

## Study of the Effect of Biosynthesized Silver Nanoparticles Using Coriandrum Sativum Leaf Extract on Some Types of Pathogenic Bacteria

Ihab Ahmad Hamood<sup>1</sup>, Shefaa Abas Hamad<sup>2</sup>, Osama Nadhom Nijris<sup>3</sup>

<sup>1</sup> Department of Continuing Education / University of Samarra

Email ID: [Ihab.ahmed.h@uosamarra.edu.iq](mailto:Ihab.ahmed.h@uosamarra.edu.iq)

<sup>2</sup> Department of Biotechnology / Faculty of Applied Sciences / University of Samarra

Email ID: [shefaa.abas@uosamarra.edu.iq](mailto:shefaa.abas@uosamarra.edu.iq)

<sup>3</sup> Department of Pathological Analysis / Faculty of Applied Sciences / University of Samarra

Email ID: [usama.n@uosamarra.edu.iq](mailto:usama.n@uosamarra.edu.iq)

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### ABSTRACT

The capacity to combat common infectious infections has been diminished by the overuse and frequent administration of conventional antibiotics, necessitating numerous medical procedures to prevent this condition. Therefore, one of the biggest threats to the effective treatment of bacterial diseases is the development of new antibacterial systems against drug-resistant microorganisms. Biocompatible nanomaterials provide potential strategies to prevent antimicrobial resistance to drugs, mainly by improving the therapeutic effect of existing antimicrobial drugs. Nanoparticles, also known as nanotechnology, have special physical and chemical characteristics, including a large surface area in relation to size, high reactivity, a functional structure, and a very small size that can be regulated, ranging from 1 to 100 nm. Coriander leaf extract was used to create silver nanoparticles, which were then thoroughly investigated using electrokinetic nanoparticles, atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FT-IR), ultraviolet and visible spectroscopy (UV-Vis), and X-ray scattering (EDX). Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were used to assess the properties. Different concentrations were prepared for the nanoparticles: 500, 400, 300, 200, and 100 µg/ml. Their inhibitory efficacy was tested against isolates of Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa* using the well diffusion method. The outcomes of the silver nanoparticle influence evaluated the efficiency of biosynthetic silver nanoparticles against bacterial isolates at five different concentrations: 500, 400, 300, 200, and 100 µg/ml. Most of the concentrations were successful in preventing the bacterial isolates from growing. The results showed that the effect of silver nanoparticles was better against the Gram-positive *Staphylococcus aureus* isolates than against the Gram-negative *Pseudomonas aeruginosa* isolates. The highest inhibitory efficacy was recorded with diameter rates of 22.00 mm for *Staphylococcus aureus* and 15.00 mm for *Pseudomonas aeruginosa*. The aqueous extract of coriander leaves has proven its ability to reduce and synthesize silver nanoparticles. When it comes to bacterial isolates, silver nanoparticles have shown the strongest inhibitory activity.

**Keywords:** Silver nanoparticles, Coriandrum sativum leaf extract, *Staph. aureus*, *Ps. aeruginosa*.

### 1. INTRODUCTION

One of the most significant issues facing the health system today is antibiotic resistance, previously found only in hospital settings, antibiotic-resistant strains are now found everywhere, antibiotic resistance spreads due to several variables, such as the use of several broad-spectrum drugs, the abuse of antibiotics in aquaculture and animal husbandry, globalization, and insufficient antimicrobial oversight, the increase in the prevalence of antibiotic-resistant pathogens suggests that there is less, According to estimates, there won't be any viable antibiotics by 2050<sup>(1)</sup>, Conventional antibiotics' ability to prevent infectious infections has been diminished by their overuse and frequent usage, necessitating a number of medical procedures<sup>(2)</sup>, Therefore, one of the biggest threats to the effective treatment of bacterial disease is the development of new antibacterial systems against drug-resistant microorganisms<sup>(3)</sup>; Biocompatible nanomaterial provide potential strategies to prevent drug resistance, mainly by improving the therapeutic effect of existing nanotechnology-based antimicrobial vector drugs, referred to as nanoparticles, they possess special chemical and physical characteristics, such their extremely small size (between 1 and 100 nm), that can be controlled and surface area, large relative to size, high interactivity and functional structure<sup>(4)</sup>,

Therefore, the development of new antibacterial systems against drug-resistant pathogens represents a major threat to the successful treatment of bacterial disease<sup>(3)</sup>. Because of their antibacterial properties, nanoparticles can circumvent traditional resistance mechanisms, such as disruption of enzymes, reduced membrane permeability, modification of target sites, increased flow by over-expression of Efflux pumps, and escape from effective antimicrobials<sup>(5)</sup>.

