

Preparation of (AgO) By Green Synthesis and Its Potential Use in Medical Fields

Maithm A. Obaid¹, Surour A. Khlaf^{2*}

¹ Department of Medical Physics, College of Applied Medical Science,
Shatrah University, Thi-Qar, 64001, IRAQ

² Ministry of Education, Al-Karkh 3, Al-Fatemiyyat Preparatory, IRAQ

*Corresponding Author: maithm1993@gmail.com

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Abstract

Since the increased drug efficacy and lower toxicity in nano-mediated drug delivery model have led to various applications in current science, the ecologically friendly synthesis regarding nanoparticles (NPs) by the green route from plant extracts has gained favour. In the presented work, we looked into the biosynthesis regarding silver oxide nanoparticles (AgO NPs) from AgO NPs leaves that are both stable and reasonably priced. The approach used for preparing AgNPs using green synthesis is demonstrated in this work to be straightforward and affordable. This was accomplished by combining an aqueous solution of silver nitrate with an olive extract, and visual spectroscopy as well as colour change were employed to demonstrate the preparation of AgNPs. Through using UV spectroscopy, the nature regarding the synthesized AgNPs was determined to be 2.5 mM. According to the research, silver nitrate aqueous extract is a good material for making AgNPs, and by modifying the factors influencing this study, more AgNPs in smaller sizes could be produced. After that, it was established that the extracted silver oxide had antibacterial characteristics, allowing it to be employed in a variety of pharmaceutical preparations for treating infections brought on by both negative and positive bacteria. As it was demonstrated to have a bactericidal effect on bacteria like the ones we utilized in this research-namely, *Staphylococcus aureus*, *Staphylococcus pneumoniae*, and *Escherichia*.

1. Introduction

The growing necessity to create environmentally benign solutions for the synthesis of materials has brought biosynthesis of Nanoparticles an emerging feature at the interface of biotechnology and nanotechnology into sharper focus [1]. The biosynthesis of inorganic material using microorganisms and plants, especially metal nanoparticles, has been extensively studied [2]. Numerous significant uses exist for nanosilver ions. In textiles, cosmetics, medical devices, home water purification systems, household appliances, and electronics it is utilized as an antimicrobial agent [3]. AgNPs have strong optical properties in addition to their antimicrobial characteristics, making them appropriate for biological sensing and imaging. Ag NPs are used in conductive inks, adhesives, and pastes for various electronic devices because they have a high degree of conductivity [4]. In a number of chemical reactions, including the oxidation of styrene, AgNPs are employed as catalysts. The synthesis of AgNPs is carried out using a variety of methods [5]. AgNPs are synthesized through a variety of techniques, including a reduction in the solutions, microwave aided synthesis, biological thermal decomposition of Ag compounds, microwave-assisted synthesis, and laser mediated syntheses (biological thermal decomposition of Ag compounds microwave-assisted synthesis, and laser mediated syntheses). The newer method is the most widely

used because it provides a single, environmentally friendly step in the synthesis of nanoparticles. When plants are used instead of microorganisms, the reduction rate of metal ions has been found to be significantly faster, and stable synthesis of metal nanoparticles has been documented. In addition, this method does not contain toxic materials or solids as by-products that are difficult to dispose of, and does not produce toxic gases. Through adjusting the pH, it is possible to regulate and control the size and structure of the NPs synthesized utilizing plants. Since antiquity, Ag salts were utilized to inhibit the growth regarding bacteria in a range of applications, such as catheters, burn work, and dental implants. It is a common fact that ions of Ag and compounds that are based on Ag exhibit strong biocidal effects and are extremely toxic to microbes. The olive plant has long been used as a food and medicinal source. When olive leaf extract (OLE) was discovered to be useful in the treatment of malaria and fever in 1854, it was the first documented medicinal usage. OLE includes compounds that have strong antimicrobial properties against fungi, mycoplasma, and bacteria. Furthermore, OLE possesses anti-inflammatory and antioxidant properties [6]. According to research the main active components in olive leaf include tyrosol, vanillic acid, caffeic acid, and rutin. With the use of aqueous OLE, we examined the synthesis regarding stable AgNPs with the use of the bioreduction process and assessed their anti-bacterial activity against isolates of drug-resistant bacteria. The study supports earlier findings on the bio-synthesis of the nano-metals from extracts of the plant leaves.

