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***RESEARCH ARTICLE* – Physics**

Bactericidal effects of silver nanoparticles prepared by green synthesis

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Abstract

This work aims to paper silver nanoparticles (AgNPs) using the green synthesis method, considered a low-cost and environmentally friendly method, and to study the structural properties of these nanoparticles by X-ray diffraction (XRD), scanning electron microscope (SEM), and atomic force microscopy (AFM) measurements. The results of XRD show that the AgNPs are polycrystalline in the dominant direction (122) (111), (200) (220) (311). By studying the surface morphology, the AFM results showed that the grain size of the AgNPs is (6.73 nm). In contrast, the SEM results showed that the AgNPs have a spherical shape, and the average particle size is (52.532 nm). The chemical signature of AgNPs was known through FTIR measurement and was found to consist of a group of organic compounds. Through biological application, AgNPs were found to be effective in killing Escherichia coli, which is usually present in contaminated water and infects the intestines of humans and animals. That allows the AgNPs to effectively reduce many human diseases caused by these types of bacteria.

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***Keywords*: AgNPs, Green synthesis, Escherichia coli, SEM, XRD, AFM.**

Introduction

NPs are minimal-size materials with diameters ranging from 1-100 nm [1].They are categorized into many classes according to their qualities, forms, and dimensions. The nanoparticle groupings include fullerenes, metal NPs, ceramic NPs, and polymeric NPs. Because of their nano-scale size with large surface area, NPs have distinct physical and chemical characteristics. Scientific reports indicate that their optical qualities vary depending on size, which imparts varied colours owing to absorption in the visible spectrum. Their particular size, shape, and structure impact their reactivity, toughness, and other characteristics. These characteristics make them ideal options for a variety of commercial and domestic applications, including catalysis, imaging, medicinal applications, energy-based research, and environmental applications. NPs are broadly classed depending on their shape, size, and chemical characteristics. Based on their physical and chemical characteristics, carbon-based NPs, metal NPs, ceramic NPs, semiconductor NPs, polymeric NPs, and lipid-based NPs are well-known types of NPs [2] .

NPs may be synthesized in a variety of ways. Nonetheless, these approaches are roughly classified into two categories: Mechanical milling, chemical etching, sputtering, laser ablation, and electro-explosion, which are all examples of top-down synthesis. Bottom-up synthesis is divided into two parts: biosynthesis (bacteria, yeast, fungus, plants, etc.) and chemical synthesis (template support synthesis, spinning, laser pyrolysis, plasma or flame spraying synthesis, chemical vapour deposition, and atomic or molecular condensation) [3]. Nanoparticles may be created via green synthesis. Green nanoparticle production has several potential uses in environmental and medicinal domains. Green synthesis aims to decrease the usage of potentially hazardous chemicals in particular. Using biological components, such as plants, is normally safe. Plants contain reducing and capping agents as well. The three most important parameters for nanoparticle synthesis are the selection of a green or environmentally friendly solvent, a suitable reducing agent, and a safe substance for stabilization. Extensive synthetic approaches have created nanoparticles, with physical, chemical, and biosynthetic mechanisms dominating. Chemical procedures are generally excessively costly and contain harmful and poisonous substances that pose various environmental dangers. For the green creation of silver nanoparticles, a silver metal ion solution and a reducing biological agent are required. Silver ion reduction and stabilization via biomolecule fusion of polysaccharides, vitamins, amino acids, proteins, saponins, alkaloids, terpenes, and phenolics [4], [5] is the most accessible and cost-effective nanoparticle synthesis method. Later, the discovery of variable-shaped silver nanoparticles (AgNPs) demonstrated their use as antibacterial agents against various microbes. AgNPs' antibacterial and anti-inflammatory characteristics have been used in the medical field [6], [7], [8] for controlling microbial infections. Silver nanoparticles

(AgNPs) are rapidly being employed in medical, food, health care, consumer, and industrial industries due to their unique physical and chemical characteristics. Among these are optical, electrical, and thermal characteristics, strong electrical conductivity, and biological qualities [9], [10], [11], [12], [13]. Water pollution is one of the most dangerous types of environmental pollution resulting from the rapid industrialization and civilization process and one of the main issues facing environmentally friendly environments, in addition to its harmful effects on health. Water pollution has changed the way of life of aquatic organisms. In addition, the number of premature deaths has increased daily as a result of water pollution [14]. This work aims to manufacture silver nanoparticles (AgNPs) using a green synthesis method and employ these particles to kill Escherichia coli bacteria.

