

Research Article

Green Synthesis of Silver Nanoparticles Using *Grewia optiva* Leaf Aqueous Extract and Isolated Compounds as Reducing Agent and Their Biological Activities

Muhammad Iftikhar,^{1,2} Muhammad Zahoor ,¹ Sumaira Naz,¹ Nausheen Nazir,¹ Gaber El-Saber Batiha,³ Riaz Ullah,⁴ Ahmed Bari,⁵ Muhammad Hanif,⁶ and Hafiz Majid Mahmood⁷

¹Department of Biochemistry, University of Malakand, Chakdara Dir Lower Khyber Pakhtunkhwa, Pakistan

²Department of Chemistry, University of Malakand, Chakdara Dir Lower Khyber Pakhtunkhwa, Pakistan

³Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour 22511, Al Beheira, Egypt

⁴Medicinal, Aromatic and Poisonous Plants Research Centre (MAPRC), College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

⁵Central Laboratory College of Pharmacy King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

⁶Riphah Institute of Pharmaceutical Sciences, Islamabad, Pakistan

⁷Department of Pharmacology, College of Pharmacy, King Saud University, PO Box 2457, Riyadh 11451, Saudi Arabia

Correspondence should be addressed to Muhammad Zahoor; mohammadzahoorus@yahoo.com

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In this study, an attempt was made to synthesize silver nanoparticles (Ag-NPs) using *Grewia optiva* leaf extract and isolated compounds. The bioreductant capacity of *Grewia optiva* leaf extract for the synthesis of Ag-NPs was assessed using various confirmatory techniques like thermogravimetric analysis (TGA), particle size analysis (PSA), energy-dispersive X-ray (EDX), X-ray diffraction (XRD) analysis, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and UV-Visible spectroscopy. The presence of various bioactive compounds in leaf aqueous extract was confirmed through HPLC analysis, and 8 compounds were identified among the different peaks present in the chromatogram. Biopotencies like antioxidant, antibacterial, and effect on hair growth were determined for extract and NPs. Antioxidant capacities were assessed through standard ABTS and DPPH methods. The antibacterial potential was evaluated in terms of zone of inhibition, minimum bactericidal concentration, and minimum inhibitory concentration of the Ag-NPs and the leaf extract against selected strains of bacteria, whereas the effect on growth of rabbit hair was studied through topical treatment for a specific period of time. Better antibacterial and DPPH and ABTS free radical inhibition was observed for the formulated Ag-NPs as compared to leaf extract. The previously isolated eight compounds from this plant's chloroform and ethyl acetate extracts were also tested for their bioreductant capacities. Out of them, the highest amount of precipitates was obtained with compound VII ((2,5-dihydroxyphenyl)-3',6',8'-trihydroxyl-4H-chromen-4'-one). The study implies that the biogenically engineered nanoscale particles could have promising biological activities in comparison to parental extract and they need to be investigated further as potential therapeutic agents to be used as antibacterial and antioxidant agents and for hair growth enhancement.

1. Introduction

Metal and metal oxide nanoparticles (MNPs and MONPs) have found their roles in almost all fields of technology including medicine. In medicine, these are used in tissue engineering and food packaging, as biosensors, and for the delivery of drugs, genes, and other biopolymers [1–3]. However, the different methods for MNP and MONP synthesis, *viz.*, physical, photochemical, and chemical, have multiple problems associated with them like high cost, cumbersome procedures, requirement of high external conditions, lower biocompatibility, and above all their toxic effect on the environment as well as in the final products for consumer use. Therefore, scientists are switching towards the greener method as an alternative to other methods of NP synthesis because these methods are simple, cost-effective, less toxic, and producible in large scale, and more importantly, the NPs synthesized by the green method have enhanced desired properties as compared to their counterparts prepared through other methods [4–7].

Green synthesis of nanoparticles (NPs) using plants is quite similar to the chemical methods with the exception that here the plants (their extracts or isolated compounds) are used as stabilizer and reducing agents instead of chemical reducing and capping/stabilizing agents. The plant and plant-derived material-based green synthesis is preferred because of its easy availability, larger-scale production, and higher stability of the produced NPs [8]. Different molecules like alkaloids, polyphenols, terpenoids, antioxidants, sugars, flavonoids, organic acids, and quinones along with low molecular weight proteins from plant extract are believed to take part in synthesis of NPs as source/s of reducing agent [7]. Many MNPs and MONPs have been effectively synthesized using extract of different parts of various plant species, and they have shown significant biological potentials like antimicrobial, photocatalytic dye degradation, antioxidant, and as nanotheranostic agent. Several studies revealed that the NPs synthesized employing plants have better biological potential as compared to those synthesized through chemical methods. The better biological potential observed is mainly due to the medicinal properties possessed by the metabolites present in the plant extract, which are integrated in the final formulation of the NPs [7–9].

Silver (Ag) metal has antimicrobial properties, and attempts have been made to prepare its NPs and investigate their clinical and other applications as well. Ag-NPs have been used in the field of sensitive biomolecular detection, diagnosis and therapeutics, catalysis, and microelectronics [10]. Moreover, Ag and Ag-NPs are used in skin cream and ointment for burns and open wounds [11]. Ag-NPs synthesized using plant extract have also been shown to have good antimicrobial, antimycotic, antioxidant, and cytotoxic activities [12–15].