2. Materials and Method

2.1 Preparation of Plant Extract

Using 9 g of AgNO_3 is added to distilled water (100 ml) and then the mixture is placed on a hot plate. Until complete dissolution is obtained, on the other hand, two grams of olive leaves are placed in distilled water (100 ml), after that, we put it on a hot plate for an hour as shown in Fig. 1. Then we put the olive leaf extract after filtering it using filter papers on the AgNO_3 solution. After a period of time not exceeding half an hour, we get nanoparticles of silver oxide, see Fig. 2 and Fig. 3.



Fig. 1 Olive plant leaves



Fig. 2 OLE and silver nitrate



Fig. 3 Silver oxide NP extract

2.2 AgNP Synthesis

A specific volume of OLE (0.20–9 ml) has been added to AgNO_3 solution for AgNP synthesis, and the volume has then been completed to 10 ml by using the deionized water. Ag^+ had a final concentration of 103 M. For two minutes, the solution was stirred. The solution changed colour from yellow to brownish-yellow to deep brown due to reduction process of Ag^+ to AgO NPs, depending on the pH, temperature, and extract concentration that were investigated. Various pH values have been used for preparing the NPs, and 0.1N NaOH or 0.1N H_3PO_4 solutions were used to modify the pH of solutions [8,9].

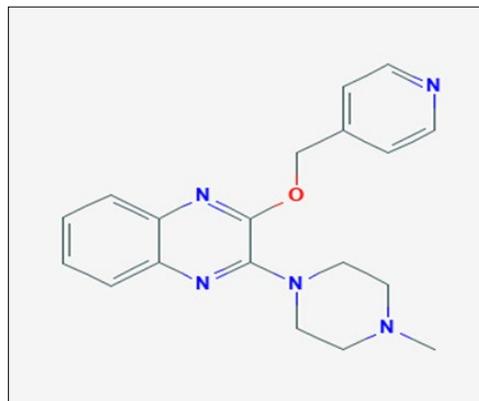


Fig. 4 Chemical structure of silver oxide

2.3 Pharmacokinetics of Silver Nanoparticles

On the other hand, a high degree of distribution to non-target tissues might result in unintended toxicity and must be avoided [9]. Predominant accumulation of medicines or NPs in the target tissue is frequently preferred for improved therapeutic benefits. Thus, for the safe and effective biological applications regarding NPs, pharmacokinetic as well as tissue bio-distribution data are essential.

2.4 Types of Bacteria

2.4.1 E-Coli

E. coli is a major cause of diarrhea in young and postweaning pigs and causes considerable losses in large-scale farms across the globe. The two primary types of diarrheagenic *E. coli* which cause enteric infections in pigs are verotoxigenic (VTEC) and enterotoxigenic (ETEC). Heat-labile toxin (LT), a high molecular weight toxin, and heat-stable toxins (STb and STa, called STII and STI), which are small, weakly immunogenic proteins, are the two main enterotoxins classes that are produced by porcine ETEC strains [4,11,12]. The edema verotoxin (VTe), also referred to as VT2 variation (VT2v) or Shiga-toxin 2e (Stx2e), is produced by porcine VTEC strains, known as Shiga toxin-producing *E. coli* (STEC) [7,13,14]. Certain strains of *E. coli*, commonly isolated from pigs suffering from edema and postweaning diarrhea (PWD), have the ability to produce both VT2e toxin and enterotoxins. As such, they are correctly referred to as ETEC/VTEC strains [5]. In the absence of enterotoxins, VT2e produced by *E. coli* causes edema illness but not diarrhea [13,14].

