

Reduction of Silver Oxide Nanoparticles Using Anethum Graveolens Leaves Extract and Assessment of Their Antibacterial and Antibiofilm Properties

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Abstract

This research aims to develop a new method for preparing silver oxide nanoparticles using *Anethum graveolens* (dill) leaf extract as a natural reducing agent, using cold plasma technology. This study was designed to evaluate the antibacterial and anti-biofilm properties of the prepared nanoparticles. Initially, dill leaf extract was prepared and used as a reducing agent and stabilizer for silver oxide nanoparticles. Cold plasma technology was used to enhance the synthesis process and provide ideal conditions for the formation of nanoparticles. The results showed that the prepared silver oxide nanoparticles possess strong antibacterial properties, as they showed remarkable activity against a variety of pathogenic bacteria such as "*Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus*". Nanoparticles also proved effective in preventing the formation of bacterial biofilms, as the highest rate of inhibition was for the Gram-positive bacteria *Staphylococcus aureus*. The study provides evidence of the effectiveness of combining plant extracts and cold plasma technology in producing nanoparticles with improved properties, which could push towards the use of these materials. In developing innovative and sustainable solutions in various scientific and applied fields.

1. Introduction

The development of novel nanomaterials with potential uses in medicine is an exciting area of research in nanotechnology [1]. The unique properties and increasing use of silver nanoparticles (AgNPs) for various nanomedicine applications have piqued significant interest among emerging nanoproducts. For a long time, people have turned to silver nitrate or silver sulfadiazine, two forms of silver, to treat burn and wound infections caused by bacteria [2]. The production of nanomaterials requires a wide range of technologies derived from several fields, including physical, chemical, and biological sciences [3]. There are now a number of uses for plasma production for example, the deposition of surface modification layers for use in electrical devices and in the creation of anti-bio or biomaterials [4]. One environmentally friendly method that has several benefits over chemical synthesis is the non-thermal plasma creation of metal nanostructures. Due to the lack of hazardous substances and the reduced processing time compared to chemical synthesis, non-thermal plasma offers a viable option to NP synthesis [5]. Atmospheric Pressure Plasma Jet is a non-thermal plasma type characterized by high-electron temperature and low gas temperature [6]. The synthesis of AgNPs has been accomplished through a multitude of physical, chemical, and biological methods [7]. On the other hand, producing AgNPs of a consistent size is challenging, and traditional physical and chemical approaches to nanoparticle synthesis have a poor yield [8]. On top of that, chemical processes can be harmful to the environment because they use toxic-reducing agents like citrate, borohydride, or other organic substances [9]. The utilization of biological methods to synthesize AgNPs offers an eco-friendly alternative, as the control of particle size and shape is a crucial factor in numerous biomedical applications [10][11]. These techniques include the synthesis of AgNPs by means of bacterial proteins, which can influence the nanoparticles' shape, size, and monodispersity through changes in growth stage, growth medium, synthesis conditions, pH, substrate concentrations, temperature, and reaction time, among other parameters [12]. Nanoparticle synthesis using traditional physical and chemical methods appears to be

both costly and risky[13]. These methods include laser ablation, pyrolysis, lithography, chemical vapour deposition, sol-gel techniques, and electro-deposition [14]. In addition, the process calls for a number of reactants, including reducing agents like sodium borohydride, potassium bitartrate, methoxypolyethylene glycol, or hydrazine, and a stabilizing agent like sodium dodecyl benzyl sulfate or polyvinyl pyrrolidone to keep metal nanoparticles from clumping together [15]. A growing demand exists for the development of simple, cost-effective, high-yield, and environmentally friendly procedures for the synthesis of nanoparticles, despite the availability of numerous methods for this purpose. Thus, it is critical to seek out non-conventional environmentally friendly ways to synthesize metal nanoparticles [16]. Synthesis of nanoparticles using biological methods is possible with a shorter reduction time and a wide variety of naturally occurring biological resources, such as plants and plant products, algae, fungus, yeast, bacteria, and viruses. Stable and abundant in solution, synthetic AgNPs are easy to work with [17]. Plants and plant products are readily available, among other natural sources, and they allow for the fairly rapid synthesis of nanoparticles [18]. On top of that, there are alkaloids, tannin, steroids, phenol, saponins, and flavonoids in the water fraction of leaf extracts. We hypothesise that the proteins, polysaccharides, or secondary metabolites in the leaf extracts can convert the Ag⁺ ions to the Ag⁰ state and produce silver nanoparticles based on the compounds found in the extracts. Nanoparticle synthesis using a variety of plants has received a lot of attention as of late [17]. Managing public health in the modern world has become more important due to the increasing prevalence of microbial resistance. Despite the development of numerous new antibiotics in the past few decades, none of them have shown any improvement in the fight against bacteria that are resistant to multiple drugs [19]. This highlights the need for new and improved methods of treating both Gram-negative and Gram-positive infections [20]. Therapeutic agent delivery using nanoparticles has been a successful application of this technology [21]. Within the realm of long-term illness diagnosis, as well as the management of burn wounds and skin infections caused by bacteria, is one possibility. AgNPs have antimicrobial, antifungal, anti-inflammatory, antiviral, anti-angiogenic, and anti-cancer properties, among other [2].

The aim of the study is to use cold plasma technology and aqueous extract of *Anethum Graveolens* leaves, which is an environmentally friendly hybrid method, to synthesize silver oxide nanoparticles and evaluate their antibacterial against *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *E. coli* and biofilm activity.

2. Materials & Methodology

2.1. Preparation of aqueous extract of *Anethum Graveolens* Leaves

Fresh dill leaves 10 g are obtained from the fields, rinsed well with distilled water several times, and cut into small pieces. The chopped leaves were placed in 100 ml of deionized water for 20 min at a temperature of 70 C° on a magnetic stirrer. After that, the filtration process was carried out in three stages, the first using medical gauze, the second using a centrifuge for the purpose of separating

sediments for 10 min, and the third using filter paper for the purpose of ensuring that the extract was filtered from any impurities and storing it at a temperature of 4 C° it was used.

2.2. Preparation of Solution for Silver Nitrate Ag NO₃

Silver nitrate is made into an aqueous solution. To achieve homogeneity, add 1.69873 g of de-ionized Ag NO₃ to 100 ml of dilute water (DI) until the concentration reaches 0.1 M. additionally, agitate the mixture using a motor. Afterwards, combine with an Ag NO₃ solution.

2.3. Preparation of Silver Oxide Nanoparticle from Aqueous Extract of *Anethum Graveolens* Leaves

The gas discharge used was argon gas to generate plasma with the application of a high voltage of around 5 kV. The aqueous solution of Ag(NO₃) at a ratio of 6:4 underwent plasma treatment for 5 minutes. Figure 1 shows that the solution turns dark brown 5 minutes after the discharge starts, which is a marker of the creation of AgO NPs. It was determined that 5 minutes was the appropriate exposure time for each concentration.

