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Studying the Effect of Silver Nanoparticles (AgNPs) Synthesized from Coriander Plant on The Biofilm of *Pseudomonas aeruginosa* Isolated from Burns

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ABSTRACT

The AgNPs used in this study were synthesized from silver nitrate using coriander plant extract as a reducing agent, and the successful formation of silver nanoparticles was confirmed by UV-vis spectrophotometer analysis and by FE-SEM and transmission electron microscopy TEM, X-ray diffraction (XRD) and energy dispersive X-ray analysis (EDX) to characterize and confirm the formation of crystalline nanoparticles. The synthesized nanoparticles showed strong activity against the biofilm formation of *Pseudomonas aeruginosa*.

INTRODUCTION

Nanoparticles can be prepared in three different ways: biological, chemical, and physical (Kango *et al*., 2013). Chemical and physical methods were prepared by toxic, costly and environmentally hazardous chemicals (Ahmed *et al*., 2016). While the biological method includes a variety of biological elements that can be harnessed for the synthesis of metal nanoparticles, these biological agents appear as environmentally friendly, clean, and non-toxic agents for the synthesis of metal nanoparticles (Singh, 2015). In this study, biotinylated silver nanoparticles with the advantages of C sativum in terms of its antimicrobial application. Coriander is used for problems in bacterial and fungal infections (Mandal and Mandal 2015).

MATERIALS AND METHODS

Bacterial swabs from burn infections of different ages of both sexes were collected for the period between first of October 2021 to the first of February 2022 from the Burn Hospital in the Medical City in Baghdad and private clinics in Samarra. After conducting morphological and biochemical tests, 20 samples were obtained. *Pseudomonas aeruginosa* was also diagnosed by studying the phenotypic characteristics of the bacteria by cultivating it on a solid MacConkey medium, as the colonies appeared in a pale color, due to its inability to ferment the sugar lactose (Forbes *et al*., 2007).

Preparation of Plant Extracts:

About 20 gm of coriander leaves were taken, washed well four times with de-ionized water to remove dust particles, and dried in the air at room temperature, then the leaves were ground into a fine powder and added to 100 ml of de-ionized water, and left for 20 minutes to boil. At 60ºC, after boiling, the leaf extract was cooled at room temperature, and filtered, and 75 ml of the yellow-transparent leaf extract was taken, which was stored at 4ºC in the refrigerator (Rhamah *et al*., 2021).

Preparation of Silver Nanoparticles:

Silver nanoparticles were prepared by dissolving 0.067 g of AgNO3 in 100 ml of deionized water. A 4 mM AgNO3 solution was made, stored in the dark to prevent oxidation, and used to prepare AgNPs. After that, 5 ml of coriander leaf extract was added to 45 ml of a solution. Silver nitrate, AgNO3 was placed on a heat plate device and a magnetic stirrer was used for an hour. After that, the color of the reaction mixture changed from transparent yellow to dark brown, indicating the formation of silver nanoparticles AgNPs. The AgNPs solution was collected and then placed in the centrifuge. For the purpose of sedimentation, the excess liquid was removed and the precipitate was taken by drying it in an electric oven at a temperature of 40ºC until it was completely dry and we obtained a silver nanopowder (Khan *et al*., 2018).

Detection of the Ability of *Pseudomonas aeruginosa* to form A Biofilm Using the Microtiter Plate Method:

The ability of *P. aeruginosa* bacteria to form a biofilm was revealed by following the method (Abdelraheem *et al*., 2020). in which the method of microtiter plate 96 was used.

