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Eco-Friendly Production of Metal Nanoparticles Through Plant Extract Besides Their Assessment of Antibacterial, and Antifungal activity

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Abstract

A green-synthesis method for silver nanoparticles (Ag NPs) is a scientific breakthrough. Using sunflower plant extracts, this approach uses metallic and botanical synergy. Naturally occurring and renewable extracts reduce, chelate, stabilize, bind, and precipitate. The Ag nanoparticles' X-ray diffraction (XRD) crystal structure was cubic. Average nanoparticle crystallite size was 31.18 nm. Energy-dispersive X-ray spectroscopy (EDX) detected silver. Field Emission Scanning Electron Microscope (FESEM) examination revealed that the particles were Ag (silver) and spherical, averaging 31.23nm. Diffuse Reflectance Spectroscopy also indicated a 2.7 eV optical gap. Using many characterization methods, nanostructured silver was synthesized during this procedure. Biological efficacy assays can evaluate hierarchically porous silver's antibacterial properties. In the previous five years, strategies against *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus mutans, Ecoli, and Candida albicans, Penicillium spp*., and *Aspergillus spp.* were essential. Data demonstrates that these structures are attractive antibacterial choices. A majority of Ag NPs are natural bacterium substitutes for *Staphylococcus aureus, pseudomonas aeruginosa, Streptococcus mutans, and Ecoli.* Also against various fungi: The immune systems of immunocompromised hosts are threatened by yeasts *such Candida albicans, Penicillium*, and *Aspergillus*.measured. It calls into doubt the study's efficacy in antibacterial and other applications.

Keywords: Green Synthesis, Nanoparticles, Silver, *Staphylococcus aureus, Pseudomonas aeruginosa, , Ecoli*.

1 Introduction

Modern public health is threatened by microbial illnesses, necessitating the ongoing quest for novel antimicrobial medications and procedures. Common bacteria connected with human ailments include Candida albicans, *Staphylococcus aureus, S. epidermidis*, *E. coli*, and *Klebsiella spp*. Disease-causing organisms, they contribute to worldwide illness and death. *C. albicans*, a fungal pathogen that exploits chances, causes most healthcare-associated infections, especially in immunocompromised patients. However, *Staphylococcus aureus* and *epidermidis*, which are resistant to conventional antibiotics, are major clinical and societal issues. *Escherichia coli* and *Klebsiella spp*. are also risky because they cause urinary tract infections and severe systemic diseases.. [12].

The rise of antimicrobial resistance and the shortage of innovative, safe, broad-spectrum antibiotics need the development of potent, broad-spectrum alternatives. Silver nanoparticles (Ag NPs) have been studied extensively due to their intriguing physicochemical properties and proven antibacterial action against most pathogens. Ag NPs change microbial cell structure and function, disrupting important cellular processes and killing bacteria. [5].Although Ag NPs have been examined significantly in their antimicrobial properties, there is a lack of a comprehensive understanding of their effectiveness against clinical pathogens such as *Candida albicans*, *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli,* and *Klebsiella.remains* crucial. Moreover, it will shed light on how these compounds act and how they can be combined with the present antimicrobial agents in order to generate a new attacking plan against multidrug-resistant infections [19].

Silver nanoparticles are tested for their antibacterial activities against *Staphylococcus aureus, epidermidis*, *Escherichia coli*, *Klebsiella species*, and *Candida albicans.*[6] which make them eligible to be used as sensor and antibacterial coating [14]. Semiconductors and nanoparticles of silver are extensively used as the reinforcing fillers; most importantly, for the superconducting applications. Their utilization in materials is one of the critical factors that defines properties and performance of different industrial applications [9]. Due to their catalytic characteristics, surface reactivity, chemical stability, and heat tolerance, silver nanoparticles have gained popularity. Their unique properties make them mysterious, intriguing, and useful in many fields. Silver nanoparticles' distinctive properties include improved surface reactivity, chemical stability, thermal stability, and catalytic capabilities that interest researchers. Nanoparticles' unique qualities explain their scientific value in nanoscience and other domains. [8]. Silver nanoparticles (AgNPs) are now nanofabricated using high-tech methods and expensive machines that can operate at high temperatures [25]. Alternative procedures are now preferred due to their cost and complexity. Universal synthesis, especially green manufacturing, is a major priority for mass producing nanostructured materials, notably silver nanoparticles. Nature-based empowerment strategies come from natural resources and are environmentally benign, financially effective, and ecologically beneficial[10] [23].

