OLEANOLIC ACID MEDIATED SYNTHESIS OF SILVERNANOPARTICLES: ANTIOXIDANT AND ANTIMICROBIAACTIVITIES

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ABSTRACT

The use of silver nanoparticles (AgNPs) in drug delivery, medical research, catalysis, and other areas may be highly advantageous. Worldwide demand for therapeutic AgNPs has prompted numerous businesses to start synthesising them. Although these materials can be produced at minimal cost, using chemical procedures poses risks. These processes have other unsettling characteristics, such as environmental pollution and the production of hazardous byproducts. Plant phytoconstituents-mediated nanomaterial synthesis is becoming more and more common as a technique for creating AgNPs that are uniform in size and shape. Oleanolic acid was utilised as a reducing agent in the proposed study to quickly create AgNPs, which could provide the framework for the industrial production of nanomaterials. Oleanolic acid was used to create AgNPs with a hexagonal shape and mean sizes of 10 nm and 60 nm, acting as a green technology. Due to the Surface Plasmon Resonance phenomenon, the optical absorption band peak of the synthesised silver nanomaterial was found to be at 440 nm. Silver was identified as the major biofabrication material via diffraction grating planes. Phytoconstituents in the root extract are what caused the crystalline peaks to develop. The capping phenomenon was thoroughly examined utilising ultraviolet-visible spectroscopy, Fourier-transformed infrared spectroscopy, scanning electron microscopy, and X-ray diffraction. As an alternative to the current chemically intensive processes, the results of this work may be applied to the industrial scale synthesis of therapeutically active AgNPs.

Keywords: Oleanolic acid, Silver, Nanoparticles, Synthesis, Characterization

1. INTRODUCTION

Since the previous two decades, nanostructured metallic particles with sizes ranging from 1 to 100 nm have been exploited as a powerful tool in a variety of practical applications due to their extraordinary physiochemical and optoelectronic capabilities. Silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs), the most common types of fine metal nanostructures, have been shown to have different physicochemical and biological activity compared to their bulk parent materials [1,2]. As a catalyst in a wide variety of oxidation processes and with a very high surface-to-volume ratio, silver nanoparticles (AgNPs) stand out. There has been a lot written about the impressive uses of AgNPs in fields including pharmacology and medical diagnostics as well as agriculture. The chemical reduction of a silver salt solution, thermal breakdown, and sonication are all potentially dangerous steps in the nanoparticle manufacturing process. Green chemistry and an appreciation of biological processes led to the creation of a fresh environmentally friendly method for synthesising NPs [3,4].

Chemical nanoparticle production is fast, but it has been hindered from being used in important fields like medicine, agriculture, and diagnostics because of the need for toxic and costly capping chemicals to maintain the particles' desired sizes [5]. The green or biological technique is easier to use than the others since it simplifies the synthesis process, allows for better size control, and may give benefits such adequate substitution of source. Plant-based biosynthesis of metal nanoparticles is undergoing a significant reorganisation for environmental protection and to reduce the usage of harmful or toxic chemicals [6].

Several plant-based and microorganism-based biological NP production strategies have been developed thus far. Because of the secondary metabolites and other active substances found in plant extracts, plants have been shown to offer inherent potential for the creation of nanoparticles. Nanoparticles with enhanced antibacterial agents against pathogenic bacterial strains and larvicidal action against mosquito vectors may be synthesised from plant components, which are regarded as sustainable and renewable resources [7]. Natural oils, fruits, leaves, crop waste, extracts of various plants including citrus peel, edible mushrooms, aloe vera, clove, coffees/teas, and algae are all employed in the production of AgNPs. Leaf extracts from several plants that are effective against malaria, chikungunya, and Japanese encephalitis mosquito vectors have been reported to be synthesised into cost-effective and environmentally friendly AgNPs [8].

