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RESEARCH ARTICLE

A comparative Study on Synthesis, Characterization and Antibacterial Activity of Green vis-a-vis Chemically Synthesized Silver Nanoparticles

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Highlights

- AgNPs were prepared by green synthesis and chemical reduction method
- Aqueous extract from *Zingiber officinale* leaf was used for green synthesis of AgNPs
- $NaBH_4$ was used for the synthesis of AgNPs by chemical reduction method
- AgNPs were characterized in terms of XRD, FTIR and UV-Vis spectroscopy
- AgNPs synthesized from green synthesis method exhibited good antimicrobial activity

Abstract

Green synthesis of silver nanoparticles is of great importance in the field of nanoscience and nanotechnology. Various phytochemical constituents present in whole or parts of plants can act as reducing, capping and stabilizing agents in the synthesis of silver nanoparticle (AgNPs) by green method. In this work, AgNPs have been successfully synthesized in the laboratory by chemical reduction and green method. $NaBH_4$ was used as a reducing agent in chemical reduction method whereas Zingiber officinale leaf extract was used as reducing and capping agent in green method. The properties of as synthesized AgNPs by both green and chemical methods have been examined by UV- Vis spectroscopy, XRD and FTIR method. Besides these, biological activities of as synthesized AgNPs were tested against Bacillus subtilis and Escherichia coli which exhibited remarkable antimicrobial activities in green method. Based on the result, it is found that AgNPs synthesized from ginger plant (Zingiber officinale) leaf extract exhibited significant antimicrobial effect than that by chemically synthesized AgNPs.

Keywords: *Zingiber officinale, green synthesis, chemical synthesis, AgNPs, XRD, antimicrobial activity*

Introduction

Synthesis and characterization of nanoparticles (1-100 nm) is exponentially growing these days because of their effectiveness, versatile applications and potency of large scale production [1]. Because of high surface to volume ratio and quantum confinement, nanoparticles show unique properties as compared to its bulk mass which trail its applications in various sectors. Since decades, numerus researches are going on in the synthesis of nanoparticles of different metals, semimetals, alloys, polymers, ceramics and composites of either constituent targeting their specific applications [2–6]. Interest on the synthesis of silver nanoparticles is increasing because of its high application potential in the field of medicine and biomedical science including antimicrobial activities against bacteria, viruses and fungi [7], reduction in bacterial growth, in dental work and wound healing [8,9], wound dressing to treat ulcers [10]. Besides these, silver NPs are profoundly used in water filter materials [11] and textiles [12].

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Various methods have been developed for the preparation of silver nanoparticles among which chemical method (CM) and green method (GM) have been adopted in this study. CM is easy and fast process with yield of fine and uniform AgNPs [13]. However, probable toxicity of the products, investment in the chemical costs impart in the search of alternative method of synthesis. In order to address the limitations of chemical synthesis method of nanoparticle synthesis, green method is in forefront. Green method is cost effective, sustainable and eco-friendly [14].

In green method, aqueous extract of different parts of plants is used [15]. Such extracts are assumed to contain various phytochemicals like alkaloids, flavonoids, polyphenols, terpenoids, steroids etc. which are responsible for the reduction metal ions and stabilization of NPs [16]. Some of the literatures regarding the synthesis of silver nanoparticles by green method are listed in the table 1.