Experimental

In this work, silver nanoparticles were prepared using the green synthesis method using the hibiscus flower. The hibiscus was ground and sieved using a micro sieve with diameters of 38 µm. Then, 2 gm of curds were added to 100 ml of deionized water, and the solution was placed on magnetic stirrer at a temperature of 50 ˚C for an hour until the dark red color of the solution was reached. An hour after preparing the solution, it was filtered. 1.7 gm of silver nitrate, at a concentration of 1 mM, was mixed with 100 ml of deionized water, and the solution was placed on a Magnetic stirrer for an hour at a temperature of 50 ˚C. To prepare nanosilver, 3 ml of hibiscus was added drop by drop to the silver nitrate prepared above. We note that it does not dissolve directly, so 5µl of hydrochloric acid was added so the solution became a light lead color. Thus, the final ratio of the solution became equal to 1:9, where the ratio of silver is 1 and the ratio deionized water 9, as shown in Fig. (1). The prepared silver nanoparticles were used to treat the most common Escherichia coli bacteria in polluted water.

$$\mathbf{AgNO_3 + 2OH \rightarrow AgO + NO_2 + H_2O}$$

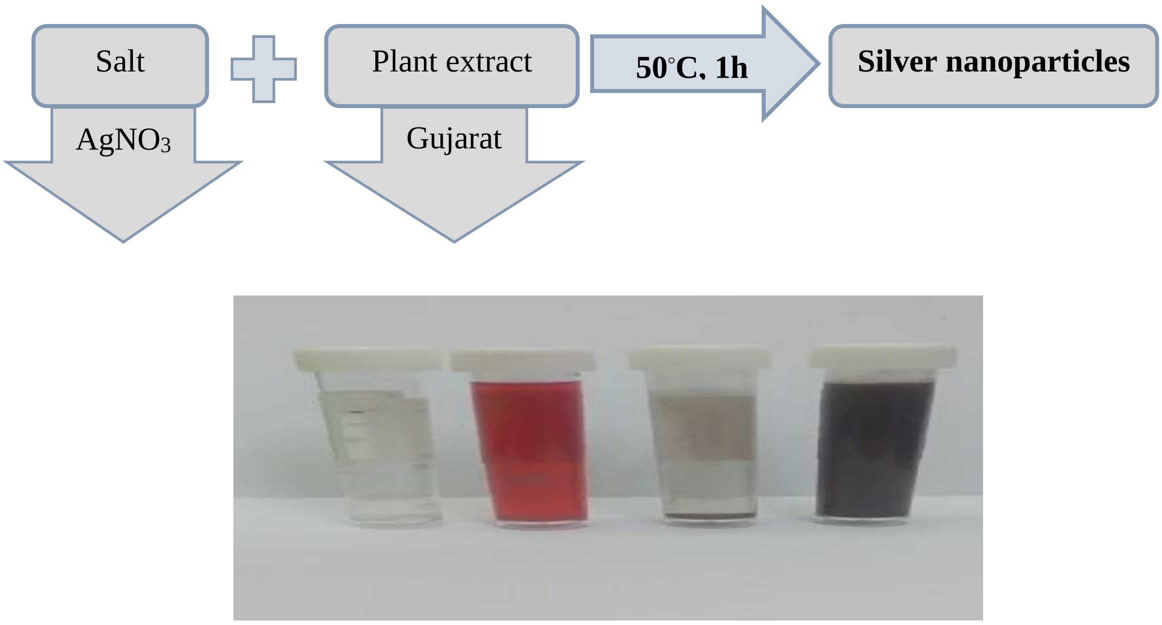


Fig. 1. Stages of preparation of silver nanoparticles

This research studies the effect of silver nanoparticles prepared by the green synthesis method on a type of bacteria present in polluted water that causes health problems in the intestines of humans and animals called Escherichia coli. This is done by preparing the Petri dish, cultivating the bacterial medium, spreading the bacteria over the entire dish, and making a hole in the middle of the dish; then, silver nanoparticles are placed in this hole, and after that, the dish is in the incubator for 24 hours at a temperature of 37◦C to determine the extent of the effect of the nanosilver in bacterial killing and its penetration into the cell wall and its viability. After the end of the incubation, the results are read as the noticed effect of silver nanoparticles on the bacteria through the appearance of a halo of inhibition in Escherichia coli.