Grewia optiva belongs to family Malvaceae and reportedly possesses antioxidant, analgesic, anticholinesterase, and antibacterial activities. Its phytochemical profile as studied by a number of authors indicates the presence of numerous biomedically important alkaloids, saponins, tannins, flavonoids, and terpenoids. The presence of these secondary metabolites makes the plant an eligible candidate to be used

in green synthesis of NPs that imparts promising bioactive potential to the synthesized NPs [16–19].

The reports on successful synthesis of Ag-NPs using different plant extracts and their enhanced biological potentials make the topic interesting to be investigated further using more medicinally important plants to improve/add to their biological potential as different plants have different phytochemical compositions. Using this idea, the inherent medicinal properties and the exciting phytochemical profile of *Grewia optiva* (which make it potentially more capable for the synthesis of Ag-NPs) promoted us to synthesize Ag-NPs using leaf extract and its isolated compounds (that were isolated previously).

2. Material and Methods

2.1. Chemicals. Silver nitrate, extra pure (AgNO_3 , 99%), was purchased from Scharlau Spain, and *Grewia optiva* leaves were collected from Tehsil Munda district Dir (L) Khyber Pakhtunkhwa, Pakistan. The leaves were thoroughly cleaned with deionized water and crushed to small pieces with the help of mortar and pestle. Of the crushed leaves, 10 g was taken into a conical flask, immersed in 100 mL of deionized water, and boiled for 20 min with constant stirring. Afterwards, filtration was carried out with Whatman paper no. 1 to obtain the aqueous extract.

2.2. Synthesis of Ag-NPs. Ag-NPs were synthesized using the standard biogenic method as described by Evanoff and Chumanov [17]. AgNO_3 solution (1 mM) was prepared by adding 0.085 mg of AgNO_3 in 100 mL of distilled water at room temperature followed by constant stirring for 5 min. The leaf extract of *Grewia optiva* was added to the solution, and it was kept shaking on the shaker for 3 h. The color changes from pale yellow to deep yellow and brown as observed indicated the successful formation of Ag-NPs. Five different solution mixtures were prepared for obtaining NPs of different shapes and sizes. The AgNO_3 solution was treated with a fixed ratio of leaf extract (1 : 6, 1 : 8, 1 : 10, 1 : 12, and 1 : 14). A PerkinElmer spectrophotometer was used to confirm the NP synthesis of silver.

2.3. HPLC Analysis of Leaf Aqueous Extract. To confirm the presence of biologically active compounds in the aqueous extract responsible for the reduction of silver ions, the aqueous extract was subjected to HPLC analysis. An Agilent 1260 system was used for the purpose. After two times of filtration, the extract was poured into HPLC vial and eluted with a mixture of solvents (methanol, acetic acid, and deionized water) in varied ratios, designated as A (10 : 2 : 88, *v/v*, respectively) and B (90 : 2 : 8, *v/v*, respectively). The compounds present were identified through comparison with available standards and literature data.

2.4. Synthesis of Ag-NPs by Isolated Compounds. In our previous studies [18, 19], we isolated 8 compounds from *Grewia optiva*, which are given in Figure 1.

The mentioned compounds were evaluated for their relative capability of Ag-NP synthesis. A very small quantity ($5\ \mu\text{g/mL}$) of each of the isolated compound was taken in

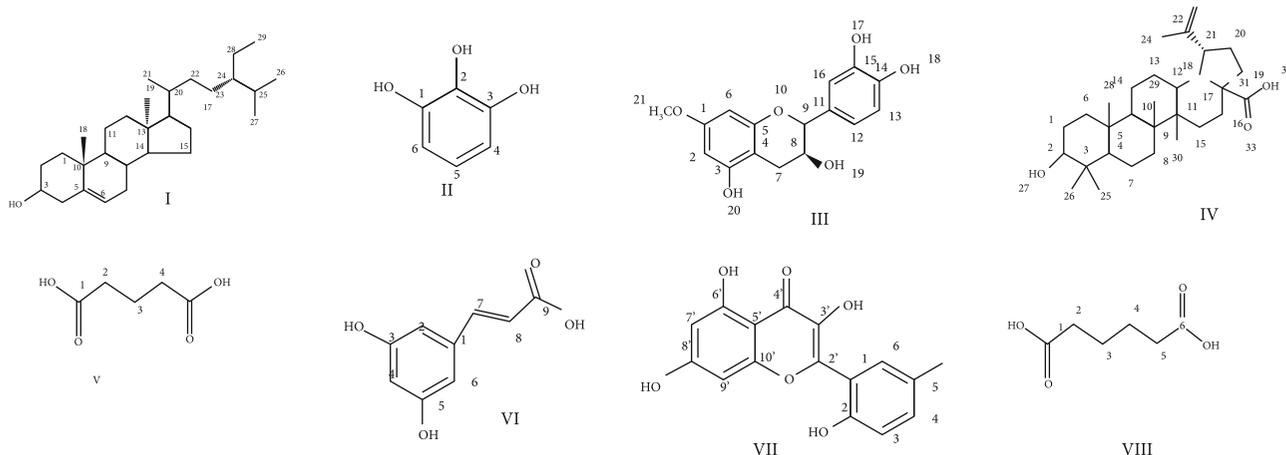


FIGURE 1: Isolated compounds from *Grewia optiva*: β -sitosterol (I), 1,2,3-benzenetriol (II), 7-O-methyl cathachin (III), betulinic acid (IV), glutaric acid (V), 3,5-dihydroxyphenyl acrylic acid (VI), (2,5-dihydroxyphenyl)-3',6',8'-trihydroxy-4H-chromen-4'-one (VII), and hexanedioic acid (VIII).

vials; a small amount of the Ag solution was added to each of the vial and allowed to rest for 30 min. Upon examination after the given time, each vial had small quantities of fabricated NPs. The eight vials containing the 8 compounds (one each in each different vial) were labelled as I-VIII, and the quantity of synthesized NPs in each vial was compared with that in the others.

