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Research Article

Fabrication of Silver Nanoparticles from *Ziziphus nummularia* Fruit Extract: Effect on Hair Growth Rate and Activity against Selected Bacterial and Fungal Strains

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Nanoparticles are extensively used in biomedical and biotechnological research. Their large surface area, excellent physical properties, high permeability, and retention effect make them ideal for biomedical applications including diagnosis and treatment. Silver nanoparticles proved to be the safest for therapeutic uses. In the present study, silver nanoparticles (AgNPs) were prepared using various ratios of *Ziziphus nummularia* fruit extract and silver nitrate solution. The nanoparticles were investigated for hair growth and antibacterial and antifungal activities. Characterization of AgNPs was done by using UV-spectrophotometer, scanning electron microscope (SEM), X-ray diffractometer (XRD), thermogravimeter (TG), energy dispersive X-ray (EDX), Fourier transform infrared spectroscopy (FTIR), and master sizer. UV-spectrophotometer results showed the best ratio 10 : 10 of *Z. nummularia* fruit aqueous extract to silver solution for nanoparticle production at 400 to 430 nm wavelength. The size of AgNPs was 40 nm as measured by SEM. Characterization of AgNPs through EDX resulted in a silver peak at 3 keV. In contrast, differential scanning calorimetry (DSC) spectra show that the AgNPs are stable up to 160°C.

The XED spectra gave 12 nm size of crystallite at 2 theta degree angle. FTIR bands for the metal oxides were recorded at 665 cm^{-1}. Weight loss of the prepared nanoparticles was observed due to moisture loss when subjected to TGA, whereas particle size distribution 0.1 μm to 0.17 μm was recorded by the master seizer. The *Z. nummularia* fruit aqueous extract-mediated AgNPs were noted highly effective against Gram-positive bacteria compared to ethanolic, methanolic, chloroform, and ethyl acetate extracts of *Z. nummularia* fruit. The Gram-negative bacteria fungal species showed less sensitivity to AgNPs. The hair growth activity was observed to be higher for AgNPs followed by minoxidil than ethanolic and methanolic extracts of *Z. nummularia* fruit. These findings have concluded that *Z. nummularia*-AgNPs have an effective hair growth activity and exhibit several applications in distinctive biomedical and pharmaceutical industries.

1. Introduction

Nanotechnology has induced a great scientific advancement in research and technology. It deals with science and technology to control matter at the molecular level. On the nanoscale level, the properties of matter are considerably different from their properties in bulk. It refers to the ability to design, characterize, produce, and apply structures, devices, and systems by controlling shape and size at the nanometer gage. Nanotechnology is the study and application of small objects which can be used across all fields such as chemistry, biology, biotechnology, physics, material science, and engineering [1].

The preparation of metallic nanoparticles has attracted attention due to their unique biotic and physicochemical characteristic related to their macroscaled counterparts. Gold, silver, zinc, iron, and copper stable dispersions of nanoparticles are very useful in microbiology, photography, catalysis, biological labeling, photonics, and optoelectronics. Silver nanoparticles are of prime importance because of their wide applications in medical devices and pharmaceutical products [2], antimicrobial properties, and low toxic effects on animal and human cells. For such applications, small particle size can enhance the antimicrobial effect. So the reactants used to prepare nanoparticles should be nontoxic or nonirritant. Therefore, a method is known as green synthesis favor well these purposes. The nanoparticles prepared through "green synthesis" are eco-friendly, nontoxic, and safe reagents [3]. Several methods are available for the fabrication of silver nanoparticles, including a reduction in solutions, chemical and photochemical reactions in reverse micelles, thermal decomposition of silver compounds, radiation assisted, electrochemical, sono-chemical, microwave-assisted process, and recently via green chemistry route [4]. The use of environmentally friendly materials such as plant extract, microorganisms (bacteria, fungi), and enzymes for the preparation of silver nanoparticles has many advantages of eco-friendliness, compatibility for pharmaceuticals, and other biomedical applications as they do not contain toxic material for the fabrication protocol. Chemical synthesis methods for the preparation of nanoparticles lead to the presence of some harmful material absorbed on the surface that may cause adverse effects in treating various ailments. Green synthesis has advantages over chemical and physical methods; for example, it is cost-effective, eco-friendly, and easy preparation for large scale synthesis. Moreover, there is no need to utilize high pressure, energy, temperature, and toxic chemicals [5].

The role of hairs is of great importance in mammals, especially in the padding of heat and community patterns. Comparatively, the human body consists of fewer hairs than other mammals. The presence of hairs on the human body has cosmetic importance rather than the existence of life. Hair loss in humans is a skin disorder. It has remained an issue for many years due to disturbance in metabolism, genetic factor, irregular secretion of hormones, and use of different drugs like immunosuppressant and antineoplastic agents. The FDA approved synthetic drugs for hair growth such as minoxidil and finasteride have been discouraged due to side effects. Therefore, searching for new sources from natural product of plant origin can play a significant role in overcoming these problems [6]. *Z. nummularia*, commonly known as Malla or Jher berry, belong to Rhamnaceae. The fruit of *Z. nummularia* has a cooling and astringent effect and can be used as an appetizer, stomachic, to treat mucous and enhance biliousness effect [7]. Different parts of *Z. nummularia* have been reported for the traditional treatment of various types of diseases [8]. however, no published data are available regarding the preparation of silver nanoparticles from its fruits. The roots, leaves, bark, and seeds of *Z. nummularia* have been focused on in all the research work up till now, and fruits have been neglected so far as a possible subject of study and analysis [9, 10].

In this study, silver nanoparticles (AgNPs) were formed by blending different amounts of fruit extract from *Z. nummularia* and silver nitrate solution. The nanoparticles were studied for their effect on hair growth and activity against pathogenic bacterial and fungal strains.

2. Materials and Methods

2.1. Fruit Collection and Preparation. Healthy and fully ripened fruits of *Z. nummularia* were collected from plants during the fruiting season and washed thoroughly with deionized water. The fruits were cut into pieces and kept in a sterile shady environment for drying. After drying, the samples were ground into the powdered form using mortar and pestle and transferred into polythene bags. Extraction from the dried plant material was done in five different solvents, i.e., water, chloroform, ethanol, methanol, and acetone. In 100 mL of each solvent, 25 g of the dried powder sample was taken in a beaker and kept on a shaker for 48 h. The solutions were then filtered using Whatman No.1 filter paper (pore size 25 μm) into conical flasks, and the filtrates were kept for drying in a shady sterile environment. Under reduced pressure, solvents were allowed to be evaporated, and the dried extracts were stored at 25°C for analysis.

2.2. Preparation of Silver Nanoparticles. Silver nanoparticles (AgNPs) were prepared via the standard method described by Turkevich et al. [9]. Aqueous extract of *Z. nummularia* fruit was used as a bioreductant to synthesize silver nanoparticles. A 0.085 g of silver nitrate was thoroughly dissolved in 100 mL of distilled water to get a 1 mM solution of $AgNO_3$. Then, silver nitrate solution was mixed with aqueous extract of *Ziziphus* fruit in the ratios of 6 : 1, 8 : 1, 10 : 1, 12 : 1, and 14 : 1. The mixture was kept on a shaker for about 4 h. The change of color from colorless to yellowish-brown was observed, indicating silver nanoparticles' formation.