Among the common elements used in nanofine technology are silver Ag, Zinc, Cu copper, aluminium Al, etc., and among the many metal nanoparticles Silver or its ionic form is the most hazardous to bacteria, and their oxides are already used as antibacterial active agents<sup>(6)</sup>.

## 2. METHODS

### Preparation of coriander leaf extract

Make the coriander plant's leaf aqueous extract using the prescribed procedure. In Mofid *et al.* (7), 10 g of dry plant powder is combined with 100 ml of hot non-ionic water (rather than distilled water) and departed in the refrigerator for 24 hours. The extract is then filtered through multiple layers of gauze to remove any remaining large plant material. Finally, using filter paper No. 1 Whatman, It is introduced at a concentration of 1 mM to the silver nano solution.

### Biosynthesis of silver nanoparticles

Adjust the pH of the mixture to 7 by mixing 10 ml of *Coriandrum sativum* leaf extract with 90 ml of nanosolution at a concentration of 1 mmol. Then, as control factors, add two laboratory vials: one with the extract only and no nano solution, and the other with the nano solution only and no plant extract.

After 35 minutes on a magnetic heater, we observed a change in color and the formation of a grey precipitate of silver nitrate AgNO<sub>3</sub> at the bottom of the lab vial. This is a sign that nanoparticles are forming. The precipitate was collected, centrifuged for ten minutes at 10,000 rpm after being washed three times with non-ionic water. After that, the precipitate was brought to a convection furnace that was heated at 105° C for five to six hours to dry the precipitate and produce the nanoparticles<sup>(8)</sup>.

### Diagnostic techniques of prepared silver nanoparticles

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were used to evaluate the characteristics of electrokinetic nanoparticles, while silver nanoparticles were thoroughly investigated using ultraviolet and visible spectroscopy (UV-Vis), atomic force microscopy (AFM), Fourier infrared transformation spectroscopy (FT-IR), and X-ray scattering (EDX).

### Preparation of nanoparticles stock solutions

Stock solutions were prepared at a concentration of 1024 µg/ml silver nanoparticles by dissolving 1024 mg in 1 ml of solvent DMSO and placing the nanoparticle solution 30 minutes in an ultrasonic bath. The remaining concentrations of 500, 400, 300, 200, and 100 µg/ml were then calculated using the dilution law<sup>(9)</sup>.

### Testing the effectiveness of silver nanoparticles against isolates of bacteria under study.

The test was carried out using a well diffusion method, where the Müller medium was prepared according to the manufacturer's instructions and then left to harden. The bacterial stranded was prepared at the age of 18-24 hours and a concentration of 15×10<sup>8</sup> cells/cm<sup>3</sup> and compared with a solution of the standard turbidity constant McFarland, the bacteria were spread by cotton swabs. The cork drill made drilling and 60 µl of concentrations of 500, 400, 300, 200 and 100 µg/ml prepared from the nanoparticles were transferred and placed in the pits and then incubated in the incubator at a temperature of 37°C For 24 hours, the diameter of the information produced by each concentration was recorded <sup>(9)</sup>.

### Statistical Analysis

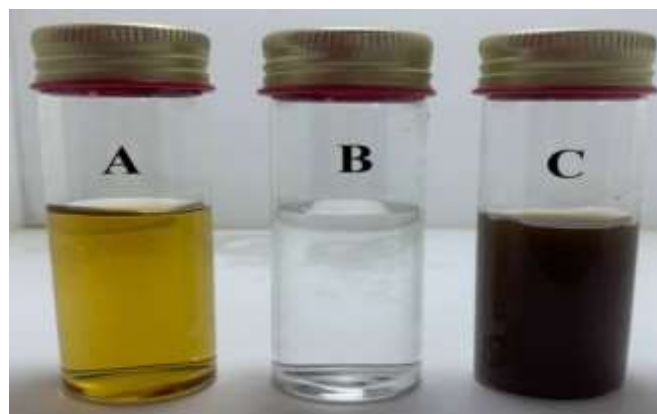
The ANOVA test of complete random design (CRD) was used to statistically assess the findings. The Dunkin' polynomial test was used to compare the arithmetic averages at a 0.05% probability level<sup>(10)</sup>.