2.5. Characterization of NPs. UV-Visible spectrum was recorded using the PerkinElmer spectrophotometer. The spectra were recorded in the range of 400-430 nm. A Hitachi S-4500 machine was used for SEM imaging. FTIR was carried out through IR prestige Fourier transform infrared spectrophotometer, Shimadzu, Japan. Diamond series TG/DTA, PerkinElmer, USA, analyzer was used for TGA where Al_2O_3 was used as a reference standard. The EDS X Sight Oxford instrument was used for EDX analysis while a Joel X-ray diffractometer JDX-3532 with Ni filter and monochromatic $\text{CuK}\alpha$ radiation having $\lambda = 1.5418\text{\AA}$ was utilized for XRD analysis.

2.6. Antibacterial Activities. The antimicrobial capacity of the extract and NPs was evaluated in terms of ZI, MIC, and MBC measurement against the selected bacterial strains. Sterilized agar culture dishes were inoculated with respective strain of bacteria; 3 mm holes were made in the media at 5 cm distance from each other and from the standard (Cephadrine). Leaf extract and the NPs were introduced into the holes through a micropipette. The culture dishes were incubated at 37°C for 24 h. ZI around each hole was measured. The whole procedure was carried out under laminar flow.

2.6.1. Determination of Minimum Inhibitory (MIC) and Bactericidal (MBC) Concentrations. Macro broth dilution method was used for the determination of MIC [20]. Solutions of the standard (Cephadrine) and Ag-NPs and leaf extract (0.02-10 mg/mL) were made in Mueller Hinton broth medium and were inoculated with selected bacterial strain (100 μL). One tube having the medium broth only was used

as negative control. All the tubes were put in an incubator at 37°C overnight. The tubes were observed visually for turbidity, and in the sequence, the first tube with no appreciable changes in turbidity was chosen as MIC.

The tube with no growths was further incubated for additional three days (four days total). After 96 h, the tube with no bacterial growth was considered as MBC.

2.7. Antioxidant Activity

2.7.1. DPPH Assay. Diphenyl-1-picrylhydrazyl (DPPH) assay was employed to calculate percent scavenging potentials of extract and NPs using standard protocol [21]. The DPPH stock solution was prepared in 100 mL distilled methanol having 10 mg of the solid DPPH in it. The absorbance of a 3 mL stock DPPH solution was adjusted to be 0.75 at 515 nm (considered as control solution). The stock solution under cover of aluminum foil was kept at room temperature in a dark place for 24 h to induce free radical formation. Afterward, 5 mg/mL stock solutions of the extract/NPs were prepared in methanol from which different dilutions in the range 1000-62.5 $\mu\text{g}/\text{mL}$ were made as well. About 2 mL DPPH stock solution and 2 mL of each dilution were incubated for at least 20 min. The same dilution for ascorbic acid (used as standard) was also prepared, and 2 mL of each dilution mixed with 2 mL DPPH was also incubated for the mentioned interval of time. Formula (1) is used to estimate the percent inhibition of DPPH:

$$\% \text{ inhibition} = \frac{A - B}{A} \times 100, \quad (1)$$

where A is the absorbance of oxidized DPPH form while B is the absorbance after mixing with extract/NPs. The experiments have been performed in triplicates, and mean values have been presented.

2.7.2. ABTS Assay. The leaf extract and synthesized Ag-NPs were also tested for their antioxidant capabilities through ABTS (2,2-azinobis-[3-ethylbenzthiazoline]-6-sulfonic acid)

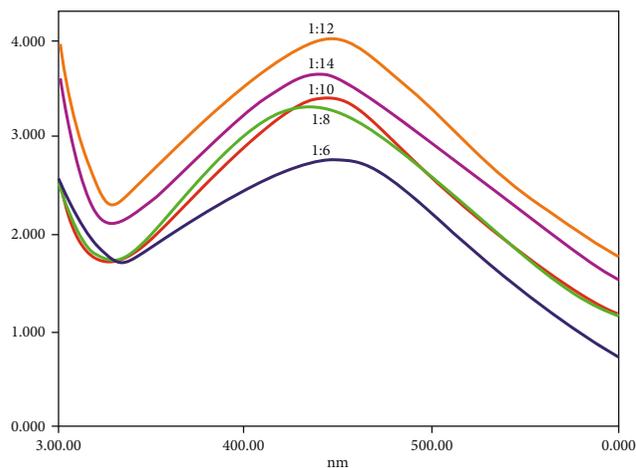


FIGURE 2: UV-Vis spectra of the synthesized Ag-NPs.



FIGURE 3: Different color patterns of different ratios of Ag and leaf extract.

assay using standard procedure [22, 23]. Different dilutions of the samples as mentioned in Section 2.7.1 were added to the ABTS stock solution (2 mL each). The incubation time of 15 minutes was provided to mixture, and the absorbance was measured at 745 nm. The extract and NP scavenging potentials were calculated using equation (1). Ascorbic acid was used as standard.