2.4. Bacterial Varieties and Analytical Tools

Sigma-Aldrich (St. Louis, MO, USA) was consulted for the acquisition of bacterial strains, reagents, bacteria, Muller-Hinton broth (MHB) or agar (MHA), silver nitrate, ampicillin, chloramphenicol, erythromycin, gentamicin, tetracycline, and vancomycin antibiotics. With the exception of the compounds listed, all of the others were bought from Sigma-Aldrich. Without exception, Sigma Aldrich of St. Louis, MO, USA, supplied all of the chemicals and culture medium.

2.5. Method for inhibiting bacterial proliferation.

Bacteria trap cells used by SWAB were dispersed over the prepared agar medium, and the diffusion technique was employed to ascertain the degree of bacterial growth suppression. The next step was to make the discs, soak them in the nanoparticle solution for a few minutes, and then put them in each of the bacterial dishes. We examined how AgO NPs inhibited bacterial growth. After incubating the plates at 37°C for 24 hours, the following day, the region of inhibition around each disc was measured using a ruler to record the findings.

2.6 Determination of the lowest concentration of AgO NPs that inhibit (*Staphylococcus aureus*) and (*Klebsiella pneumoniae*, *coli*, *Escherichia*)

The broth microdilution method was used to evaluate the minimum inhibitory concentration (MIC) of AgO NPs. In a nutshell, 96 well microtiter plates with flat bottoms had wells prepared and filled with AgO NPs at double serial concentrations of 0.05, 0.025, 0.125, 0.00625, 0.0015625, 0.00078125, 0.000390625, 0.000195313, 0.00097656, and 0.00048828) µg/ml. All wells were then supplemented with 10 µl of (*Staphylococcus aureus*) and (*Klebsiella pneumoniae*, *coli*, *Escherichia*) suspension that had

been diluted overnight and adjusted to a 0.5 MacFarland turbidity standard. They also made sure to have both positive and negative controls on hand.

2.7. Tissue culture plate technique for biofilm activity determination

To find out if AgO NPs could stop biofilm formation, this test was conducted. The colorimetric measurements of the crystal violet that sessile cells incorporate form the basis of the test. To summarize, a single bacterial species (1×10^6 /ml) was added to 180 μ L of sterile, 96-well flat-bottom polystyrene TCPs. Various concentrations of AgO NPs were introduced after 24 hours of culture. A further 4 hours of incubation at 37°C was applied to the cell culture plates. In the combination tests, bacteria were exposed to antibiotics at sublethal doses or to specific antibiotics together with AgO NPs. Following incubation, the medium was taken out and the wells were rinsed three times with 200 μ L of sterile distilled water in order to eliminate any bacteria that did not adhere. After waiting 45 minutes for the wells to air dry, 200 μ L of a crystal violet solution in water with a concentration of 0.1% (v/v) was applied to each well. Next, 300 μ L of sterile distilled water was used to wash the wells five times in order to eliminate any remaining stain. Two hundred microliters of 95% (v/v) ethanol were used to dissolve the dye that was incorporated by the adhering cells. Utilizing a microtiter ELISA reader, the absorbance of every well was assessed at 595 nm. An indicator of bacterial adhesion to surfaces and, by extension, biofilm activity, was the absorbance difference between the treated and control wells. The mathematical formula (1) that was used to determine the percentage of biofilm activity inhibition was [22]:

$$\text{Biofilm inhibition rate (\%)} = \left(\frac{\text{Control OD} - \text{Treated OD}}{\text{Control OD}} \right) \times 100 \dots \dots (1)$$

Triplicate runs of each experiment were carried out. Means $\pm$ SD are used to express the data.

2.8. Characterization of silver oxide nanoparticles

The sample of the decreased AgO NPs solution was taken, and 5 mL of the water-based portion was used for UV-vis spectroscopy. Using a ratio of (4:6) ml, the reduction of pure Ag ions was tested using dill leaf extract. A JASCO UV-visible absorption spectrophotometer, which has a resolution of 300 to 1000 nm, was used for the analysis. We used the deposition (drop casting) approach to generate a thin layer of AgO NPs for XRD research, and then we recorded the diffraction pattern using Cu-K α radiation at a wavelength of 1.78Å. Scanning was done in the 20–90° range at a rate of 0.02°/min for 20 minutes with a viewing time constant of 2. To prepare the grids for SEM examination, a carbon-coated copper grid was sprayed with a drop of AgO NPs solution and then dried under a light. A 200 keV ultra-resolution scanning electron microscope (JEOL-2010) was used to observe and document the nanoparticles' structural organization, which includes their size and form.

3. Results and Discussion

The production of AgO NPs using dill leaf extract was effectively accomplished in a single step. Figure 1 demonstrates that the AgO NPs were decreased by the dill leaf extract after 1min of the reaction, resulting in a yellow tint. With longer exposure to cold plasma, the color of the AgO NPs changed dark brown, indicating the creation of AgO NPs. Subsequent analysis of the produced nanoparticles provided more evidence to substantiate the earlier findings.

3.1. UV-visible Spectra of Silver Oxide Nanoparticles Synthesis

The effective fabrication of nanosilver oxides was confirmed by analyzing the visible color shift using UV-visible absorption spectroscopy. The progression of the interaction between the metal ions (Ag+) and the leaf extract was observed for a duration of 5 minutes. The wavelength of the nanoparticle extract was determined by UV-visible spectroscopy in order to verify the presence of silver oxides. The peaks shown in Fig. 2 unequivocally indicate the existence of the surface plasmon resonance phenomena. Additionally, an ultraviolet absorption band ranging from 405 to 420 nm was detected. Optical characterization was used to get data on the physical characteriztics of nanoparticles, including their absorption and band gap energy. The band gap energy is the most crucial factor among optical properties. Graphing empirical absorbance data is a standard method used to ascertain the optical band gap energy of nanoparticles. Planck's law, as represented by Eq. (2) [23] was used to directly determine the band gap energy from λ_{max} :

$$E_g = \frac{hc}{\lambda_{max}} = \frac{1240}{\lambda_{max}} \dots \dots \dots (2)$$

The energy gap, denoted as E_g , is obtained using the following variables: h for Planck's constant (6.626×10^{-34} J.s), c for the speed of light (3×10^8 m/s), and λ for the wavelength, which may be found by extrapolating the linear portion of the absorbance spectrum.