Hashem *et al* (2022). discusses green silver nanoparticle synthesis and Bacillus thuriengistsen. These 32.7 nm nanoparticles with high efficacy antifungals against *aspergilli* strains are promising for treating *aspergillosis*[7].

Silver nanoparticle production and characterisation are the focus of this study. We investigate green synthesis methods using sunflower plant extracts to solve this challenge. This research advances nanotechnology and promotes clean, environmentally friendly nanomaterial creation [4]. An novel approach is used to analyze this green Nanosilver by carefully processing the silver nanoparticles and synthesizing it.[21].

This research will demonstrate that silver nanoparticles may be employed as alternative antibacterial agents against medically essential infections. This knowledge can assist solve the complex problem of antimicrobial resistance by improving antibiotic formulation. Recently, nanoparticles have received interest for their extensive physicochemical features, including strength, exceptional thermal conductivity, great damping, strong mechanical qualities, surface reactivity, and chemical and thermal durability.

2 Experimental

2.1 Silver NPs Preparation

Silver nanoparticles (Ag NPs) were successfully synthesised by reducing $AgNO3 \cdot 6H2O$. The synthesis utilized only chemical reagents sourced from the Aldrich Chemical Corporation. In the experimental procedure, 1gm of silver nitrate was dissolved in 30 ml of deionized water. Subsequently, 20 ml of sunflower plant extract was incrementally added to the mixture under continuous agitation using a magnetic stirrer while the temperature was maintained at 10°C through an ice-water bath. This stirring led to a brown precipitate, indicative of silver nanoparticle formation, within approximately 15 minutes, and the process was extended for an additional 15 minutes to ensure completion. Throughout the synthesis, the pH was meticulously maintained at 9. Following the reaction, the precipitate underwent filtration. This was succeeded by four cycles of centrifugation, each lasting 5 minutes at a velocity of 5000 revolutions per minute, after which the supernatant was discarded [26]. The resultant product was then left to reach equilibrium at ambient temperature. Annealing of the synthesized particles was conducted at two elevated temperatures, 600°C and 250°C and room temperature, to achieve the desired structural properties [11].

2.2 Prepare of Mueller Hinton Agar

Muller-Hinton (M-H) medium is made up of 20 mL of the powder which is added into 1 L of distilled water that is dithered on burner with shaking. The M-H would be sterilized by autoclaving it at 121°C for 15 minutes, which is the actual time. After that, I started the second pouring procedure as the thermometer registered 50 °C. Subsequently, I flipped the mould over and waited for about 15 minutes before refrigeration at 4 °C.[2]

2.3 Characterization

The (FE-SEM) field emission scanning electron microscopy were used in the morphological investigations. The crystal structure and chemical composition were examined using both X-ray diffractometer (XRD) and EDX, energy-dispersive X-ray spectroscopy. The optical features - including absorbance coefficient and energy gap were discovered in the DRS. However, the characterized samples underwent a detectability test in which their response and recovery times were measured to assess their comprehensiveness. These analytic operations served as a foundation for the operational details of its structure, morphology, light effects and bactericidal nature**.**

3. Result and Discussion

3.1 X-Ray Diffraction Analysis

The Ag nanoparticles were examined for their crystal structure with (XRD). The interplanar distance and size of the particles, with respect to their structure, were well established with the help of the average crystalline size calculated from the Debye–Scherer equation [3]. All these methods made a very important contribution toward acquiring only a complete understanding of the crystalline properties and atomic arrangements of the synthesized sample [24].The Debye–Scherer equation:

$$D = \frac{0.9\,\lambda}{\beta \cos\theta}$$

D: is the crystallite size (nm), λ: is the X-ray wavelength, θ: is the Bragg diffraction angle (degree), and β: is the Full Width at Half Maximum (FWHM) of the diffraction peak. Equation (2) measured the distance between the inter-planar spacing (d).

$$n\lambda = 2dsin\theta$$

Where n: is the order of the diffraction peak [15]. Figure (1) displays the X-ray diffraction (XRD) patterns of nano-sized silver at room temperature (R.T) as well as at 250°C and 600°C. The patterns reveal seven distinct sharp peaks at specific 2θ angles (100) , (111), (200), (311), (202) and (311). Notably, same diffraction peaks were observed at room temperature as well 250°C and 600°C , indicating specific structural features is same at all temperature but differ in intensity.

