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# Formulation of Silver Nanoparticles from Grewia Asiatica Leaf Extract and their Biomedical Applications

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Nanotechnology is a well-advanced area and several types of nanoparticles have been synthesized. Different types of nanoparticles have been synthesized so far using different techniques. It is most practical to produce nanoparticles from plant extracts for use in medicine and other applications. In this study, silver nanoparticles were synthesized by *Grewia asiatica* leaf extract. Synthesized nanoparticles were characterized by different techniques including Ultraviolet-visible (UV) spectroscopy, X-ray diffraction (XRD), Dynamic light scattering (DLS), and Scanning electron microscopy (SEM). Results showed a characteristic peak of silver nanoparticles in UV-region at 425nm, while DLS analysis revealed the z-average size of synthesized particles i.e., 461.6nm and -28.4mV zeta potential. SEM analysis revealed the amorphous surface morphology. The antibacterial and antioxidant activity of silver nanoparticles were also evaluated. Silver

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nanoparticles showed significant antibacterial activity against both gram-positive and gram-negative bacteria. A maximum zone of inhibition of 16mm diameter was observed against *Bacillus subtilis* and *Klebsiella pneumonia.* While results of antioxidant activity showed a 43.27µg/ml IC50 value. In conclusion, *Grewia asiatica* leaf extract is a good source to synthesize silver nanoparticles. Also, green synthesized silver nanoparticles could act as potential antibacterial and antioxidant agents.

*Keywords: Nanotechnology; biomedical application; Grewia sp.; extract.*

## 1. INTRODUCTION

Nanotechnology is the branch of science that provides nanomaterials with dimensions and standards of less than 100 nanometers, or which includes the manipulation of specific atoms and molecules [1]. Nanotechnology can create new goods and materials that are crucial in all spheres of existence [2]. In nanotechnology, there are two approaches: a top-down approach and a bottom-up approach. The top-down technique is the reduction of large materials into tiny (nano) sizes without changing their original properties or behaviors [3]. The process of creating big structures out of molecules or atoms through self-assembly is known as the "bottom-up technique." Generally, there are 3 methods of nanoparticle synthesis physical, chemical, and biological. Physical and chemical methods are expensive due to the use of energy and chemicals. These methods are also harmful to the environment. Biological methods or green synthesis in which plant materials or microbes are utilized for the synthesis of nanoparticles are more economical, safe, and easily scalable [4,5]. Green synthesis is advantageous since it employs chemically inert synthetic processes [6,7]. An important stem attachment of a plant's vascular system, a leaf is made primarily for photosynthesis and is frequently expanded laterally above ground. Chlorophyll is often green in color since it is found in the outer leaf tissues and provides food for the entire plant. Although certain leaves may have distinctive colors as a result of pigments in vegetation that mask the green chlorophyll. Depending on the climate, leaves' length, breadth, and structural characteristics can alter. Even the smallest alteration increases a plant species' chances of survival in a specific environment [8]. *Grewia asiatica* is sometimes known as falsa or phalsa. According to [9], it alludes to the flowering plant genus Malvaceae. It was first found in Varanasi, and then Buddhist professors took it to other Asian countries. Phalsa is a small tree as well as a shrub that grows no higher than 8 meters. It has broad, rounded leaves that are 15–18 cm long. It has a petiole that is 1 to 1.5 cm long. The blossoms grow in groups known as cymes. Each bloom measures about 2 cm in length and has five large (12 mm) sepals with five narrower (4-5 mm) petals. When fully developed, the plant's fruit is a tasty drupe with a diameter of 5 to 12 mm that darkens from violet to black. Its leaves are antimicrobial, antiplatelet, antiemetic, antioxidant, antibiofilm, and also anticancer activities. Due to having all these characteristics it is widely used in medicine and food. *Grewia asiatica* is widely distributed in South Asian countries and is important as a medicinal plant and edible fruit. Its fruits contain vitamins, minerals, proteins, antioxidants, tannins, flavonoids, cyan colors, and a variety of other substances. There are differences between *Grewia asiatica's* tall and dwarf varieties [10-12].