2.3. Characterization of Silver Nanoparticle. Characterization of AgNPs was carried out by UV-Vis spectral analysis through UV-Vis spectrophotometer UV-2450 (Shimadzu). Infrared spectra were obtained using a Fourier transform infrared spectrometer (I.R. Prestige Fourier transform infrared spectrophotometer, SHIMADZO, Japan) ranging 4000-600 cm^{-1}. XRD analysis was performed using Joel X-ray diffractometer JDX-3532 with coat JCPDS no. 003-1018 and Ni filter, using monochromatic CuKα radiation of wavelength 1.5418 Å. The X-ray generator was operated at 40 kV and 30 mA. The scanning range $2\theta/\theta$ was selected. The scanning speed of 10 min^{-1} was employed for precise determination. Hitachi S-4500 SEM machine was used for scanning electron microscopic (SEM) analysis. EDX Sight Oxford instrument was used for EDX analysis. The thermal gravimetric analysis used the Diamond Series TG, PerkinElmer, USA, analyzer using Al_2O_3 as reference. The particle size analyzer gives an order about the size of the synthesized silver nanoparticles formed by the leaf extract of *Ziziphus nummularia*.

2.4. Antibacterial Assay. *S. aureus* (ATCC 6538), *E. coli* (ATCC 35218), *Streptococcus pyogenes* (ATCC 19615), *Pseudomonas aeruginosa* (ATCC 125668), *S. pneumoniae* (ATCC 6303), *Streptococcus faecalis* (ATCC 9790), *Proteus vulgaris* (ATCC 6380), and *Proteus mirabilis* (ATCC 14153) were obtained from Sarhad University microbiology lab. The agar well-diffusion was followed to determine antibacterial activity. Nutrient agar (N.A.) plates were swabbed (sterile cotton swabs) with 8 h old-broth culture of respective bacteria. A sterile cork borer was used for making wells of about 10 mm diameter and about 2 cm apart in each of these plates. A stock solution of each plant extract was prepared at a 1 mg/mL concentration in different plant extracts, viz., methanol, ethanol, petroleum ether, and water. Around 100 μL of various plant solvent extracts was syringed into the wells and left to diffuse for 2 h at room temperature. Control experimentations, including inoculums without plant extract, were carried out. The incubation of plates was carried out for bacterial pathogens at 37°C for 18-24 h. The activity index was also calculated, and the diameter of the inhibition zone (mm) was also measured. The experiment was repeated three times for maintaining triplicates. The readings were taken in three different fixed directions for each replicate, and the average values were noted [11].

2.5. Antifungal Assay. *Candida albicans* (ATCC 60193), *Cryptococcus neoformans* (ATCC 14115), and *Aspergillus niger* (ATCC 6275) were obtained from Sarhad University microbiology lab. A well-diffusion method was followed to determine the antifungal activity. Potato dextrose agar (PDA) plates were swabbed (sterile cotton swabs) with 8 h old-broth culture of respective fungi. Wells (10 mm diameter and about 2 cm apart) were made in each plate using a sterile cork borer. A stock solution of each plant extract was prepared at a 1 mg/mL concentration in different plant extracts, viz., methanol, ethanol, petroleum ether, and water. About 100 μL of different concentrations of plant solvent extracts was added sterile syringe into the wells and allowed to diffuse at room temperature for 2 h. Control experimentations, including inoculums without plant extract, were carried out. The incubation of plates was carried out for bacterial pathogens at 37°C for 18-24 h. The activity index was also calculated, and the diameter of the inhibition zone (mm) was also measured. The experiment was repeated three times for maintaining triplicates. The readings were taken in three different fixed directions for each replicate, and the average values were noted [12].

2.6. Determination of Minimum Inhibitory Concentration (MIC). The extracts of *Z. nummularia* were very effective as antimicrobial agents. Later on, the MIC and MBC values were also determined for each strain by applying tests. The extracts were diluted to give the final concentrations of 75, 37.5, 18.8, 9.4, 4.7, 2.4, 1.2, 0.6, 0.3, and 0.15 mg/mL. 100 μL of 105 CFU/m of the microbial strains was inoculated in tubes with an equal volume of nutrient broth and plant extracts. The tubes were incubated aerobically for 24-48 h at 37°C. Three control tubes were maintained for each strain (organism control, extract control, and media control). The least concentration of the extract (highest dilution) that did not produce visible growth (no turbidity) in the initial 24 h as compared to the control tubes was considered as preliminary "MIC." The dilutions that showed no turbidity were further incubated for 24 h at 37°C. The lowest concentration that did not show any visual turbidity after the overall incubation period of 48 h was considered the final "MIC."

2.7. Determination of Minimum Bactericidal Concentration (MBC). The value of MBC was found out by subculturing the test dilution (which did not show any visual turbidity) on newly prepared nutrient agar media. Further incubation of plates was carried out for 18-42 h at 37°C. The highest dilution that yielded no single bacterial colony on the nutrient agar plates was taken as MBC [13].

2.8. Minimum Fungicidal Concentration (MFC). The MFC determination was carried out initially by choosing those tubes that did not show any growth during "MIC" determination. An inoculating loop full from each tube was subcultured onto agar plates free from the extract and incubated at 37°C for further 24 h. That minimum concentration, at which there was no growth observation, was considered the "MFC" [14].

2.9. Hair Grow Activity. Four rabbits of almost the same weight were taken and kept in the animal house for one week. All the rabbits were offered the same diet. After one

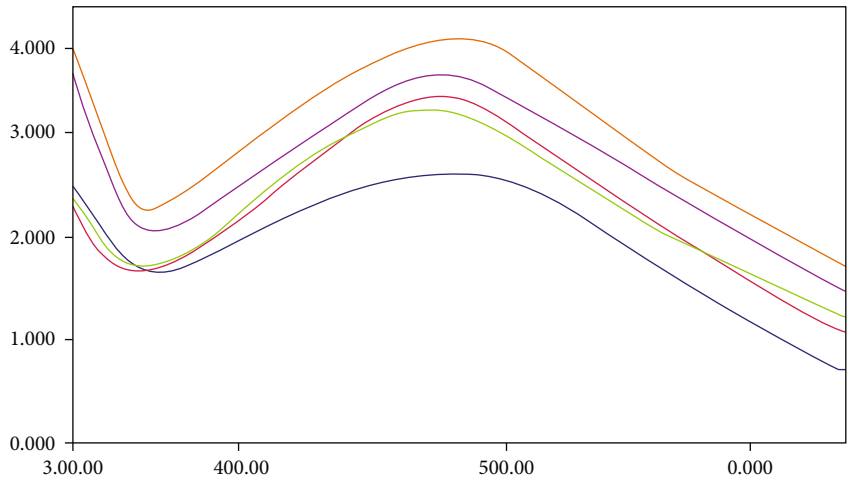

Figure 1: UV-spectrophotometer spectra of silver nanoparticles of different ratios of *Ziziphus nummularia* fruit.