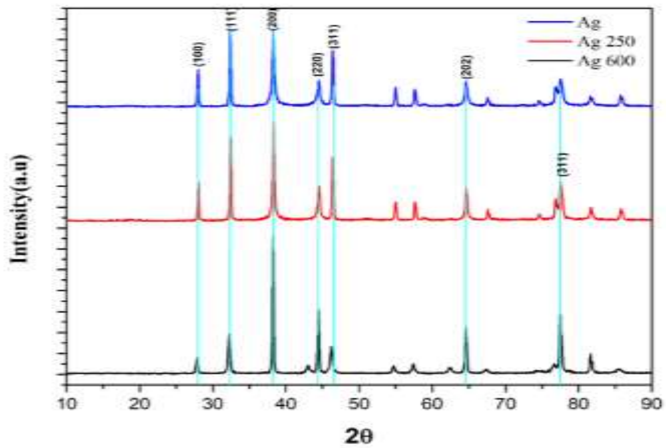


Figure 1: X-ray diffraction (XRD) patterns Ag NPs at room temperature, 250℃ and 600 ℃.

Results were agreeing and match with standard ICCD file no, , (96-100-0055) and (96-900-6748) respectively. The analysis revealed that the prepared nano-sized silver exhibited a cubic structure and possessed a polycrystalline nature. The crystallinity of the samples was quantitatively determined by calculating the Full Width at Half Maximum (FWHM) values. These results indicated a high degree of crystallinity.

predominantly in a single crystal form. By applying the Scherrer equation, the crystal size of AgNPs was determined at room temperature (RT),250℃, and 600℃, utilizing the Miller coefficients. The average crystal size was calculated to be 31.18 nm, as documented in table (1). Notably, the results indicated a correlation between temperature and crystal size, revealing an increase in particle crystallinity with rising temperatures.

Table 1: Structural properties of Ag NPs at R.T,250 ℃, and 600 ℃.

2θ (Deg.)	**FWHM (Deg.)**	**crystallite size (nm)**	**Average crystallite size**
27.84	0.30	26.98	29.19
32.24	0.34	24.16	
38.24	0.22	37.86	
44.43	0.26	33.28	
64.58	0.33	28.73	
77.53	0.42	24.18	

3.2 Surface Morphology

Field The Field Emission Scanning Electron Microscopy (FE-SEM) technique was used to carefully investigate the shape, surface morphology and crystallinity of the obtained silver nanoparticles [17]. Figure (2) shows the nanometer dimension silver particle shapes at 600°C after calcination on FE-SEM images whose images were magnified. the FESEM image showed that the green method samples contained small spherical particles with uniform distribution with an average size of 31.23 nm. This result supports similar findings from the synthesis of silver nanoparticles in previous research, which demonstrate the reliability and consistency of the results obtained in this study.[18].