In this study, the leaf extract of *Grewia asiatica* was used as a source to produce silver nanoparticles.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

Healthy, fresh, and diseased-free leaves of *Grewia asiatica* (falsa) were collected from a nearby garden in Multan, Pakistan. Washed them well with tap water first to remove dust, then 3 times washed with distilled to get rid of any pollutants. After two weeks of shade drying, the leaves were ground into a fine powder.

**Extract preparation:** Plant leaf solution was prepared by taking 5g of leaf powder in a flask and adding 200ml of sterile distilled water. The mixture was boiled for 1 hour in the water bath at 100℃ and then cooled down at room temperature. Then Whatman filter paper #1 was used to filter the leaf extract. Filtered leaf extract was centrifuged at 3800 rpm for 10 minutes. The supernatant was carefully separated and used for the further process [13].

**Preparation of solution of $AgNO_3$:** Silver nitrate is an inorganic substance with the chemical formula $AgNO_3$. For the preparation of a stock solution of 1M $AgNO_3$, 3.396g of $AgNO_3$ was added to 25 ml of deionized water or distilled water. Dilutions were prepared from this stock solution.

**Silver nanoparticle synthesis**: To prepare the silver nanoparticle from leaf extract, 20mM of $AgNO_3$ was mixed with leaf extract in a 12:1 ratio respectively. Kept in a shaking incubator for two hours in the dark, the color changes were observed after two hours. Then kept in an incubator at 37℃ for 24 hours [14].

**Drying of silver nanoparticles**: After synthesis, silver nanoparticles were purified by repeated centrifugation at 15000rpm for up to 20 minutes. Nanoparticles were then washed thrice with deionized water. Purified nanoparticles were dried by keeping them in a hot air oven. For this, we poured nanoparticles into a glass petri plate and kept this in a hot air oven at 100℃ for 24 hours. After 24 hours, dried silver nanoparticles were stored at room temperature [15].

## 2.2 Investigation of Physical Characteristics of Silver Nanoparticle

**UV-visible spectroscopy:** UV- spectroscopy is used to determine the light's scattering and absorption as it passes through a substance. UV light is a useful tool for detecting and researching nanomaterials because nanoparticles exhibit visual characteristics that are highly related to their shape, dimension, quantity, and refractive index of the light close to the nanoparticle's surface. A spectrometer for UV-visible absorption with a resolution of 1 nm between 200 and 800nm for the confirmation of nanoparticles was used. Which works on the principle of surface Plasmon resonance. For UV- analysis, 100µl of test samples were taken. Water was taken as blank [16].

**Dynamic light scattering (DLS):** It is a method from which we can guess the hydrodynamic diameter of nanoparticles. It is also useful for finding the zeta potential or zeta size and polydispersity index of nanoparticles. DLS is mostly used to analyze nanoparticles. Examples include calculating the sizes of proteins, latex, colloids, and nanogold. The method can be used to measure particles smaller than a nanometer in size, however, it works best for submicron particles [17].

**Scanning electron microscopy (SEM):** The surface morphology of green synthesized silver nanoparticles was evaluated by scanning electron microscopy [18]. SEM magnifies 20-50,000,000 times more and has a resolution of 5-25Å. It creates an image of the specimen that is in its path using an electron beam. When the beam strikes the specimen, an image is created that contains information about the object's surface, its structure, and a quantitative analysis. It delivers high-resolution versions of all the data. SEM can be used to demonstrate 3-D graphs and also to assist in characterizing.

**Dynamic light scattering analysis:** Average particle size was determined by using a particle analyzer, there would be different sizes of nanoparticles. By adjusting the resultant reaction temperatures, leaf broth concentration, and $AgNO_3$ concentration, the average size of the particles could be adjusted from 15 to 500 nm [19]. The silver nanoparticle size is revealed by the dynamic light scattering size distribution histogram.