2.4.2 Klebsiella Pneumoniae

K. pneumoniae was extensively distributed throughout surface water, soil, sewage, plants, and mammal mucosal surfaces. Owing to its wide range of virulence factors, *K. pneumoniae* infections typically have a significant impact on the development of bloodstream infections, pyogenic liver abscesses, and pneumonia in the mammals [1,2]. The spread of *K. pneumoniae* in domestic animals not just endangers livestock productivity, yet has the potential to affect public health due to the fact that such animals can serve as a reservoir for strains of the bacteria that are resistant to drugs. Despite the fact that antibiotic therapy is a commonly utilized treatment for *K. pneumoniae* infections, antibiotic resistance in pathogenic bacteria from environmental sources and animals that produce food is acknowledged as global public health concern [3]. Selecting sensitive antibiotics for treatment is challenging because isolates of *K. pneumoniae* often exhibit drug resistance properties [4]. Recently, ciprofloxacin was utilized extensively for treating *K. pneumoniae* infections. It was noted as well that *K. pneumoniae* is getting a higher resistance to the fluoroquinolones [5,6]. Target enzymes DNA gyrase and topoisomerase IV, both of which are highly important for the replication of DNA, are inhibited by fluoroquinolones [14]. According to several researchers, the primary mechanism underlying fluoroquinolone resistance in *K. pneumoniae* is altered ions in quinolone-resistance-determining region (QRDR) of DNA gyrase (*gyrA* & *gyrB*) and topoisomerase IV (*parE* & *parC*).

2.4.3 Staph. Aureus

A wide variety of infectious illnesses, including skin infections, endocarditis, bacteremia, food poisoning, and pneumonia, are caused by the Gram-positive bacterium *S. aureus*. Following the initial rise to prominence as nosocomial infection, the epidemiologically separate clones of bacterium appeared in the community settings. Several virulence factors that are expressed by *S. aureus* aid in infection establishment through the promotion of the tissue attachment, invasion, and evasion of host immune response. Due to the fact that *S. aureus* can get resistant to a number of different antibiotic kinds, it is an organism that is difficult to treat. Methicillin-resistant *S. aureus* (MRSA) bacteria strains were known to spread and emerge, which results in high rates of illness, mortality, and higher treatment expenses. For many years, vancomycin was the go-to drug for treating such strains, yet the evolution of resistance limited its practical use. The U.S. FDA approved newer anti-MRSA medications, which provided clinicians with a break. Though the pathogen can swiftly develop resistance to newer medications when they are introduced in clinics, efforts to identify new antibiotics and non-antibiotic ways to combat MRSA must not be undermined.

2.4.4 Streptococcus Pneumonia

A member of the Streptococcaceae family of bacteria, *S. pneumoniae* is typically present in the nasopharyngeal as well as oropharyngeal mucosa of the healthy individuals. It is the bacterial pathogen most frequently linked to pneumonia and acute otitis media, and it ranks second in importance when it comes to occurrences of meningitis in children younger than two. Pneumococcus is the cause of 25% to 40% of meningitis cases in Europe and US [1,2]. Since the invention of antimicrobial medications like penicillin and sulphonamide in the early 20th century, the death rate from pneumococcal infection has dramatically dropped. Pneumococci resistant to penicillin have been initially identified in New Guinea during 1960s. After a decade, resistant instances have been reported from Spain and Africa [4,5]. The main causes of the formation of resistant strains are infections acquired in the community as well as repeated exposure to antimicrobial drugs, particularly in youngsters [14]. Reports of pneumococcus infections as well as penicillin-resistant *S. aureus* have increased during the past few years. For treating pneumococcal meningitis, penicillin has traditionally been the preferred drug. [6,10] Depending on the rates of resistance to the penicillin and other anti-microbial medicines, clinical suspicion, and quick lab tests like the Gram stain and latex agglutination test of cerebrospinal fluid (CSF), the first empirical treatment is chosen. Due to the significant prevalence of penicillin-resistant strains of *S. pneumoniae* around the world, it is necessary to first isolate the infection, determine etiological agent, and do in vitro susceptibility testing before determining whether to continue the initial empirical therapy. When pneumococcus is susceptible to penicillin, this antibiotic is still the drug of choice [15].