The change in the color of the reaction medium is attributed to the presence of reducing agents inside the leaf extract. The color of silver nanoparticles is a result of the stimulation of surface plasmon oscillation inside the nanoparticles. This change in color is caused by the redox reaction between the leaf extract and $AgNO_3$. Silver nanoparticles (AgO NPs) possess unbound electrons, resulting in the absorption of surface plasmon resonance. This occurs when the electrons in the metal nanoparticles vibrate in sync with the light wave. The user's text is[24]. Figure 1 shows that the AgNPs generated had a distinct and solitary surface plasmon resonance (SPR) band at 415 nm, indicating that the silver ions were successfully reduced to metallic silver.

3.2. X-Ray diffraction analysis of silver oxide nanoparticles synthesis

In addition, XRD examination verified the existence of silver oxide nanoparticles. In Fig. 3, we can see the XRD patterns of the leaves extract-synthesized silver oxide nanoparticles. From the lattice planes, a handful of Bragg reflections with 2θ values of 27.5° , 28.7° , 38.20° , 43.55° , 53.78° , and 74.90° have been noted; these may be attributed to the silver (110), (210), (111), (200), (142), and (311) faces equally. Also, there are several 33.54° , 35.48° , 39.54° , 54.87° , and 67.24° reflections from the lattice levels that may be indexed to the faces of silver oxide (002), (132), (111), (202), and (002) respectively. The XRD pattern makes it quite evident that the oxides and silver nanoparticles created in this mixture are crystalline and possess a face-centered cubic (FCC) crystal structure. The existence of Ag colloids in the sample was verified by the XRD pattern. The (210) facets of Ag were thought to be responsible for the high diffraction peak at 30.01° . Particles composed entirely of pure silver are clearly indicated by the strong diffraction peak at a 2θ value of 30.01° relative to the (111) lattice plane of silver FCC. The crystalline and amorphous organic phases may be associated with the peaks at 2θ values of 19.55° , 22.45° , 24.98° , 42.10° , 46.21° , 59.36° , 57.25° , 70.12° , and 77.25° [25][3]. A comparison was made between the acquired spectra and standard JCPDS data for the location and strength of the Bragg peak. According to the findings, the molecular structure is cubic. The crystallization of the bioorganic phase was also indicated by the observation of certain unassigned peaks (*) [24]. Using the Debye-Scherrer Eq. (3)[26], [27] the size of the Ag/AgO NPs' crystallites was determined:

$$D = \frac{k\lambda}{\beta \cos\theta} \quad \dots \dots (3)$$

The variables in this equation are the crystallite size (D), the X-ray wavelength (λ), the Bragg's angle location, the full width at half maximum (FWHM) of the peaks at the θ diffraction angle, and a constant k that depends on the crystallite form (0.94). The average crystalline sizes of Ag NPs are 27.2 nm and for AgO NPs are 28.7 nm.

3.3. SEM and EDX analysis of silver oxide nanoparticles synthesis

The surface shape and content of the constituent elements were characterized using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX). The surface topography of the AgO NPs that were biosynthesized in a green environment was examined using a scanning electron microscope (SEM) with a 2 μm magnification area and a 5.0000 magnification power. Inspection of the adsorbent's surface was carried out using a scanning electron microscope. Figure 4 shows that the biosynthesized AgO NPs were uniformly formed and distributed. They may be found in size ranges of 40–132 nanometers, either as tiny particles or as clusters of many particles. Figure 5 shows that the average diameters for AgO NPs were 132 nm.

Element types (qualitative) and analyte concentrations (quantitative) in a sample element can be determined by EDX analysis. The produced nanoparticles' contents were characterized quantitatively and qualitatively by EDX analysis. The chemical composition of AgO NPs produced from *Anethum graveolens* leave extract is shown in Fig. 6. Silver accounted for the largest weight percentage observed

(31.1% in nanoparticles), according to the data in Table 1, which reveal the percentage of EDX element contents of AgO NPs biosynthesized from plant leave extracts. An additional the components in the *Anethum graveolens* leave extract may be C (29. 7%), Ca (1.4%), Rb (0.7%), O (37. 0%). Figure 7 show the chemical mapping image of silver oxide nanoparticles from aqueous extract of *Anethum Graveolens* leaves.

Table 1
Element percent of silver oxide nanoparticles prepared from aqueous extract of *Anethum Graveolens* leaves.

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
C	29.7	0.7	8.1	0.2
O	37.0	1.2	13.4	0.4
Ca	1.4	0.1	1.3	0.1
Rb	0.7	0.2	1.3	0.3
Ag	31.1	0.2	75.9	0.4

3.4. Antibacterial Activity

The results of an investigation into the inhibition effects of AgO NPs produced with *Anethum Graveolens* leave extract at 0.1 M concentrations against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Klebsiella pneumoniae* and *Escherichia coli*) bacterial species were listed in Table 2. The most bacteria with a thick peptidoglycan cell wall are more susceptible to the influence of silver ions, which can penetrate this wall easily, and this is why Ag/AgO NPs inhibit Gram-positive bacteria (*S. aureus*) at a faster rate. The efficacy of silver oxide against Gram-negative bacteria is reduced because these bacteria have an extra layer of lipids and polysaccharides that protects them from the direct influence of silver ions. Nanoparticles' ability to inhibit bacterial growth has been the subject of numerous theories. This is because nanoparticles share many characteristics with bacteria, including their size, stability, and the fact that they allow for longer interactions with bacteria due to higher concentrations of the growth medium. As the surface-to-volume ratio in the nanostate increases millions of times, this leads to a change in the overall structural and optical properties[28]. There are stomata, or nanoscale holes, in the membranes of the vast majority of bacterial cell types. However, for nanostructures to inhibit bacterial growth by interfering with normal cell function, they need to be stable enough to cross or permeate these membranes [29]. Figure 8 shows the nature of antibacterial effect of AgO NPs against different type of bacteria.

Table 2
Tests on gram-positive and gram-negative bacteria to determine the inhibitory effectiveness of AgO NPs.

Bacteria	Inhibition Zone (mm)
<i>Escherichia coli</i>	12
<i>Klebsiella pneumoniae</i>	11
<i>Staphylococcus aureus</i>	14

3.5. Influence AgO NPs on Biofilms Activity

The MIC of AgO NPs was summarized in the results shown in Table 3. The Sub-MIC of AgO NPs was determined at a concentration of 0.05 M. This concentration was utilized for additional in vitro and spectroscopic investigations.

Table 3
MIC of AgO NPs against Gram-positive and Gram-negative bacteria

Bacteria	AgO NPs	
	MIC	Sub-MIC
<i>Klebsiella pneumoniae</i>	0.1	0.05
<i>Escherichia coli</i>	0.1	0.05
<i>Staphylococcus aureus</i>	0.1	0.05

Figure 9 shows that *K. pneumoniae*, *E. coli*, and *S. aureus* treated with AgO NPs significantly reduced biofilm development. Treatment with 0.05 M of AgO NPs resulted in the greatest inhibition percentage (59.31%) against *S. aureus*, followed by *E. coli* (58.89%) and *K. pneumoniae* (48.31%), as listed in Table 4.

