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RESEARCH ARTICLE

Antimicrobial Potential of Green Synthesized Silver Nanoparticles From the Fruit of *Azadirachta indica* a High-Valued Medicinal Plant

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ABSTRACT

The activity and stability of nanoparticles synthesized from plants; green-synthesized nanoparticles are obtaining more consideration from scientists. This work aimed to synthesize the green silver nanoparticles (AgNPs) using the fruit extract of *Azadirachta indica* A. Juss. The fruit extract acts as both a capping and reducing agent. The physicochemical properties of synthesized nanoparticles were studied through x-ray diffraction (XRD), FT-IR, UV-vis, scanning electron microscopy (SEM), and EDX. Furthermore, antioxidant properties and antibacterial activity of the nanoparticles were also studied. The reaction was preceded under sunlight, and the color of the reaction mixture changed from colorless to dark brown, indicating the production of Ag nanoparticles. A UV-vis analysis revealed an absorption peak of 429 nm, which was measured after 30 min of reaction. The XRD spectroscopy results suggest that the nanoparticles are crystalline with an average diameter of 36.50 μm. SEM images show that the majority of the AgNPs are spherical and fairly distributed. The obtained AgNPs showed efficient antibacterial activity against gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus*) bacterial species. Surprisingly, the synthesized Ag nanoparticles from *A. indica* fruit by the DPPH assay method show 55% antioxidant activity.

1 | Introduction

The technologies in green chemistry, which are environmentally friendly, are increasing worldwide. Silver is among the most commercialized nanoparticles with 500 tons of silver nanoparticle (AgNP) production/year, and it is expected to increase in the coming few years [1]. Green chemistry is now a new trend in the synthesis of many chemical products, including NPs, which significantly reduces environmental risk by removing potentially hazardous ingredients from methods of production that are harmful to human health. A better platform for the synthesis of other nanomaterials, such as AgNPs, is therefore provided via the green synthesis of metal nanoparticles (AgNPs). Physical synthesis, chemical synthesis, and biological approaches

have all been used for the stabilization and production of AgNPs thus far. However, in addition to physical and chemical approaches, biological methods that are more cost-effective and environmentally benign have received a lot of attention for the production of AgNPs. In this way, a variety of knowledge studies have been explained about the production of Ag NPs applying a variety of biological materials, for example, algae, protozoa, bacteria plants, fungi, and so on [2]. In nanoparticles, the metal nanoparticles gained more importance in a few decades due to their captivity properties. Metal nanoparticles are mostly present in the form of oxides, hydrides, sulfides, fluorides, etc. The metal nanoparticles were first introduced by Michael Faraday in 1857. After that, it became a topic of interest for research work for their vital properties in various areas of life [3]. The traditional synthesis of nanoparticles is very toxic, thus it is our need to minimize the risks of toxicity by introducing a synthesis method that can be nontoxic and may be eco-friendly. So, green synthesis of nanoparticles are most easy, eco-friendly, and useful method as compared to other chemical methods. The term green shows the plants, and these various plants can be used as a forerunner, for the production of nanoparticles of any metal. This way, production is attached to the plant as a biotechnology. Different chemicals are used for the reduction of metal ions in the chemical method, which is very dangerous and environmentally harsh. That is why the green route substitutes the chemical method, in which no need to use any type of chemical and environmentally friendly for the reduction of the metal. Since plants possess biomolecules like polyphenols, reducing sugars, phenolic acids, etc., which act as both capping and reducing agents. Green synthesis has more importance over chemical and physical methods due to its environmentally friendly, nontoxicity, and easiness of characterization. The best thing for the production is that results are obtained within minutes or hours. This method has a great approach for the synthesis of nanoparticles to the safe and eco-friendly, which is acceptable in nanotechnology and environmentally friendly over chemical and physical methods that are toxic, environmentally polluted, and more expensive, and green syntheses are considered more recently in these years for the synthesis of nanoparticles of gold and copper. Various microorganisms like bacteria and fungi are used to produce nanoparticles, that is, gold, zinc, copper, silver Ni, etc. [4]. Different green methods are available by using nanoparticle synthesis, like from vitamins, from enzymes, from bacteria, from plant extract, and microwave-assisted method. At this time, we synthesize NPs through the green synthesis method. Plants have both bimolecular and phytochemicals that work as reducing agents and species. And have various metabolites, safe and easy to handle. Since the extract of plants has capping and reducing agents, which are responsible for metal ion reduction to nanoparticles [5]. With excellent antibacterial properties, AgNPs also seem to be immune to antibiotics. These antibacterial compounds perform better against bacteria than other nanomaterials due to their big AgNP site and crystallographic structure. This work reported how well AgNP inhibited *E. coli*. As a result of AgNPs creating holes in the cell wall and attaching viruses to the cell wall, the cells die. He conducted a similar study on *E. coli* using particles smaller than AgNPs, and the bigger surface area led to greater effectiveness. Furthermore, the shape as well as size of the nanoparticles has a role in their antibacterial action [6]. This study was conducted to synthesize green AgNPs using the fruit extract of *Azadirachta indica* A. Juss. and its application against selected bacterial strains.