Results

After preparing silver nanoparticles using the green synthesis technique, their structural and morphological properties were measured by XRD, AFM, and SEM. Measuring the X-rays indicates that the silver nanoparticles are polycrystalline, as well as the appearance of silver nanoparticles in the dominant direction (122) (111), (200) (220) (311) as shown in table (1) with an FCC structure of the synthesized silver nanoparticles [15], as well as silver oxides (111) (211) as in Fig. (2) The organic compound present in the extraction of hibiscus flower forms the unstable crystalline band [16]. AFM measurement, as in Fig. (3), indicates that the average grain size (6.73nm), roughness average (13.58 nm), and root Mean Square (1.59 nm) are shown in Table (2). As for the particle size, it was measured through the SEM technique, and it was found that the average particle size of the silver particles is (52.532nm) of a spherical shape [17], as shown in Fig. (4).

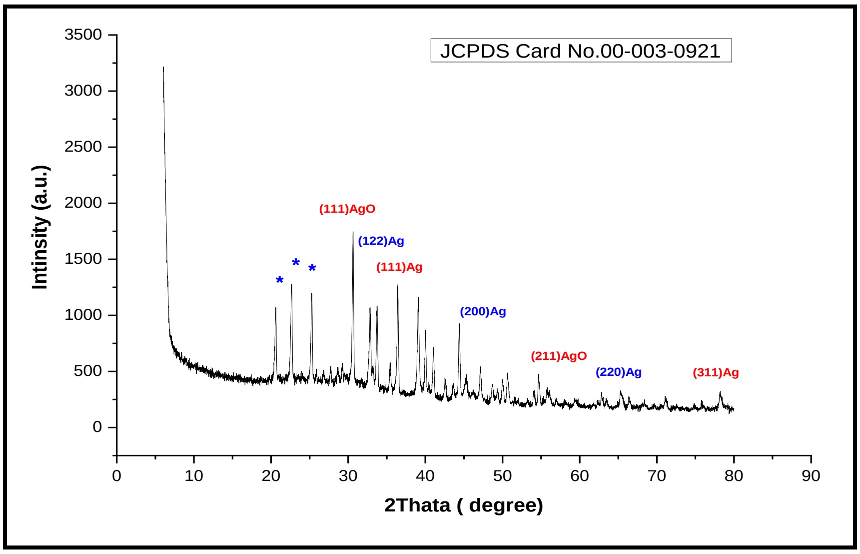
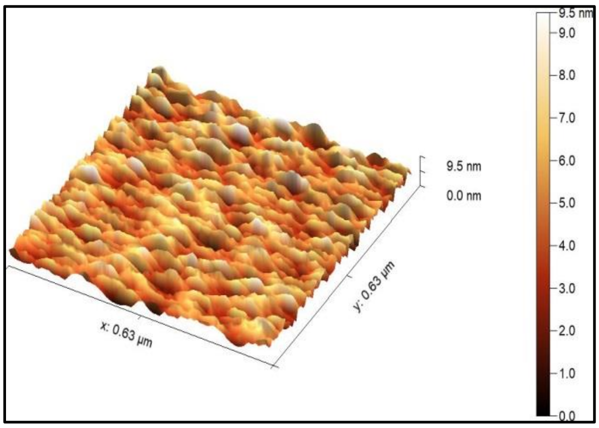


Fig. 2. XRD of Ag

Table 1. Ag XRD measurements

No.	Pos. (2θ°)	Rel. Int. (%)	Crystallite Size only (nm)
1	20.6315	46.57	0.026666
2	22.7025	55.57	0.024203
3	25.2998	56.47	0.007767
4	30.6323	100	0.015812
5	32.8434	51.43	67.9
6	33.2146	12.88	0.016348
7	36.4509	63.57	0.005403
8	39.0793	62.76	0.019543
9	44.391	48.75	0.009238
10	54.0479	7.89	39.9
11	65.2924	9.87	0.01384
12	75.9284	3.63	33.5