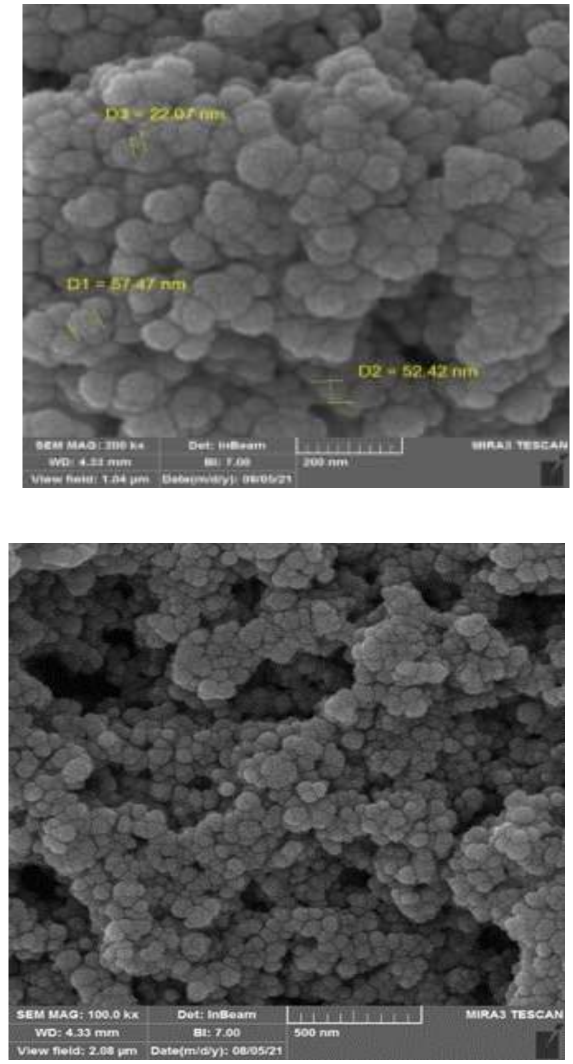


Figure 1: The surface morphology of the silver nanoparticles (Ag NPs) at 600°C at varying magnifications: (top) 200 nm and (bottom) 500 nm.

3.3 EDX analysis

Figure (3) displays the EDX spectra utilized to validate AgNP production. The presence of Ag L emission series peaks in the

EDX spectrum confirmed the stoichiometry of the synthesized nanoparticles. A near-perfect stoichiometric ratio of the Ag element was observed [20]. The accompanying table in the graph provides quantitative data on both weak and strong absorption peaks in the Ag region, aligning consistently with previous findings. Additionally, the table presents the normalized concentration in weight percentage (W%) and atomic weight percentage (A%) of the Ag element, as illustrated in the attached table within Figure (3).

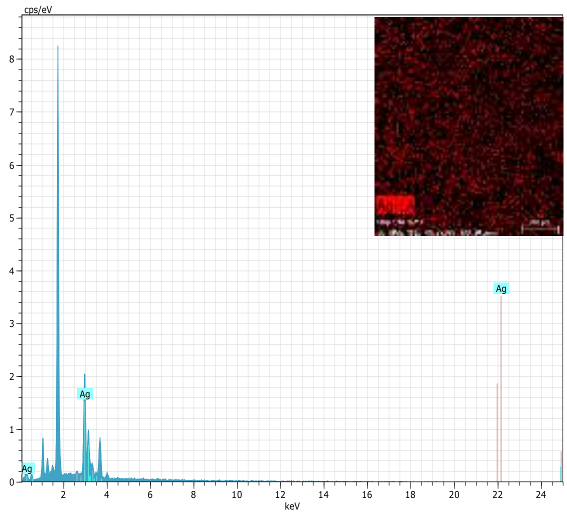


Figure 3: The EDX spectrum confirms the presence of elemental Ag.

Table 2: The elemental composition of Ag NPs.

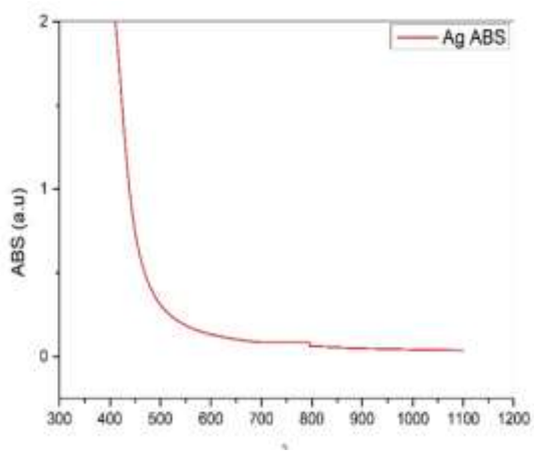
Element	Weight %	Atomic %
Ag	31.22	100

3.4 UV-visible Analysis

The result in figure (5), show the absorbance spectrum curve of the synthesized Ag sample is depicted concerning wavelength. The results indicate remarkably high absorbance at short wavelengths, signifying high energy levels relative to the wavelength. This unique property is a distinctive characteristic of nano-sized materials. The significance of this finding lies in the potential applications of absorbance, particularly in areas involving visible light and extending into the ultraviolet spectrum. This property holds substantial promise for diverse applications within these spectral ranges [1, 22]. Additionally, employing Diffuse Reflectance Spectroscopy (DRS) analysis, the optical energy gap was computed using the Tauc method based on equation (3):

$$(\alpha h\nu)^2 = (\alpha E)^2 = B^2(h\nu - E_g) \qquad (3)$$

This calculation involved setting the constant value to (1/2) and subsequently formulating the equation accordingly. This methodological approach ensured precise determination of the optical energy gap, a crucial parameter in understanding the electronic properties of the synthesized material. And from drawing the graphic relationship between $(\alpha h\nu)^2$ and the photon energy (hν), by extending the straight part of the curve to cut the photon energy axis, it gets the value of the energy gap, the allowed transition at the point $((\alpha h\nu)^2 = 0$ as shown in the figure (1.4), Where it appeared worth approximate (2.7eV).