Oleanolic acid (3β-hydroxy-Olean-12-en-28-oic acid) is a pentacyclic triterpene. More than 120 plant species, including major commercially and socially significant crops like olive (*Olea europaea* L.), contain this chemical [9]. Leaves and fruits have cuticle waxes on their epidermis, and these waxes include oleanolic acid. The oleanolic acid found in olive leaves may make up as much as 3.5% of the leaf's dry weight. The pharmacological effects of oleanolic acid are varied. Oleanolic acid is hepatoprotective, has anticancer and antiviral activities, and has a low toxicity profile. Weak anti-HIV and anti-HCV effects *in vitro* were shown using oleanolic acid, although more strong synthetic analogues are being researched as potential medicines [10].

2. MATERIALS AND METHODS

2.1. Chemicals

Silver nitrate and Oleanolic acid were purchased from Sigma-Aldrich (Germany) company from a local vendor. The other chemicals employed during the study were of analytical grade and procured from HiMedia (India). For the experiment, double distilled water (Borosil® apparatus) was utilized.

2.2. Synthesis of Silver Nanoparticles

2.2.1. Preparation of Silver Nitrate Solution

For the synthesis of AgNPs, silver nitrate ($AgNO_3$) solution of strength 1 mM was prepared by dissolving 0.01698 g of solute in 100 mL of double distilled water.

2.2.2. Preparation of Oleanolic acid Solution

For the synthesis of AgNPs, oleanolic acid solution of strength 1 mM was prepared by dissolving 0.04567 g of solute in 100 mL of double distilled water.

2.2.3. Preparation of Nanoparticles

1 mM of oleanolic acid (5.0 mL) solution was added to 45 mL of 4.0 mM $AgNO_3$ solution at room temperature (25–28°C). The change in color after 30 min showed a rapid reduction of $AgNO_3$ and continued the observation in color change till 24 hr.

2.3. Characterization

2.3.1. UV-Vis Spectroscopic Study

UV-Vis Spectrophotometer (double beam Shimadzu® UV-1800, Kyoto, Japan) in the range of 200-800 nm was used to examine the development of ionic silver's transformation into its corresponding element type. The use of the distilled water served as a standard. To verify the AgNPs' production, their absorption peak was calculated [11].

2.3.2. X-ray Diffraction Analysis

The researchers wanted to verify the presence of AgNPs, look into how different bioreductants function, and learn more about the AgNPs' structural makeup. Powder X-ray diffraction analysis (P-XRD) using an X-ray diffractometer (ULTIMA-III, RIGAKU, Japan) was performed on the AgNPs by placing a sample of the material in an aluminium crucible. The procedure was performed at 40 kV using monochromatic CuK-radiation with a scanning speed of 4/min and an angle range of 10-90 degrees. Careful notation and analysis of the diffractogram were performed [12].

2.3.3. Scanning Electron Microscopy

The scanning electron microscope (JOEL-JSM 6390 SEM equipment) was used to capture the micrograph at an acceleration voltage of 20 kV. The experiment was conducted by scattering the sample over a double tape wedge secured with an aluminium stub and then placing the whole thing into a scanning electron microscope (SEM) chamber. The shape of the AgNPs was shown by randomly scanning the sample [13].

2.3.4. Zeta potential measurements

The strength of an NP's surface charge is proportional to how stable it is. The AgNPs were combined using a magnetic stirrer after being diluted with distilled water at a ratio of 1:1000 (v/v). The Zetasizer (Mettler Toledo, UK) was used to calculate the zeta potential [14].

2.3.5. Fourier-transform infrared spectroscopy

The FTIR absorption spectra of the AgNPs were recorded between 4000-400 cm-1 using a Fourier transform infrared spectrophotometer (Perkin Elmer® GX-FT-IR, USA), following a

potassium bromide dispersion procedure. Twenty scans per second were performed at a resolution of 0.15 cm^{-1} to image the compounds [15].