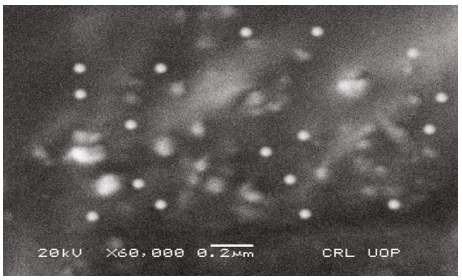

Figure 2: SEM image of silver nanoparticles of *Ziziphus nummularia* fruit extract at 60,000 magnification level.

week, the right limb of rabbits was shaved with a razor in a dimension of 1×3 inch. Then, the shaved area was massaged with minoxidil, ethanolic, and methanolic extracts of *Z. nummularia* and silver nanoparticles three times a day throughout the experimental periods. The size length of hairs was measured by the scale and noted in photographs [15]. All rabbits throughout the experimental study provided the same diet and environmental conditions.

3. Results and Discussion

Z. nummularia aqueous fruit extract was used as a reducing agent to synthesize silver nanoparticles and characterized using various sophisticated instruments. The prepared silver NPs were analyzed by UV-spectrophotometer, SEM, XRD, TGA, DSC, EDX, and particle size analyzer. Further, the prepared AgNPs were evaluated for antimicrobial and hair growth activities.

3.1. Analysis of Silver Nanoparticles by UV-Spectrophotometer. Figure 1 shows peaks of different sizes and shapes of silver nanoparticles confirmed at a region of 460 to 485 nm. The silver nanoparticle formations depend on the concentration of silver and plant extracts. The maximum absorbance of silver nanoparticles depends on Ag concentration concerning plant extract [16, 17]. The maximum absorbance was noted with a ratio of 1 : 12 of plant extract and silver nitrate solution indicated by the uppermost orange line, which is the optimum ratio peak, while the other lines show minimum absorbance specified for nanoparticles larger in size than the orange line. Namratha and Monica [18] studied that variation in the absorption peaks of the synthesized silver nanoparticles might be the difference in the morphology of the nanoparticle. Analysis of silver nanoparticles by scanning electron microscopy white patches as shown in Figure 2 indicates silver nanoparticles' formation using *Z. nummularia* fruit extract. The silver nanoparticles formed are round; however, some white large dull patches can also be seen that represents an accumulation of the particles. The round and uniform silver nanoparticles have been noted with a diameter of 40 nm. The SEM results indicated that *Z. nummularia* fruit extract acts as a strong reducing agent, resulting in spherical and uniform Ag nanoparticles. However, the large white spot may be an aggregation of the nanoparticles. Our results showed particle size contrast [13], where the observed particle sizes ranged between 4 nm and 6.5 nm.

3.2. Analysis of Silver Nanoparticles by EDX. EDX spectroscopy is used for elemental analysis, as depicted in Figure 3. The EDX spectra show the high intensive silver peak, which represents the formation of silver nanoparticles. The spectra indicated the silver nanoparticles peak at 34 keV, whereas carbon and oxygen peaks are also present, representing the mixed precipitation in the plant extract. It has been reported that oxygen and carbon peaks are due to the presence of biomolecules attached to the surface of the silver nanoparticles [19, 20]. Priya et al. [21] also studied plant-mediated silver nanoparticles. They reported the presence of silver, carbon, and oxygen peaks when analyzed through EDX. Jiang et al. [22] suggested that Ag is only the major element present in the NPs under study. Our results are also in concordance with the study of [14], which observed silver, carbon, and oxygen peaks in *Z. nummularia* fruit aqueous extract-mediated silver nanoparticles characterized by EDX.

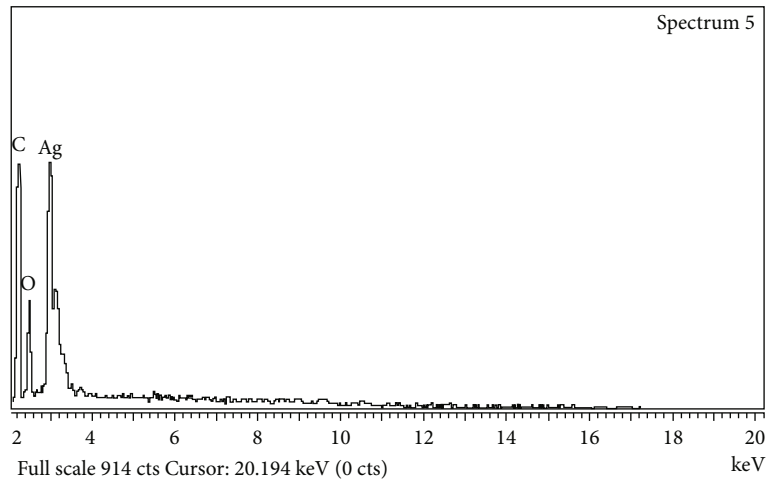

Figure 3: EDX spectra of silver nanoparticle of *Ziziphus nummularia* fruit.

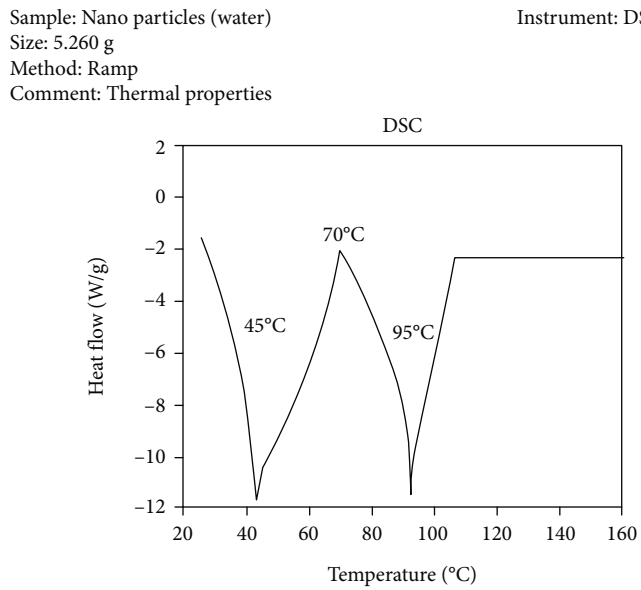

Figure 4: DSC pattern of silver nanoparticles of *Ziziphus nummularia* fruit.

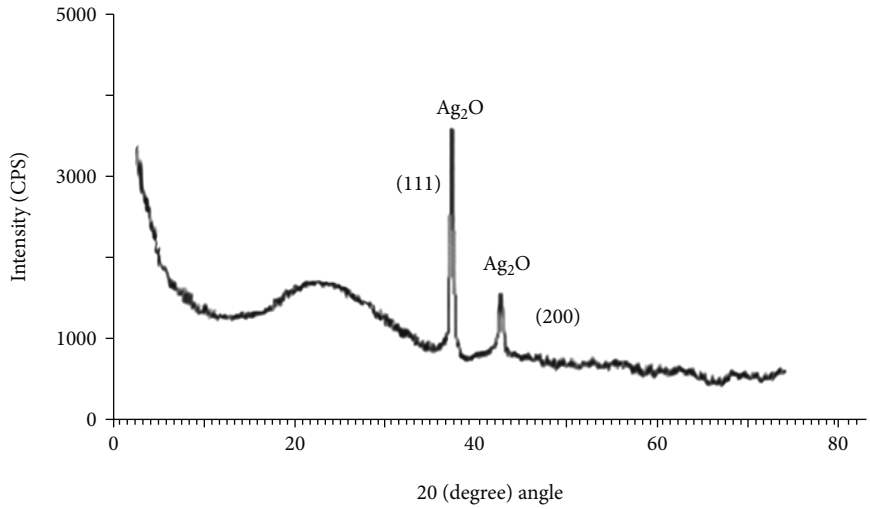

Figure 5: XRD spectra of silver nanoparticle of *Ziziphus nummularia* fruit.