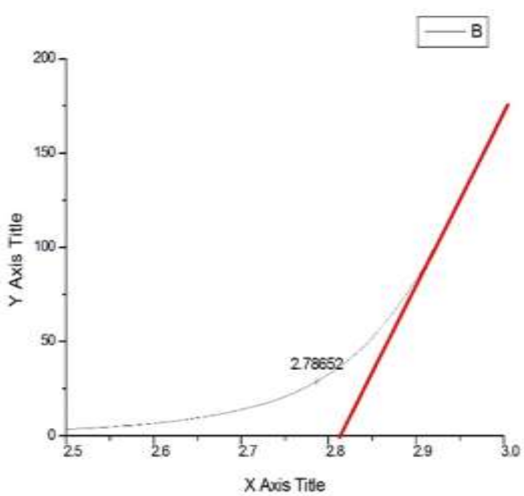


Figure 2: Absorbance spectrum and energy gap for Ag.

3.5 Antimicrobial Activity

The result show in Figures (5,6,7, and 8) and Table (2) demonstrate the inhibitory Effect of Ag NP samples (N1) on the Pleiotropic microorganisms used in testing. The size of the inhibitory zones indicates the antibacterial activity of Ag nanoparticles against four bacteria species, i.e., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans* and *Escherichia coli*. The findings are related to the effectiveness of Ag NPs as antibacterial agents towards both universal types of bacteria, including Gram-positive and Gram-negative ones. The obtained inhibitory zones denote the ability of the NPs-based samples (N1) to hinder the growth of the tested bacterial strains. The spectrum of severely inhibited zones on the colony of various bacterial species signifies their diverging reactivity towards Ag NPs. These findings reinforce the need for intensive research based on the mode of action for the antibacterial activity of Ag NPs.

The well diffusion assay of already synthesized Ag NP (N1) exhibits anti-bacterial activity against gram positive and gram-negative bacterial species on the range. The findings show the prospect of Ag NPs as potent antibacterial agents are currently the highest priority area for continued research and development of their clinical and industry applications. The Samples (N1) and the test organisms were incubated overnight at 37°C before measuring and recording the average zones of inhibition diameter.

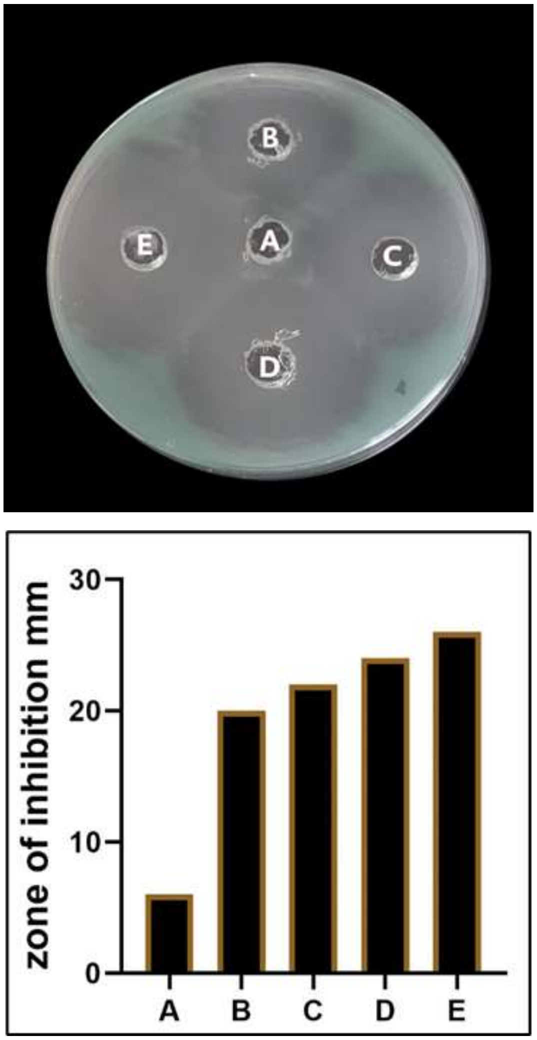


Figure 3: Antibacterial activity of (N1) against *Pseudomonas aeruginosa* . A, Control. B, 12.5 µg/ml. C, 25 µg/ml. D, 50 µg/ml. E, 100 µg/ml.