3. Results and Discussion

3.1 XRD Measurement

AgO NPs: XRD analysis, strong peaks of the thin film (AgO nano structure) in a polycrystalline structure are shown by XRD analysis in Fig. 5. The XRD pattern of AgO NSs placed on a glass substrate is displayed in Fig. 5. Through comparing with the conventional pattern (JCPDS), it displays a large peak at (26.52) on the 2theta scale associated with rhombohedral AgO, which provides proof of the creation of AgO.

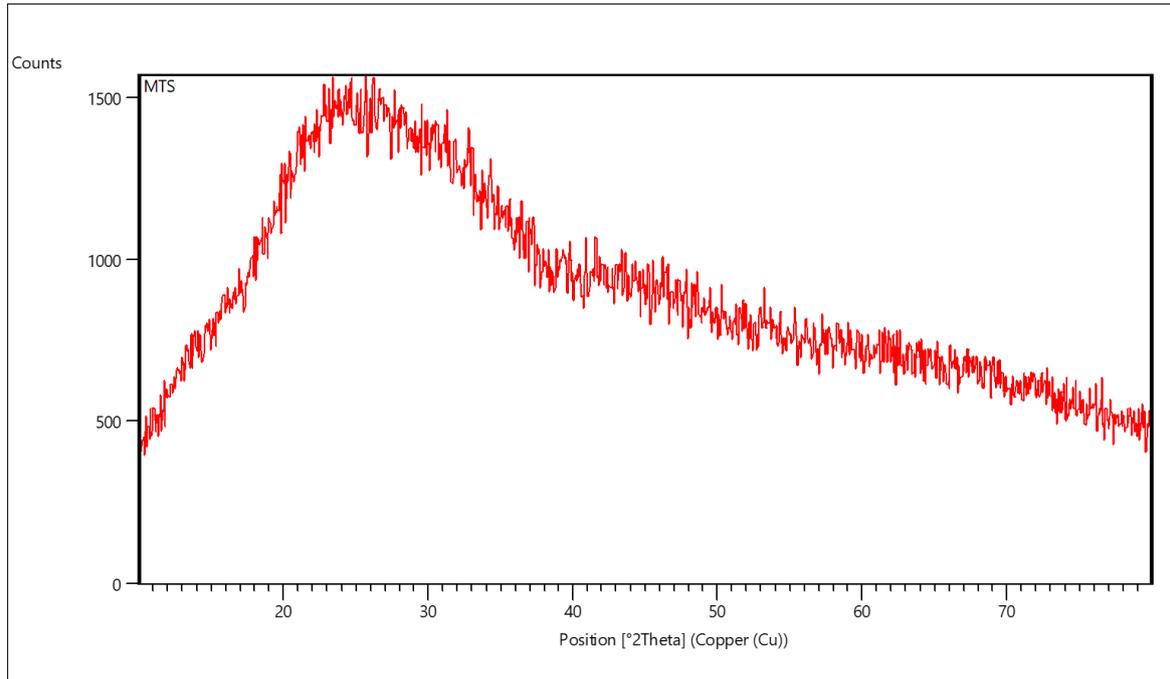


Fig. 5 XRD pattern of AgO NPs

To calculate the grain size (GS) [16], one method is to use the Scherrer formula and the width of peak that occurs in the patterns of XRD on Fig. 5:

$$GS = \frac{k\lambda}{\beta \cos\theta} \quad (1)$$

In which β represent the full width at half maximum (FWHM) for angle of diffraction at highest value of the peak of intensity (θ), λ represent the incident x-ray wavelength, and k represent a shape factor. Equations 2 and Equation 3 can be utilized for the determination of the size of the produced AgO NPs, the dislocating density η value, and the micro strain value (δ) [17].

$$\eta = \frac{\beta \cos\theta}{4} \quad (2)$$

$$\delta = \frac{1}{G_s^2} \quad (3)$$

Table 1 The values of the FWHM, grain size, the strain (δ) and dislocating density (η) of AgO

