the mean crystallite size of the structure has been calculated at about 27 nm, as shown in table (3).

Tables (3): The crystallite size of Ag NPs Prepared by green syntheses

2 theta (degree)	FWHM (deg)	2 theta (Rad.)	FWHM (Rad)	D (nm)
28.1349	0.1968	0.246	0.003	41.599
32.5492	0.3149	0.284	0.005	26.271
38.3444	0.6298	0.335	0.011	13.349
46.4846	0.2755	0.406	0.005	31.371
55.1087	0.3149	0.481	0.005	28.445
57.7715	0.3149	0.504	0.005	28.802
76.9592	0.4809	0.672	0.008	21.134

The AFM 3D images showed that silver nanoparticles were formed in a uniform distribution and no accumulation was seen. Ag NPs particle size distribution is shown by AFM as granularity accumulation distribution. The average particle diameter of the created silver nanoparticles is 65, 75, and 85 nm at 8.10, 7.75, and 7.95, respectively. Figure (2) showed that the synthesized silver nanoparticles particle diameter ranged from 35 to 125 nm. This large diameter may be the result of the particles' sedimentation, which causes their absorbance to decrease. As a result, the extract from *Musa acuminata* leaves provided the best AgNO3 concentration (Molarity) for the synthesis of Ag NPs. 75 nm was the average diameter Table (4).

Table (4): The size of the Ag NPs produced by Musa acuminate extract

Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumuaction (%)	Diameter (nm)<	Volume (%)	Cumulation (%)
35	3.20	3.20	65	8.10	7.55	95	8.50	4.1
45	4.90	4.50	75	7.75	7.60	105	7.15	3.64
55	9	9	85	7.95	7.90	125	4.4	2.35