2 | Experimental Section

2.1 | Sample Preparation

One-milliliter solution of silver nitrate was prepared by using 17.0 mg of $AgNO_3$. It was dissolved in a sufficient amount of distilled water to make the total volume of the solution up to 100 cm^3. The color of silver nitrate was colorless. *A. indica* fruit was taken from the *A. indica* plant, it was cleaned with tissue paper and then washed with double-distilled water. Then it was dried. Then the fruits were peeled off carefully. Now this fruit was mixed in a sufficient amount of double-distilled water in the beaker to make a total volume of 300 cm^3. It was boiled for 30 min gently to make the extract of *A. indica* fruit. This solution was cooled for 1 h to make it normal. Now it was filtered gently by using Whitman's filter paper No. 1 and stored at 4°C. The color of extract was dirty white (ghost white). Synthesis of AgNPs was done by mixing the 100 cm^3 of silver nitrate solution and 50 cm^3 of *A. indica* extract in the 2:1, respectively. Now it was stirred to homogenize, it was colorless. This solution was kept in sunlight for 25–30 min. A change in color was observed from colorless to dark brown.

2.2 | Spectroscopy of the Synthesized Nanoparticles

X-ray diffraction (XRD) of a catalyst is the most known and suitable technique for studying the principal crystallographic characteristics of the nanoparticles [7]. To carry out XRD, we used the x-ray diffractometer of Shimadzu model XRD-6100 equipped with monochromatic Cu K-α. We used a current of 30 mAv, a voltage of 40 kV, and an x-ray line of $\lambda = 1.5418$ °A. The XRD pattern was recorded at 2θ range of 4°–90° at a scanning rate of 0.02/min. The Scherer equation was used for the calculation of the size of nanoparticles.

$$D = \frac{k\lambda}{\beta \cos\theta} \tag{1}$$

where "k" denotes the shape factor and its value is 0.9, "λ" represents the wavelength of radiations, and "β" indicates the full width of half of the maximum intensity in radians [7].

We also conducted scanning electron microscopy (SEM) to study various crystallographic characteristics of nanoparticles. We used the scanning electron microscope Nova Nano SEM 450 model. The scanned pictures of nanoparticles have been captured at the voltage of 20 kV. UV-visible spectrometry is an important tool used for the determination of particle formation and the study of characteristics. Moreover, it is well understood that the shape, size, free electron density, antiparticle interactions, and the surrounding medium affect the spectrum surface plasmon resonance of the nanoparticles [8]. All these indicate that UV-vis is an important technique to monitor the injection of electrons and the aggregation of nanoparticles. For UV-vis, we used the

Jasco V-530 spectrophotometer. For performing spectrometry, 190–1500 nm range was set.

2.3 | DPPH Free Radical Scavenging Assay

The spectrophotometric technique used to determine antioxidant activity was modified by Brand-Williams et al. [9].

Dispersing nanoparticles in distilled water at various concentrations, 5, 10, 15, 20, 40, 60, 80, and 100 μg/cm^3, was prepared. The nanoparticle solutions were continuously sonicated for 3–4 h at room temperature. A 0.004% DPPH solution was created by dissolving 0.06 g of DPPH in 150 mL of ethanol and resting for 30 min.