2.4. Biological activities

2.4.1. Antioxidant activity

2.4.1.1. 1,1-diphenyl-2-picrylhydrazyl radical scavenging capacity

Researchers looked into extract's ability to quench 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. To start, we made a 1 mg/mL stock solution of whole-plant extract. An equivalent volume of AgNPs (100 µg/mL) was added to a 0.1 mM DPPH solution in methanol. The sample aliquot was left to incubate at room temperature for 30 minutes. Ascorbic acid was used as a reference substance when measuring absorbance at 517 nm [16].

2.4.1.2. *In vitro* reducing capacity

Experiments were performed in triplicate to establish the reducing power of AgNPs. Plant extract (100 µg/mL) was combined with 0.2 M phosphate buffer (pH 7.4) and 1% potassium ferricyanide in a final volume of 2.5 mL. After that, it was left to incubate at 50 degrees Celsius for 20 minutes. After 10 minutes of incubation, the mixture was centrifuged at 3000 rpm and 2.5 mL of trichloroacetic acid was added. Absorbance was determined at 700 nm after combining the top solution layer (2.5 mL) with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%). The enhanced reducing power was reflected in the elevated absorbance of the reaction mixture [17].

2.4.2. Antimicrobial activity

AgNPs were tested for their antibacterial efficacy *in vitro* using *Escherichia coli*, a highly pathogenic bacterium. The MIC values were established by evaluating several chemicals in relation to the antibacterial ciprofloxacin. AgNPs' antibacterial efficacy was examined in a petri dish using the disc diffusion technique and the Muller Hinton Agar standard. Bacterial cells were produced by spreading them onto Muller Hinton agar plates in a laminar flow cabinet after being cultivated in nutrient broth for 24 hrs at 37°C. The AgNP was soaked onto sterile discs of Whatman filter paper No. 1 (6 mm in diameter) that had been pretreated with dimethylsulfoxide (DMSO). After the bacterial plates had been prepared, the discs were put on top. AgNPs' zone of

inhibition was measured to be 24 mm in diameter after being incubated at 37°C for 24 hrs. Standard antibiotic ciprofloxacin was utilised as a positive control, while a disc laced with DMSO served as a negative control. There were three separate trials of the tests [18].

3. RESULTS AND DISCUSSION

3.1. Characterization of AgNPs

3.1.1. UV–Vis study

In order to identify AgNPs, oleanolic acid precipitated the Ag^+ ions from $AgNO_3$ into their equivalent elemental form. Over the course of 24 hrs, the colour of the solution changed from clear to dark brown as $AgNO_3$ was reduced to Ag^+ **(Figure 1A)**. After 24 hrs, there should be no more noticeable colour change in the solution, indicating that the reduction and AgNPs production have been completed. UV-Vis absorption observations at hourly intervals corroborated the colour shift, and absorbance at 440 nm suggested elemental silver form, which peaks at about 24 hrs.

Inspiring by the interaction with electromagnetic fields, the SPR is a coupled oscillation of free electron conduction. The stimulation of the SPR peak **(Figure 1B)** may account for the transformation of the clear solution into a dark brown hue during the reduction. The particle's local refractive index, as well as its size, shape, and the total charge transfer between the reducing medium and the particle, might affect where in the UV-Vis spectra the band associated with SPR appears. The formation of a new absorbance band in the visible region with a maximum at max = 380-750 nm confirmed the initiation of AgNPs production and the advancement of silver ion reduction in solution. The presence of oleanolic acid as a natural capping agent was also indicated by a shift in the SPR band to a strong absorption peak at 440 nm (after 24 hrs) [19].