1. BH broth was present in a large suspension of the heart and brain fluid, as the sample was diluted and compared with McFarland's standard solution, then incubated at a temperature of 37 ℃ for a period of 24 hours.
2. 200 µl of BHI broth containing 2% glucose without bacteria were placed in the first three wells of the calibration dish, and negative control was returned.
3. 150 µl of BHI broth containing 2% glucose were distributed into the holes of the microtiter plate, then the holes were inoculated with 50 microliters of the bacterial suspension prepared in the first section, after which three replications were made for each isolate.
4. Close the calibration dish, then cover it with Parafilm to prevent contamination, and incubate at a temperature of 37ºC for 24 hours. After fortification, the contents of the pits were removed, then washed three times with phosphate-buffered saline (PBS) with an acidity of 7.2 to get rid of bacterial cells, then left to dry at laboratory temperature. .
5. The attached live cells were fixed by adding 200 µl of absolute methanol to each hole and left for 15 minutes, then the contents of the holes were removed and left to dry.
6. 200 µl of Crystal Violet dye at a concentration of 1% was added to each hole and left for 20 minutes, after which the dye was discarded and washed three times with PBS to get rid of the remaining formula, then left to dry in laboratory temperature.
7. Add 200 µl of 96% ethanol to each well.
8. Optical Density (OD) was evaluated using the ELISA device and at Wavelength (630) nanometers.

Detection of the Effect of Silver Nanoparticles on Biofilm Formation of *Pseudomonas aeruginosa* by MTP Method:

1. BH broth was present in a large amount of BH broth, as the sample was diluted and compared with McFarland's standard solution, then incubated at a temperature of 37 ℃ for a period of 24 hours.

2. Addition of AgNPs silver nanoparticles At a concentration of 64 µg/ml.
3. The same steps that were previously used to detect the ability of bacteria to produce a biofilm.

RESULTS AND DISCUSSION

Characterization of AgNPs:

1. Ultraviolet-Violet Spectroscopy:

The formation of green AgNPs synthesis was demonstrated by the visible color change (light green to dark brown) after the completion of the reaction between the CS extract and silver nitrate. The resulting solution showed a maximum constant λ at 454 nm, confirming the orderly size and shape of the AgNPs synthesized as in Figure 1.

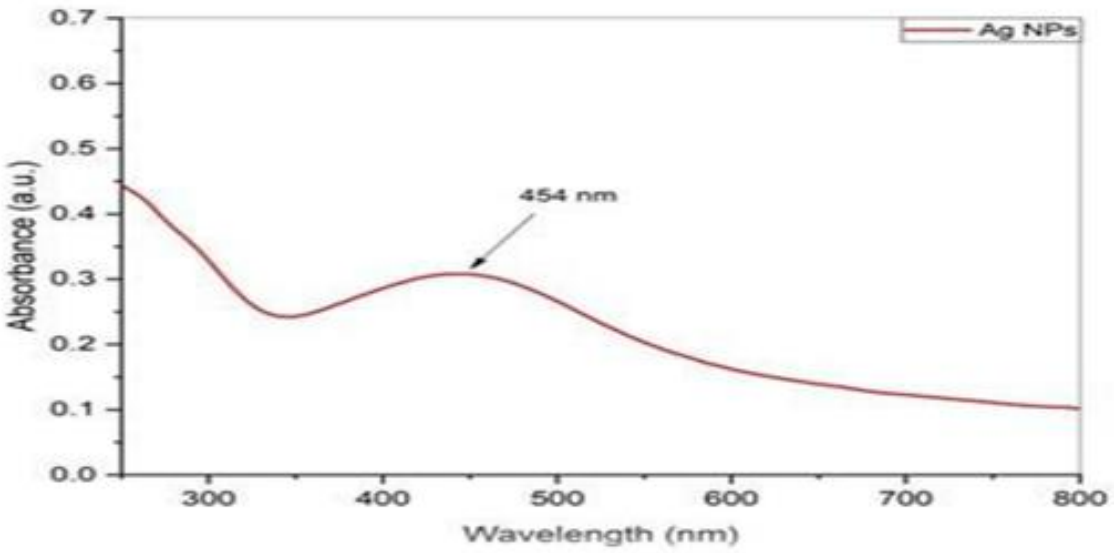

Fig. 1: UV-visible spectrum of AgNPs

2. Scanning Electron Microscopy (SEM):

The SEM image of the as-synthesized high-density green AgNPs confirmed the evolution of the silver nanostructures, as in the Figure 2. The SEM micrographs of the NPs obtained in the filter showed that the AgNPs were spherical in shape and well distributed in the solution without aggregation. The SEM images showed that the nanoparticles are spherically shaped and measure between 32.08 and 43.28 nm (Ibraheem *et al*., 2020).