In these eight test tubes, we add 1 cm^3 of AgNPs solution and mix with 3 cm^3 of double-distilled water in each test tube so that solution becomes dilute.

At 95°C, test tubes had been gestating for 90 min. The absorbance of these sample solutions was measured, and means sample values were obtained. The standard solution was ascorbic acid. The following formula was used.

$$\%\text{age scavenging} = (AB\,(c) - AB\,(s)) \,/\, (AB\,(c)) \times 100$$

where $AB(c)$ is the absorbance of the control and $AB(s)$ is the absorbance of the test compound.

2.4 | Antimicrobial Activity Screening

Antimicrobial testing of nanoparticles was conducted via the Kirby disk diffusion method.

2.4.1 | Media Preparation

In a clean 250 mL Erlenmeyer flask, 3.8 g Muller Hinton Agar (MHA) (Merk) was dissolved in 50 mL distilled water, and the volume was raised to 100 mL. The flask was cotton plugged, and the media was autoclaved at 121°C, 15 psi for 30 min.

2.4.2 | Disk Preparation

Whatman's Filter paper No.1 disks of 6 mm were made using a punching machine and autoclaved at 121°C, 15 psi for 30 min. The autoclaved disks were placed in a sterile plate and 10 μL of sonicated AgNPs fraction was poured on each disk. Some of the disks were also loaded with 10 μL of sterile distilled water for negative control. All the disks were then dried in laminar air flow for 25 min so that the disk absorbed maximum extract.

2.4.3 | Inoculums Preparation

Sterile nutrient broth (20 mL) in two separate 100 mL Erlenmeyer flasks was taken and inoculated with two different indicator strains (*Staphylococcus aurous* and *Escherichia coli*) separately under a sterile environment. Both flasks were incubated at 37°C in a shaking incubator for 2 h to attain the bacterial growth 0.5 McFarland standard.

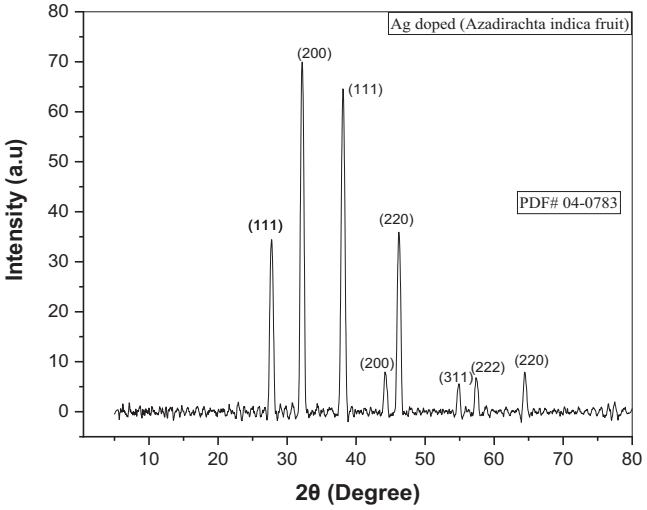

FIGURE 1 | X-ray diffraction (XRD) analysis of green silver nanoparticles.

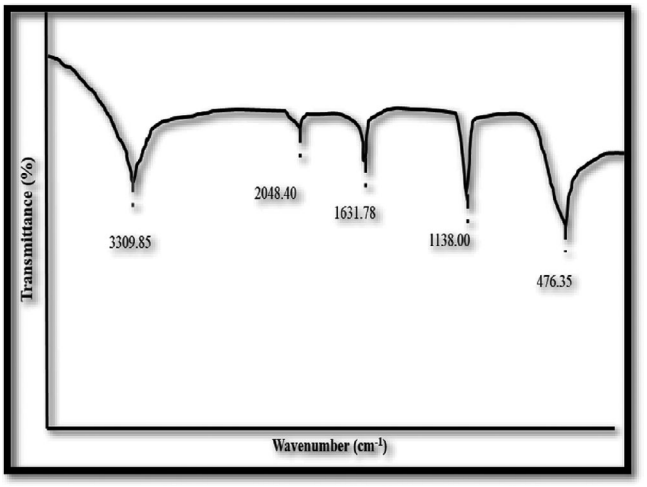

FIGURE 2 | FT-IR nanoparticles of silver spectra.