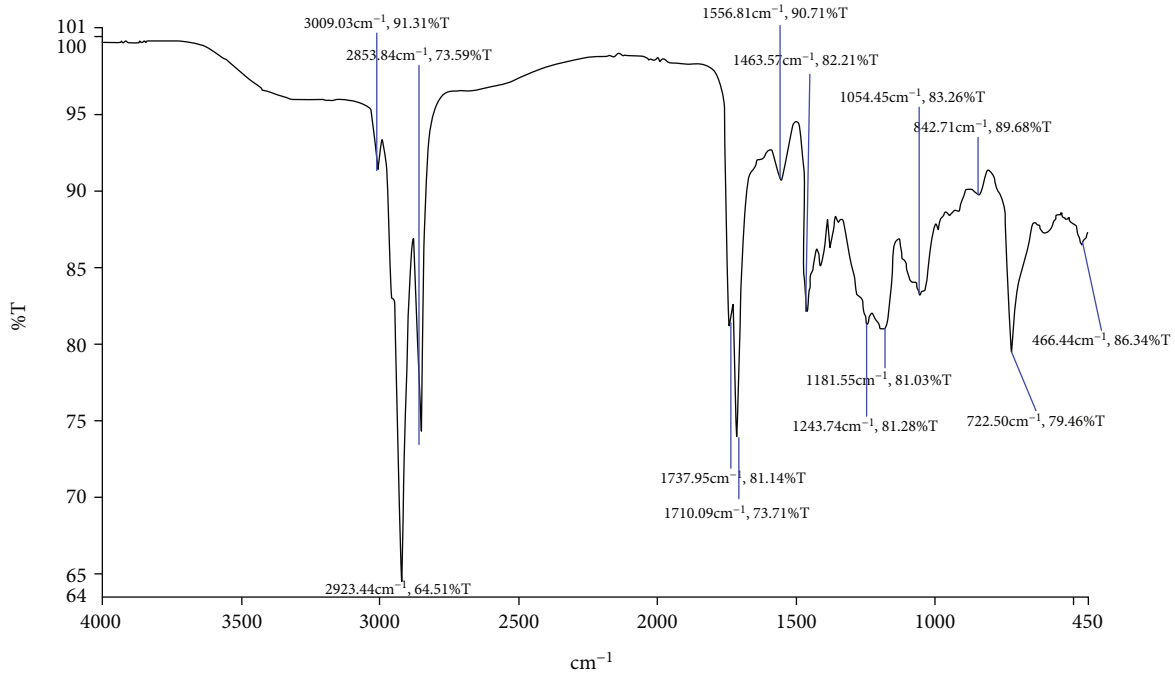

Figure 6: FTIR spectra of silver nanoparticle of *Ziziphus nummularia* fruit.

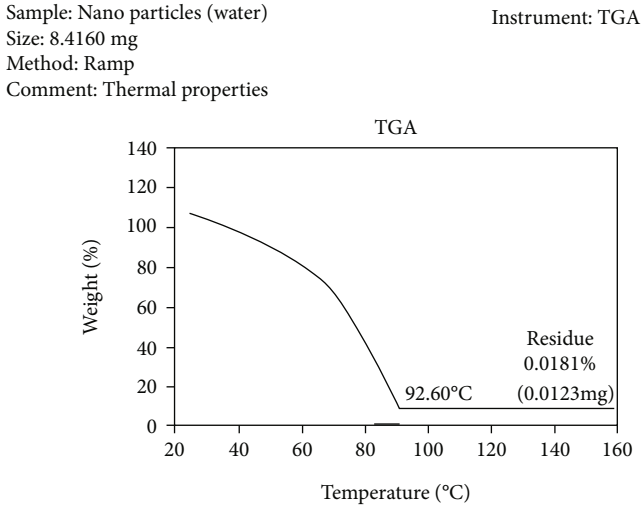

Figure 7: TGA peak pattern of silver nanoparticle of *Ziziphus nummularia* fruit.

3.3. Analysis of Silver Nanoparticles by DSC. DSC has been used to study the isothermal behaviour of silver nanoparticles. Figure 4 shows the DSC spectra of silver nanoparticles with temperatures ranging from 20°C to 160°C. The DSC spectra clearly show various exothermic peaks at 45, 70, 95, and 99°C. These peaks indicated that the gradual loss of water started at a temperature of 45°C and ended at 99°C, whereas above 99°C, the silver nanoparticles were found stable up to 160°C. Our results are nearly in agreement with Farhat et al. [16], where AgNPs of *Z. nummularia* leaf extract were stable up to 93°C.

3.4. Analysis of Silver Nanoparticles by XRD. The XRD spectra of silver nanoparticles at 2θ (degree) angle are shown in Figure 5. The diffraction peaks at 37 and 43 represent the correspondence indices 111 and 200, respectively. The correspondence peaks indicated the typical face-centered cubic structure of silver nanoparticles. Sulochana et al. [23] investigated the XRD technique of *Andrographis paniculata* leaf extract's silver nanoparticles. The diffraction peaks of silver nanoparticles at 37 and 44 confirmed the indices 111 and 200, respectively. The particle size of the prepared silver nanoparticles was noted at 15 nm by using the Deby Scherer equation as listed below.