2.8. Hair Growth Activity. To observe the hair growth effect of extract and NPs, three rabbits were selected and the right limb of each was shaved in a length of $1 \times 3''$. The shaved area of one rabbit was massaged with distilled water as a control, one with Ag-NPs, and the other one with the extract thrice a day. The massage was continued for two weeks, and the results were recorded in the form of photographs.

2.9. Statistical Analysis of the Data. All the results obtained are given as mean \pm SEM, and the data was statistically analyzed for significant difference between the different groups using ANOVA (one-way analysis of variance) followed by post-hoc Bonferroni's test.

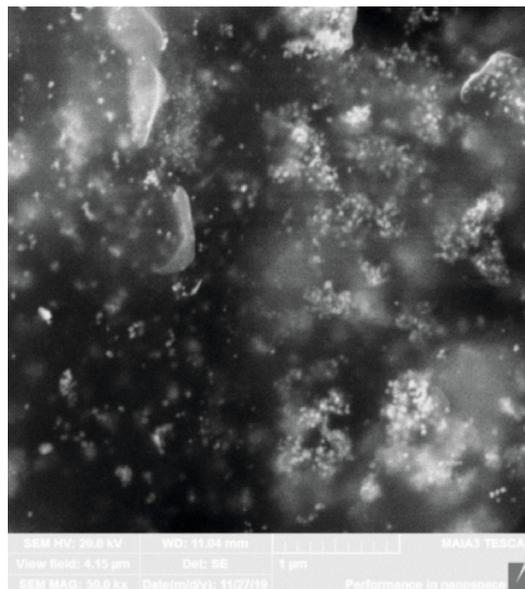


FIGURE 4: SEM photograph of Ag-NPs.

3. Results and Discussion

In view of the hazards associated with the chemical methods of synthesis of NPs, a biogenic method was used in this study. In literature, different plant extracts and isolated compounds have been successfully used as reducing agents in the synthesis of metal nanoparticles. Synthesis of NPs using plant extracts incorporate medicinal properties in the fabricated NPs. That is why majority of NPs synthesized through green route reported in the literature possess high biological potential of a given bioactivity (e.g., antibacterial, antioxidant, and cytotoxic) in comparison to those synthesized by other methods. Different types of bioactive compounds from plants have been reported that can interact with inorganic substances and can potentially be used in nanotechnology in preparation of NPs [7]. In the present study, Ag-NPs were synthesized using *Grewia optiva* leaf extract and its isolated compounds as source of capping and reducing agent. After their characterization, their potential bioactivities were tested as described below.

3.1. Optimized Ratio of the Ag-NP Formation. Plant extracts are a mixture of a number of compounds, some of which could act as reducing/stabilizing agents. Such compounds can change the oxidation state of metal ions as well. Fixed amount of extract and varied concentrations of AgNO_3 (1 mM) were mixed together to find out the optimized ratio for NP synthesis. The variation in intensities of colors of the solutions observed during the experiment was due to the reduction of Ag by compounds present in the extract. The observed changes in color intensities confirm the formation of Ag-NPs. The given ratio of 1:12, with the sharpest peak as can be observed in Figure 2, was chosen as the optimized ratio. The observed color pattern of the studied ratios is given in Figure 3.

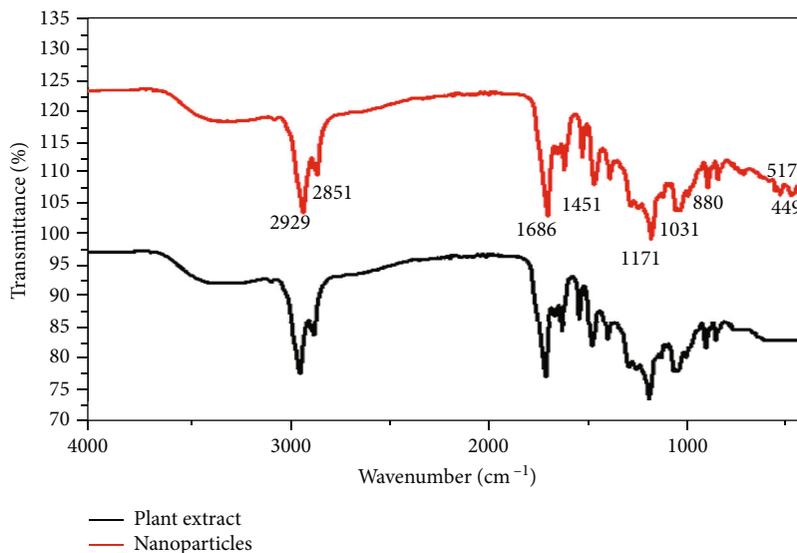


FIGURE 5: FTIR spectrum of extract and Ag-NPs.