2Theta (deg)	Gs (nm)	D (nm)	$\delta \times 10^{14}$ lines.m ⁻²	$\eta \times 10^4$ lines· m ⁻⁴
26.5234	34.08	0.25	1.058	0.85

3.2 Optical Properties

AgO is shown to be transmitted in Fig. 6. The wavelength at which the minimum transmittance is recorded is 360 nm, indicating that the corresponding electron changes occurring within the sample are the cause of the ultraviolet absorption. It is also evident from this figure that the transmittance increases with wavelength (390 nm). The material has strong absorbance in the visible region (390-600 nm); thus, it will be effective there. However, in the region that is above that value, the material's behavior is similar to a window. The highest transmittance value is in IR region and is stable there.

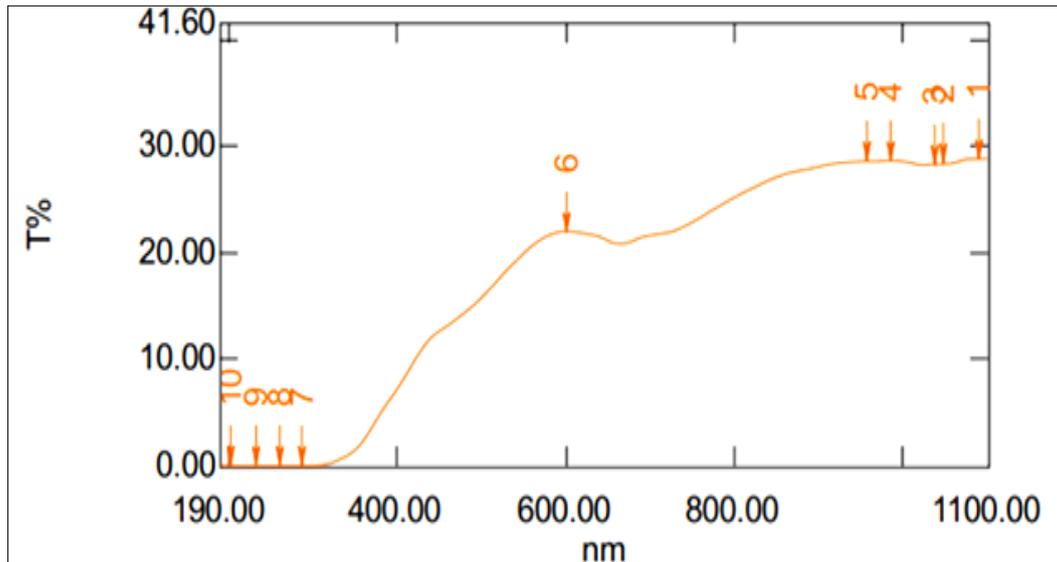


Fig. 6 Transmission spectrum of AgO

3.3 Fourier Transform

Fig. 7, the Fourier transform infrared spectroscopy (FTIR) at 500–4000 cm^{-1} has been utilized in order to examine chemical bonds as well as functional groups of colloidal AgO NPs generated by olive plant leaves, as shown in Fig. 7. At 3251.08 cm^{-1} , the bands in colloidal AgO NPs are evident. The peak is associated with the stretching vibration of hydroxyl group (H-bonded O-H stretch) [18,19]. The amines group C-N signal at 2129.41 cm^{-1} suggests the production of metal–oxygen bonds [20]. On the surface of AgO NPs, the O-H bending vibration of adsorbed water molecule is 1631.78 cm^{-1} .