$$D = \frac{K\lambda}{\beta \operatorname{Cos}\theta}, \tag{1}$$

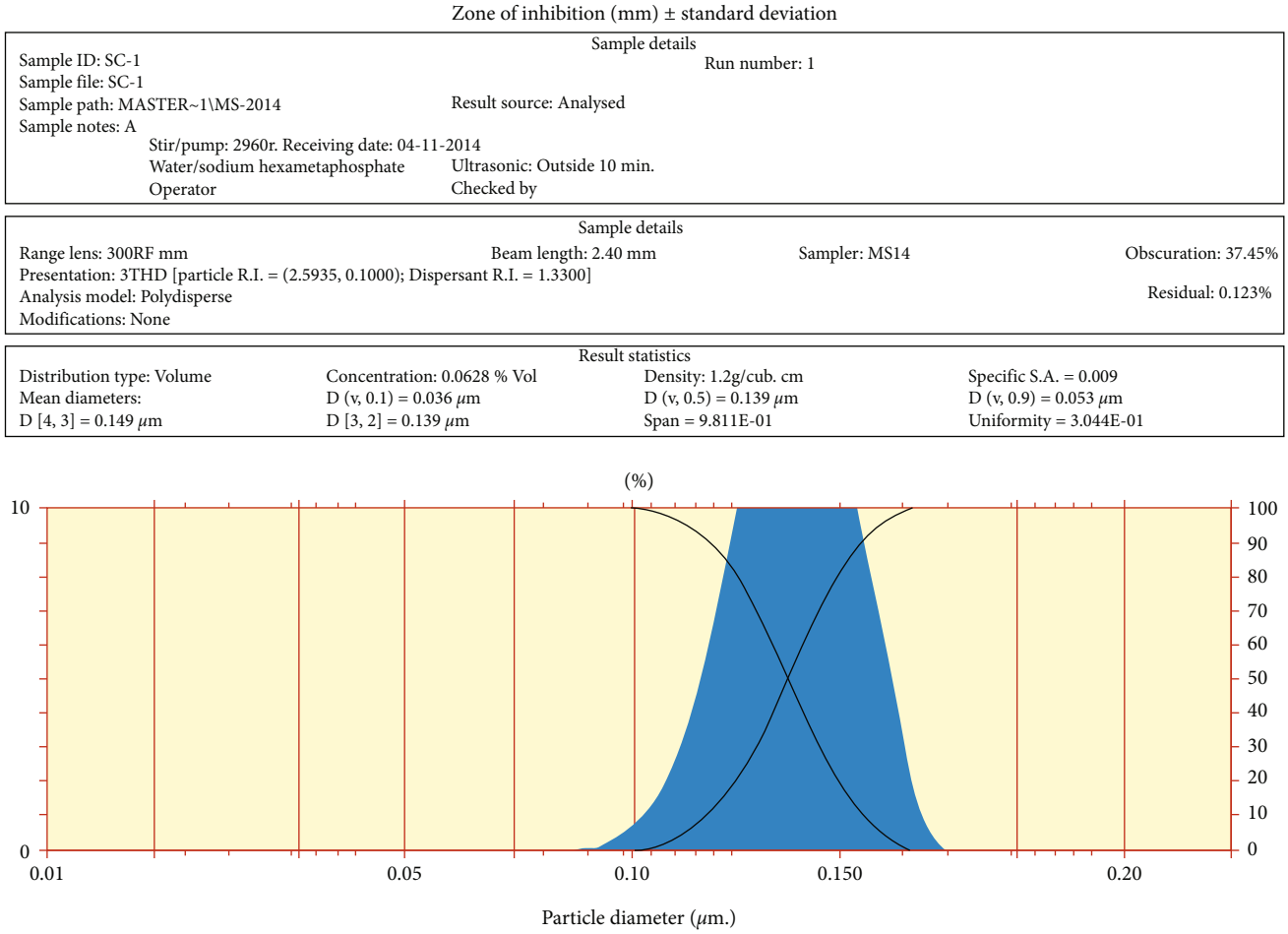

Figure 8: MSA pictogram of silver nanoparticle of *Ziziphus nummularia* fruit.

Table 1: The antibacterial activity of different extract of *Ziziphus nummularia* fruit and silver nanoparticle against Gram-positive bacteria.

	Aqueous	Methanol	Ethanol	Chloroform	Ethyl acetate	Ag nanoparticles
S. aureus (ATCC 6538)	11.66 ± 1.52	13.66 ± 1.52	13.33 ± 2.51	06.33 ± 0.57	06.66 ± 1.15	16.66 ± 1.52
S. pyogenes (ATCC 19615)	13.00 ± 2.00	16.13 ± 1.15	15.66 ± 2.08	10.66 ± 0.57	06.25 ± 0.57	20.00 ± 1.00
S. pneumonia (ATCC 6303)	06.33 ± 1.15	09.63 ± 1.15	08.66 ± 2.00	04.10 ± 0.57	04.33 ± 1.52	12.00 ± 1.00
S. faecalis (ATCC 9790)	12.54 ± 1.52	13.00 ± 2.64	10.38 ± 0.57	05.00 ± 1.00	05.66 ± 1.52	17.00 ± 2.00

Table 2: The antibacterial (Gram negative) activity of different extract of *Ziziphus nummularia* fruit and silver nanoparticle (mean ± SD).

Gram-negative bacteria	Zone of inhibition (mm) ± standard deviation					
	Aqueous	Methanol	Ethanol	Chloroform	Ethyl acetate	Ag Nanoparticles
E. coli (ATCC 35218)	06.24 ± 1.00	08.73 ± 0.57	07.39 ± 0.57	04.00 ± 1.00	03.66 ± 0.57	18.66 ± 1.15
P. aeruginosa (ATCC 125668)	06.51 ± 0.21	07.15 ± 0.57	06.54 ± 1.15	03.78 ± 1.00	02.99 ± 1.00	13.00 ± 1.00
P. mirabilis (ATCC 14153)	07.33 ± 1.52	07.07 ± 1.00	06.00 ± 1.00	03.71 ± 1.15	02.68 ± 0.57	14.00 ± 1.00
P. vulgaris (ATCC 6380)	06.87 ± 1.54	06.61 ± 0.57	07.08 ± 0.32	03.43 ± 0.57	03.19 ± 1.15	10.00 ± 1.00

Table 3: The antifungal activity of different extract of *Ziziphus nummularia* fruit and silver nanoparticle (mean ± SD).

Fungi	Zone of inhibition (mm) ± standard deviation					
	Aqueous	Methanol	Ethanol	Chloroform	Ethyl acetate	Ag nanoparticles
C. albicans (ATCC 60193)	13.00 ± 1.73	14.33 ± 1.53	12.12 ± 1.53	03.00 ± 1.73	08.74 ± 1.15	14.67 ± 1.53
C. neoformans (ATCC 14115)	11.31 ± 1.15	15.54 ± 2.08	13.00 ± 1.73	07.33 ± 0.58	03.67 ± 1.53	23.67 ± 1.53
A. niger (ATCC 6275)	05.60 ± 1.15	10.11 ± 2.08	11.41 ± 0.58	06.67 ± 0.58	03.57 ± 0.58	21.00 ± 1.73

Table 4: MIC and MBC of ethanolic and methanolic extracts of *Ziziphus nummularia* and silver nanoparticles (μg/mL).

Gram-positive bacteria	Ethanolic extract (μg/mL)		Methanolic extract (μg/mL)		Silver nanoparticles (μg/mL)	
	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus (ATCC 6538)	250	500	230	475	100	200
*S. pyogenes (*ATCC 19615)	200	500	210	425	100	150
S. pneumonia (ATCC 6303)	300	550	275	510	150	250
S. faecalis (ATCC 9790)	300	600	280	525	100	300

Table 5: MIC and MBC of ethanolic and methanolic extracts of *Ziziphus nummularia* and silver nanoparticles (μg/mL).

Gram-negative bacteria	Ethanolic extract (μg/mL)		Methanolic extract (μg/mL)		Silver nanoparticles (μg/mL)	
	MIC	MBC	MIC	MBC	MIC	MBC
E. coli (ATCC 35218)	300	600	310	630	200	375
P. aeruginosa (ATCC 6303)	250	500	280	540	200	350
P. mirabilis (ATCC 14153)	250	450	290	510	150	300
P. vulgaris(ATCC 6380)	300	500	280	490	250	400

Table 6: MIC and MFC of ethanolic and methanolic extracts of *Ziziphus nummularia* and silver nanoparticles (μg/mL).

Fungi	Ethanolic extract		Methanolic extract		Silver nanoparticles	
	MIC	MBC	MIC	MBC	MIC	MBC
C. albicans (ATCC 60193)	350	600	325	570	200	400
C. neoformans (ATCC 14115)	300	600	270	550	150	250
Aspergillus niger (ATCC 6275)	300	550	250	510	200	300

where D is the mean size, K is the constant (0.94) proportionality, λ is the wavelength (1.54060 Å) of X-ray, β is the excess line broadening, and θ is the Bragg angle.

$$B = B - b, \tag{2}$$

where B stands for line width (radian) and b is instrument line broadening (radian). The crystallite sizes were found to be 20-60 nm using the above formula. Geethalakshmi et al. [24] reported that *Trianthema decandra* extract mediated silver nanoparticles and was characterized by XRD at 2 theta, ranging from 10 to 80. According to the study, the average size of prepared silver nanoparticles was 15 nm, ranging from 10 to 50 nm. The diffraction pattern corresponds to no impurities present; this proves that pure silver nanoparticles were prepared. Similar XRD pattern reports were observed in the *Eclipta prostrate*, *Tribulus terrestris*, and *Prosopis juliflora* extracts for synthesized AgNPs [23].