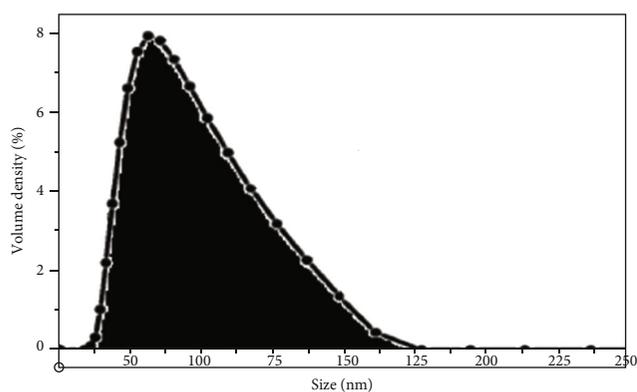


FIGURE 6: PSA spectrum of Ag-NPs.

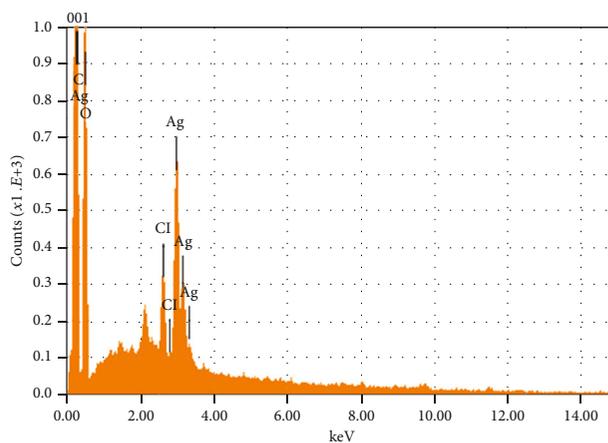


FIGURE 8: EDX spectrum of Ag-NPs.

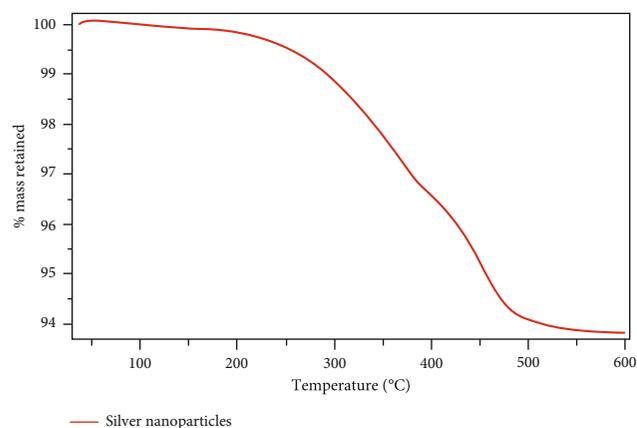


FIGURE 7: TGA of Ag-NPs.

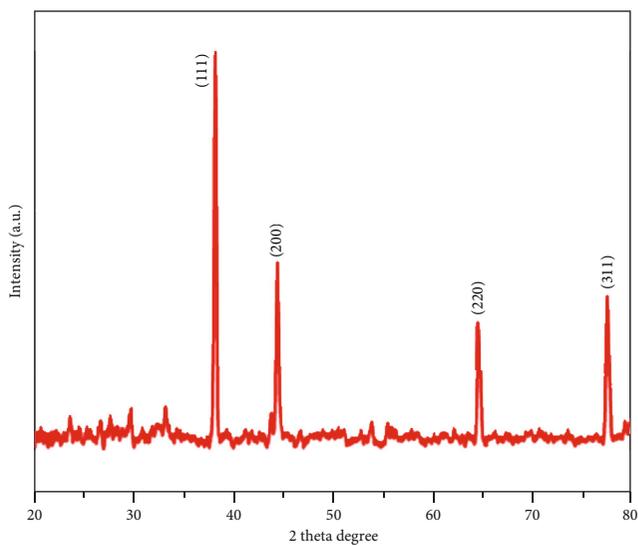


FIGURE 9: XRD photograph of Ag-NPs.

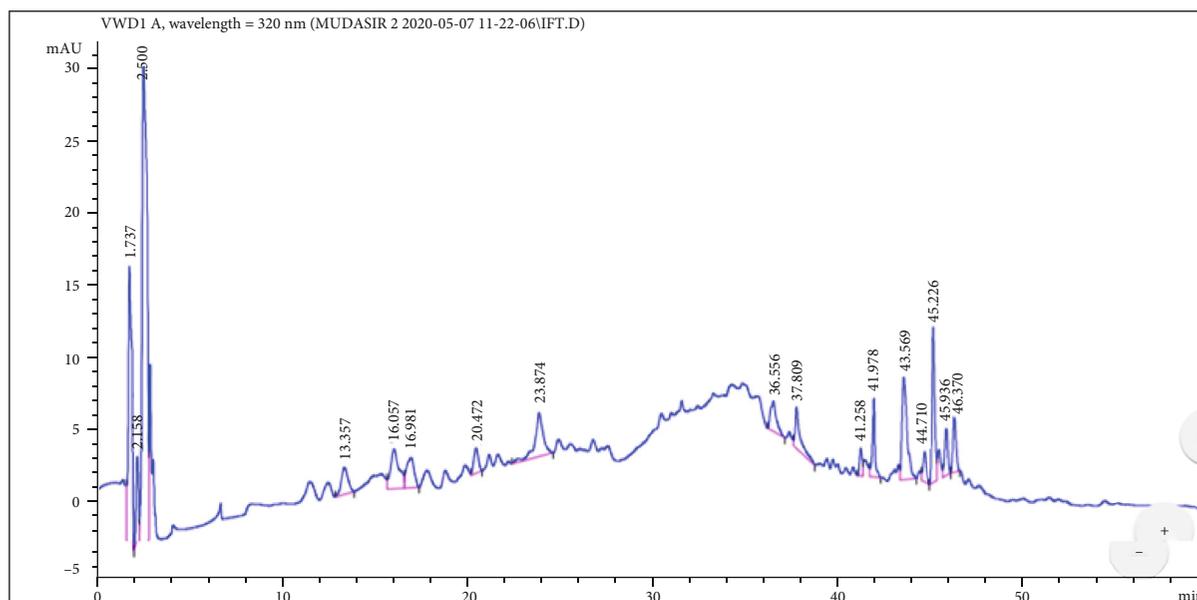


FIGURE 10: HPLC chromatogram of aqueous extract.