Table 1. Table showing the plant extract for the synthesis of AgNPs

S.N.	Name of Plant	Plant part	Characterization method	Size of AgNPs	Applications	Ref.
1.	*Scabiosa atropurpurea*	Fruits	XRD, TEM	40-50	Antioxidant Antimicrobial Cytotoxicity	[17]
2.	*Moringa oleifera*	Leaves	TEM	15-29	Antibacterial Antioxidant	[18]
3.	*Piper retrofractum*	Fruits	FESEM, EDX, TEM	1-5	Antibacterial	[19]
4.	*Zingiber officinale*	Stems	UV, XRD	10-20	Dye degradation	[20]
5.	*Coccinia indica*	Leaves	SEM, TEM	8-48	Antibacterial	[21]
6.	*Kalanchoe pinnata*	Leaves	UV, XRD	40	Dye degradation	[20]
7.	*Azadirachta indica*	Leaf	UV, XRD, TEM	2-14 nm	Band gap Antioxident Antimicrobial	[22]
8.	*Alpinia ofcinarum*	Rhizome	XRD, FETEM	20-80	Photocatalytic dye degradation	[23]
9.	*Shorea robusta*	Leaves	EDX, AFM, TEM	12-37	Dye degradation	[24]
10.	*Punica granatum*	Seeds	XRD, HRTEM	10-35	Photocatalytic dye degradation	[25]
11.	*Berberis asiatica*	Roots	UV, XRD	9	Antibacterial	[26]
12.	*Citrus limon*	Peels	UV, TEM	9-20	Antimicrobial	[27]
13.	*Azadirachta indica*	Leaves	UV, XRD, TEM	11-35	Dye degradation	[28]
14.	*Camellia japonica*	Leaves	UV, HRTEM	12-25	Electrocatalytic and photocatalytic dye degradation	[29]
15.	*Lantana camara*	Flowers	UV, XRD, TEM	53	Antioxidant Dye degradation	[30]

It has been reported that AgNPs can be synthesized using ginger rhizomes extract [31]. The polar group/ functional group in the phytochemicals of rhizomes is responsible for the reduction and stabilization of nanoparticles [32].

Ginger (*Zingiber officinale*) is a green herbaceous plant. It has yellowish-green flowers with purple lining and grass-shaped green leaves. It is hypothesized that the aqueous extract of *Zingiber offcinale* leaf contains various phytochemicals including alkaloids, tannins, flavonoids, terpenoids, and steroids[32]. Those phytochemicals will reduce aqueous silver ion to AgNps and stabilize them too. So, this study aims to synthesize AgNPs by above mentioned two methods and to compare the findings between them.

Materials and Methods

Materials

Materials and equipments used in this reserch work are as follows: Silver nitrate (Qualigens, AR grade), sodium borohydride (Lobachimie, AR grade), magnetic stirrer (Unilab, India), double beam Uv-visible spectrophotometer (LT2802), FTIR (Perkin Elmer 10.6.2), XRD (Rigaku Multiflex Diffractometer, Japan), Mueller Hinton Agar, Bacterial Cell line (ATCC6051, ATCC8739, ATCC2091).

Preparation of AgNPs by Chemical Reduction Method

50 mL of 0.1 N aqueous sodium borohydride was added dropwise to the 50 mL (0.1 N) aqueous silver nitrate solution with continuous stirring at room temperature. The color change of the reaction mixture was noted, and then the solution was centrifuged and the yield was collected. The collected nanoparticles were washed with distilled water and then by ethanol followed by drying in vacuum.

Preparation of Extract

Fresh leaves of Zingiber *officinale* were collected and washed with distilled water. The clean leaves were cut and crushed in mortar and pestle. The paste was then mixed with distilled water and stirred for an hour at laboratory temperature. The muslin cloth was used to filter the mixture and followed by filter paper. The filtrate was collected which was the desired aqueous extract.

Preparation of AgNPs by Green Synthetic Method

For the preparation of AgNPs, the extract to precursor ratio was initially optimized. For the optimization of reaction time, the optimized reaction mixture was continuously stirred at 60 °C for 0.5, 1, 1.5, and 2 hours. The color change of the solution symbolized the formation of silver nanoparticles and then it was centrifuged and collected. The collected sample was washed with distilled water followed by ethanol and dried in vacuum.

UV-Vis Spectroscopic Study

Synthesized AgNPs were characterized by ultraviolet visible (UV-Vis) spectrometric measurement for which double beam UV-Vis spectrometer (LT-2802) was used. Spectra of the samples were recorded over the range 200-700 nm at the resolution 5 nm.