3.5. Analysis of Silver Nanoparticles by FTIR. Fourier transform infrared spectroscopy of AgNps is shown in Figure 6. An infrared study was carried out to ascertain the nature and purity of the Ag nanoparticles. The infrared spectrum consists of two regions, i.e., fingerprint and functional group regions. The absorption band for organic compounds is observed in the functional group region. At the same time, metals normally show absorption spectra in the fingerprint region, resulting from the atomic vibration of the molecules. The peak detected at 3443.22 cm^{-1} show the O-H group,

Table 7: Correlations among antibacterial (Gram positive) activity of *Ziziphus nummularia* fruit extract in different solvents and in combination with silver nanoparticles. The symbols * and ** show the significance of a correlation.

	Aqueous	Methanol	Ethanol	Chloroform	Ethyl acetate	Silver nanoparticles
Aqueous	1.00					
Methanol	0.89**	1.00				
Ethanol	0.63	0.91**	1.00			
Chloroform	0.60	0.89**	0.95**	1.00		
Ethyl acetate	0.86**	0.86*	0.79	0.62	1.00	
Silver nanoparticles	0.94**	0.99**	0.83*	0.84*	0.83*	1.00

Table 8: Correlations among antibacterial (Gram negative) activity of *Ziziphus nummularia* fruit extract in different solvents and in combination with silver nanoparticles. The symbols * and ** show the significance of a correlation.

	Aqueous	Methanol	Ethanol	Chloroform	Ethyl acetate	Silver nanoparticles
Aqueous	1.00					
Methanol	-0.57	1.00				
Ethanol	-0.85*	0.50	1.00			
Chloroform	0.20	0.42	0.24	1.00		
Ethyl acetate	-0.15	0.00	0.64	0.63	1.00	
Silver nanoparticles	0.63	-0.39	-0.95**	-0.47	-0.85*	1.00

Table 9: Correlations among antifungal activity of *Ziziphus nummularia* fruit extract in different solvents and in combination with silver nanoparticles. The symbols "*" and "**" show the significance of a correlation.

	Aqueous	Methanol	Ethanol	Chloroform	Ethyl acetate	Silver nanoparticles
Aqueous	1					
Methanol	0.91**	1.00				
Ethanol	0.84*	0.99**	1.00			
Chloroform	-0.52	-0.12	0.03	1.00		
Ethyl acetate	0.64	0.26	0.11	-0.99**	1.00	
Silver nanoparticles	-0.39	0.03	0.18	0.99**	-0.96**	1.00

which result from the stretching and deformation of water molecules adsorbed to the surface of the metal. Similarly, the peaks were observed at 1646 cm^{-1}, 1458 cm^{-1}, 1140 cm^{-1}, 1056 cm^{-1}, and 7787.6 cm^{-1}, representing other functional groups present in the synthesized particle. FTIR stretching vibrations showed that the biomolecules such as alkaloids, flavonoids, and phenols in bark extract were responsible for reducing, capping, and stabilizing silver nanoparticles. A similar trend was also observed in the synthesis of AgNPs using *Artocarpus heterophyllus* Lam [25] and *Abelmoschus esculentus* [26, 27] seed extracts.

3.6. Analysis of Silver Nanoparticles by TGA. By increasing the temperature and employing the thermal gravimetric analysis, % weight loss of AgNPs was detected, as shown in Figure 7. The experimental conditions for gravimetric analysis were set at a temperature range of 200°C to 1600°C to determine the decomposition of the prepared silver nanoparticles. It was found that the decomposition started at 25°C. At the same time, the size of the particles was noted to have been decreased up to 92.60°C. The initial sample size was 8.416 mg which gradually decreased to 0.0123 mg due to extensive loss of moisture contents at a temperature of 92.60°C. Kasthuri et al. [28] analyzed the TGA of the phyllanthin extract loaded with gold and silver NPs when heated from 35 to 800°C. The initial weight loss observed at 150°C was attributed to the water molecules present. In our study, no further weight loss occurs beyond 92.60°C as proposed by Farhat et al. [16] for *Z. nummularia* fruit extract-mediated silver nanoparticles.

3.7. Analysis of Silver Nanoparticles by MSA. Master sizer is mainly used to measure the particle size in the nanometer range. The synthesized silver nanoparticles of *Z. nummularia* fruit extracts are shown in Figure 8. Further analysis of the synthesized particle confirmed their size range from 0.10 μm to 0.17 μm. On the mass medium diameter analysis, 50% of the particles were small in their size and diameter of 0.9 micrometres. Jeyanthi et al. [29] prepared *Dracaena mahatma* leaf extract-mediated silver nanoparticles and

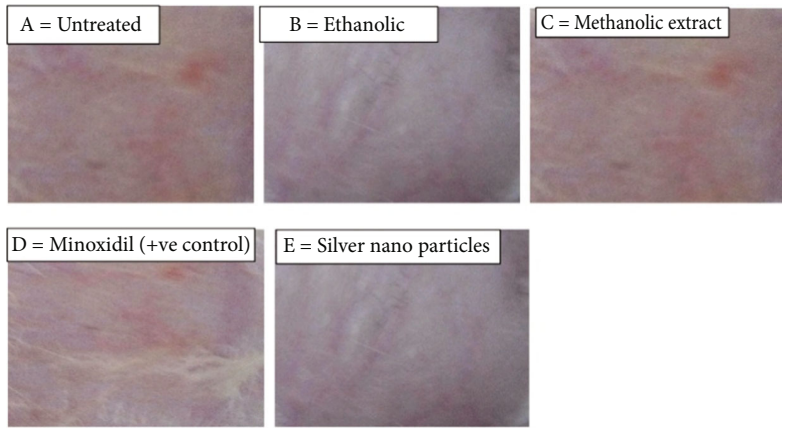

Figure 9: Shaved areas of rabbit skin.

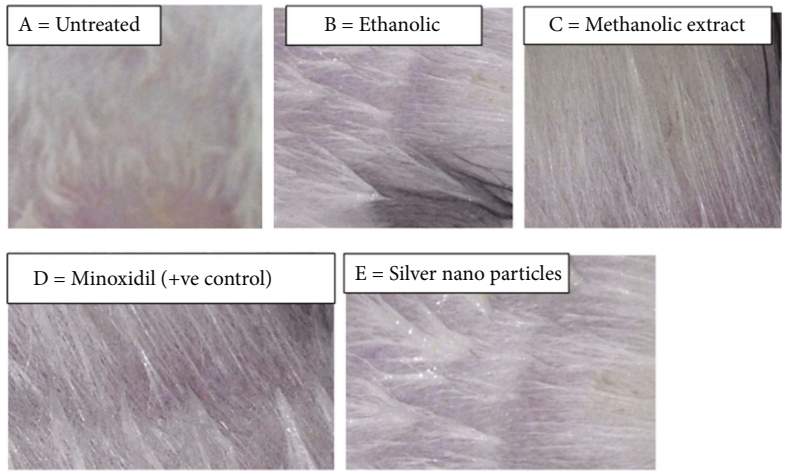

Figure 10: Air growth on the shaved area of rabbits in response to various extracts on the 7th day.

confirmed that average particle size obtained 0.87 micrometres through master size analyzer. In contrast, the average particle size of the synthesized nanoparticle 108 nm was reported by Tugçe et al. [30].