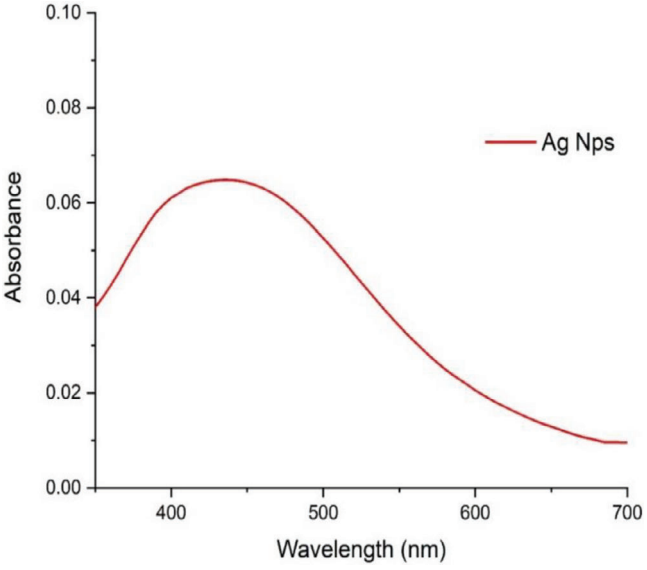

FIGURE 3 | UV-vis spectrum of green synthesized silver nanoparticles.

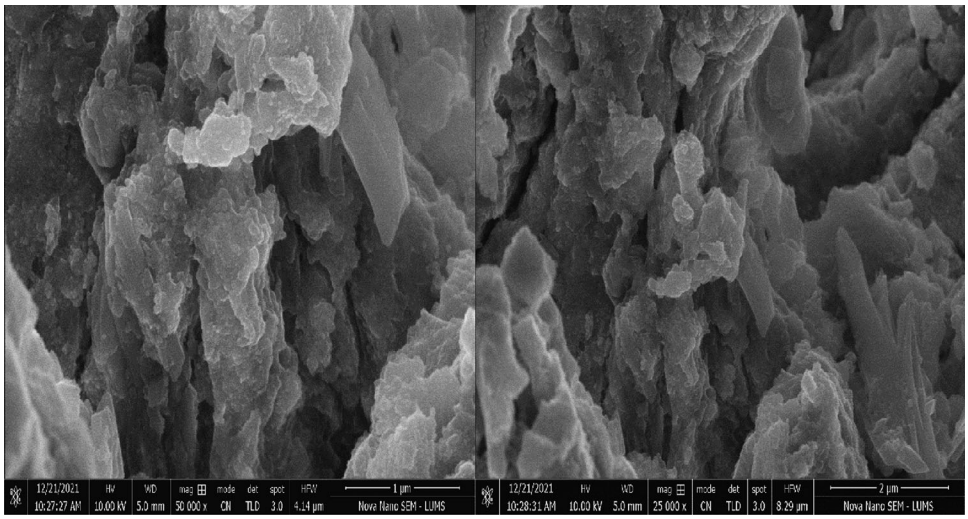

FIGURE 4 | Scanning electron microscopy (SEM) of silver nanoparticles (AgNPs) at different scales.

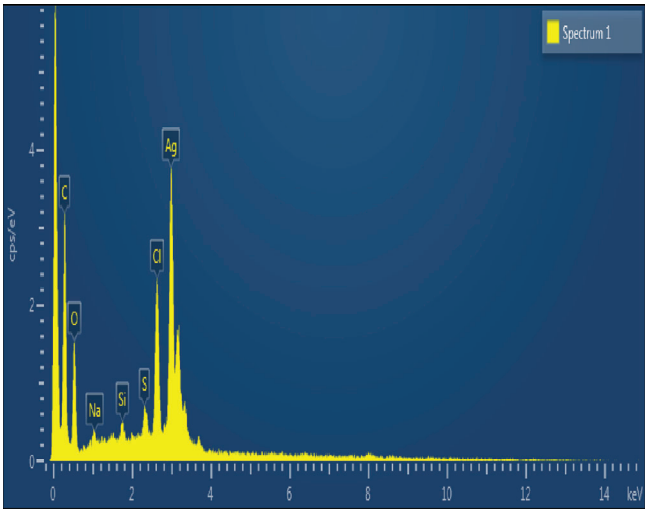

FIGURE 5 | EDX results showed the presence of silver metal.