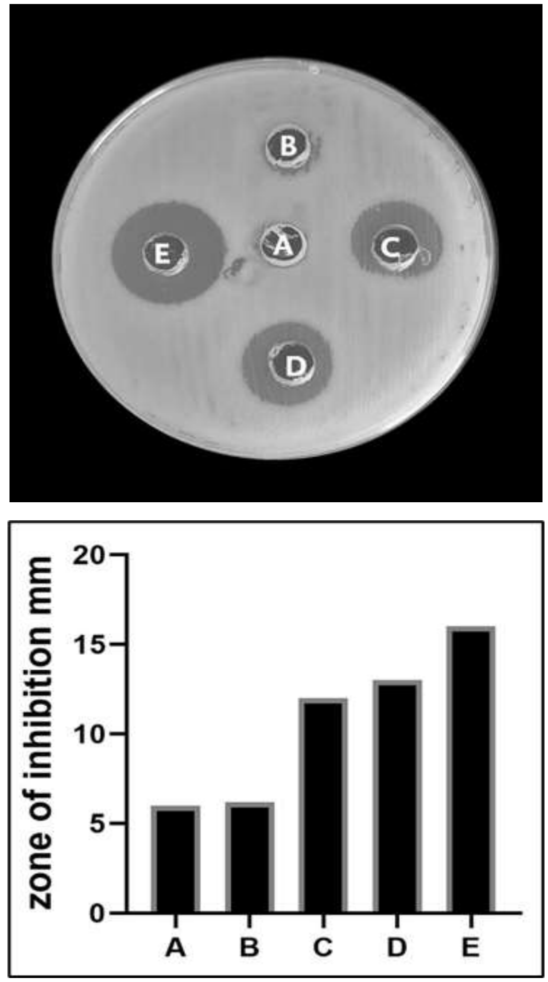


Figure 4: Antibacterial activity of (N1) against *Staphylococcus aureus* A, Control. B, 12.5 µg/ml. C, 25 µg/ml. D, 50 µg/ml. E, 100 µg/ml.

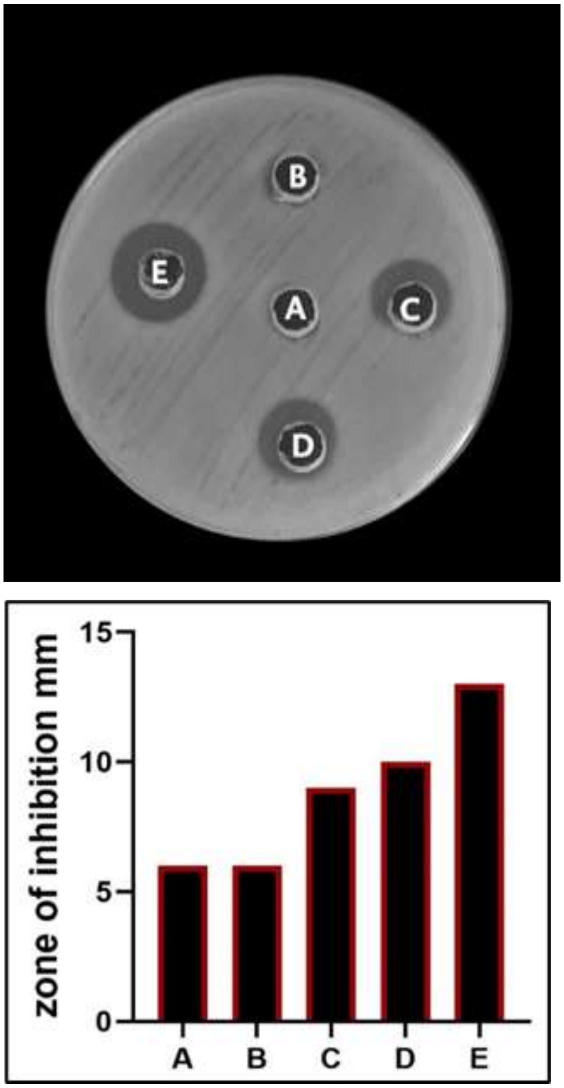


Figure 5: Antibacterial activity (N1) against *Streptococcus* mutans. A, Control. B, 12.5 µg/ml. C, 25 µg/ml. D, 50 µg/ml. E, 100 µg/ml.