Fourier Transformed Infrared Spectroscopic Study

AgNPs synthesized by chemical method as well as green synthesis method may be inherently doped with some sorts of group or capping agents in green synthesis method. Functional groups present in the organic compounds were assessed by FTIR measurement. FTIR spectrometric determination of extract as well as AgNPs were carried out using Perkin Elmer Spectrometer 10.6.2 version in the Department of Chemistry, Amrit Campus, Kathmandu, Nepal. The background correction was carried out using isopropanol. All the spectral data were recorded from 4000 - 450 cm^{-1} at the cutoff range with 4 cm^{-1} resolution.

X-ray Diffraction Study

Crystallite structure and the grain size of the AgNps were determined by using powder x-ray diffraction (PXRD) technique. Diffraction patterns of AgNPs synthesized by both methods were obtained using Rigaku Multiflex Diffractometer at Nepal Academy of Science and Technology (NAST), Lalitpur, Nepal. A monochromated X-ray of wavelength 1.5406 Å from radiation source CuKα was subjected to the sample at the ambient temperature over the 2θ (10-80°) region at the scan rate of 2°/min (40 kV, 20 mA). The crystallite domain size of AgNPs was calculated using Debye-Scherer's formula.

$$\text{Grain Size} = \frac{k\,\lambda}{\beta\,cos\,\theta} \quad (1)$$

Where, k is constant and its value is 0.94, β is the full width at half maximum in radius [β] = FWHM × π /180, **λ** is wavelength of x-ray whose value is 1.5406×10^{-10} m, and **θ** is Bragg's angle.

Antimicrobial Study

Kirby-Bauer Agar Diffusion method was used for testing of antimicrobial activity as per the protocol mentioned elsewhere [33]. In this method, the average diameter of zone of inhibition (ZOI) produced by AgNPs in the reference of positive control on the agar surface against particular pathogenic bacteria was measured for the estimation of the antimicrobial activity of the AgNPs.

Sterile Muller-Hinton Agar plates prepared for this experiment were dried for the removal of excess moisture from the surface of the media. Then, 5µL of the standard culture of bacteria was placed at the center of labeled plates. The agar surface was lightly touched with the help of a bent glass rod and the plate was turned to spread the culture over the agar surface. Then the plates were rotated at an angle of 60° for uniform distribution. The inoculated plates were covered with the lids and left to dry for some minutes.

The disc of filter paper of diameter 5 mm was placed on the surface of the media on the Petri plates and was labeled properly. Then, 10 mg of each sample of Ag NPs synthesized by chemical and green methods were loaded on the respective discs with the help of tweezers. The plates were covered with lids and were kept at room temperature for 30 minutes to allow proper diffusion of the nanoparticles into the media. The plates were then incubated overnight at 37 °C. After that, the zone of inhibition (ZOI) produced by the antibacterial activity of plant extract was observed and the diameter of the inhibition zone was measured by the use of a scale and was noted.

Result and Discussion

UV- visible Spectroscopic Study

The UV-Vis spectra of chemically synthesized AgNPs are shown in figure 1. The hump-shaped absorbance peak around 430 nm is due to the presence of AgNPs.