Table 4

Biofilm formation by *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli* strains and the effect of AgO NPs prepared from aqueous extract of *Anethum Graveolens* leaves.

Bacterial type	Before treatment				After treatment				
	1st OD	2nd OD	3rd OD	Mean	1st OD	2nd OD	3rd OD	Mean	Inhibition rate %
<i>Klebsiella pneumoniae</i>	0.673	0.683	0.616	0.657	0.318	0.373	0.334	0.341	48.10 ± 1.638
<i>Escherichia coli</i>	0.673	0.632	0.687	0.664	0.3	0.33	0.19	0.273	58.89 ± 1.7149
<i>Staphylococcus aureus</i>	0.647	0.611	0.644	0.634	0.245	0.262	0.268	0.258	59.31 ± 1.703

Multiple investigations have shown that AgO NPs exhibit antibiofilm action against human infections, suggesting that they might be used as an alternative to antibiotics. Smaller AgO NPs have a greater surface contact area with microbes, which increases their activity, but the shape and size of nanoparticles also have a role. In this study, 132 nm in average diameter spherical AgO NPs are used. Because of their complex composition, aqueous extracts stabilize AgO NPs and may even function as protective coating agents. To guarantee the stability of the AgO NPs and prevent their aggregation, the present preparations do not need any further coating procedures, in contrast to previous known techniques for aggregating AgO NPs. According to some sources, AgNPs have the potential to hinder bacterial biofilm formation by interfering with the biofilm's primary components or by physically damaging the biofilm. Nanoparticles' effects on microbes and the biofilms they create may be broadly classified into two main groups. It starts by damaging the cytoplasmic membrane and other parts of the cell directly [30]. Additionally, the production of reactive oxygen species (ROS) has the potential to increase the permeability of bacterial membranes and render proteins, RNA, and DNA currently present in the cell inactive [17][31]. On top of that, AgO NPs have the potential to stop biofilm development in its tracks by reducing quorum sensing in bacteria [32]. The release of silver ions from nanoparticles is responsible for their bactericidal effect, which greatly reduces the viability of bacterial cells[33]. To combat staphylococci, they alter intracellular adenosine-5'-triphosphate (ATP) levels, inhibit bacterial DNA replication, and degrade bacterial cytoplasmic membranes, among other ways of damaging bacterial cells. Phosphate, sulfhydryl, and carbonyl groups—electron donors—are common in proteins and cell membranes, and silver ions bind strongly to these groups. Protein thiol groups may be bound to these molecules, altering the proteins' three-dimensional structure and blocking their active binding sites [34]. Their one-of-a-kind shape and many ways of interacting with bacterial cell membranes give them the unique capacity to inhibit bacterial growth. A variety of reactive oxygen and nitrogen species (RONS) can only be produced by non-thermal atmospheric pressure plasma sources, which are crucial to the effectiveness of this application. One of the most important steps in making nanomaterials is interacting plasma with liquid, as RONS produced by plasma may change the chemical composition of a liquid [35]. Reactive oxygen and nitrogen species (RONS) such as hydroxyl radical, superoxide, ozone, singlet

oxygen, nitric oxide, and hydrogen peroxide are the most common byproducts of plasma-liquid interactions [22].

Conclusions

The current study indicated that AgO NPs inhibited biofilm formation in (*Staphylococcus aureus*) and (*Klebsiella pneumoniae, coli, Escherichia*) through the repression of genes related to biofilm formation. AgO NPs were prepared using cold plasma technology with *Anethum Graveolens* leave extract, which resulted in a slight reduction in the severity of all tested biofilms. Plant extracts include flavonoids, polyphenols, and other naturally occurring chemical substances that may interact with nano-sized silver ions to stabilize and minimize their toxicity. Although larger granule size may reduce some aspects of the bioactivity of nanoparticles, encapsulation with plant extracts can provide additional benefits, such as improved biocompatibility and reduced toxicity. In this study, increasing the particle size of the nanoparticles may have slightly affected their ability to inhibit biofilm formation, but they were still effective to a certain degree.

Declarations

Conflicts of interest

No conflict of interest among the authors

Ethical Approval

Not apply.

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Author Contribution

“The idea for the manuscript and the experimental work was by the author R.S.M., the author N. Kh wrote the main text of the manuscript, N. Kh., R.S.M. Prepared Figures 1 to 3, and R. S.A. Obeah prepared Figure 5. The author, A.M.D, revised the language of the manuscript. All authors reviewed the manuscript.”

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

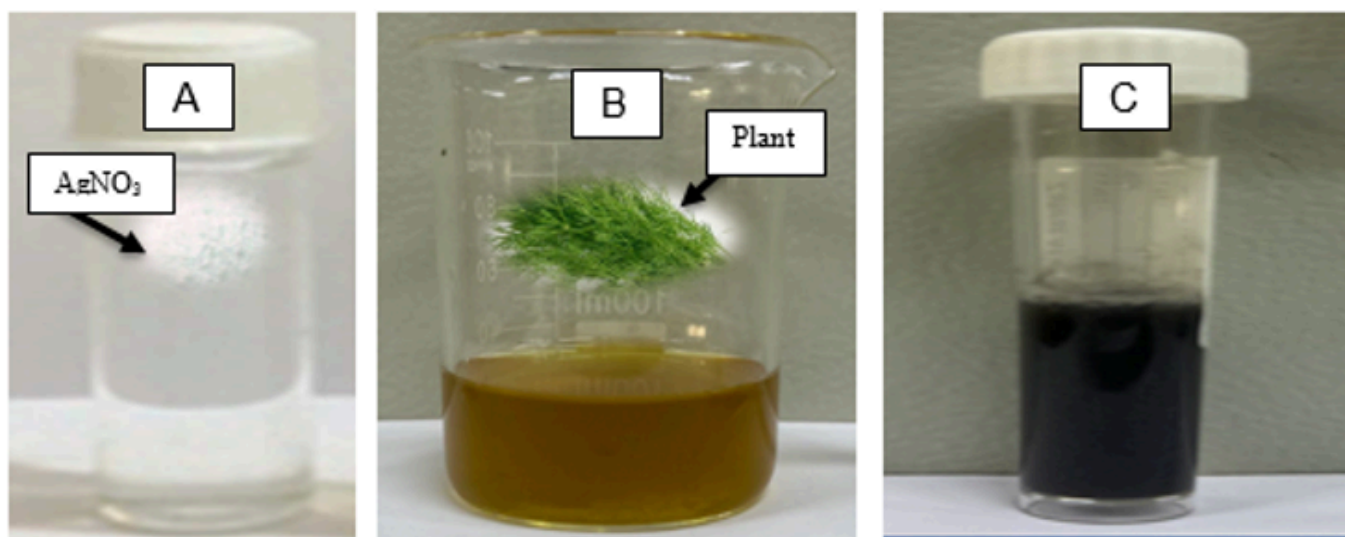


Figure 1

Synthesis of AgO nanoparticles prepared from aqueous extract of *Anethum Graveolens* leaves. **(a)** AgNO_3 solution, **(b)** aqueous extract, and **(c)** AgO NPs.