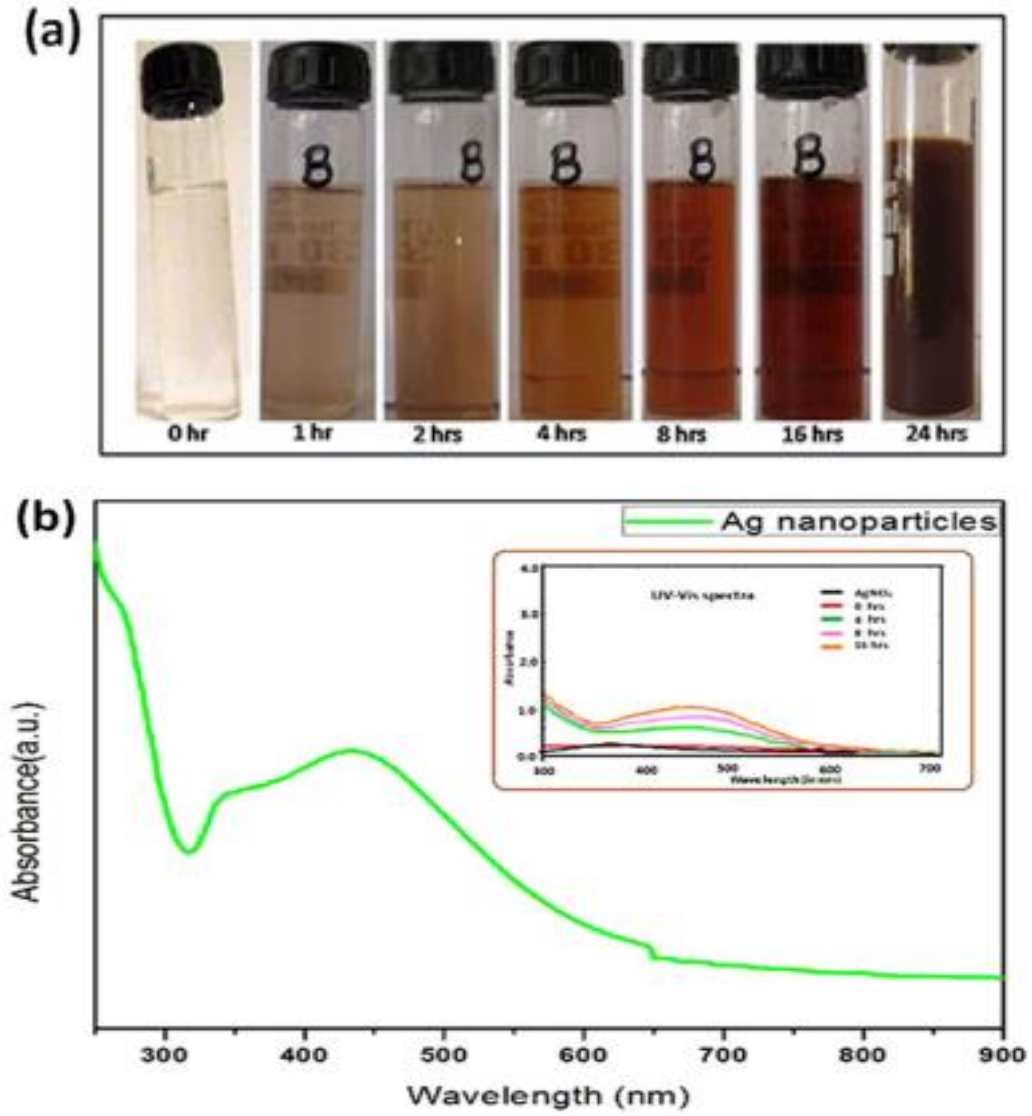

Figure 1. Color change of reaction mixtures UV–Vis absorbance spectra of as prepared AgNPs at different time intervals: (A) Visual observation (B) UV-Vis absorption spectra.