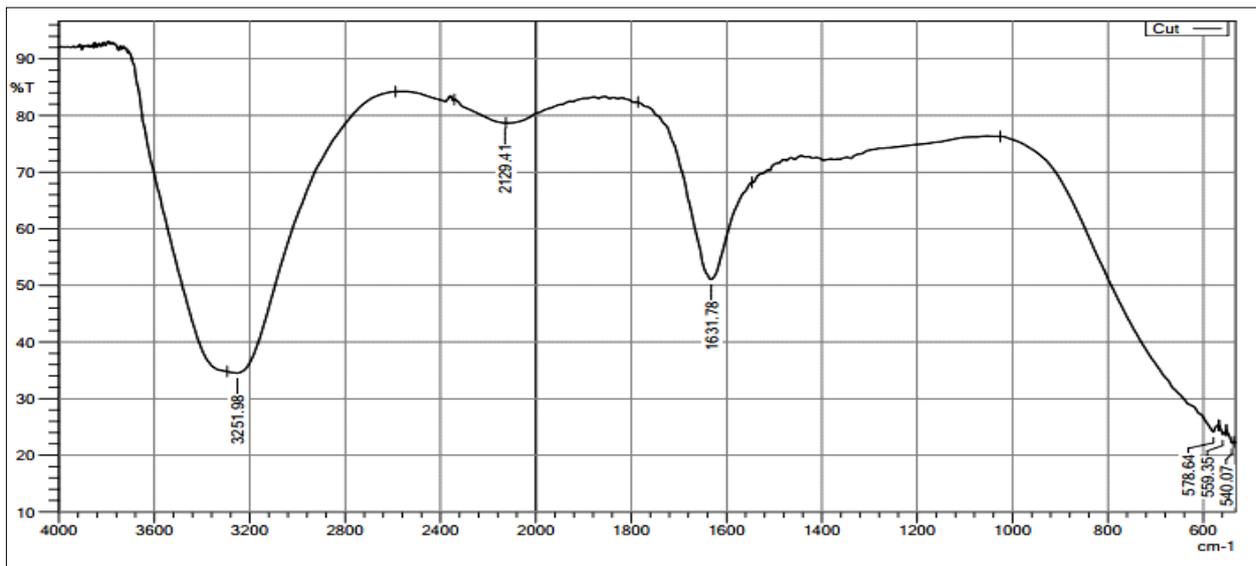


Fig. 7 FTIR spectra of AgO

3.4 Scanning Electron Microscopy (SEM)

With the use of SEM method, the silver oxide solution made from silver nitrate as well as olive leaves was evaluated for concentration (2M). The forms (4) displayed characteristics of the surface of the solution. They had a regular, uniform shape and a hexagonal structure, however occasionally they gave the impression of having a spiral structure. The produced solution has a nanostructure larger than 200 nm, according to the results.