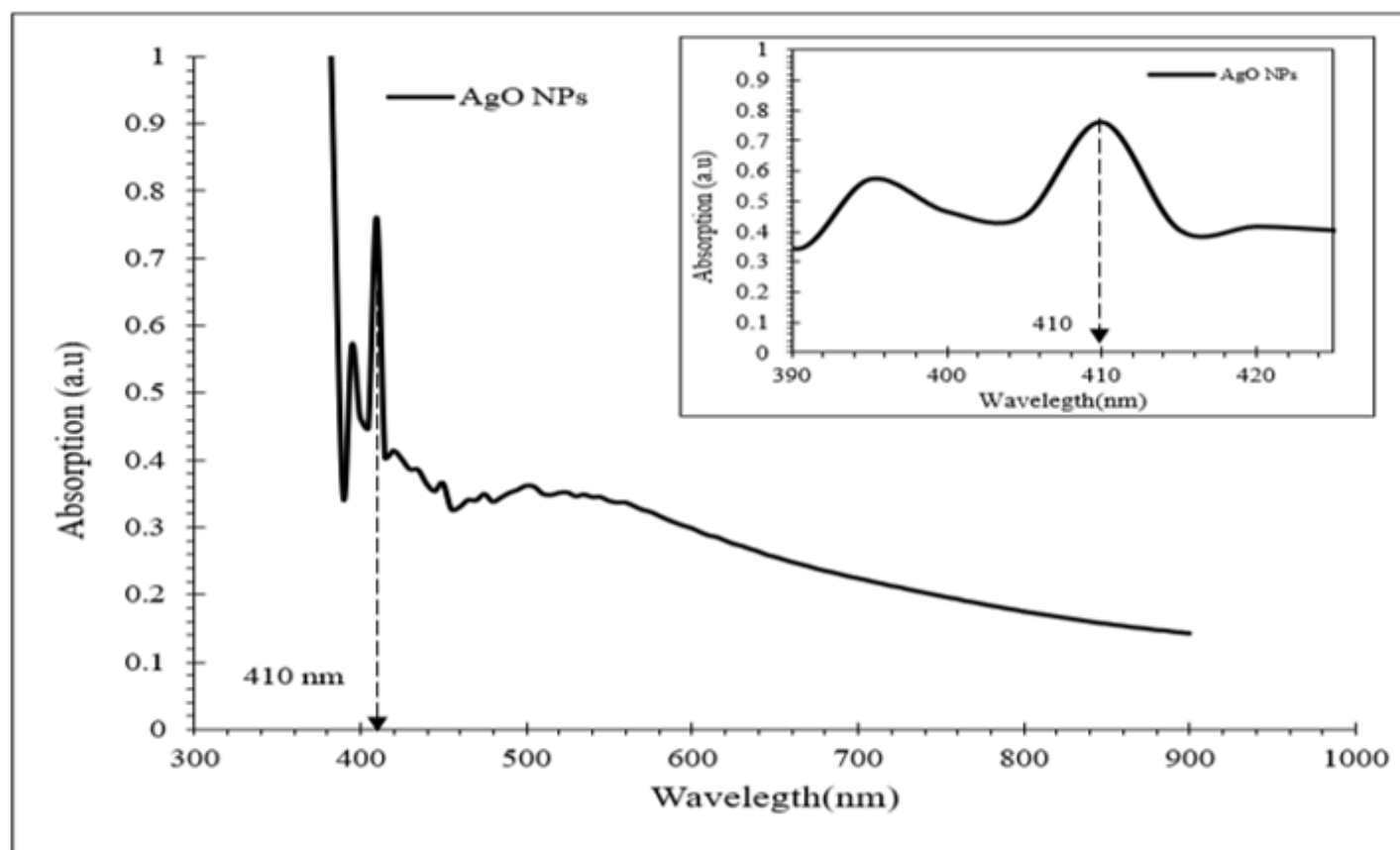


Figure 2

UV-visible spectra of silver oxide nanoparticles prepared from extract of *Anethum Graveolens* leaves.