3.1.2. SEM analysis

The purification and characterisation of the synthesised AgNPs were carried out according to established procedures. The fine scattering in solution shown by these NPs is explained by their spherical form and the organic layer enveloping the biogenic AgNPs, as seen in **Figure 2** of the corresponding SEM picture. Additionally, a modest degree of aggregation was seen with a size distribution of NPs between 10 and 60 nm. It reveals the hexagonal structure of AgNPs on a plane. The crystalline structure of the AgNPs was further validated by the brilliant circular point in the targeted region electron diffraction pattern.

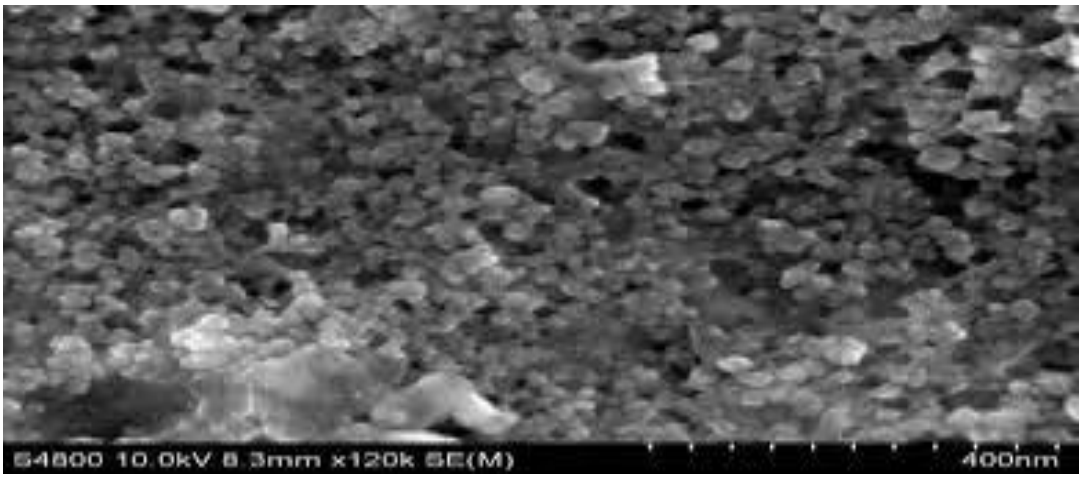

Figure 2. Particle size of nanoparticles.

3.1.3. FTIR analysis

The studies suggested that the hydroxyl & carbonyl groups or other similar active groups (C=O, –OCH, C–O, etc.), attached to the major secondary metabolites or amino acids and peptides have stronger ability to bind metals, and the metabolites, proteins could form a coating around metal NP. The identification of the possible active group of biomolecule (oleanolic acid) extract or the presence of stabilizing organic molecules/agents accountable for the reduction of Ag^{+} ions were done by the FTIR measurements. Since, solution of oleanolic acid was prepared in aqueous solution, the –OH group might play an important role during formation of AgNPs. FTIR spectra of Ag-NPs are depicted in Figure 3 showed C=C vibration of alkenes, C=C stretching and –C–H vibration of aromatic ring, presence of –OCH3, C=O, and C–O group.

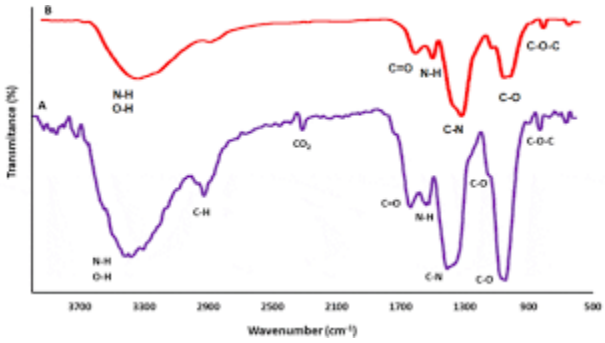

Figure 3. FTIR spectrum of nanoparticles: (A) Oleanolic acid (B) AgNPs.