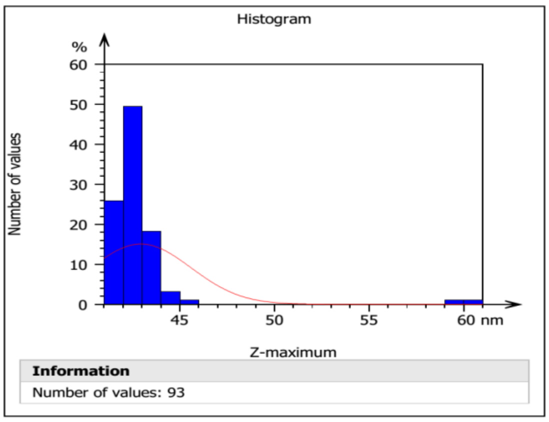


Fig. 3. AFM of Ag

Table .2. The results of AFM measurements

Samples	Average grain size (nm)	Roughness average (nm)	Root Mean Square (nm)
Ag	6.73	13.58	1.59

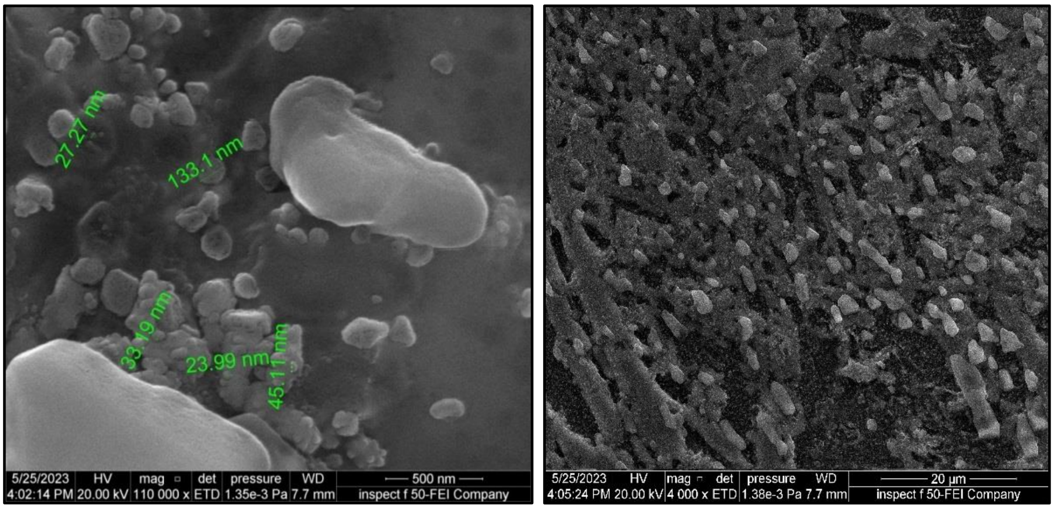


Fig. 4. SEM of Ag

From measuring FTIR, it was found that the silver nanoparticle spectra were recorded in the range of (483.91-3852.07 cm^{-1}). Several significant absorption peaks can be observed. The IR spectra of AgNPs show broad absorption bands at a range of (3745.08-3446.32 cm^{-1}) which corresponds to O-H stretching bands that attributed to compound phenolic, and at range (2923.86-2064 cm^{-1}) and (1383.98,1357.07 cm^{-1}) which corresponds C-H that attributed to compound alkane[18], and at range (1635.89 cm^{-1}) which corresponds C=C that attributed to compound conjugated alkane, and at scope (1083 cm^{-1}), (1031.39 cm^{-1}) which corresponds C-O that attributed to compound carboxylic and at the field (745.86 cm^{-1}), (483.91cm^{-1}) which corresponds Ag-O that attributed to compound metal oxide [17] . Table (3) also showed FTIR that the wave number is a function of the transmittance, as shown in Fig (5).