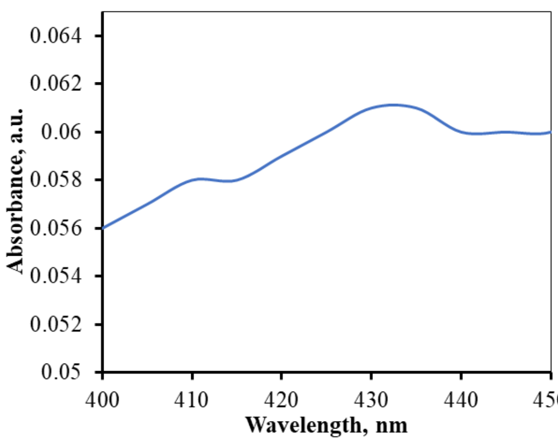

Fig 1. UV-Visible spectra of chemically synthesized AgNPs

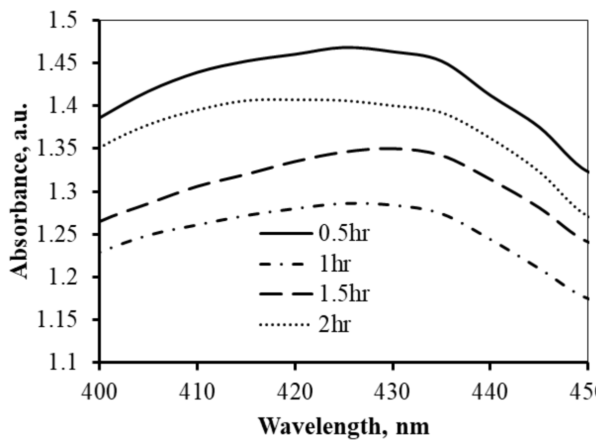

Fig 2. UV-Visible spectra of AgNPs synthesized by green method

The UV-Vis spectra of the AgNPs synthesized by the green method are shown in figure 2. The maximum absorbance peak due to the formation of AgNPs is around 430 nm. The broad and uniform peak is seen in the UV-Vis spectrum of the synthesized AgNPs by the green method. In green synthesis, the effect of the stirring time was also studied. The formation of AgNPs was observed from the very beginning. Formation of nanoparticles was enhanced on increasing the stirring time. It is observed that the absorbance peak due to the AgNPs at ½ h to 2 h remains almost similar indicating the complete reduction of precursor solution to nanoparticle within ½ h. The strong absorbance peak of AgNPs around 430 nm is also reported [34].

FTIR Spectroscopic Study

In this study, FTIR spectroscopic study was performed to assess the presence of any functional group onto AgNPs. The AgNPs synthesized by green synthesis method are likely to be capped with some phytochemical molecules onto its surface. Thus, FTIR spectra of such NPs give the absorbance of such adsorbed phytochemicals. The very weak intensity of absorbed peaks also supports this. The broad peak due to stretching vibration of O-H and N-H was appeared around 3300 cm^{-1} (Figure 3). Absorbance peak around 2900 cm^{-1} is due to the presence of aromatic C-H or alkene stretching vibrations. Similarly, the absorbance peaks at 1636, 1545, 1383, and 947 cm^{-1} are due to stretching and bending vibrations of C=O, C-N, C-O, and O-H, respectively [35–38]. The presence of such functional group in the silver nanoparticles could be attributed to the adsorbed phytochemical moieties in the process of green synthesis of nanoparticles. In the similar FTIR spectra of AgNPs synthesized by chemical reduction process, these absorbance peaks are absent. However, weak peaks at 1449 and 870 cm^{-1} are present due to stretching and bending of metal hydride bonds.