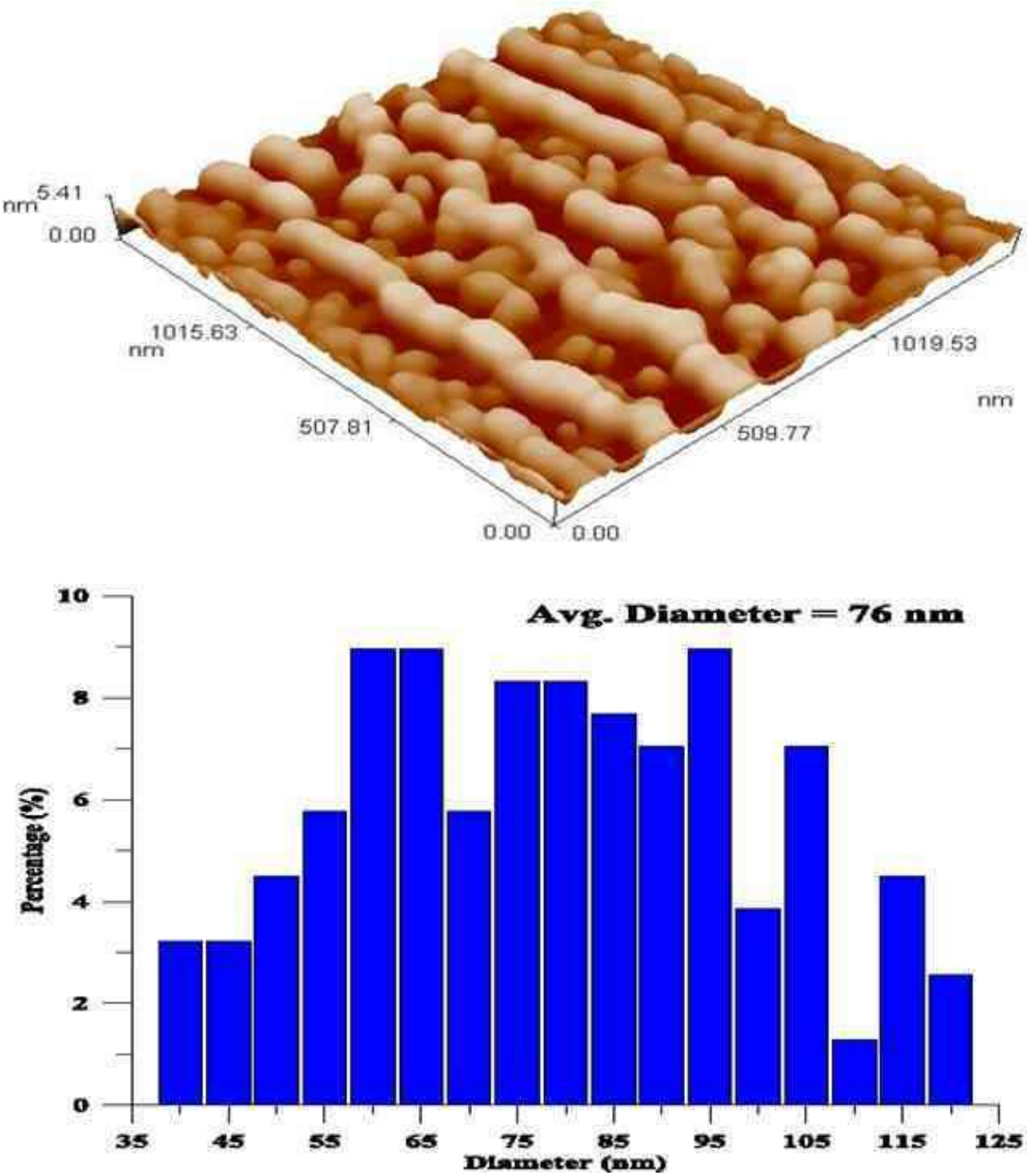

Figure (2): AFM images with the nanoparticles size distribution of Ag NPs synthesized using *Musa acuminata* extracts.

Scanning electron microscopy (SEM) is a type of electron magnifying lens that takes images of a sample while using an active electron to examine it. It was possible to see the size, shape, and distribution of green synthesized silver nanoparticles using a scanning electron microscope. They created 30-70 nm Ag nanoparticles. Figure (3) showed that the particles were spherical with a smooth surface area with the size of Ag NPs as follows: (104.63, 55.83, 63.04, 89.90, 94.32, 73.07, 97.18, 56.56, 49.58, 94.08, 76.08, 99.16, 64.65, 75.26, 65.41, 121.3, 77.63, 66.13, 196, and 48.32 nm) average size was 83.40.

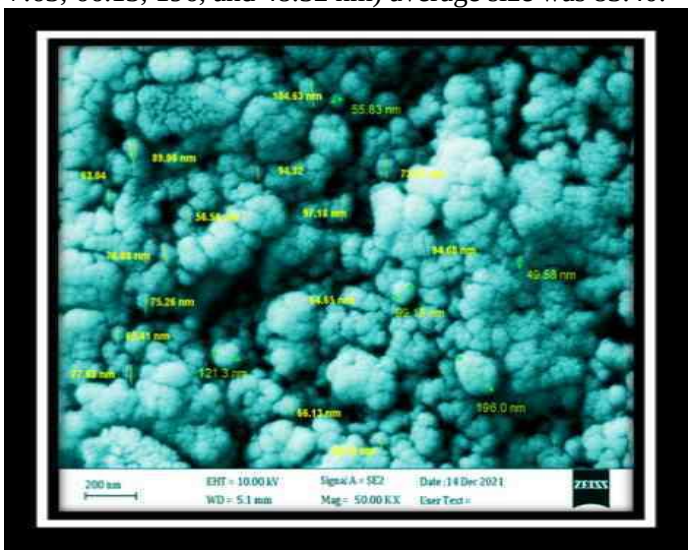

Figure (3): The SEM images of Ag nanoparticles

The Ag NPs significant characteristics can be found in their absorption character spectra. The UV-Visible spectra were a good method for characterizing Ag NPs formation and production and were very helpful for Ag NPs analysis. Figure (4) Showed the UV-visible spectrum with the peak of the Surface Plasmon Resonance (SPR) at 426 nm, which corresponds to Ag_2O NPs. This wavelength described the color of Ag_2O NPs, which is close to dark brown [9].

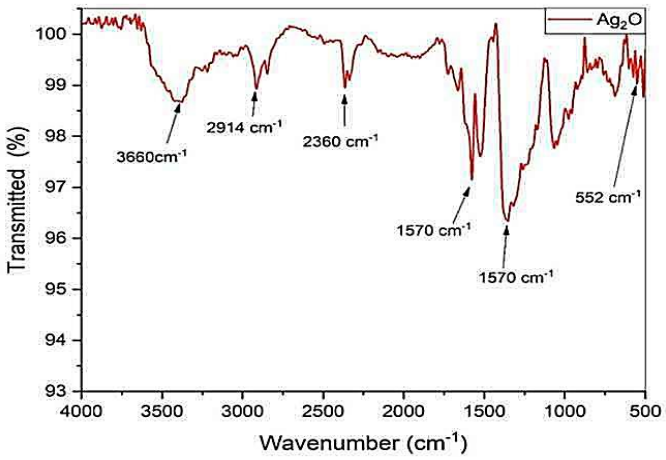

Figure (4): Uv-Visible spectroscopy of Ag NPs prepared by green syntheses.