TABLE 1: Identification of bioactive compounds in aqueous extract.

Peak	Retention time (min)	Proposed identity of compound	Peak area %	Identification reference
1	1.737	Malic acid	15.82	Standard
2	2.157	Gallic acid	9.09	Standard
3	2.500	Ascorbic acid	33.97	Standard
4	13.37	Morin	2.45	Standard
5	16.06	Ellagic acid	4.16	Standard
6	20.472	Chlorogenic acid	1.56	Santos [33]
7	23.874	Quercetin-3,7-di-O-glucoside	4.92	Santos et al. [33]
8	36.55	Hydroxybenzoic acid	2.37	Standard

TABLE 2: ZI of Ag-NPs and leaf extract against different bacterial strains.

Bacterial strain	Zone of inhibition (mm): mean \pm SEM		
	Aqueous extract	Ag-NPs	Cephadrine
<i>E. coli</i>	9.00 \pm 1.20***	16.00 \pm 0.40***	23.00 \pm 0.20
<i>S. aureus</i>	9.00 \pm 0.99***	18.00 \pm 0.20***	24.00 \pm 1.20
<i>S. typhi</i>	10.00 \pm 1.32***	19.00 \pm 1.10 ^{ns}	21.00 \pm 0.60
<i>S. pneumoniae</i>	10.00 \pm 0.21***	18.00 \pm 1.20***	25.00 \pm 0.30

*** $P < 0.001$ and ^{ns} $P > 0.05$ as compared to the standard Cephadrine.

3.2. Biogenic Reduction/Stabilization of Ag-NPs. Ag-NPs were synthesized through the biogenic method in this study where the bioreductant/stabilizer source/s from the aqueous leaf extract were utilized to reduce silver ions (Ag^+) and generate the atomic silver (Ag) [24]. The reduced Ag then induces the reduction of the remaining Ag^+ in the solution acting as a nucleation center resulting in the formation of Ag clusters. During the reaction, the resulting mixture passed through different color transitions from pale yellow to deep

yellow and brown until the stabilization of all Ag^+ . Figure 3 shows the color pattern of mixtures of different concentrations of the AgNO_3 solution against the fixed ratio of leaf extract. The brown color of the solution is due to the surface plasmon resonance [25], which depends on the size and shape of NPs and dielectric constant of the medium. As stated earlier by Zielińska et al. [25], only free electrons carry plasmon resonance in metals like Ag, Cu, and Au, and first group elements in the visible region result in the production of different colors.

3.3. Formation of NPs by Isolated Compounds. In our previously reported studies [18, 19], 8 compounds were isolated from *Grewia optiva*. The ability of each of the isolated compound for NP fabrication was also investigated. The pattern of the precipitate formation in the vials containing the isolated compounds in terms of decreasing order was observed as VII>III>VI>II>IV>I>V>VIII. The higher percentage of precipitate formation was observed in the vial containing compound VII followed by that containing compounds III and VI. The chemical structures of the compounds with the highest yields indicate their phenolic nature, and the

TABLE 3: The MIC and MBC of leaf extract, Ag-NPs, and standard in $\mu\text{g/mL}$.

Bacterial strain	Aqueous extract		Ag-NPs		Standard	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>S. typhi</i>	125.00 \pm 2.20***	245.00 \pm 1.85***	70.00 \pm 1.90***	90.00 \pm 2.00***	60.00 \pm 1.11	70.00 \pm 1.50
<i>E. coli</i>	220.00 \pm 1.20***	460.00 \pm 1.34***	95.00 \pm 1.11***	190.00 \pm 1.20***	65.00 \pm 1.23	75.00 \pm 1.60
<i>S. pneumoniae</i>	190.00 \pm 1.00***	380.00 \pm 0.90***	70.00 \pm 1.00***	130.00 \pm 1.32***	50.00 \pm 1.20	50.00 \pm 1.00
<i>S. aureus</i>	250.00 \pm 1.20***	450.00 \pm 1.00***	95.00 \pm 0.30***	160.00 \pm 1.20***	45.00 \pm 1.32	60.00 \pm 0.40

MIC and MBC values where *** $P < 0.001$ as compared to the standard Cephradine.

presence of multiple hydroxyl groups could be responsible for the reduction of Ag ions at a higher rate than in the other vials.

3.4. SEM Analysis of Ag-NPs. The SEM image was taken to study surface morphology and to determine the size of Ag-NPs formed at optimum concentration ratio of *Grewia optiva* leaf extract and AgNO_3 solution. The image taken is shown in Figure 4; the white color spots in the image confirmed the synthesis of Ag-NPs. They are spherical in shape, uniform, and in the range of approximately 30 to 65 nm in diameter. The highest concentration of Ag-NPs as can be seen is in the range of 35 nm in diameter. The capping agent used for the stabilization of NPs was not in direct interaction even in the accumulated aggregates showing that only the extracts have performed the reduction. In the SEM image, there are some aggregates of greater size of the Ag-NPs which have formed due to the agglomeration of smaller NPs.