3.8. Antimicrobial Activities. Nowadays, multiresistant bacterial strains have been developed, resulting in increased morbidity and mortality. Due to the high cost and unwanted effects of commercially available antibiotics, searching low-cost and potentially active compounds is needed that can act as new antimicrobial agents. Therefore, new antibiotics from other sources, especially plant origin with known antimicrobial activity, are required [30–33]. Table 1 shows that the zone of inhibition 11.66 mm was produced against *S. aureus* (ATCC 6538) by aqueous extract followed by 13.66 mm and 13.33 mm by methanolic and ethanolic extracts, respectively. Chloroform and ethyl acetate extract was noted less effective, having 6.33 mm and 3.66 mm zone of inhibition, respectively.

In contrast, a silver nanoparticle of *Z. nummularia* fruit extract was noted highly effective with a 16.66 mm zone of inhibition. *Streptococcus pyogenes* (ATCC 19615) showed high resistance against ethyl acetate extract, having a 6.25 mm zone of inhibition; however, methanolic extract was found highly effective, having a 16.13 mm zone of inhibition against Streptococcus pyogenes followed by a 20.00 mm zone of inhibition produced by *Z. nummularia* fruit extract AgNPs. *Streptococcus pyogenes* (ATCC 19615) showed high resistance against chloroform and ethyl acetate extract with 4.10 mm and 4.33 mm zone of inhibition, respectively. The same bacteria showed less resistance against *Z. nummularia* fruit extract AgNps having a 12.00 mm zone of inhibition. In the case of *Streptococcus faecalis*, the methanolic extract was found effective, having a 13.00 mm zone of inhibition; however, the silver nanoparticle of *Z. nummularia* fruit extract was noted highly effective with a 17.00 mm zone of inhibition.

Table 2 reveals that ethyl acetate extract of *Z. nummularia* fruit extract was less effective with a 3.66 mm zone of inhibition against *E. coli*, followed by a 4.00 mm zone of inhibition produced by chloroform extract. AgNps of *Z. nummularia* fruit extract had the maximum zone of inhibition of 18.66 mm. *Pseudomonas aeruginosa* (ATCC 125668) was noted to be highly resistant to chloroform and ethyl acetate extract with 3.78 and 2.99 mm zone of inhibition. Aqueous and ethanolic extracts showed nearly the same activity of

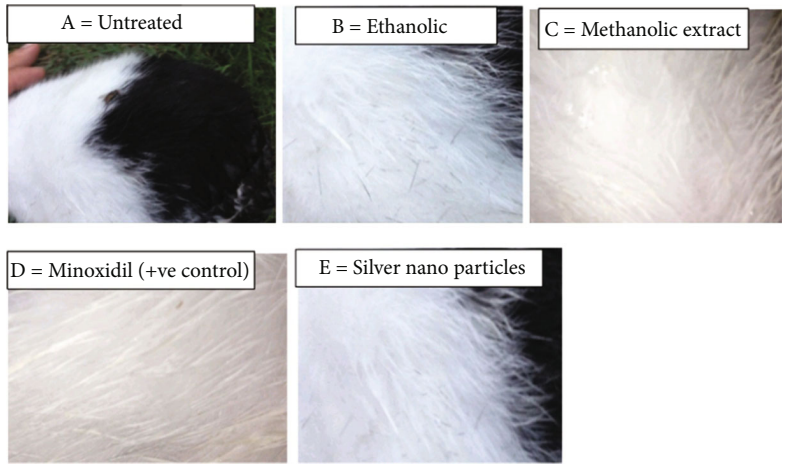

Figure 11: Investigation of hair growth on the shaved area of rabbits in response to various extracts on the 14th day.

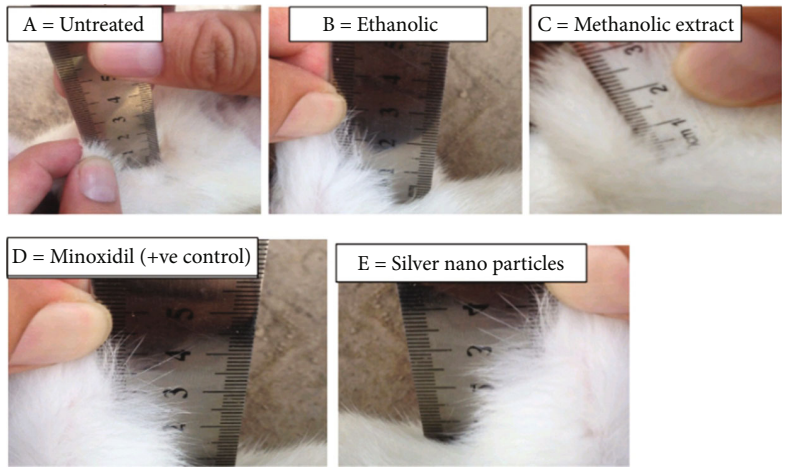

Figure 12: Analysis of hair growth in response to various extracts on the 21st day.

Table 10: Measurement of newly grown hairs of rabbits in response to different solvent extracts and *Ziziphus nummularia* fruit-mediated silver nanoparticles.

Time period	Length of hair measurement in centimeter (cm) Groups				
	Untreated	Ethanolic extract	Methanolic extract	Minoxidil (positive control)	Silver nanoparticles
Day 0	0	0	0	0	0
Day 7	0.6	0.7	0.7	0.9	0.9
Day 14	1.4	1.9	1.8	2.8	3.1
Day 21	2.4	3.3	3.5	4.5	4.8

6.51 and 6.54 mm zone of inhibition against the same bacteria. The prepared silver NPs were found highly effective with 13.00 mm zone of inhibition. The highest zone of inhibition, 14 mm, was recorded against *Proteus mirabilis* (ATCC 14153) by silver NPs, while the zone of inhibition of all the extracts was in the range of 2.68 to 7.33 mm. The least zone of inhibition, 3.19 mm of ethyl acetate, was recorded against *Proteus vulgaris*(ATCC 6380) whereas the highest zone of inhibition, 10.00 mm of silver NPs, was measured against *Proteus vulgaris* (ATCC 6380). Taking together, the *Z. nummularia* fruit-mediated silver nanoparticles were found highly effective against various types of tested bacteria and possessed maximum antibacterial activity. Previous studies conducted to examine plant-mediated silver nanoparticles [23] also report the maximum antimicrobial potential of silver nanoparticles.