Fourier transform infrared spectroscopy (FTIR) of Ag_2O NPs has been analyzed as shown in figure (5), the FTIR technique is based on the mode of atoms vibration and gives us information about the surface chemistry of Ag_2O NPs. The functional group of Ag_2O NPs prepared by green synthesis showed multi-active bonds. The range of the FTIR spectrum from 500 – 4000 cm^{-1} showed the bands at 3493 cm^{-1} refer to the alcohol stretching (OH group) and 2912 cm^{-1} assigned to the methylene bonds (CH group). According to other studies metal-oxygen stretching frequency normally appears at 500-600 cm^{-1}. Thus in this Figure weak bands appear at 552 cm^{-1} this result was close to [10]. referring to Ag-O vibration and coordinated water as Ag_2O NPs. Silver nitrate and phytochemical elements of the leaf extract are responsible for the other bands.

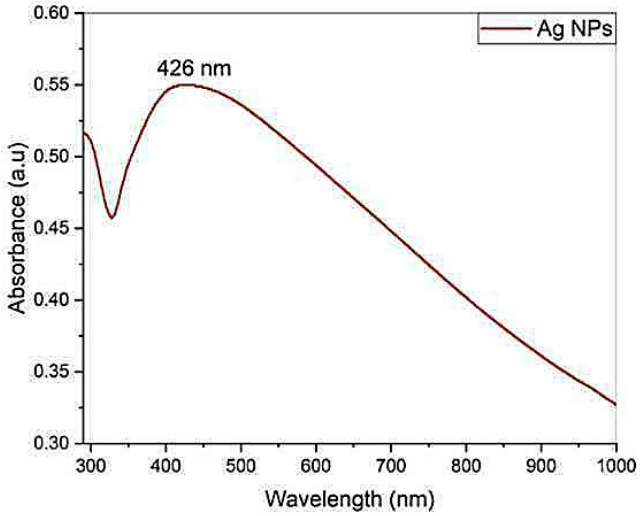

Figure (5): the Ag NPs FTIR spectrum following green synthesis.

Ag NPs' antibacterial activity against pathogenic bacteria

Using an agar well diffusion assay silver nanoparticles biosynthesized from *Musa acuminata* isolates demonstrated antimicrobial efficacy against the MDR bacteria *E. coli*, *S. aureus*, *S. epidermidis*, *K. pneumonia*, and *P. aeruginosa* isolates.

Silver nanoparticles showed the highest diameter of inhibition zone at concentration of 200 mg/ml of *S. aureus*, *S. epidermidis*, *P. aeruginoas*a, *K. pneumonia* and *E. coli* reaching 20, 24, 20, 15, 20 and 18 mm respectively, while the Ag NPs recorded at concentration 12.5 mg/ml lowest areas of inhibition zone against the same isolates reaching 18, 15, 11, 14 and 12 mm respectively as shown in table (5) and figures (6), (7), and (8). These results were close to **Singh *et al.*** [11].

Table (5): Antibacterial activity of Ag NPs on bacterial growth

Isolates	Average of inhibition zone diameter(mm)				
	Concentration 12.5 mg/ml	Concentration 25 mg/ml	Concentration 50 mg/ml	Concentration 100 mg/ml	Concentration 200 mg/ml
E. coli	12	14	17	17	18
K. pneumonia	14	15	16	17	20
P. aeruginosa	11	12	13	14	15
S. epidermidis	15	17	17	18	24
S. aureus	18	18	18	19	20