3.5. FTIR Spectroscopy. FTIR analysis shows the nature and purity of MNPs. There are two regions of IR spectrum: the functional group region and fingerprint region. The absorption bands of the organic compounds are normally in the functional group region while the peaks which appeared in the fingerprint region indicate the presence of metals. The peaks that appeared at 2929 cm^{-1} and 2852 cm^{-1} are the main peaks for C-H while the one that appeared at 1686 cm^{-1} is for C=O. The peaks at 1451 , 1171 , 1023 , 880 , 828 , and 699 cm^{-1} represent different functional groups present in the NPs and extract which are due to multiple compounds present in the plant extract (Figure 5) which has been predicted through HPLC analysis as described below. The slight displacement in the peaks of FTIR spectrum of NPs as compared to the extract curve is due to the interaction of metal ions with different functional groups present. The peak which was observed at 517 cm^{-1} and 401 cm^{-1} is for silver oxide and is in close agreement with the reported FTIR in the study of Singho and his colleagues for Ag-NPs [26–28].

3.6. PSA Studies. The size of the synthesized NPs was determined with the help of a particle size analyzer as shown in Figure 6. From the results of PSA, its size was found to be in the range of 50 to 75 nm. Based on mass median diameter, 50% of the particles were found to be smaller ones. The results obtained are in close agreement with the study of Mustafa et al. [29].

3.7. TGA Studies. This analysis shows thermal stability of the NPs and gives information about the decrease in mass, i.e.,

percent weight loss with the increase in temperature. The results are presented in Figure 7. The Ag-NPs were heated up to 600°C . At the beginning, the sample mass was constant (10.814 mg), but at 200°C , gradual mass loss was observed with increase in temperature which was due to the removal of moisture and pyrolysis of bioorganic compounds present in the sample. At 500°C , the sample size reduced to 0.0812 mg, no further weight loss was observed with the increase in temperature, and the mass of the sample remained constant. The results are in close agreement with the reported studies in the literature [30, 31].

3.8. EDX Analysis. The EDX analysis confirmed the presence of elemental Ag in the sample which indicates the formation of Ag-NPs. The graph shows Ag absorption peak at 3.7 keV. The other peaks (Figure 8) indicate the presence of C, O, and Cl which may probably have come from plant extract [32].

3.9. XRD Analysis. XRD analysis is usually performed (range 20 – 80 at 2θ) to differentiate crystalline and amorphous nature of a substance as well as to determine the size of the synthesized Ag-NPs. Figure 9 shows the XRD spectra of the synthesized Ag-NPs. The synthesized NPs showed high intensity peaks at 38° , 44° , 64° , and 77° representing Bragg's reflection plans of 111, 200, 220, and 311, respectively. These are the peak positions of the face centered cubic crystals of Ag [31, 32]. X-ray diffraction pattern revealed the absence of impurities and indicated the successful synthesis of pure Ag-NPs as well.

3.10. HPLC Fingerprinting of Aqueous Extract. HPLC analysis of aqueous extract was performed to confirm the presence of biologically important compounds in it. The typical chromatogram is given in Figure 10. Although there are many peaks in this chromatogram, only those which were identified based on comparison of their retention time with those of reported compounds in literature or with that of the available standards were presented. The identified compounds are presented in Table 1. Ascorbic acid was present in high amount followed by malic acid. Gallic acid was also present in appreciable amount. Almost all of these compounds are polar in nature which indicates their successful isolation in aqueous media.

3.11. Antibacterial Activities of Extract and Ag-NPs. The antibacterial activity of the extract and the synthesized NPs was investigated against *Salmonella typhi*, *E. coli*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* using agar well diffusion method.

TABLE 4: Percent DPPH and ABTS scavenging potential and IC₅₀ values of leaf extract of *Grewia optiva* and Ag-NPs.

Sample	Dilutions ($\mu\text{g/mL}$)	%DPPH scavenging mean \pm SEM	IC ₅₀ ($\mu\text{g/mL}$)	%ABTS scavenging mean \pm SEM	IC ₅₀ ($\mu\text{g/mL}$)
Aqueous	1000	76.91 \pm 0.90***	60	68.83 \pm 0.89***	70
	500	73.49 \pm 0.83***		65.78 \pm 0.99***	
	250	68.84 \pm 0.67***		65.11 \pm 0.50***	
	125	64.67 \pm 1.40***		50.55 \pm 0.20***	
	62.5	50.76 \pm 0.12***		45.67 \pm 0.10***	
Ag-NPs	1000	86.88 \pm 1.00***	46	78.55 \pm 1.20***	48
	500	76.98 \pm 0.90*		75.56 \pm 0.80***	
	250	75.68 \pm 0.80***		78.90 \pm 0.45 ^{ns}	
	125	71.57 \pm 1.00***		63.23 \pm 0.67 ^{ns}	
	62.5	66.39 \pm 0.24***		54.67 \pm 0.20**	
Ascorbic acid	1000	92.32 \pm 0.40	28	90.34 \pm 0.30	30
	500	80.21 \pm 0.80		83.20 \pm 0.89	
	250	72.45 \pm 0.30		76.45 \pm 1.00	
	125	66.72 \pm 0.12		64.34 \pm 0.23	
	62.5	62.50 \pm 0.09		58.24 \pm 0.12	

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, and ^{ns} $P > 0.05$ as compared to ascorbic acid.