Fig 2: FE-SEM scanning electron microscopy of AgNPs.

3. TEM Scanning Electron Microscopy:

To analyze the morphology and size distributions of silver AgNPs, TEM analysis was performed using a 77.500 kx magnification. The results of the enlarged image (Fig.3) showed that the majority of the NPs have spherical shapes at 250 nm.

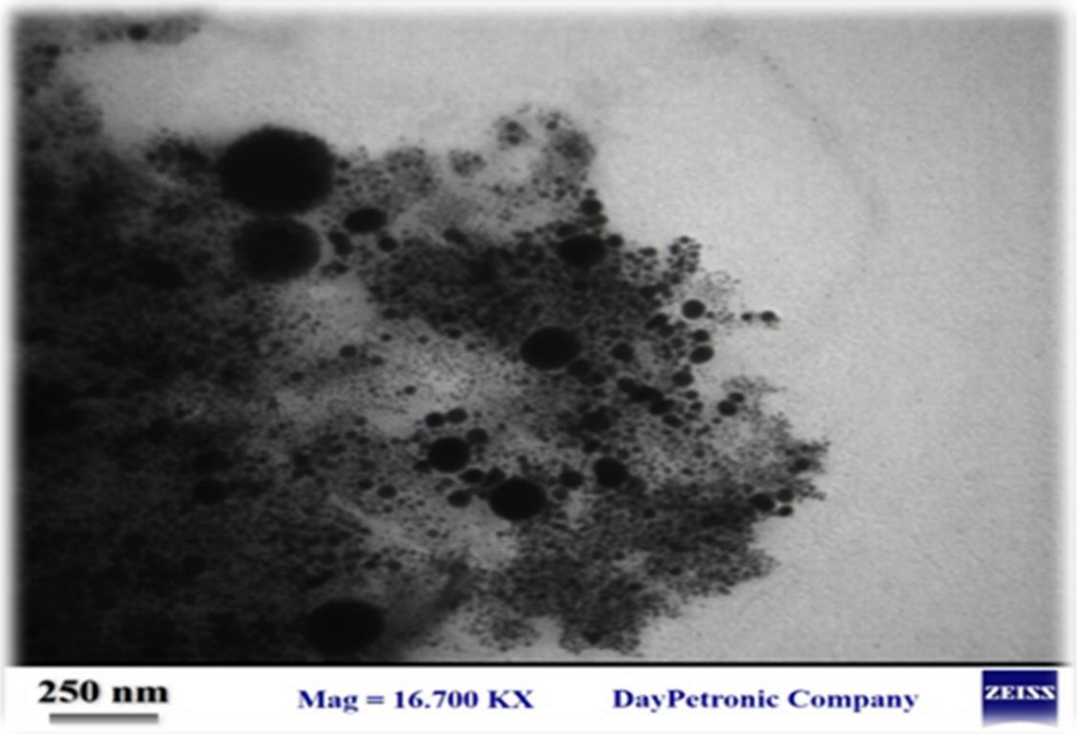

Fig. 3: AgNPs under TEM electron microscopy

4. X-ray Diffraction (XRD) Analysis:

The XRD pattern of AgNPs at 2θ showed four peaks, 32.12º, 38.04º, 46.21º, and 64.18º, corresponding to (101), (111), (200), and (220), respectively. The crystal size (D) of silver AgNPs was calculated using Scherrer equation (1) for the highest peak in the XRD pattern using K = 0.9 ($D = 0.9\lambda / \cos\theta$). The D of AgNPs is about 36.3 nm (Fig .4).