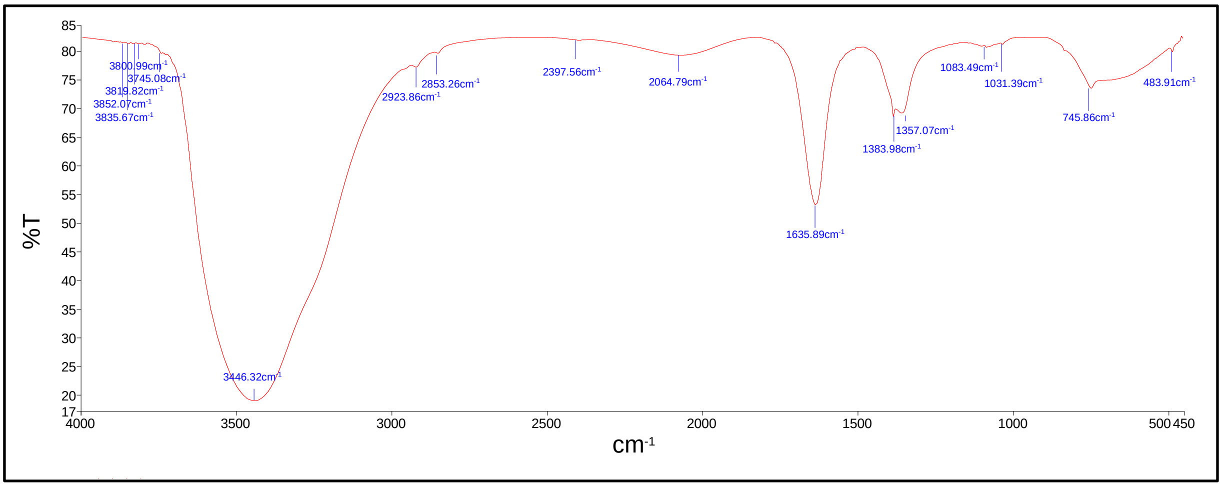


Fig. 5. FTIR of Ag

Table 3. FTIR measurement results

Peak Number	Wavenumber(cm^{-1})	Transmittance (a.u)	Group	Compound Class
1	3852.07	81.55	O-H	phenolic
2	3835.67	81.56	O-H	phenolic

3	3819.82	81.54	O-H	phenolic
4	3800.99	81.43	O-H	phenolic
5	3745.08	79.87	O-H	phenolic
6	3446.32	18.82	O-H	phenolic
7	2923.86	77.46	C-H	alkane
8	2853.26	79.90	C-H	alkane
9	2397.56	82.15	C-H	alkane
10	2064.79	79.53	C-H	alkane
11	1635.89	53.27	C=C	conjugated alkene
12	1383.98	68.78	C-H	alkyne

Through the biological application, it was observed that silver nanoparticles prepared by the green synthesis method effectively killed Escherichia coli bacteria in polluted water. Fig. (6) shows the inhibition zone, which was 12 mm. They are gram-negative bacteria commonly found in the intestines of animals and humans that are too small to be seen without a microscope [19]. AgNPs release Ag+ ions, which can accumulate on the cell walls and membranes of microorganisms and enter the cytoplasm. Inside the cell, Ag+ ions generate reactive oxygen species (ROS), which are the main factor of antimicrobial activity, including (1) inhibition of DNA synthesis, (2) inhibition of mRNA synthesis, (3) cell membrane destruction and extravasation. Cell components, (4) inhibition of protein synthesis, (5) inhibition of cell wall synthesis, (6) mitochondrial damage, and (7) inhibition of the electron transport chain. These effects eventually lead to cell death. In addition to their ability to release silver ions, AgNPs can kill bacteria themselves [20].

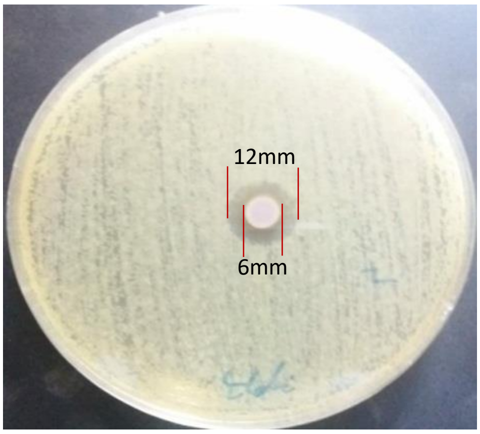


Fig. 6. Effect of Ag on Escherichia coli

Conclusions

Green synthesis methods and physical and chemical methods can prepare nanoparticles effectively. The green synthesis technique is a simple and low-cost, and available method that is environmentally friendly and does not produce toxic fumes. Silver nanoparticles prepared using the green synthesis method kills Escherichia coli bacteria in polluted water. That will allow the use of Silver nanoparticles to purify contaminated water.

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