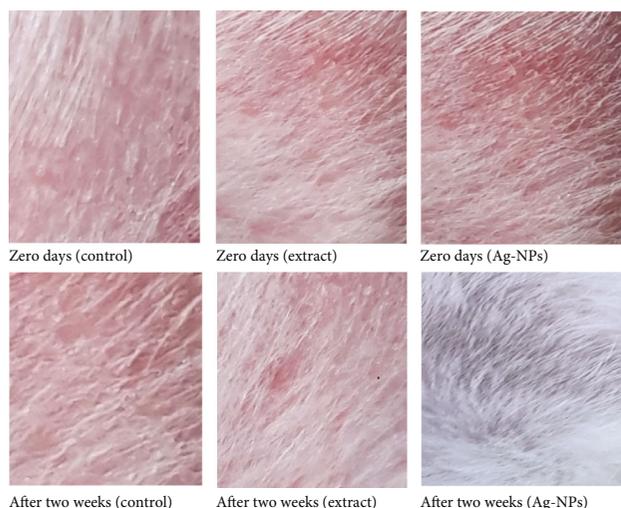


FIGURE 11: Hair growth potential exhibited by extract and NPs.

3.12. Determination of ZI. The obtained results are shown in Table 2. The leaf extract of plant showed less inhibition zone as compared to the prepared Ag-NPs. Against *E. coli*, Ag-NPs showed 16 mm zone of inhibition whereas leaf extract showed 9 mm ZI against the same bacterial strain. Against *Staphylococcus aureus*, the NPs produced 18 mm zone of inhibition while leaf extract produced ZI of about 9 mm. The ZI of Ag-NPs against *S. typhi* was 19 mm while for extract it was 10 mm. Similarly, *S. pneumoniae* was restricted to ZI of 18 mm while extract produced ZI of about 10 mm. Cephadrine was used as positive control.

3.13. MIC and MBC of Extract and NPs. The MIC and MBC values are shown in Table 3 for *Grewia optiva* leaf extract and fabricated Ag-NPs. As can be seen from the results in the

given table, the values of MIC and MBC have improved and comparable to those of the standard in case of Ag-NPs as compared to plant extract. The highest activity was observed against *S. typhi*, by both the aqueous extract and the Ag-NPs with MIC values of 125 and 70 $\mu\text{g/mL}$ and MBC values of 245 and 90 $\mu\text{g/mL}$, respectively.

3.14. Antioxidant Activities of the Leaf Aqueous Extract of *Grewia optiva* and the Formulated Ag-NPs. Free radical scavenging activities and the IC₅₀ values of the extract and Ag-NPs as evaluated through DPPH and ABTS assays are shown in Table 4. Higher and promising antioxidant activity compared to the aqueous extract was obtained for Ag-NPs. The observed activity was concentration dependent as well. Ascorbic acid was used as positive control.

3.15. Hair Growth Activities. The hair growth activity was carried out on the limbs of rabbits as shown in photographs (Figure 11). The experiments were undertaken to check the hair growth potential of the aqueous leaf extract and the synthesized NPs. From the photographs, it is clear that the rabbit limb massaged with Ag-NPs have far better hair growth compared to those massaged with distilled water and leaf aqueous extract. These results are in close agreement with one of our previously reported studies on Ag-NPs [34].

4. Conclusions

Grewia optiva is a source of some highly important secondary metabolites of biological importance that make it a good candidate to be used as a reducing agent in the synthesis of Ag-NPs. In the study, the aqueous extract and the compounds previously isolated from different fractions of the leaf extract of *Grewia optiva* were used as a source of reducing and stabilizing agents for the synthesis of Ag-NPs. Ag-NPs

were obtained in good yield for almost all ratios of the test substances used as reducing agents. The highest yield was obtained for compound VII which is a flavonoid followed by the compounds III and VI that are polyphenols in nature. The results obtained are consistent with several studies where flavonoids and polyphenols were found to have greater participation in the fabrication of NPs compared to other secondary metabolites. An appreciable extent of antioxidant potential as evaluated through DPPH and ABTS assays was observed both for the aqueous leaf extract and the Ag-NPs. ZI, MIC, and MBC of Ag-NPs and the extract were determined to measure their antibacterial capability, and promising results were obtained for NPs as compared to extract. The propitious antibacterial activity of the synthesized NPs, especially against *S. typhi*, suggests that these could be studied further as an antibacterial candidate alternative to available antibiotics to overcome the drug resistance problem. Moreover, the effect of NPs and the extract was monitored on the growth of rabbit's hair as well. The rate of hair growth was found higher for NPs as compared to extract. A good level of antioxidant capability of prepared Ag-NPs in comparison to extract was also observed. However, further toxicological studies are needed in this connection to use the prepared NPs clinically for the studied biological effects.

Data Availability

The data associated with this publication is totally presented in the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

MZ and SM wrote the paper. MI performed the experiments. NZ, RU, GEB, MH, and AB proofread the paper and helped in laboratory work. All authors have read and agreed to the published version of the manuscript.

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