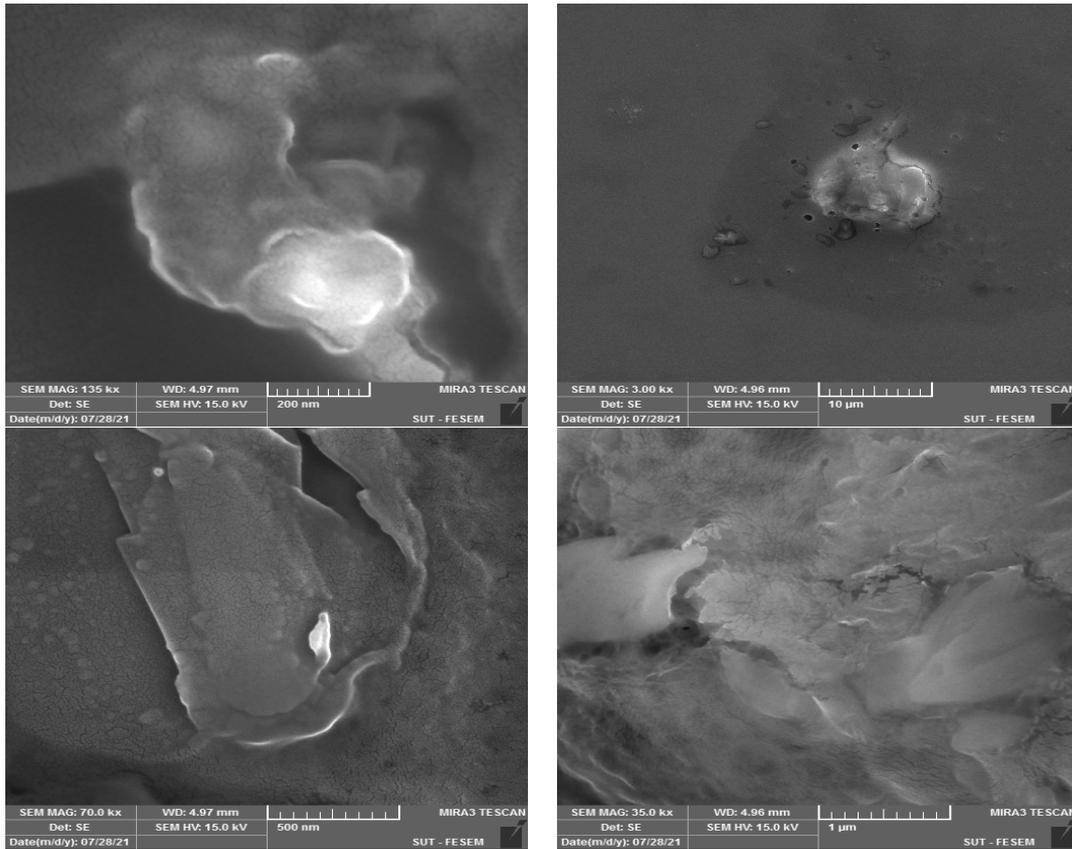


Fig. 8 SEM images of AgO

3.5 Antibacterial Mechanism of AgNPs

Different antibacterial actions were suggested in Fig. 5, even though the precise mechanism of anti-bacterial properties of Ag NPs has not been fully elucidated. The mechanism by which AgNPs kill microbes is that they could continuously emit silver ions [7]. Ag ions could adhere to cell wall and to a cytoplasmic membrane because of electro-static attraction and affinity for the sulphur proteins. The bacterial envelope can be disrupted because of adhering ions' increased permeability of the cytoplasmic membrane [8]. Respiratory enzymes could get inactive after the uptake of free ions of Ag into the cells, which produces reactive oxygen species (ROSs), yet halts adenosine triphosphate synthesis [9]. The alteration of the Deoxyribonucleic acid (DNA) and disruption of the cell membranes can both be caused by ROSs, which could be a major agent. Given the significance of phosphorus and sulphur as components of DNA, the Ag ions' interaction with those elements can lead to issues with cell division, DNA replication, or even eradication of the micro-organisms. Furthermore, Ag ions could prevent synthesis of new proteins. AgNPs are capable of destroying bacteria on their own besides releasing the ions of silver. Following the adherence to the surface of the cells, AgNPs are capable of gathering in pits developing on the wall of the cell [10]. the denaturation of the Cell membrane could be a result of AgNP accumulation. Due to their nano-scale size, the AgNPs can pass through the walls of the bacterial cells and alter cell membrane structure [10]. The denaturation of the cytoplasmic membrane can tear organelles and potentially result in cell lysis. The intrinsic characteristics of Ag NPs and the surrounding media are synthetic and processing parameter examples affecting the efficiency of the dissolution [20]. The Ag NPs are considered more dangerous for the Gram-negative bacteria [20], which have a thinner cell wall compared to the gram-positive ones. The thickness of the cellular wall could lead to hindering the ability of the NPs to enter the cells. The distinct anti-bacterial AgNP effects on the gram-negative and -positive bacteria are an indication of the fact that anti-bacterial impact is dependent on AgNP uptake [20]. AgNPs that are smaller than 10 nm are known to lead to a direct change to the permeability of the cells, penetrate bacteria cells, and harm cells. Researchers have discovered that when AgNPs with a similar dose kill all of the planktonic bacteria, 100% viability loss of bacteria did not happen in the bio-film [18]. Because of its intricate structure, the biofilm can tolerate the AgNPs.