## 2.3 Biological Activities of Silver Nanoparticles

**Antibacterial activity:** The antibacterial activity of green synthesized AgNPs was determined using the agar well method [20], by measuring the zone of inhibition, minimum inhibitory concentration (MIC), and minimum bactericidal concentration test (MBC) against the selected bacterial strains. For this purpose, fresh cultures of *Salmonella enterica*, *Escherichia coli*, *Bacillus substilis*, *Klebsiella pneumonia,* and *Staphylococcus aureus* were prepared in nutrient broth [21]. Then, nutrient agar plates were prepared by adding 2.8g of nutrient agar into 100 ml of distilled water. The media was autoclaved at 121℃. When the media cooled down then poured in the sterilized petri plates in the laminar air flow. Be careful during pouring, the area should be sterile from any type of contamination. After the solidification of the media, wells were made on agar plates with steel borer. Each bacterial culture was swabbed uniformly on the individual plate by using a sterile cotton swab. Now with the help of a micropitte added 100ul of nanoparticles, the drug (ciprofloxacin) as a standard or as the positive control, and leaf extract as a negative control in their respective wells. Plates were allowed to incubate at 37°C for 24 hours. After 24 hours, the zone of inhibition was determined and measured using a scale.

The minimum inhibitory and minimum bactericidal concentration of greens synthesized were also evaluated using the microdilution method [22] against *Staphylococcus aureus*, *Salmonella enterica,* and *Pseudomonas aeruginosa*.

**Anti-oxidant Activity:** The in vitro antioxidant activity of the silver nanoparticles was evaluated using a DPPH (2,2-Dimethyl-1-picrylhydrazyl) free radical scavenging assay [23]. To perform this activity, prepare a 0.1mM solution of DPPH in methanol. Prepared dilutions of nanoparticles at different concentrations such as 10 µg-1000 µg/ml. Dilutions of ascorbic acid (standard) were also prepared similarly. 100ul of test samples were mixed with 100ul of DPPH solution in 96 well plates and incubated at 37℃ in the dark. Distilled water was taken as negative control and methanol was taken as blank. After 30 minutes, absorbance was measured at 517nm. The percentage of DPPH inhibition was calculated as follows.

% age inhibition= [(Abs. of control- Abs. of sample- Abs. from blank)/absorbance of control] ×100.

## 3. RESULTS

### 3.1 Green Synthesis of Silver Nanoparticles

The reaction mixture's color changed after 24 hours of incubation, turning from light yellow to dark brown. The production of silver nanoparticles and light absorption were confirmed by these color changes as shown in Fig. 1.

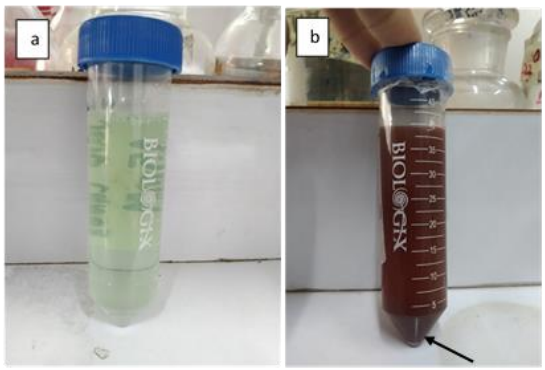


**Fig. 1. Synthesis of silver nanoparticles (a) only $AgNO_3$ salt (b) reaction mixture having $AgNO_3$ and leaf extract**

### 3.2 Drying of Silver Nanoparticles

The nanoparticles of silver were manufactured, and liquid silver nanoparticles were generated. Liquid nanoparticles were heated to a temperature of 100°C and then dried to produce dried nanoparticles. Silver nanoparticles that had been dried in a hot air oven for 24 hours had a dark brown color (Fig. 2). Further characterization was done using these dried silver nanoparticles.

**Fig. 2. Dried silver nanoparticles**

### 3.3 Characterization of Silver Nanoparticles

After the synthesis of silver nanoparticles, nanoparticles were characterized with a UV spectrophotometer, DLS, SEM, and XRD.

### 3.4 Investigation by UV-spectrophotometer

The formation of silver nanoparticles using the leaf extract was verified using UV-spectrophotometer examination in the spectrum of 200 to 800 nm. A wide peak was visible at 375 nm in the *Grewia asiatica* leaf extract. Similar to this, certain silver nanoparticles displayed two separate peaks at the wavelengths 325 nm and 425 nm, which are silver nanoparticle-specific peaks (Fig. 3). It operates according to the surface plasmon resonance theory. When UV rays hit the chamber's surface, molecules start to vibrate. Light at a certain wavelength is absorbed as it strikes the silver nanoparticles. This light absorption resulted in the generation of a particular peak. This particular peak was confirmed in the creation of the AgNPs.