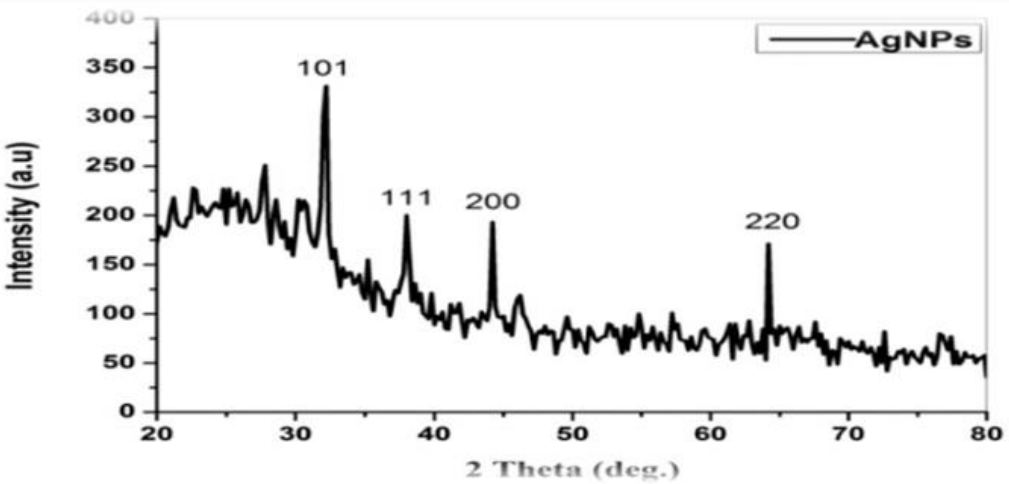

Fig. 4: X-ray diffraction (XRD) of AgNPs

5. Energy Dispersive X-Ray Analysis EDX:

Energy dispersive X-ray analysis (EDX) showed that the weight percentage of Ag in AgNPs was 60.4% of the total constituents of the sample, with only small proportions of carbon (C), oxygen (O), sulfur (S), and the presence of sodium (Na), thought to be The elements that bind to the surface of the Ag-NPs originated from the phytochemicals present in the plant extract and are shown in the figure along with the typical silver peaks, confirming the formation of CS-AgNPs using the CS extract (Fig .5).

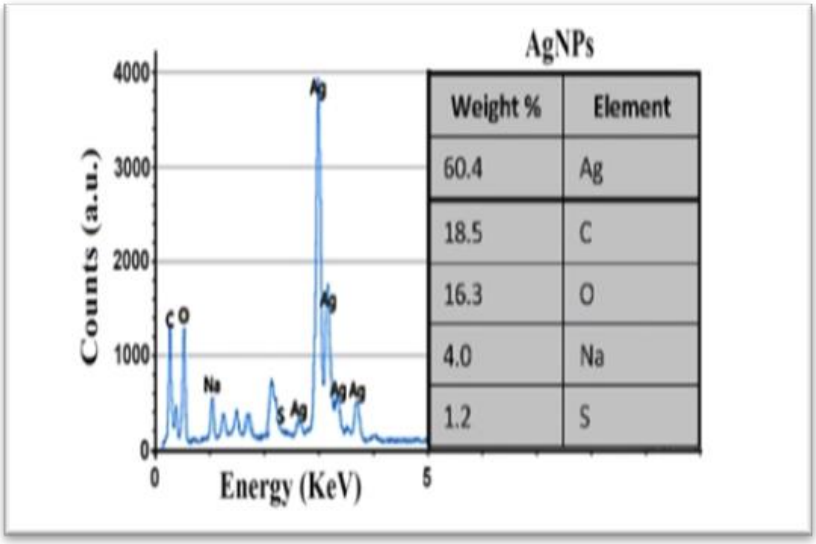

Fig. 5: Energy dispersive X-ray analysis EDX.