3.6 Antibacterial Activity of AgO NPs

After being synthesized with hot water OLE as a stabilizing and reducing agent, AgNPs were reported and their anti-bacterial activity against the drug-resistant bacterial isolates was assessed. Investigations are conducted into effects of pH, temperature, contact time, and extract concentration on AgNPs' form and reaction rate. Studies have shown that nanomaterials, which have high surface area/volume ratio, unique physicochemical and promising antimicrobial properties, may reduce side effects, overcome antibiotic resistance, increase overall pharmacokinetics through controlled drug release, and are effective in diseases. Unlike most drug molecules, they are infectious by overcoming anatomical barriers (blood-brain barrier) due to their physical properties and particle size. Although several studies have shown that antimicrobial nanoparticles are more effective than the constituent antibiotics alone, there are some challenges in translating this technology into clinical. The data showed that as temperature rose in the basic medium, the rate at which nanosilver was formed increased dramatically. The inhibition zone diameters of positive control (antibiotic) for the synthesized -AgONPs were determined as 30 for *E. coli*, while it was 38 mm for *Streptococcus*, it was 32 mm for *Klebsiella*, it was 32 mm for *Staphylococcus* respectively.

Gram-positive bacteria (*B. subtilis*) and Gram-negative bacteria were used as benchmarks to quantify the effectiveness of anti-bacterial activity with regard to inhibition zones (mm). Using well diffusion technique, the antibacterial potential regarding aqueous OLE and synthesized AgNPs was evaluated. When applied to multidrug-resistant strains of *P. aeruginosa*, *E. coli*, and *S. aureus*, AgNPs at concentrations of (2.5) mg/ml greatly suppressed bacterial growth. This investigation showed that the aqueous OLE has an impact on the concentrator utilized for the preparation of the Ag nanoparticles. Therefore, at lower concentrations, AgNPs demonstrated broad spectrum anti-bacterial activity and could be a useful substitute therapeutic strategy in the future.

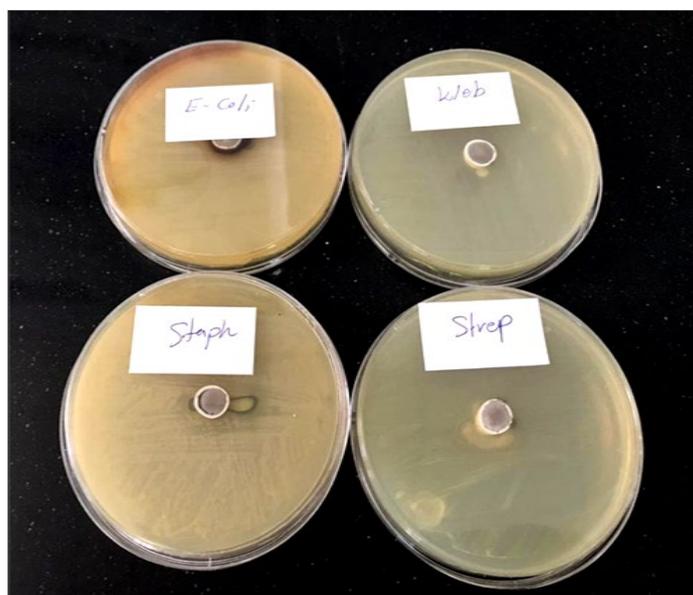


Fig. 9 Types of bacteria

Table 2 The samples with particle value

Sample	E.coli	Klebsiella	Staphylococcus	Streptococcus
MTS	30	32	32	38

4. Conclusion

This study successfully demonstrated the green synthesis of silver oxide nanoparticles (AgO NPs) using olive leaf extract (OLE). The synthesized AgO NPs exhibited strong antibacterial activity against both Gram-positive and Gram-negative bacteria, including drug-resistant strains such as *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. The nanoparticles were characterized using XRD, FTIR, SEM, and UV-Vis spectroscopy, confirming their structural and optical properties. The findings suggest that AgO NPs synthesized via green methods have significant potential for use in medical applications, particularly as antimicrobial agents. Future research should focus on optimizing synthesis conditions, evaluating long-term toxicity, and exploring applications in drug delivery systems.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

Maithm A. Obaid: Conceptualization, Methodology, Data Collection, Writing – Original Draft. Surour A. Khlaf: Supervision, Validation, Writing – Review & Editing. Both authors reviewed and approved the final version of the manuscript.

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