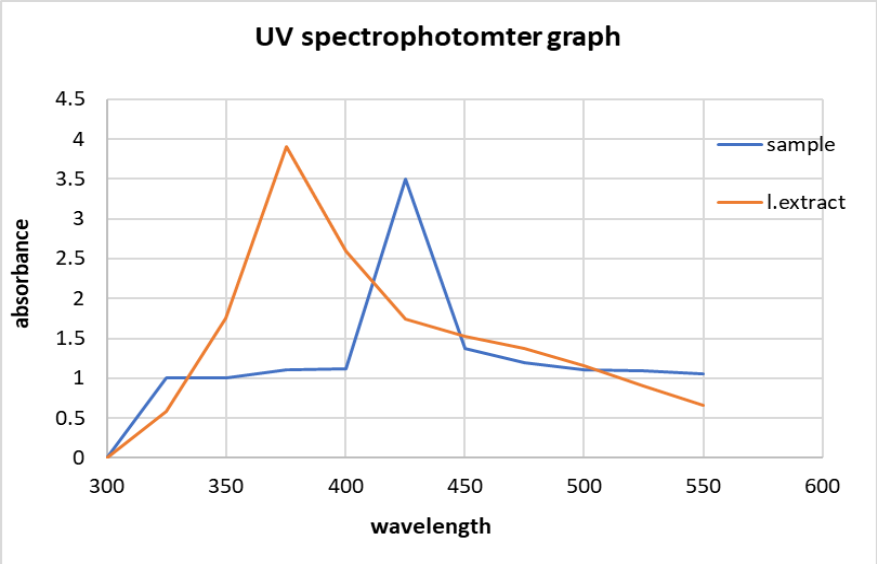


**Fig. 3. UV-Spectrophotometric analysis of Silver nanoparticles (blue line represents AgNPs, while the orange line represents leaf extract**

### 3.5 Scanning Electron Microscopy (SEM)

The diameter and surface form of silver nanoparticles were determined using SEM. SEM findings were captured at various magnifications, such as X30, X40, as well as X50. Silver nanoparticles showed an amorphous form (Fig. 4).

### 3.6 Dynamic Light Scattering (DLS)

While other methods were used to measure the actual diameter of nanoparticles, this analytical technique is utilized to estimate the hydrodynamic diameter. As illustrated in Fig. 5, silver nanoparticles made from *Grewia asiatica* leaf extract were subjected to DLS, and the following findings were obtained. The z-average size was 461.6nm. AgNPs zeta potential was 28.4mV and their polydispersity index was 0.335.

### 3.7 X-ray Diffraction Analysis

The crystalline nature of the synthesized AgNPs was investigated by XRD and the corresponding XRD diffractogram is shown in Fig. 6. The XRD peaks at 40ºC and an intensity of 1600 planes of silver nanoparticles.