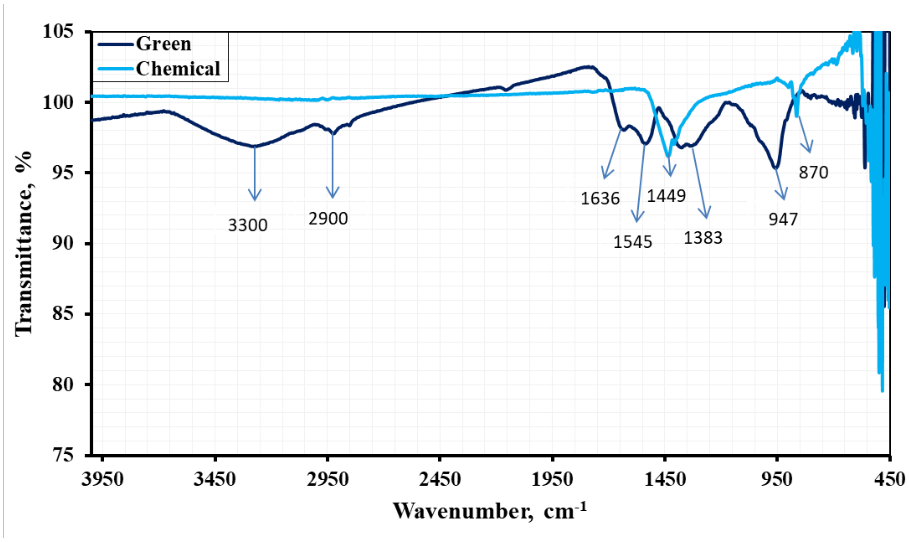

Fig 3. FTIR spectra of AgNPs synthesized by green synthesis method

X- ray Diffraction Study

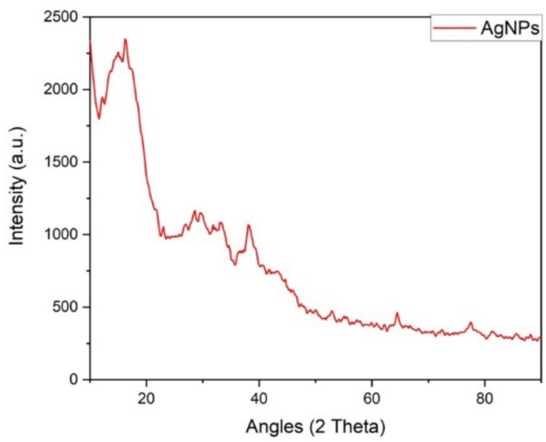

Fig 4 XRD of AgNP synthesized by ginger leaf extract

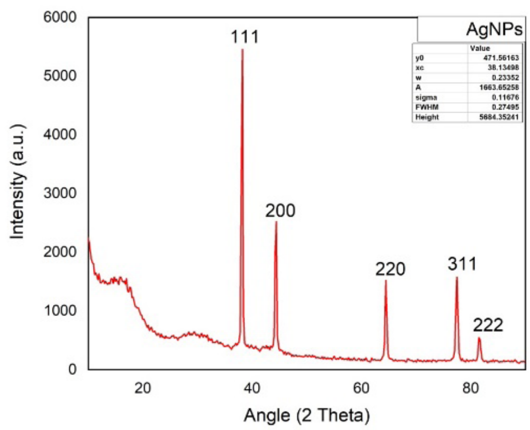

Fig 5. XRD of AgNP synthesized by chemical reduction

The XRD spectra of the AgNPs synthesized by green as well as chemical reduction method are shown in figures 4 and 5, respectively. The silver nanoparticles synthesized using green leaf extract exhibited less sharp peak compared to those prepared using sodium borohydride. These spectra reveal that the AgNPs synthesized by the green synthesis method may have contained several organic moieties as well as agglomerated form. As there are very low-intensity peaks in the spectrum indicating the formation of NPs. However, the intensity is very low, the size of the NPs cannot be calculated [32,39]. The XRD peaks at 38.04°, 64.36° and 77.22° can be ascribed to the 111, 220, and 311 crystalline structures of the face-centered cubic (fcc) crystal of as synthesized silver nanoparticles. The peaks showed the composition of nanoparticles as of silver. However, several other peaks present in the XRD patterns could be due to the residual phytochemicals present in the AgNPs.

But the XRD spectra of chemically synthesized AgNPs are very clear with high-intensity peaks. All observed peaks concerning 2θ values known for AgNPs act for the 111, 200, 220, 311, and 222 have angles of 38.24°, 44.24°, 64.48°, 77.70°, and 81.26 ° respectively (Fig. 5). Crystalline planes due to Bragg's reflections due to crystallite plane of AgNPs were undertaken to calculate the crystallite size using the Scherrer's formula, d spacing calculation

$$n\lambda = 2d \sin\theta \quad (2)$$

$$d_{hkl} \text{ (in Å)} = n\lambda / 2 \sin\theta \quad (3)$$

Where n=1 and λ for Cu source=1.54Å

The d_{hkl} spacing value (in nm) of chemically synthesized AgNPs is given here in Table 2. The average grain size of the chemically synthesized AgNPs calculated from Scherrer's formula is found to be 31.92 nm (Table 3). From the XRD-pattern of chemically synthesized AgNPs, it has been concluded that particles are present in a face-centered cubic system [40].