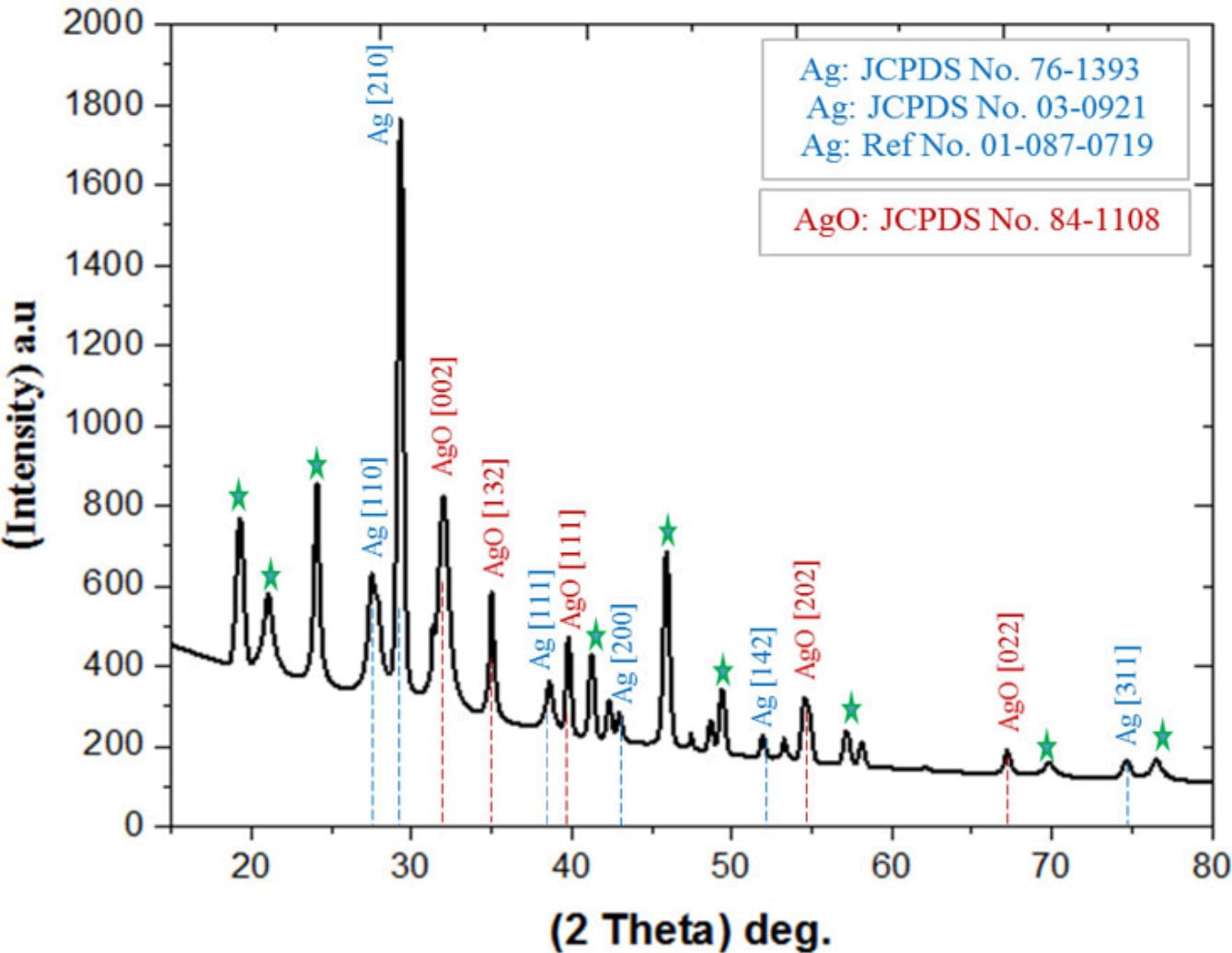


Figure 3

XRD Pattern of silver oxide nanoparticles prepared from aqueous extract of *Anethum Graveolens* leaves.

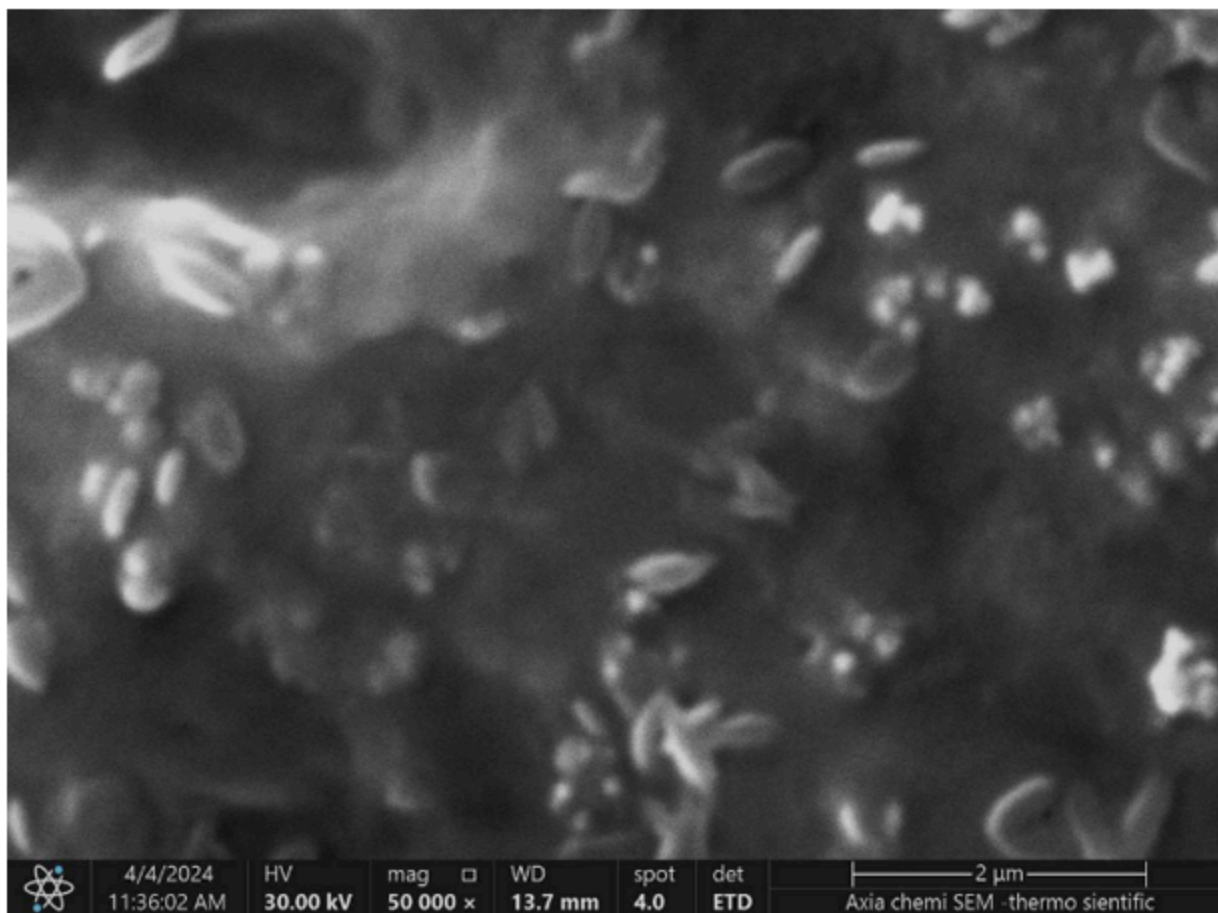


Figure 4

SEM analysis of silver oxide nanoparticles prepared from aqueous extract of *Anethum Graveolens* leaves.