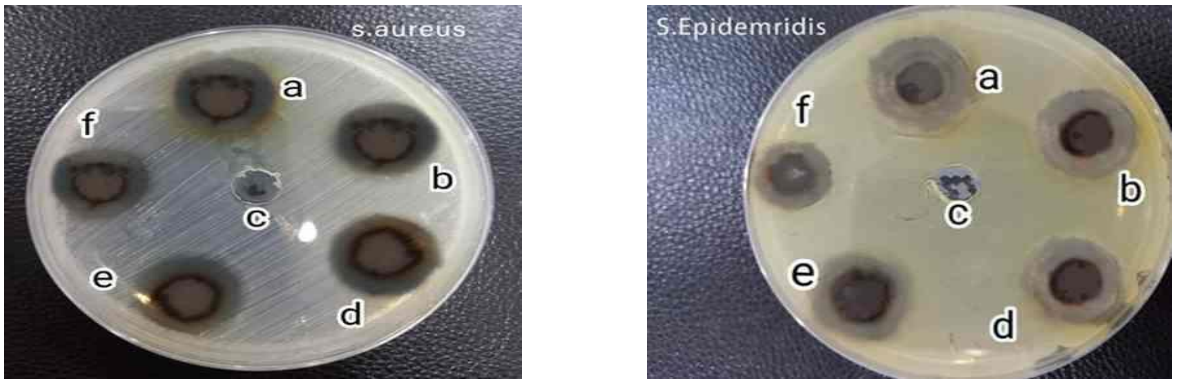

Figure (6): Ag NPs antibacterial activity by agar well diffusion method against *S. aureus* and *S. epidermidis*. The concentrations (200, 100, 50, 25 and 12.5) mg/ml are denoted by the letters (a, b, d, e, and f), respectively (control).

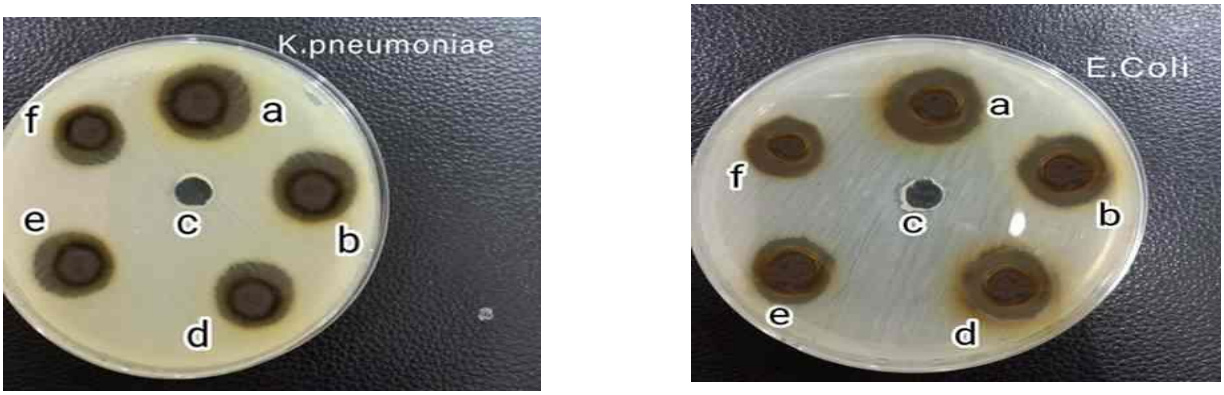

Figure (7): Ag NPs' antibacterial activity by the agar well diffusion method against *K. pneumonia* and *E. coli*. The concentrations (200, 100, 50, 25 and 12.5) mg/ml are denoted by the letters (a, b, d, e, and f), respectively (control).

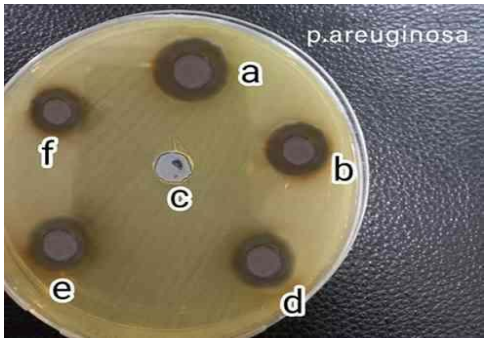

Figure (8): Ag NPs' antibacterial activity against *P. aeruginosa* as measured by the agar well diffusion method. The concentrations (200, 100, 50, 25 and 12.5) mg/ml are denoted by the letters (a, b, d, e, and f), respectively (control).

CONCLUSIONS

In our research, we discovered that *Klebsiella pneumonia* was the most common infection in diabetic foot patients. The silver nanoparticles' identity was confirmed by X-ray diffraction (XRD) analysis; the mean crystallite size of the structure was estimated to be about 27 nm for Ag NPs.

The application of highly regarded medicinal plants to steer the green synthesis of nanoparticles that acquire a variety of pharmacological properties is driven by the current excitement around the world in the use of eco-friendly and affordable resources. As a result, Ag NPs were created and examined using XRD, FT-IR, UV, AFM, and SEM. Additionally, the idea behind creating these nanoparticles was to combine green synthesis, which enhances biological properties, with a natural plant that is non-toxic and chemically inert, as well as to increase the drug's surface area to produce a more potent and effective therapeutic effect.

Therefore, it is advised that the Ag NPs is more potent than the plain extract, in comparison. Ag NPs could be used as potent nanomedicines that are ideal substitutes for the crude extract in the treatment of diabetes mellitus. But more research into pharmacokinetics is required.

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