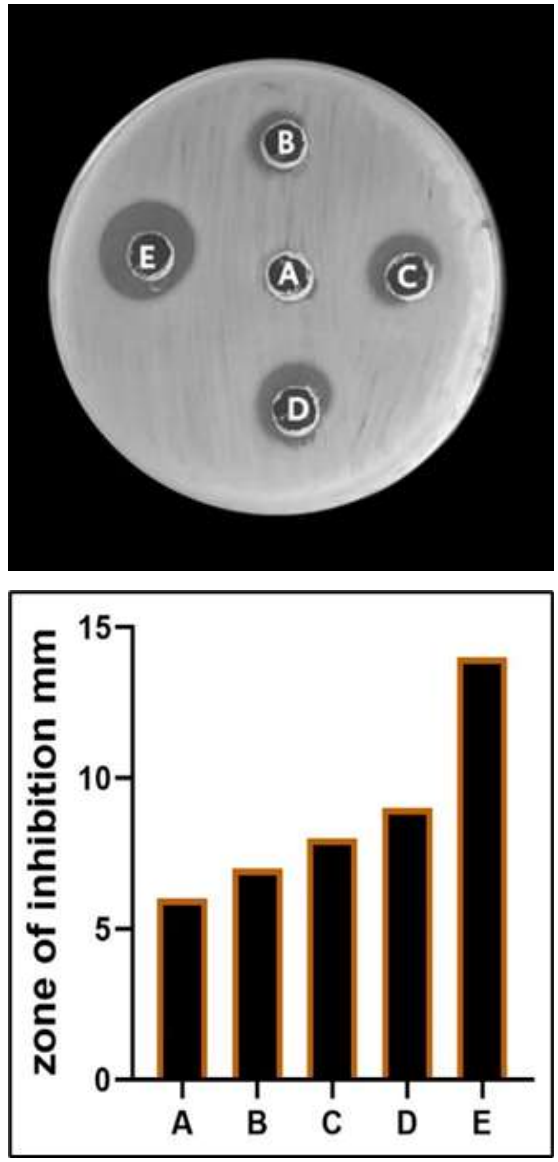


Figure 6: Antibacterial activity(N1)against *Ecoli*. A, Control. B, 12.5 µg/ml. C, 25 µg/ml. D, 50 µg/ml. E, 100 µg/ml.

Table 2 The inhibition zone Ag NP's formed by *Staphylococcus aureus, pseudomonas aeruginosa*, *Streptococcus mutans and Ecoli*.

Antibacterial analysis (Zone of inhibition (mm)						
sample		A	B	C	D	E
Staphylococcus aureus	N1	6	6.2	12	13	16
pseudomonas aeruginosa		6	20	22	24	26
Streptococcus mutans		6	6	9	10	13
Ecoli		6	7	8	9	14

Silver possesses notable potential in diverse biological applications, serving as an effective antifungal and antibacterial agent against antibiotic-resistant bacteria. Its roles extend to infection prevention, facilitation of wound healing, and mitigation of inflammation, even at minimal doses. Despite ongoing research, the precise mechanism underlying the bactericidal effects of silver nanoparticles remains incompletely understood. Current investigations suggest that these nanoparticles may bind to bacterial cell membranes, disrupting vital functions such as permeability and respiration. The efficacy of particle binding to bacteria appears to correlate with the available surface area for interaction. Moreover, some nanoparticles penetrate the cell and affix to DNA, thereby suppressing genes essential for critical metabolic processes. Notably, smaller nanoparticles, with their elevated surface area to volume ratio, exert a more pronounced bactericidal effect compared to larger counterparts. The escalating resistance of bacteria to conventional antibacterial agents presents a formidable challenge in both the pharmaceutical industry and the treatment of infectious diseases. Novel bacterial strains exhibiting high levels of resistance, encompassing both gram-positive and gram-negative varieties, have emerged. Consequently, there is an escalating demand for unidentified antibacterial compounds to combat the proliferation and dissemination of multidrug-resistant bacterial strains[16].