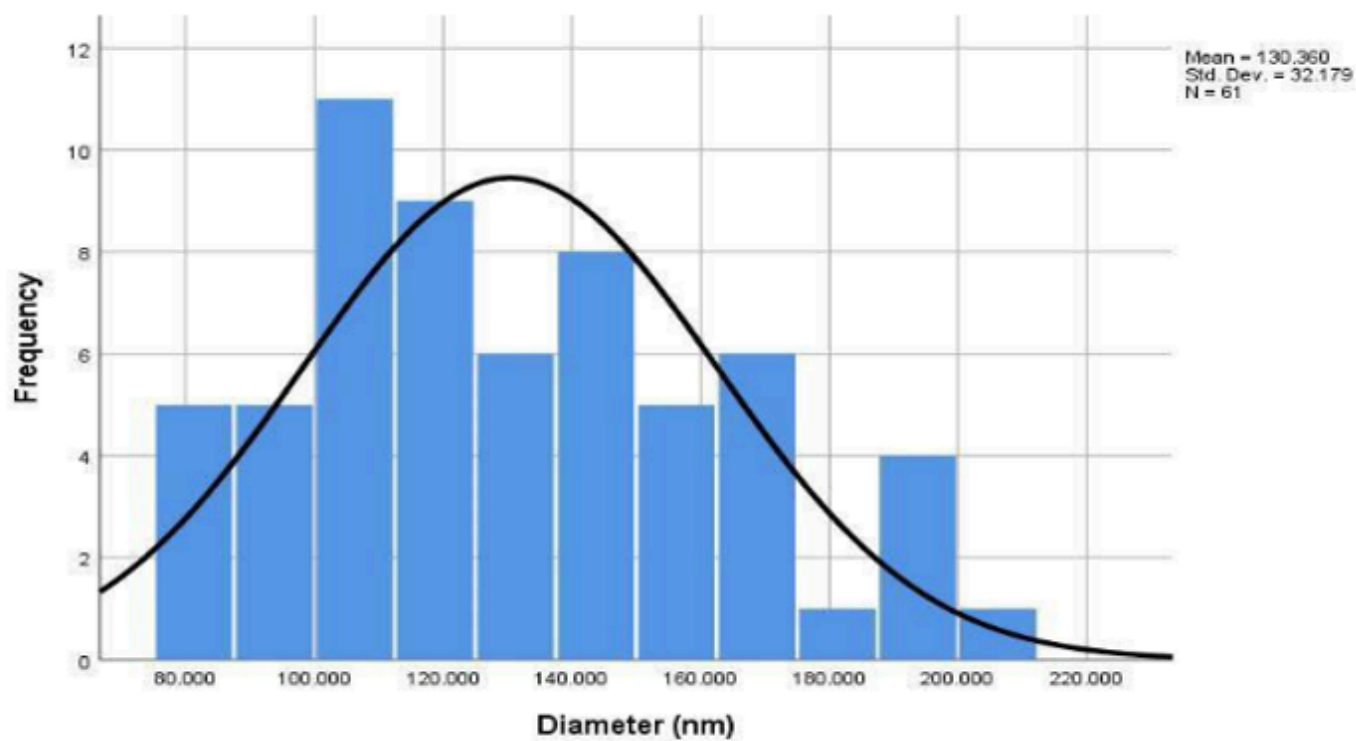


Figure 5

Average diameter of particles histogram for silver oxide nanoparticles prepared from aqueous extract of *Anethum Graveolens* leaves.

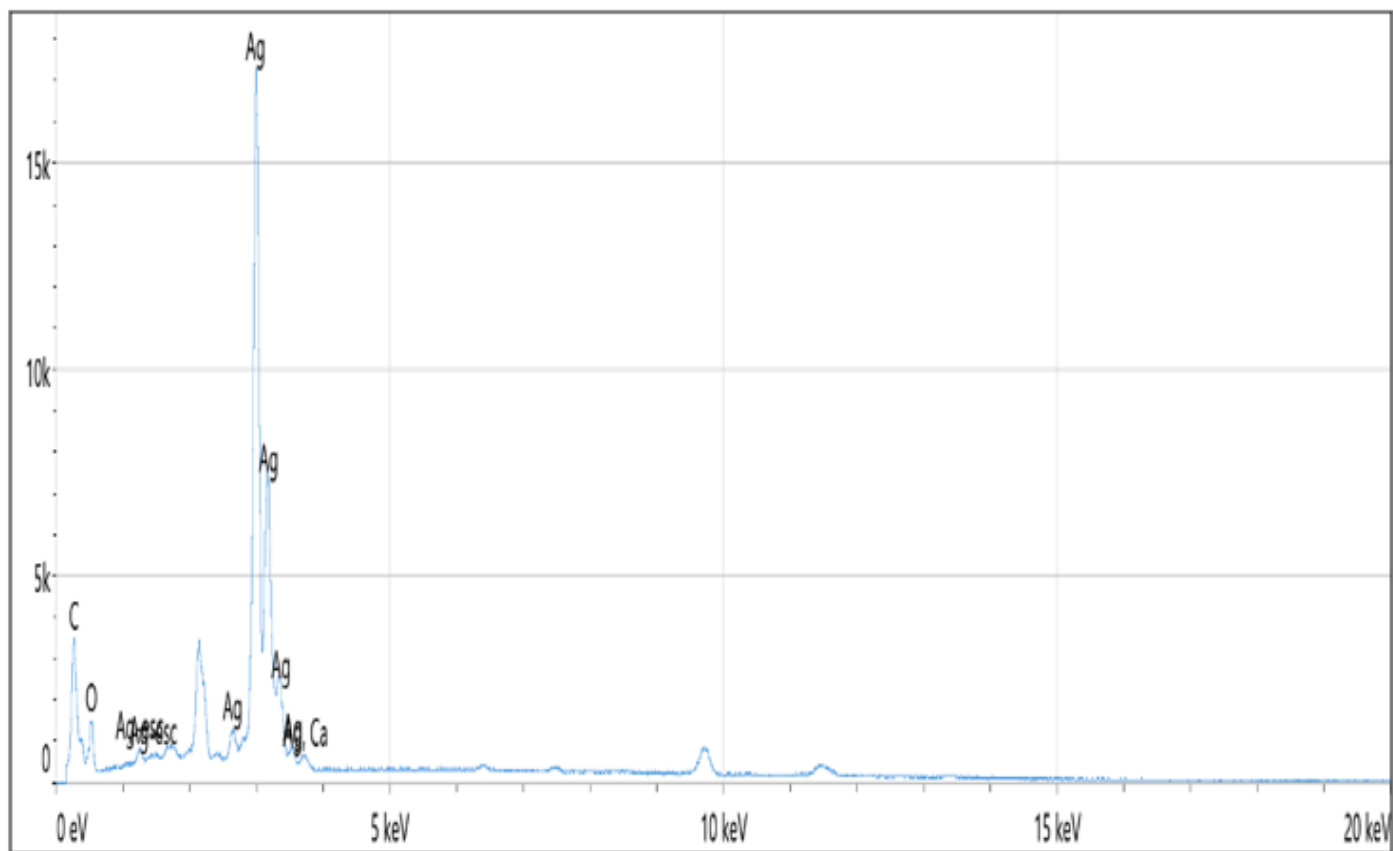


Figure 6

EDX analysis of silver oxide nanoparticles prepared from *Anethum Graveolens* leaves.