Table 3 represents the antifungal activity of different extracts of *Z. nummularia* fruit and the silver NPs of *Z. nummularia* fruit extract. It shows that the methanolic extract and AgNPs were found highly effective against *Candida albicans* (ATCC 60193) having 14.33 and 14.67 mm zone of inhibition followed by 13.00, 12.12, 8.74, and

3.00 mm zone of inhibition of aqueous, ethanolic, ethyl acetate, and chloroform extracts, respectively. Methanolic and ethanolic extracts of *Z. nummularia* fruit were effective with 15.54 and 13.00 mm zone of inhibition against *Cryptococcus neoformans (*ATCC 14115). However, the highest zone of inhibition was 23.67 mm of AgNPs against the same fungi. In the case of *Aspergillus niger* (ATCC 6275), the methanolic and ethanolic extracts of *Z. nummularia* fruit produced a nearly same zone of inhibition (10.11 and 11.41 mm). In contrast, the highest zone of inhibition, 21.00 mm of AgNPs, was recorded against *Aspergillus niger* (ATCC 6275). The values of the zone of inhibition of other extracts of *Z. nummularia* fruit were in the range of 3.57 to 6.67 mm. Our results showed that silver nanoparticles were highly effective against various tested fungal species. Tugçe et al. [30] reported that silver nanoparticles possess an effective antifungal property against *C. albicans*, *C. kefyr*, and *A. niger*. The present study emphasizes using the medical plant to synthesize silver nanoparticles with an antifungal effect.

Table 4 shows the MIC and MBC values of ethanolic and methanolic extracts of *Z. nmmularia* fruit and silver nanoparticles of the respective plant fruit extract. According to this data, the MIC value 250 μg/mL of ethanolic extract was noted against *Staphylococcus aureus* (ATCC 6538), whereas the MBC value 500 μg/mL was recorded against the same bacteria. The methanolic extract was found more effective than ethanolic extract having 230 μg/mL and 475 μg/mL MIC and MBC values. However, the AgNPs were highly effective with 100 μg/mL MIC value and 200 μg/mL MBC value. The MIC and MBC values 200 μg/mL and 500 μg/mL of the ethanolic extract were recorded against *Streptococcus pyogenes* (ATCC 19615). The prepared silver nanoparticles were found highly effective than ethanolic and methanolic extracts having MIC value 100 μg/mL and MBC value 150 μg/mL. In the case of *Streptococcus pneumonia* (ATCC 6303) and *Streptococcus faecalis* (ATCC 9790), methanolic extract was more effective than ethanolic extract; however, both the bacteria showed less resistance against silver nanoparticles.

Table 5 reveals that silver nanoparticle was highly effective against *E. coli* (ATCC 35218) having 200 μg/mL and 400 μg/mL MIC and MBC values, respectively. *Pseudomonas aeruginosa* (ATCC 125668) showed more resistance against ethanolic and methanolic extracts and less resistance against the AgNPs with 200 μg/mL MIC and 250 μg/Ll MBC values. The MIC and MBC values of methanolic extract were noted higher than ethanolic extract; however, the prepared nanoparticle was found highly effective against *Proteus mirabilis* (ATCC 14153) than ethanolic and methanolic extracts. The ethanolic and methanolic extracts were less effective against *Proteus vulgaris* (ATCC 6380). In contrast, the prepared nanoparticle was more effective than ethanolic and methanolic extracts with 250 μg/mL MIC and 400 μg/mL MBC values.

The MIC and MFC values 350 μg/mL and 600 μg/mL of the ethanolic extract were measured against *Candida albicans*, whereas the MIC and MFC values of methanolic extracts were recorded 325 μg/mL and 570 μg/mL (Table 6). *Cryptococcus neoformans* (ATCC 14115) showed less resistance against the prepared AgNPs than ethanolic and methanolic extracts. The MIC and MFC values of ethanolic extract were measured higher than methanolic extract against *Aspergillus niger* (ATCC 6275); however, the prepared nanoparticle was more effective with 200 μg/mL and 300, respectively, against *Aspergillus niger* (ATCC 6275).

3.9. Correlation among Antimicrobial Activities of Solvents. Correlations calculated for antimicrobial activity of *Z. nummularia* fruit extract in different solvents (aqueous, methanol, ethanol, chloroform, and ethyl acetate) and an aqueous solution of silver nanoparticles were informative (Table 7). The antimicrobial activity of methanolic extract of *Z. nummularia* fruit demonstrated a significant positive correlation with the other solvents and the aqueous solution of silver nanoparticles. A mixture of silver nanoparticles and *Z. nummularia* fruit extract showed significantly positive correlations with the fruit extracts in different solvents such as aqueous, methanol, ethanol, chloroform, and ethyl acetate. The highest significant positive correlation ($R^2 = 0.94$) was found between methanolic extract and silver nanoparticles.

In combination with silver nanoparticles and an aqueous solution, fruit extracts demonstrated negative correlations (Table 8) with the antimicrobial activities of the *Z. nummularia* fruit extracts in the other solvents. *Z. nummularia* fruit extracts in ethanolic solution showed strong negative correlations with the fruit extract in aqueous solution ($R^2 = -0.85$) and the aqueous mixture of silver nanoparticles and the fruit extract ($R^2 = -0.95$). A highly significant positive correlation ($R^2 = 0.99$) was observed (Table 9) between chloroform and silver nanoparticles, while strong negative correlations were noted for ethyl acetate with chloroform ($R^2 = -0.99$) and silver nanoparticles.

3.10. Hair Growth Activity. Hair growth activity of minoxidil, methanolic extract, ethanolic of *Z. nummularia* fruit, and silver nanoparticle is shown in Figures 9–12. The photographs taken after one week indicated that the shaved area of rabbits massaged by silver nanoparticles gave good results compared to ethanolic and methanolic extracts. The length of newly grown hairs in the area massaged by minoxidil and silver nanoparticles was found maximum than that of ethanolic and methanolic extracts of *Z. nummularia* fruit (Figures 9–12). The hair growth activity of *Z. nummularia* leaf extract and its respective silver nanoparticles have also been studied by Kaya et al. [14]. All the results for hair growth activity were presented in photographs. As shown in Table 10, maximum hair growth was recorded on the 21st day in groups D and E, which were treated with minoxidil and silver nanoparticles, respectively. However, groups B and C treated with ethanolic and methanolic extracts were found less effective as full hair growth did not occur there. The hair growth values in untreated group A (negative control) were less than all treated groups. A similar study was conducted by Deepa et al. [31] using different groups of rabbits treated with Amla, methi, and Neem hair oil in different concentrations. It was reported that each hair oil (Amla, methi, and Neem) in 7.5% concentration was highly effective

than 3.5 and 4.5% concentration in combination to achieve the full hair growth.

4. Conclusions

Z. nummularia aqueous fruit extract-mediated silver nanoparticles were prepared and confirmed through UV-spectrophotometer and FTIR. The size of the particles was measured at 40 nm through SEM, whereas XRD confirmed the crystalline nature of the particles. The prepared nanoparticles were found stable from 90°C to 160°C when analyzed by DSC, and EDX confirmed the intense sharp peak of the sliver. In conclusion, the size silver nanoparticles of 40 nm were noted to be highly effective against both Gram-positive and Gram-negative bacteria and different tested species of fungi compared to other extracts of *Z. nummularia* fruit. Moreover, the prepared silver nanoparticles exhibited the highest hair growth activity, followed by minoxidil. However, the toxicological study of the prepared silver nanoparticles is highly recommended before their use on a commercial level. Furthermore, clinical trials are required to determine its therapeutic effect on various bacteria.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication.

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