## 3. RESULTS AND DISCUSSION

### Biosynthesis of silver nanoparticles

In the biosynthesis of AgNPs, coriander leaf extract was employed as a reducing and stabilizing agent, the study's findings revealed a dark brown color shift, which is indicative of the nanoparticle synthesis process and could be brought on by the surface plasmon resonance resonance absorption strip (LSPR) appearing, which is a feature of metal nanoparticles such as silver, gold, zinc and copper <sup>(11)</sup>, one of the most important reasons for the use of biosynthesis of nanoparticles is cheap, safe for the environment, non-hazardous, easy to work and low toxicity <sup>(12)</sup>, The nanoparticles were obtained in the form of powder after drying and its weight was taken and then its properties were studied using ultraviolet and visible technology, atomic force microscopy, Transmission electron microscopy, scanning electron microscopy, infrared spectroscopy, and X-ray

diffraction, and our results of these tests mentioned recently came to agree with the findings of Hashim *et al.* <sup>(13)</sup> who succeeded in synthesizing silver nanoparticles by extract of coriander leaves. Additionally, it is consistent with several studies that have stated that plant extracts are directly and significantly involved in the biosynthesis process and the creation of nanoparticles by lowering the plant metal ion and facilitating the creation of nanoparticles since they function as reducing agents, and reducing agents that participate in the reduction process include many natural water-soluble products such as alkaloids, phenolic compounds, terpenoids, flavonoids and coenzymes <sup>(14)</sup>, many plants are used in green synthesis to produce Nanomolecules such as alfalfa, aloe vera, amala, capsicum annuals, geraniums, coriander and tea<sup>(15)</sup>.

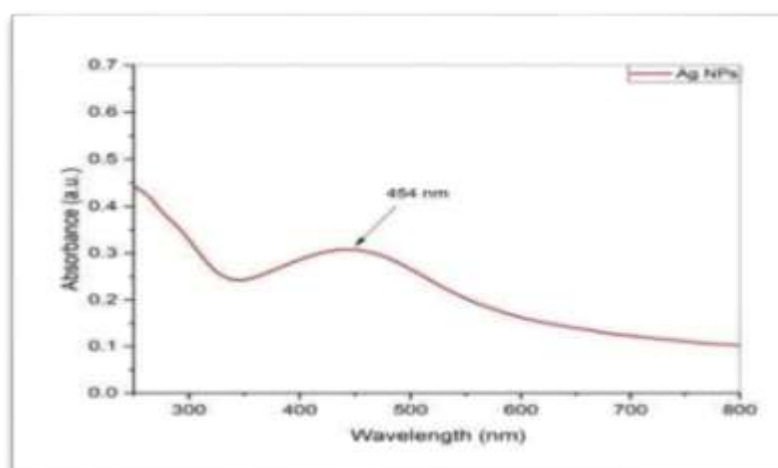


**Figure 1: Biosynthesis of silver nanoparticles by *Coriandrum sativum* leaf extract**

## Characterization of Nanoparticles

### UV-visible spectrum of silver nanoparticles

The stability of nanoparticles in the colloidal fluid was studied by means of a UV-Vis spectrometer, and Figure 2 shows the visible and visible UV spectroscopy of silver nanoparticles produced using extract from coriander leaves, by utilizing UV-Vis spectroscopy to measure the maximum wavelength  $\lambda_{max}$  of the silver nanosolution, it was discovered that the maximum wavelength of the silver nanoparticle solution is 454 nm, and the results of this study are spent with the results of the Hashim *et al.* study <sup>(13)</sup>.