2.5 | Disk Diffusion Assay

Autoclaved MHA (10 mL) was poured into two sterile Petri plates in a laminar airflow cabinet while maintaining the sterile conditions. After the solidification of the media, respective bacterial cultures were inoculated on it using sterile swabs. The inoculation of bacteria was done in such a way as to promote the growth of a bacterial lawn on the whole surface of the media. Finally, sterile forceps were used to place nanoparticle-loaded disks on the surface of inoculated media. For negative control disks loaded with water and for positive control, standard antibiotic disks were placed similarly. Both plates were incubated at 37°C for 24 h after which the zones of inhibition were measured.

2.6 | Antioxidant Activity

The DPPH is used to measure the antioxidant power of AgNPs. In DPPH, N is a central radical that shows a peak at 695 nm,

TABLE 1 | Total antioxidant assay of AgNps.

Sample	Concentration	Absorbance at 695 nm
DPPH	0.004%	0.987
Ascorbic acid	05 μg/cm^3	0.489
	10 μg/cm^3	0.482
	15 μg/cm^3	0.478
	20 μg/cm^3	0.469
	40 μg/cm^3	0.454
	60 μg/cm^3	0.441
	80 μg/cm^3	0.423
	100 μg/cm^3	0.409
Silver nanoparticles	05 μg/cm^3	0.979
	10 μg/cm^3	0.953
	15 μg/cm^3	0.941
	20 μg/cm^3	0.929
	40 μg/cm^3	0.899
	60 μg/cm^3	0.873
	08 μg/cm^3	0.857
	100 μg/cm^3	0.841

the result of DPPH shows the percentage absorption of FRS action of concentrations, which is at 5, 10, 15, 20, 40, 60, 80, and 100 μg/cm^3.

3 | Results and Discussions

3.1 | XRD Spectroscopy of Nanoparticles

The XRD gives only one diffraction distribution that has a weak value and is pointed out at 44 and onward to 50°, which shows the characterized crystalline nature of AgNPs [9, 10]. The

TABLE 2 | Antibacterial activity.

Bacteria	Silver nanoparticle	Antibiotics	Water
		a **Positive control (+)**	***b*** **Negative control (−)**
E. coli (gram −ve)	8 mm	11 mm	–
S. aureus (gram +ve)	12 mm	17 mm	–

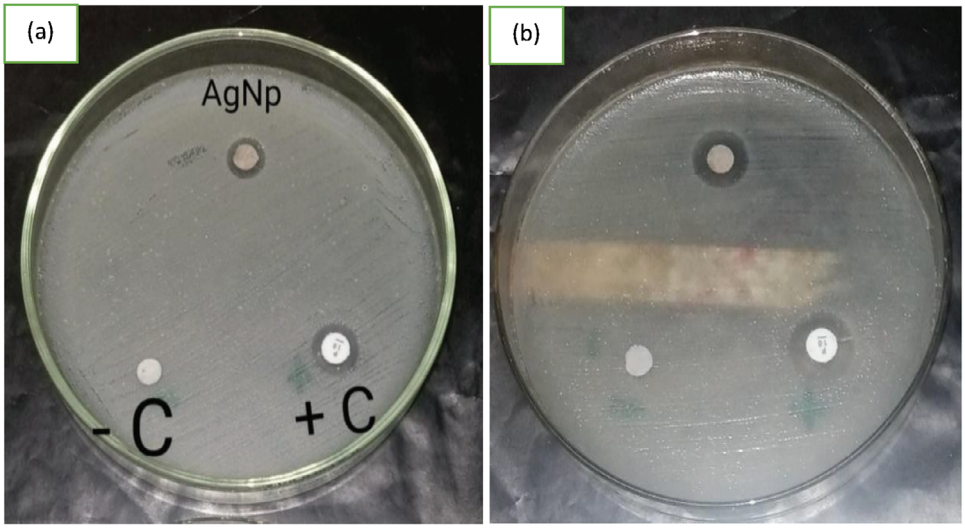

FIGURE 6 | (a, b) Antibacterial activity of silver nanoparticles (AgNPs) using *E. coli* (*a* = gram negative, *b* = gram positive).

peak of diffractions was broad but had low values of intensity, which indicates that the obtained product has a good amount of crystallites of nanosized (Figure 1).