3.1.4. XRD analysis

Powder X-ray Diffraction examination showed that oleanolic acid was present, which is crucial for the production of AgNPs. The (111), (200), (220), (311), and (222) planes all have prominent peaks at 2θ of 38.9, 45.6, 66.6, 77.6, and 82.7 in the diffractogram **(Figure 4)**. The first two peaks, which reflect capping phenomena, were caused by bioorganic molecules present on the surface of the AgNPs. The planes showed that silver was used extensively during biofabrication. The macromolecular structure of oleanolic acid may help explain why the diffractogram shows such a high intensity (count). The computed lattice constant was found to be consistent with previous estimates. The cell volumes of the synthesised AgNPs were surprisingly small [20].

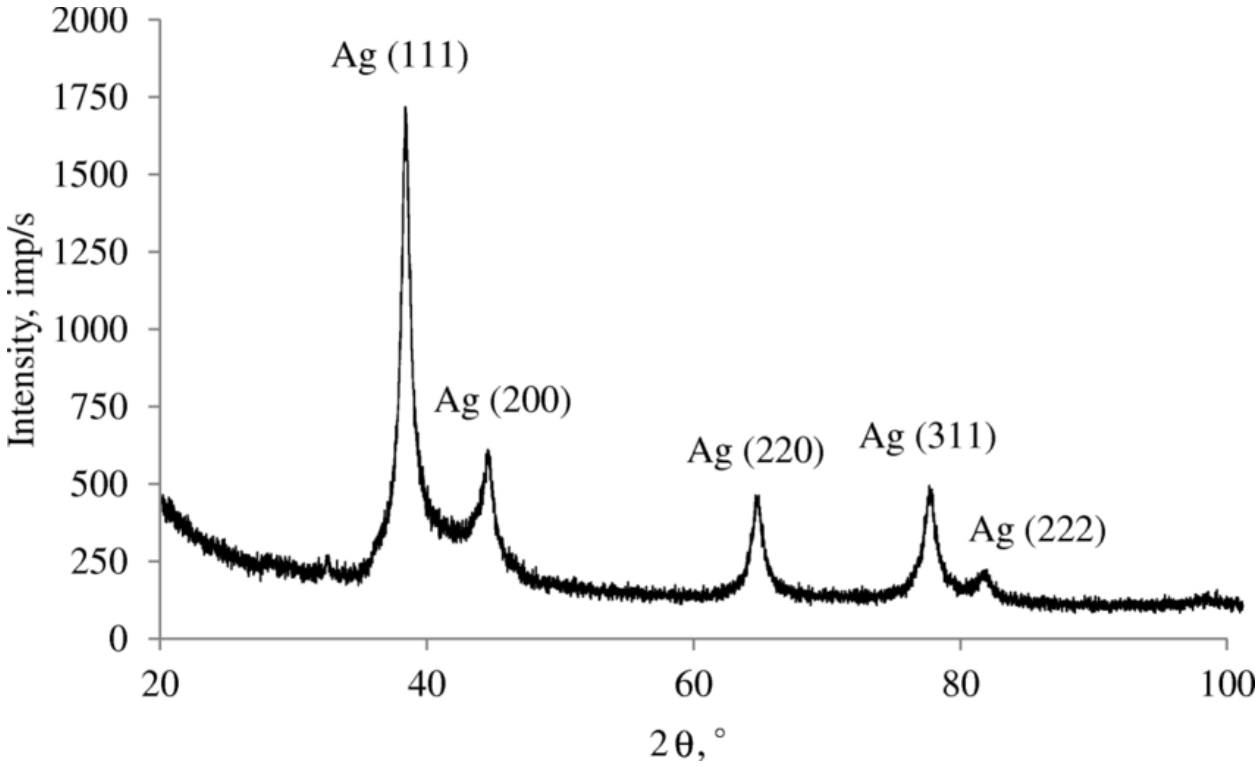

Figure 4. X-ray diffractogram of AgNPs.