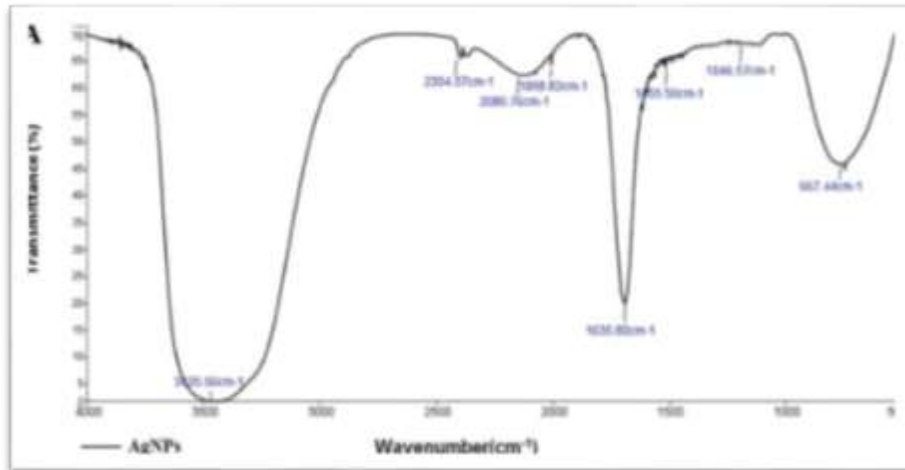
**Figure 2: UV spectrum – visible to silver nanoparticles**

### -FT-IR of silver nanoparticles

A Furay Infrared Conversion spectrometer was used to analyze the coriander leaf extract-synthesised nanoparticles in order to look into the active aggregates that were present in the extract and contributed to the reduction and stability of nanomolecules; infrared spectrometry is a crucial tool for observing functional groups that are known as functional groups

that participate in the stabilization (synthesis) of nanoparticles<sup>(16)</sup>, this measurement is employed in the study of nanostructure and protein aggregation as well as the synthesis of carbohydrates and nucleic acids, which appear as curves showing chemical groups<sup>(17)</sup>. The infrared furé spectrum for the silver nanoscales being studied is shown in Figure 3, which are located between 400 and 4000 cm. Peaks of 2354.57 and 2080.76, 1958.82, 1635.80, 1455.50, 1046.57, and 667.44 cm were detected.

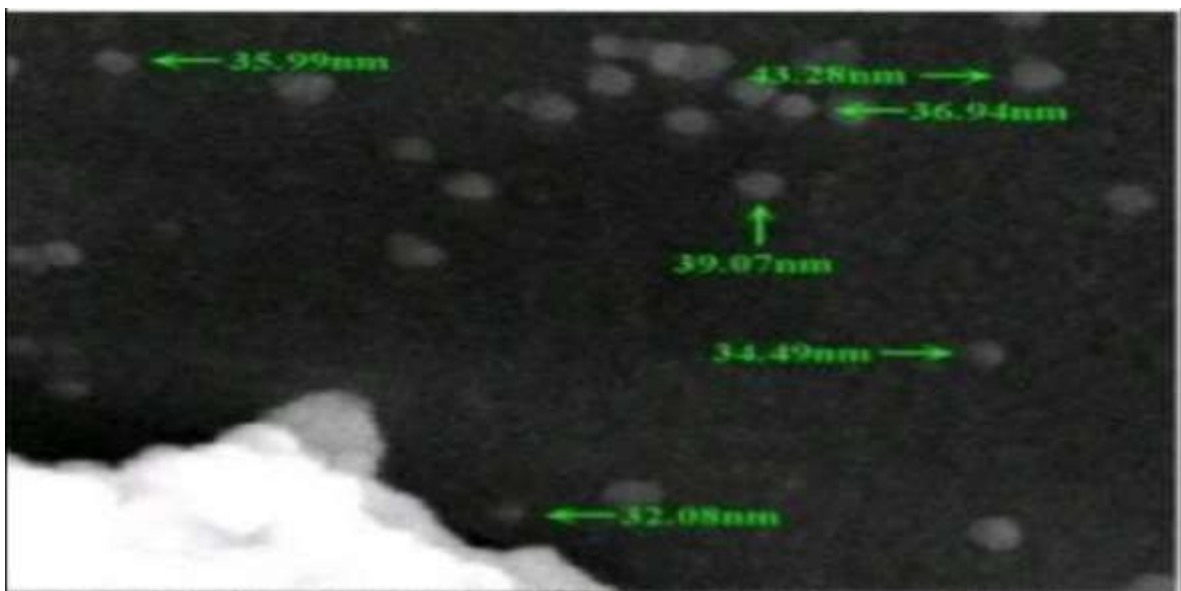
Because part of the H-O is not generated during the oxidation process when some moisture is absorbed on the highly reactive surface of the silver nanoparticles, the peak at 3435.56 cm (1-to H-O (hydroxyl group)) is set. These results are consistent with study result of Abas<sup>(19)</sup>.