The antifungal effect of Nanosilver prepared through a green method was investigated on three different species of fungi*: Candida albicans, Penicillium spp.*, and *Aspergillus spp*. The results, as depicted in Figures 9, 10, and 11, demonstrated the antifungal properties of silver particles. In the antifungal activity assay, colloidal nanosilver samples at a concentration of 0.4 molars were employed. Specifically, 0.4 molars of colloidal nanosilver samples were tested against Penicillium spp. and Aspergillus spp., while the same concentration was individually used against *Candida albicans*. The results indicated a substantial inhibition zone diameter when using 0.4 molars of colloidal Nanosilver samples against *Candida albicans*, surpassing the inhibition zones observed for the other two fungal strains. It is to be noticed that, there is a considerable decrease in the inhibitory zone diameter with the rising of concentration of colloidal Nanosilver samples [7, 13].

Figure 7: The antifungal activity of Ag NPs against *Candida.*

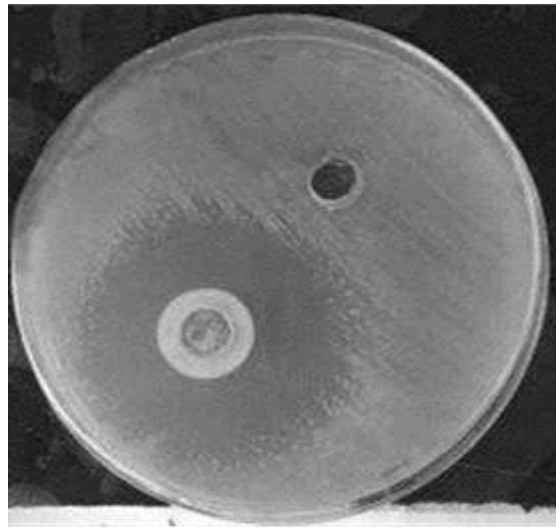


Figure 8: Antifungal activity test of Ag NPs against *Penicillium spp.*

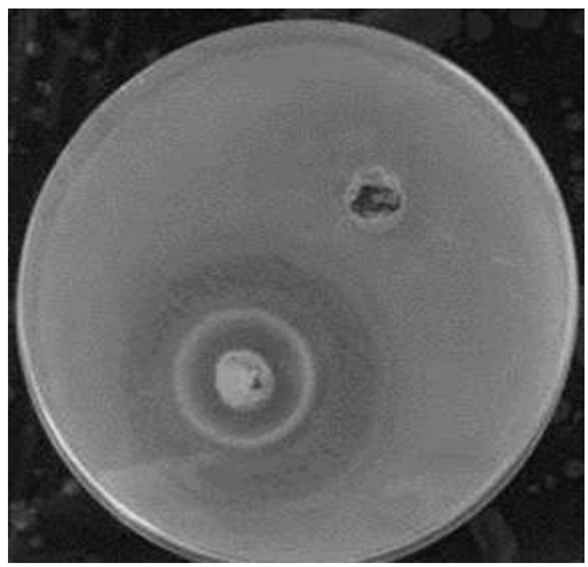


Figure 9: Antifungal activity test of Ag NPs against *Aspergillus spp*.

4 Conclusion

In conclusion, this investigation has successfully demonstrated the synthesis of silver nanoparticles utilizing a green synthesis approach by using sunflower plant extract acting as a natural reducing agent.

Structural analysis through XRD and FE-SEM techniques revealed the nanoparticles' unique cubic structure and confirmed the synthesis of silver nanoparticles with a high degree of crystallinity and a defined spherical shape. High-temperature annealing led to changes in particle morphology, underlining the effectiveness of using eco-friendly, renewable, and cost-effective materials in nanoparticle production. The implications of this research are significant, particularly in the context of antimicrobial applications. The observed inhibition zones against the types of bacteria used. also against the different species of fungi: *Candida albicans, Penicillium spp.*, and *Aspergillus spp* . underscore the potential utility of these silver nanoparticles as effective antimicrobial agents. Moving forward, further investigations into the specific mechanisms underlying their antimicrobial activity and optimization of synthesis parameters hold promise for advancing their practical applications in various fields, including biomedicine and environmental remediation.

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