The present study reports the formation of bioactive Ag-NPs synthesized in a cost-effective and natural way from coriander extract containing macromolecules such as lignin, glycosides, aldehydes and phenolic compounds with effective antimicrobial capabilities. The reduction of silver ions by plant extracts is characterized by the appearance of a yellow color solution and brown reflecting the formation of AgNPs**.**

Detection of the ability of *Pseudomonas aeruginosa* bacteria isolates to produce biofilms using the microtiter plate method (MTP)

Biofilm production is one of the most important virulence factors for bacteria, and the production of this biofilm is the first step to initiating inflammation and then the emergence of disease, as it provides self-protection for bacteria and this is a key point in maintaining infection. Pathogenic bacteria can form biofilms on medical devices, and it is also noted that they differ in their ability to form biofilms and in the thickness of this biofilm depending on the genus and species producing them, and depending on environmental conditions surrounding the organism such as temperature and pH (Sauer and Camper, 2001). This assay is a quantitative assay for the detection of biofilm production. It gives a numerical value of the absorbance at a wavelength of 630 nm using an ELISA reader to determine the amount of biofilms formed by adhering to the surfaces of titration plates. Absorbance represents the thickness of the biofilm composed of bacterial isolates compared to control 0.147 =OD c (Table 1).

Table 1: Numbers and percentages of biofilm-producing isolates**.**

=0.147) OD c(level of biofilms compared to			Biofilm production
Weak NO.(%)	Middle NO.(%)	Strong NO.(%)	
3(%15)	(%25) 5	(%60)12	Produced
(%0) 0	(%0) 0	(%0) 0	Not produced

It followed the accurate standard panel method for detecting biofilm production described by (Tang *et al*., 2011). The results shown in Table 1 showed that all isolates of *Pseudomonas aeruginosa* produced biofilms with different categories, 12 (60%) were strong, 5 (25%) medium, and 3 (15%) weak for biofilm production, and this is consistent with Hameed's result (2018), whose results showed that all isolates of *Pseudomonas aeruginosa* are biofilm-producing, while the result of the current study does not agree with Lima *et al.* (2018), which were 7.5% strong, 27.5% medium, 42.5% weak, and 22.5% non-producing biofilms.

Table 2: Effect of AgNPs silver nanoparticles on the biofilm of *Pseudomonas aeruginosa***.**

=0.071)OD c(level of biofilms compared to		Silver nanoparticles
Non-disinhibited NO.(%)	Inhibited NO.(%)	
(%15) 3	(%85)17	AgNPs

This test is a quantitative test to discover the production of thin biofilms after treating the isolates by silver nanoparticles AgNPs, for which the percentage of biofilm formation was measured as in the previous Table1, which shows the percentage formed by each isolate and the absorption value was used at a wavelength of 630 nm using ELISA reader to determine the percentage of inhibition of biofilms formed by the isolates by adhering to the surfaces of calibration plates. The value of absorption represents the percentage of inhibition compared to the control sample, which was 0.071 nm. The results showed through Table 2: effect of silver nanoparticles AgNPs on biofilm formation of *Pseudomonas aeruginosa* bacteria and inhibition percentages of 17 (85%) and 3 (15%) that did not inhibit The use of innovative nano-antibacterial method could be promising to combat biofilm infections while overcoming bacterial resistance. As described by (Wang *et al*., 2018).

Silver nanoparticles found that in the initial phase of the reaction, they attach to the bacterial cell wall, after which they enter the bacterium and kill the bacterial cell by destroying the membrane and the results showed the potential for using AgNPs as an alternative to the conventional antimicrobial agents currently in use (Salomoni *et al*., 2017). There are reports describing AgNPs-mediated DNA damage due to ingress of Ag+ ions between purine and pyrimidine base pairs. This event leads to breakdown of the DNA double-helical structure followed by the phenomenon of disrupted replication (Parmanik *et al*., 2016). The mechanism of cell death induced by nanosilver is that silver may disrupt several bacterial cellular processes, including disulfide bond formation, metabolism, and these changes may lead to increased production of reactive oxygen (ROS) and increased food permeability that can stimulate group activity. wide range of antibiotics against Gram-negative bacteria in different metabolic states (Fayaz *et al*., 2010).

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