**Figure 3: Infrared Furay Spectrum of Silver Nanoparticles**

#### -Scanning electron microscope for silver nanoparticles (SEM)

SEM micrographs of the NPs obtained in the filter showed that the AgNPs in solution without aggregation were spherical and well distributed; Figure 4 showed that most NPs were spherical and that AgNPs had a smooth surface and the dimensions were from 32.08 nm to 43.28; these differences in size may be due to SEM images that generally reflect the mineral core (molecular centres). The current investigation was successful in producing positive results, and these findings align with those of Hashim *et al.*<sup>(13)</sup> where they found the size of the silver nanoparticles biosynthesized by coriander leaf extract to range from 30-50 nm.



**Figure 4: Image from SEM scanning electron microscope with magnification of 200 nm of prepared silver nanoparticles**

### X-ray diffraction of silver nanoparticles

These results confirm that the material being studied is extremely pure and tiny in nature. The average size of silver nanoparticles was calculated using the Debye-Scherrer equation. In order to compare the observed peaks with the worldwide X-ray diffraction database JCPDS, this criterion provided significant support for the presence of nanoparticles.

$$D_{hki} = \frac{k \times \lambda}{\beta_{hki} \times \cos \theta_{hki}}$$

$$K = 0.94$$

$$\lambda = 1.05418 \text{ \AA}$$

$$\beta_{hki} = \text{deg} \times \frac{\pi}{180}$$

Where D: granular size,  $\lambda$ : wavelength of radiation,  $\theta$ : X-ray diffraction angle,  $\beta$ : width at mid-apex. The crystal structure of silver nitrate nano powder prepared by X-ray diffraction technique and the peak locations shown in Figure 5 were diagnosed, showing an average grain size of silver nanoparticles of 37.4 nm.

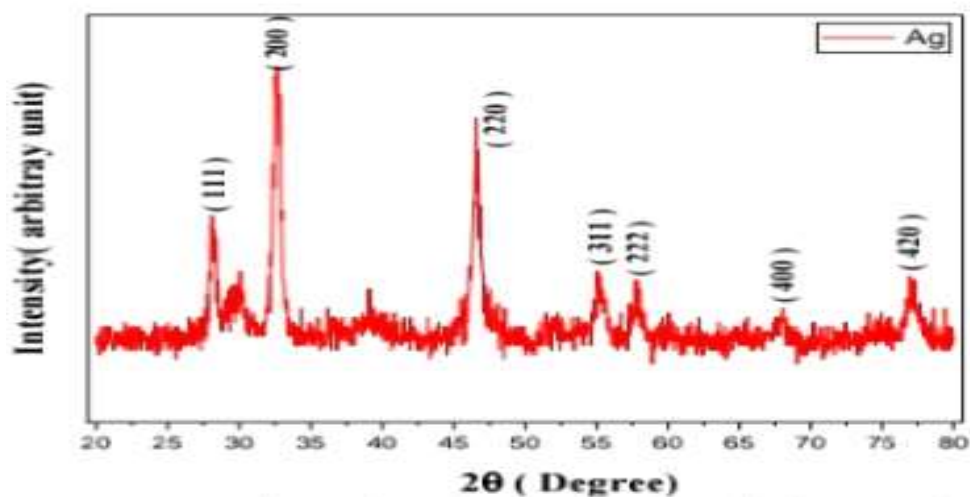


Figure 5: X-ray diffraction of silver nanoparticles

### -Energy- dispersive of silver nanoparticles (EDX)

The X-ray energy dispersion measurement indicates that there are very large amounts of silver nanoparticles. As seen in Figure 6, the beam in areas 2 and 4 denotes the presence of prepared silver granules. The results indicate that the sample contains approximately 63.2% silver, which is significantly higher than the percentage of 39.76% obtained by Garcidueñas-Piña *et al.*<sup>(20)</sup>.