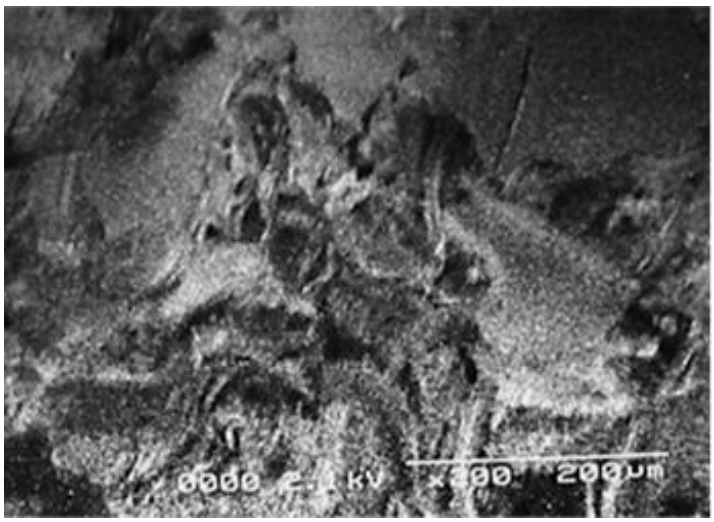

**Fig. 4. Scanning electron microscopy of silver nanoparticles**

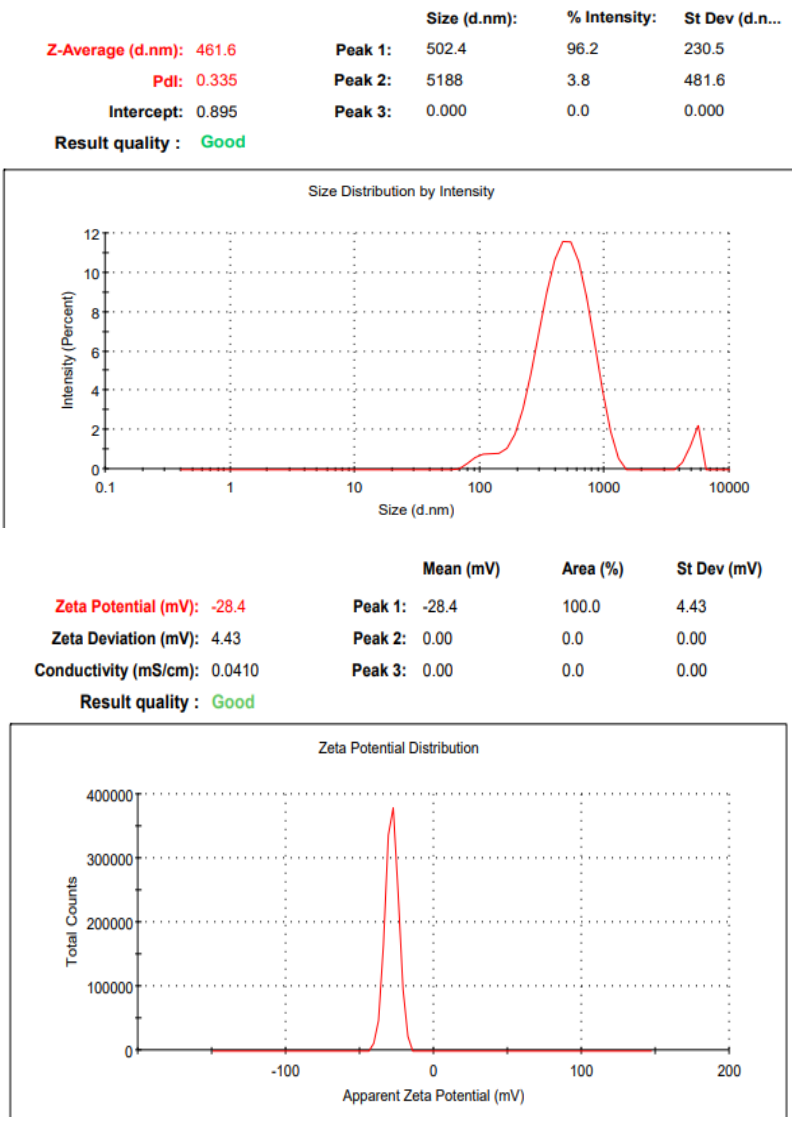


**Fig. 5. DLS results of silver nanoparticles**

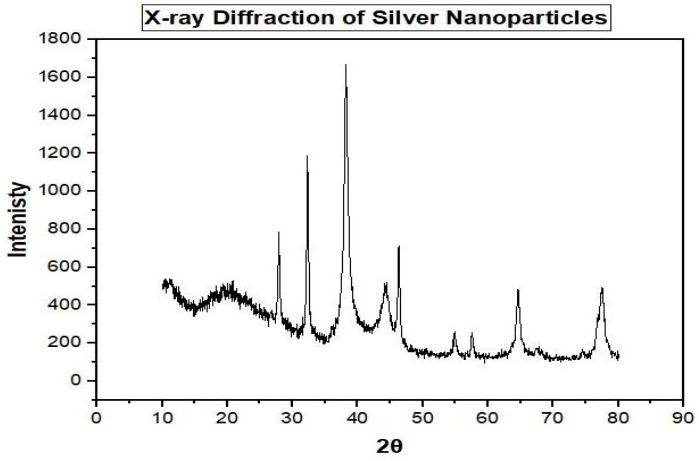


**Fig. 6. Graph showing peaks of silver nanoparticles**

### 3.8 Antibacterial Activity

It evaluates the effectiveness of silver nanoparticles made from the leaves of *Grewia asiatica* in combating various bacterial strains. The effectiveness of silver nanoparticles against various bacterial strains was found to be good. Compared to leaf extracts, silver nanoparticles made from *Grewia asiatica* exhibit a larger zone of inhibition. In comparison to leaf extract, silver nanoparticles demonstrated a 15mm zone of inhibition against the bacteria *Staphylococcus aureus* and, a 16mm zone of inhibition against *Klebsiella pneumonia* and *Basilica subtilis*. While 15mm and 14mm zones of inhibition were observed against *Salmonella enterica* and *Escherichia coli*, respectively. Table 1 and Fig. 7 show the antibacterial activity results.

### 3.9 MIC and MBC

Table 2 shows the MIC and MBC of green synthesized silver nanoparticles. It was observed against each selected bacterial strain the MIC of nanoparticles and drug was 5mg/ml i.e., 5mg/ml concentration of both nanoparticles and drug (ciprofloxacin) inhibited the growth of bacteria. However, this concertation was not enough to completely kill the bacteria.

**Table 1. Antibacterial results of AgNPs against different bacterial strains**

| Bacterial strain | Zone of inhibition of drug | Zone of inhibition of sample |
|---|---|---|