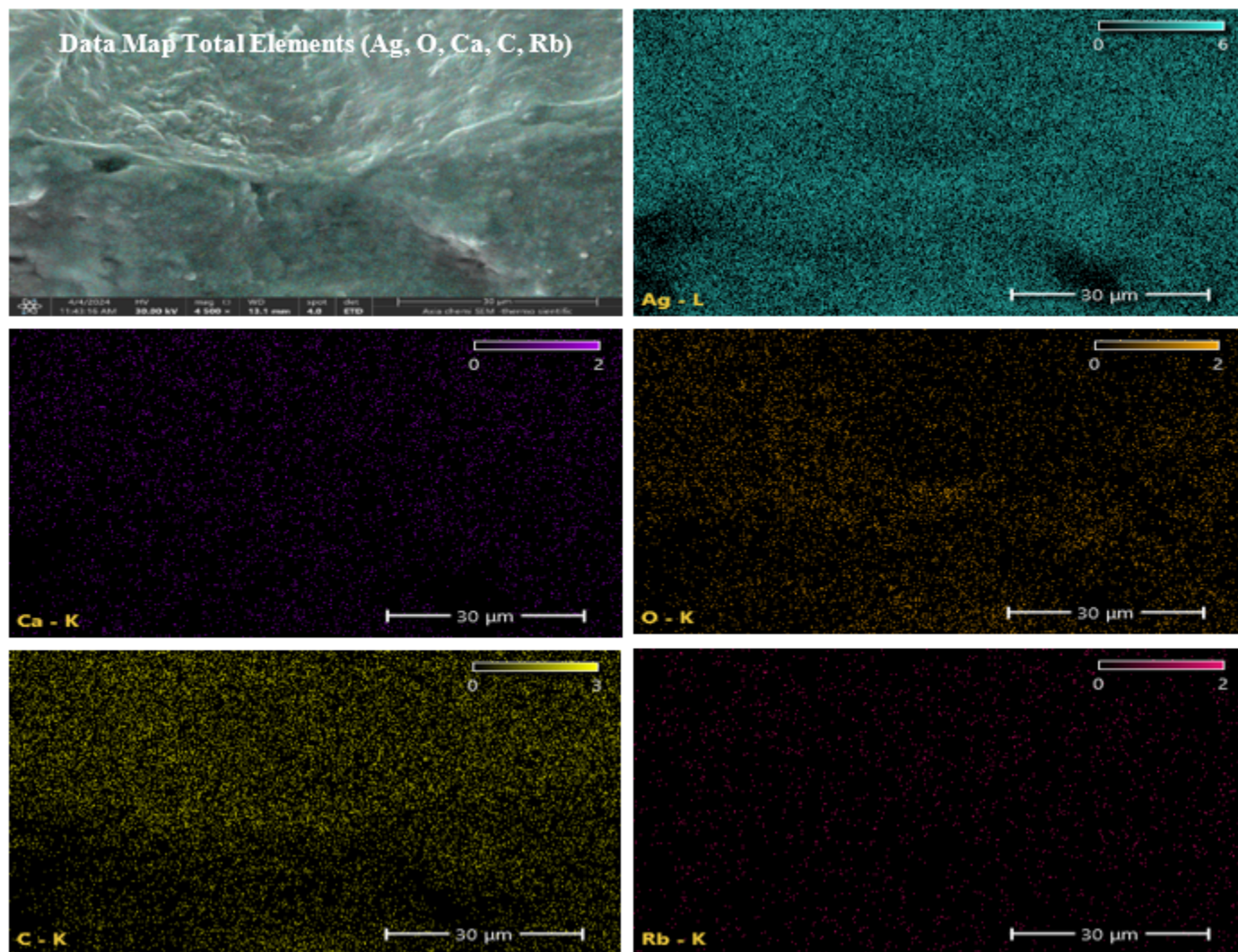


Figure 7

Chemical mapping image of silver oxide nanoparticles prepared from extract of *Anethum Graveolens* leaves.

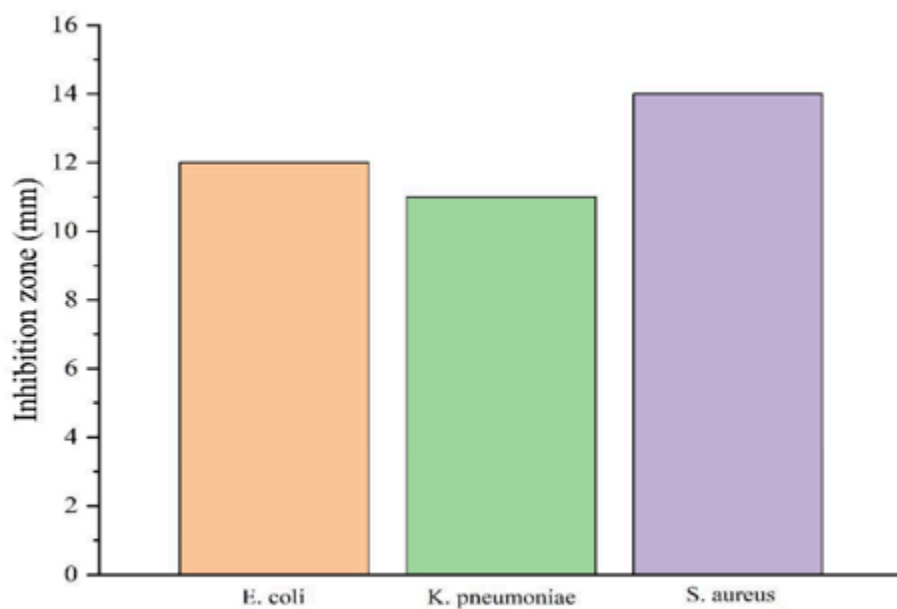


Figure 8

Effect of silver oxide nanoparticles on the inhibition bacterial (*S.s aureus*, *K. pneumoniae*, *E. coli*).

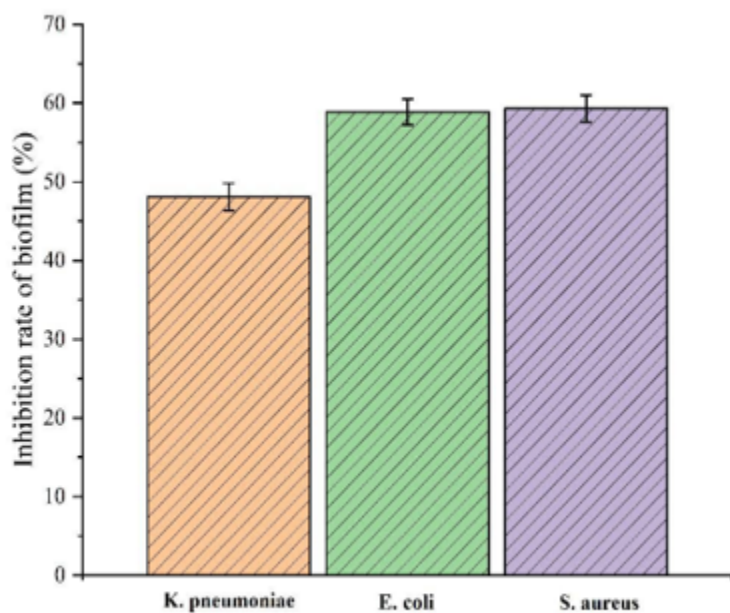


Figure 9

Effects of AgO NPs against biofilm *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*