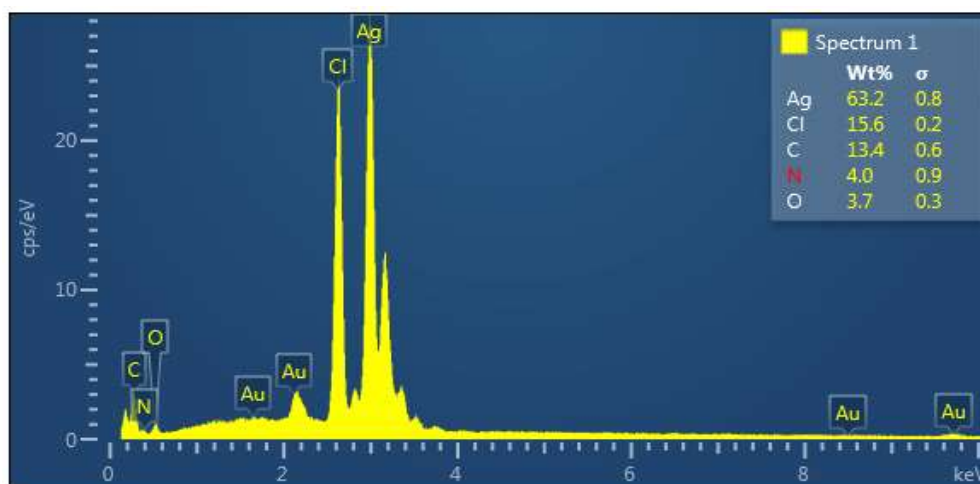


Figure 6: EDX energy dispersion of silver nanoparticles

#### -Transmission electron microscope for silver nanoparticles (TEM)

TEM investigation was carried out with a magnification level of in order to examine the size distributions and morphology of silver nanoparticles (AgNPs).77,500 kx, the results of the magnified image revealed that most NPs have spherical forms with varying dimensions at 80 nm and 40 nm. As seen in Figure 7, a spherical halo of 2.5 microns and 250 nm also emerged in spherical minutes.

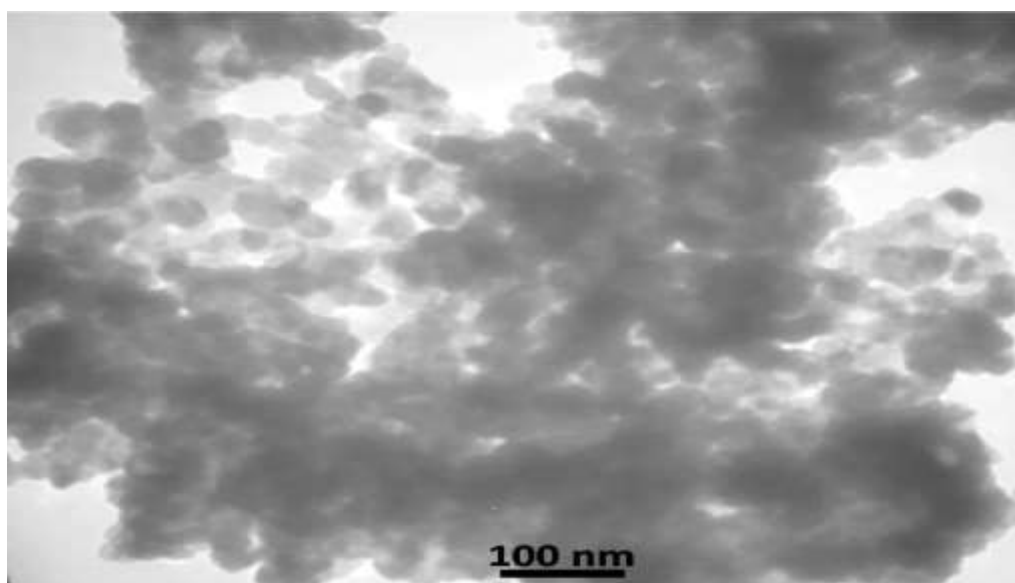


Figure 7: Silver nanoparticles are collected under a transmitting electron microscope at a magnification power of 100nm

#### -Inhibitory efficacy of AgNPs biosynthesized against bacterial isolates.

As Table 1 shows, silver nanoparticles showed the highest inhibitory activity against the positive bacteria *Staph. aureus*, with an inhibition diameter of  $2.00 \pm 22.00$  mm at a concentration of 500  $\mu\text{g/ml}$  and a minimum inhibition diameter of  $1.53 \pm 9.67$  mm at a concentration of 100  $\mu\text{g/ml}$ . In contrast, silver nanoparticles recorded an inhibitory activity towards the negative bacteria *Ps. aeruginosa*, with an inhibition diameter of  $1.00 \pm 15.00$  mm at a concentration of 500  $\mu\text{g/ml}$  and the lowest inhibitory efficacy at a concentration of 200  $\mu\text{g/ml}$ , with an inhibition diameter of  $1.15 \pm 9.33$  mm.