| *Salmonella enterica* | 20mm | 14mm |
| *Escherichia coli* | 19mm | 15mm |
| *Bacillus subtilis* | 19mm | 16mm |
| *Klebsiella pneumonia* | 19mm | 16mm |
| *Staphylococcus aureus* | 20mm | 15mn |


**Fig. 7. Antibacterial activity of silver nanoparticles against different bacterial strains**

**Table 2. Minimum Inhibitory concentration (MIC) and Minimum Bactericidal concentration (MBC) of silver nanoparticles and antibacterial drug (Ciprofloxacin)**

| | ***Staphylococcus aureus*** | | ***Salmonella enterica*** | | ***Pseudomonas aeroginosa*** | |
|---|---|---|---|---|---|---|
| | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** |
| **AgNPs** | 5mg/5ml | + | 5mg/5ml | + | 5mg/5ml | + |
| **Drug** | 5mg/5ml | + | 5mg/5ml | + | 5mg/5ml | + |

**Table 3. Antioxidant activity of silver nanoparticles**

| **Concentration** | **%age inhibition of ascorbic acid (standard)** | **IC50 value (µg/ml)** | **%age inhibition of silver nanoparticles** | **IC5O value (µg/ml)** |
|---|---|---|---|---|
| 10µg/ml | 32.094 | | 29.49 | |
| 25µg/ml | 40.241 | 43.27 | 36.67 | 55.3 |
| 50µg/ml | 49.939 | | 45.95 | |
| 75µg/ml | 62.131 | | 53.15 | |
| 100µg/ml | 71.634 | | 61.34 | |

## 3.10 Anti-Oxidant Activity

An anti-oxidant activity of the silver nanoparticles as well as standard ascorbic acid at concentrations between 10 and 100µg/ml was calculated. The UV analysis was used to determine the standard and silver nanoparticles' percentage of inhibition. Silver nanoparticles and ascorbic acid (standard) had IC50 values of 55.3 µg/ml and 43.27 µg/ml, respectively. AgNPs' ability to act as antioxidants was evident.