3.1.5. Zeta potential

The zeta potential was found to be -19.3 mV which indicated that AgNPs are highly physically stable. Generally, zeta potential more negative than -30 mV or more positive than +30 mV are normally considered stable. The zeta potential was observed to be in the partial negative, which may be due to the presence of carboxylic acid, hydroxyl, and methyl groups in oleanolic acid.

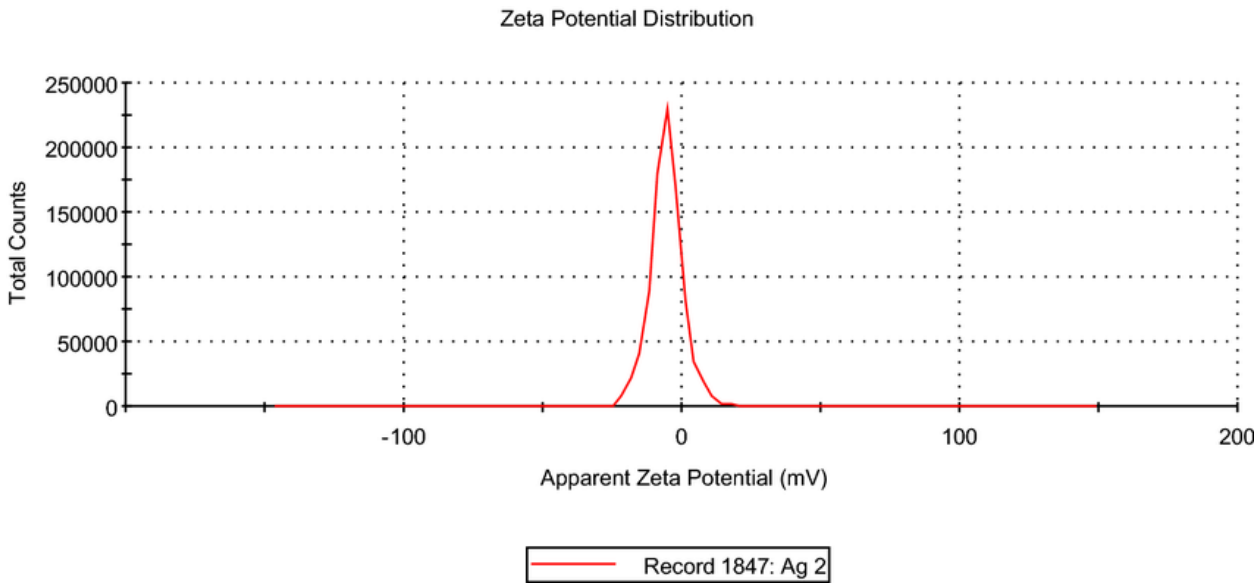

Figure 5. Zeta potential of AgNPs.

3.2. Biological activities

3.2.1. Antioxidant activity

An antioxidant activity was shown by the AgNPs by its potential in scavenging DPPH radical (42.73%) **(Figure 6)**. The reducing power of extract is based on the potential to reduce iron from ferric (Fe^{3+}) to ferrous form (Fe^{2+}), which was demonstrated by the intensity of absorbance. AgNPs displayed an absorbance of 0.582, which was less than that of absorbance shown by ascorbic acid ($p<0.001$) **(Table 1)**.

Table 1. *In vitro* antioxidant and reducing activities of AgNPs.

Treatment	Concentration (µg/mL)	% inhibition[#] (n = 3)	Absorbance (n = 3) (700 nm)
AgNPs	100	42.73 ± 0.51**	0.582 ± 0.005***
Ascorbic acid[$]	100	84.38 ± 0.97**	1.386 ± 0.002***

[#]Anti-oxidant activity was carried out as per DPPH assay method. All values represent mean ± SEM of n = 3; **p<0.01; ***p<0.001. [$] Standard reference for anti-oxidant activity.