3.1.1 | FT-IR and UV-Vis Spectroscopy of Nanoparticles

A Shimadzu FT-IR spectrometer was used to conduct FT-IR analysis of the nanoparticles. The instrument's wavelength range was selected to be between 4000 and 400 cm^{-1} in wave number. After different skimming readings, the apparatus sample was applied using a plunger and spectra. The peak at 3309.85 cm^{-1} in the graph shows the presence of hydroxyl groups, which confirms the presence of water [11]. The alkynes family found in the extract's phyto-constituents was involved in the peak at 2048.40 cm^{-1}. The peak at 1631.78 cm^{-1} shows the presence of amide associated with C═O stretching vibration. The observed peak at 1138 cm^{-1} denotes C—O—C linkage, or —C—O bonds. The peak at 476.35 cm^{-1} shows the presence of Ag–O bond stretching as shown in Figure 2. The FT-IR measurements were carried out to identify the possible biomolecules for reducing agents for the Ag nanoparticle synthesis from extracts [12]. In the UV-Vis spectrum, a peak at 429 nm in the visible region was observed (Figure 3). It is the confirmatory peak of surface plasmon resonance (SPR) and was recorded for AgNPs. Size, shape, and particle interaction all affect the distinctive SPR peak of AgNPs [13]. AgNP's visible spectrum SPR peak ranges from 380 to 450 nm [14].

3.2 | SEM and EDX Analysis of Nanoparticles

The size, shape, and structure of nanoparticles are measured using a scanning electron microscope (Nova NanoSEM 450). It shows that the majority of the AgNPs are spherical and fairly distributed, but some of the NPs were observed to have randomly oriented structures, as seen in Figure 4a–c. The average diameter of the spherical, homogeneous nanoparticles is 36.50 μm [15].

EDX determines the elemental composition of the sample using an energy-dispersive spectrometer. Results show the presence of silver metal clearly along with some other impurities like sodium in the form of minerals, etc. EDX clearly shows that the sample contains silver metal, and other peaks show that some impurities are also present in the sample [16]. The other peaks in the graph are due to the sample containing some other impurities, but the major peak is due to the presence of silver metal, as shown in Figure 5.

3.3 | Antioxidant Activity

The DPPH is used to measure the antioxidant power of AgNPs. In DPPH, N is a central radical that shows a peak at 695 nm, the result of DPPH shows the percentage absorption of FRS action of concentrations, which is at 5, 10, 15, 20, 40, 60, 80, and 100 μg/cm^3. Results of different concentrations, which are shown in Table 1, revealed that by increasing concentrations of the sample standard

absorption is decreased. The highest percentage absorption for ascorbic acid (standard) is 0.489 and for AgNPs is 0.979 [17, 18].

3.3.1 | Antibacterial Activity

In antimicrobial activity, the size of AgNPs shows a diameter of 8 mm with *E. coli* (gram-negative) and 12 mm with *S. aureus* (gram-positive) [19]. Results show that it gives 11 and 17 mm diameters with positive control and no result with the negative control group (Table 2). It revealed that the sample showed a great effect with positive control but no effect with negative control (Figure 6) [20, 21].

4 | Conclusions

This study demonstrates the synthesis of AgNPs using the fruit extract of *A. indica*. Furthermore, the DPPH and antimicrobial activity of the synthesized nanoparticles were also studied. The average particle size from SEM micrographs was measured to be around 36.50 μm. EDX shows the presence of elemental silver with minor traces of impurities. The UV-visible spectroscopy confirms the maximum absorption at 429 nm. The antioxidant activity of AgNPs shows the effective activity at low concentration, and the increase in concentration results in a surprising decrease in absorption. However, the antibacterial activity samples have effective results with positive outcomes but give negative results with negative outcomes of bacterial species.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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