## 4. DISCUSSION

After the synthesis of nanoparticles, nanotechnology has attained great importance due to its emerging properties. Science has gained more importance due to the synthesis of nanoparticles. There are many types of nanoparticles such as metallic, polymers, ceramics, and lipids. The most important nanoparticles are metal nanoparticles. Metal nanoparticles are more useful in our daily lives. These metallic nanoparticles can be synthesized by plants, bacteria, and also from fungus extracts. Metallic nanoparticles are used in the animal sciences, pharmaceutical industries, agriculture, and the field of food sciences [24]. Gold (Au) and silver (Ag) nanoparticles are the primary nanoparticles [25]. Nanoparticles synthesized from plants are more valuable as compared to others such as those synthesized from bacteria and fungus. Plant-based nanoparticles are also not as costly as others. It required less time for the synthesis of nanoparticles. There are some other methods such as chemical and physical methods but these methods are not as valuable as the green method [26]. Because it may a have harmful impact on human health and also on the environment. The synthesis of silver nanoparticles from leaf extract is more advantageous as we can easily take leaves from any nearby garden and we can prepare leaf extract easily in the laboratory. The *Grewia asiatica* leaves are antibacterial, antioxidant, and medically more important. Other methods such as the synthesis of nanoparticles from the bacteria and fungus require proper biosafety and also have handling, specific temperature, and storage issues but in the case of leaf extract synthesis of nanoparticles does not need any of these factors [27]. The salt, leaf extract, concentration of metal, pH, and temperature are more important for the synthesis of nanoparticles. The size, shape, and stability of nanoparticles may change due to changes in these factors. There are many techniques and spectrophotometer analysis through which we can confirm the formation of nanoparticles but the color changes are the major sign which we can see from our naked eye. In the case of nanoparticle synthesis, the time of incubation is also important. If we give incubation of 24 hours to a reaction mixture then color change occurs, but after 48 hours proper formation of nanoparticle occurs. We can use many types of plants for nanoparticle synthesis, maybe using plants in the form of extract or the form of powder. Mostly plant extracts are used to synthesize different types of nanoparticles. For example, lemon extract and turmeric curcumin have been used to synthesize manganese nanoparticles [28]. We have employed leave extract to create AgNPs for our experiments. The initial sign of the production of AgNPs was an

alteration in color. Within two to four hours of incubation, the quick transition from light brown to dark brown was visible. It might be because biomolecules in the extract are bound to the surface of both the NPs and absorbent, which encourages NP creation. The entire reaction was left in the incubation for one day, during which time a striking color change was noticed. The generation, size, and shape of NPs are significantly influenced by the pH, For the production of the AgNPs, neutral pH has been used in numerous investigations [29]. AgNPs' formation has been confirmed by ultraviolet (UV) radiation measurement, and various AgNP properties were seen using SEM, DLS, and XRD. The results of the UV spectroscopy validated the creation of AgNPs. It was proven that AgNPs had formed when the peak at 425nm was noticed. Biomedical applications including antibacterial as well as anti-oxidant properties of AgNPs were also evaluated. Synthesized AgNPs have shown good anti-bacterial potential against some bacteria such as a zone of inhibition of 16mm against *Bacillus subtilis* and *Klebsiella pneumonia* were observed. Additionally, the minimum bactericidal concentration (MBC) and the minimum inhibitory concentration (MIC) were also studied. The minimum inhibitory concentration (MIC) for the AgNPs against three isolates of pathogenic bacteria, including the bacterium *Staphylococcus aureus*, *Salmonella enterica*, and *Pseudomonas aeruginosa* was 5mg/ml. AgNPs also showed some moderate antioxidant action. AgNPs had an IC50 of 55.3 µg/ml while ascorbic acid had a value of 43.29 µg/ml. Several previous studies have also reported similar results which supports our findings [23, 30, 31].

## 5. CONCLUSION

This research used the green method for silver nanoparticle production as its foundation. Green synthesis is economical, accessible, and environmentally beneficial. In this study, plant leaf extract is employed as a reducing agent and stabilizing agent. Because the reaction's color changes after 2 to 4 hours of incubation, which verifies the synthesis of AgNPs, this procedure requires less time to provide results. The findings of various characterization analyses, including UV spectroscopy, XRD, DLS technique, and SEM, confirmed the formation of AgNPs. Green synthesized AgNPs had excellent antibacterial action as well and clear zones were observed against different bacterial strains. The AgNPs also have anti-oxidant potential and showed an IC50 value of 55.3µg/ml. Antibacterial and anti-oxidant activities showed the potential biomedical action of AgNPs.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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