3.2.2. Antimicrobial activity

AgNPs showed moderate anti-microbial activity against *E. coli*, with a zone of inhibition (ZOI) 16.3 mm with MIC of 12.5 µg/mL **(Table 2)**. However, it was lower as compared to standard

ciprofloxacin, which exhibited 29.6 mm at MIC value of 6.25 µg/mL. In contrast, AgNPs exhibited poor anti-microbial activity against *S. aureus* with ZOI of 10.4 mm at MIC of 50 µg/mL **(Figure 6)**.

Table 2. Anti-microbial activity of AgNPs.

SAMPLES	*E. coli*	*S. aureus*
AgNPs	16.3 ± 0.66*** (12.5)	10.4 ± 0.97*** (50.0)
Ciprofloxacin[#]	29.6 ± 0.57*** (6.25)	28.1 ± 0.41*** (6.25)

All values represent mean ± SEM of n = 3; ***p<0.001. Zone of inhibition of test compounds against microbes are measured in mm. Values inside the bracket represents the minimum inhibitory concentration (MIC). [#] Standard reference for anti-bacterial activity; [$] Standard reference for anti-fungal activity.

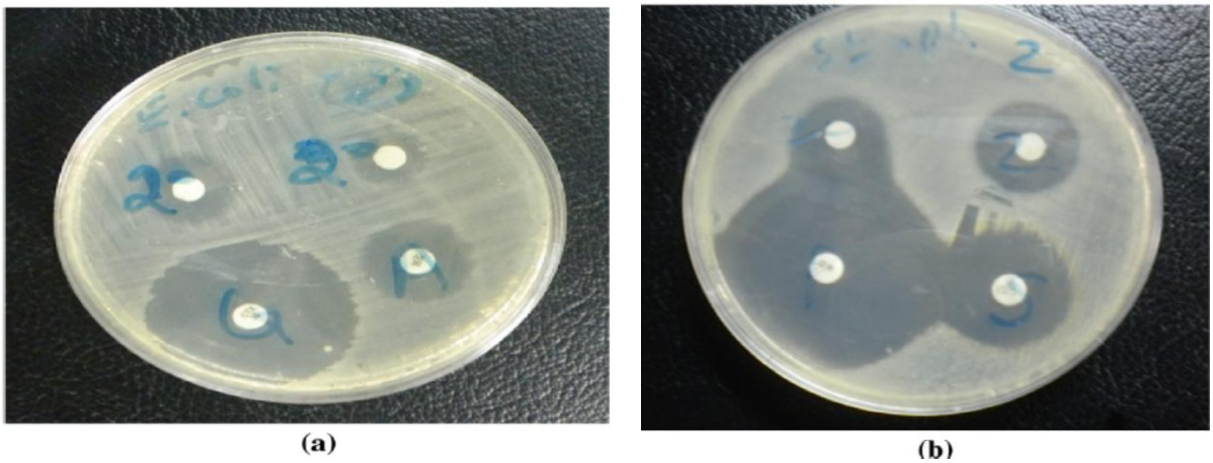

Figure 6. Antimicrobial activity of AgNPs against (a) *E. coli* (b) *S. aureus*.

CONCLUSION

This work investigated a unique environmentally friendly method of synthesising AgNPs. An environmentally friendly silver nanomaterial with a flower-like structure and an average size of 10 to 60 nm was produced using oleanolic acid leaf extract. The optical absorption band peak of the synthesised silver nanomaterial is located at 440 nm as a result of the SPR phenomenon. Silver was identified as the major biofabrication material via diffraction grating planes. Phytoconstituents in the root extract are what caused the crystalline peaks to develop. The capping phenomenon was also confirmed by in-depth examination utilising UV, FTIR, SEM, and XRD methods. This technology may be used in the large-scale commercial synthesis of therapeutically effective AgNPs in the future, providing a potential greener option to the currently employed conventional chemical synthesis.

CONFLICT OF INTEREST

No conflict of interest is declared.

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