**Table 1: Effect of silver nanoparticles on bacterial isolates**

Co. AgNPs	Average $\pm$ mean	
$\mu\text{g/ml}$	<i>Ps.aeruginosa</i>	<i>Staph. aureus</i>
500	15.00 $\pm$ 1.00 a	22.00 $\pm$ 2.00 a
400	12.67 $\pm$ 0.58 b	18.67 $\pm$ 3.06 b
300	11.00 $\pm$ 1.00 b	15.33 $\pm$ 1.53 c
200	9.33 $\pm$ 1.15 c	10.33 $\pm$ 0.58 d
100	0.00 $\pm$ 0.00 d	9.67 $\pm$ 1.53 d

\*Different letters indicate a significant difference

According to research, silver is highly effective against *Ps. aeruginosa* biofilms, and silver nanoparticles (AgNPs) had an inhibitory diameter of 17.75 mm at a concentration of 100  $\mu\text{g/ml}$ . These findings are in line with those of Saleh *et al.* <sup>(21)</sup> who showed the effectiveness of silver nanoparticles against *Staph. aureus* by recording inhibitory diameters of 15.25 mm but at a concentration of 100 mg/ml and an inhibition diameter of 13.25 mm at a concentration of 50 mg/ml<sup>(22)</sup>. The results of the Kathiresan and Kanimozhi study<sup>(23)</sup> proved that silver nanoparticles have inhibitory effectiveness against *staph.aureus*, *Strep. pyogenes* and *K. pneumoniae*.

Silver ions' positive charge the negative charge of bacteria can react with  $\text{Ag}^+$ , since the  $\text{Ag}^+$  ion attaches itself to functional groups that harm membranes and cause ROS to be released, which has an antibacterial effect, this reaction results in structural alterations in DNA, proteins, and cell walls<sup>(24)</sup>, it was found that valued positive bacteria are more susceptible to the antibacterial effects of nanoparticles (NPs) than are valued negative bacteria. This result is associated with the highly permeable walls of positive bacteria that form covalent connections with proteins and other surrounding constituents, allowing foreign particles to pass through, while the walls of non-porous negative bacteria function as barriers that stop nanoparticles from entering and penetrating<sup>(25)</sup>.

Although there are several ways to demonstrate that nanoparticles can inhibit microbial growth, the basic idea is the same: because the nanoparticles' positive charge is linked to the bacteria's negative charge on the cell membrane, particles accumulate on the membrane's surface, changing its chemical and physical characteristics and causing damage that impairs the envelope's ability to perform essential functions like permeability, osmotic regulation, and the transfer of breathing electrons<sup>(26)</sup>, and that free radicals are produced when silver nanoparticles build up in the plasma membrane, interfering with the membrane's capacity to penetrate the cell and function<sup>(27)</sup>, they release positively charged ions after entering the nanoparticles, which, by attaching to the nanoparticles and destroying DNA, can bind to the bacterial ribosome and stop the replication of the microbe's genetic material or prevent protein synthesis, they can also inhibit respiratory chain enzymes and cell proteins by attaching to the thiol group (SH) of proteins, which causes the cell to lose its cellular components and die<sup>(28)</sup>, as it can interact with any organic compound in the surface of the bacteria wall and destroy it, leads to damage and death of the cell wall and can interact with the plasma membrane, thus affecting surface chemistry and function and reducing ATP levels and primary energy particles that affect cell membrane stability<sup>(29)</sup>.

**Conclusions:** The aqueous extract of coriander leaves has demonstrated its ability to reduce and synthesize silver nanoparticles, and silver nanoparticles have recorded inhibitory activity against bacterial isolates under study.

**Recommendations:** Examine the effects of biosynthetic nanoparticles on organs like the liver, kidneys, and others, as well as how they affect laboratory animals' histology and functionality, to ascertain the compounds' toxic effects on those tissues, their accumulation sites, and their disposal mechanisms.

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