Table 2. d spacing value of synthesized Ag nano particles by chemical method

Plane	2θ(in degree)	θ(in degree)	d_{hkl} (in Å) = nλ / 2 sin θ n=1 and λ for Cu source=1.54Å
111	38.24	19.12	2.35
200	44.24	22.12	2.04
220	64.48	32.24	1.44
311	77.70	38.85	1.23
222	81.26	40.63	1.18

Table 3. Characterization parameters for chemically synthesized AgNPs as per XRD data

Plane	2θ (in Degree)	β (FWHM, in degree)	β=FWHM (rad)	D=0.9λ/βcosθ (in nm)
111	38.13498	0.27495	0.004795	31.92

Antibacterial Activity

Various literatures have reported the good antibacterial activities of the AgNPs synthesized from green route. The effectiveness of the antibacterial activity of the AgNPs may depend on the size of NPs as well as the route of the synthesis [3]. In this work, the antibacterial activities of AgNPs synthesized by chemical reduction method and green synthesis method were performed against two American Type Culture Collection (ATCC) bacteria using Agar well diffusion method and the results are tabulated in table 4.

Table 4. Inhibition zone shown by AgNPs synthesized by chemical method and green synthesis method

Test Organism (Bacteria)	Reference Culture	Type	ZOI (cm) exhibited by AgNPs synthesized by	
			Chemical Method	Green Method
Bacillus subtilis	ATCC 6051	Gram-positive	0.45	0.70
Escherichia coli	ATCC 8739	Gram-negative	0.55	0.60

In the table 4, silver nanoparticles exposed by the green synthesis method when spread on gram-positive bacteria *Bacillus subtilis* and gram-negative bacteria *Escherichia coli* incubated for 12 h obtained a zone of inhibition (ZOI) of 0.70 cm and 0.60 cm, respectively concerning standard used as Kanamycin with 1.05 cm as a zone of inhibition. Similarly, chemically synthesized nanoparticles when spread on the bacteria *Bacillus subtilis* and *Escherichia coli* incubated for 12 h obtained a zone of inhibition of 0.45 and 0.55 cm respectively. These results reflected that the nanoparticles synthesized through green route are more effective for both Gram positive and Gram negative bacterial control. This could be due the size of nanoparticles as well as the adsorbed phytochemicals on the NPs surface. The zone of inhibition shown by AgNPs synthesized by chemical method and green synthesis method are represented in the Figure 6 and 7.

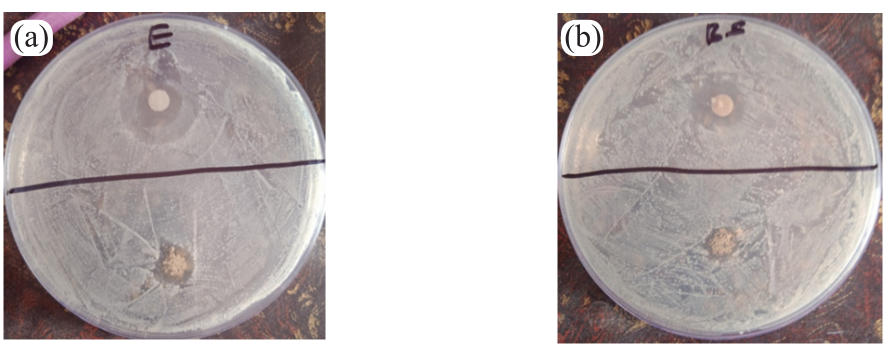

Fig 6. Antibacterial activity shown by chemically synthesized AgNPs on microorganisms; (a) Escherichia coli (gram-negative bacteria) and (b) Bacillus subtillis(gram-positive bacteria).

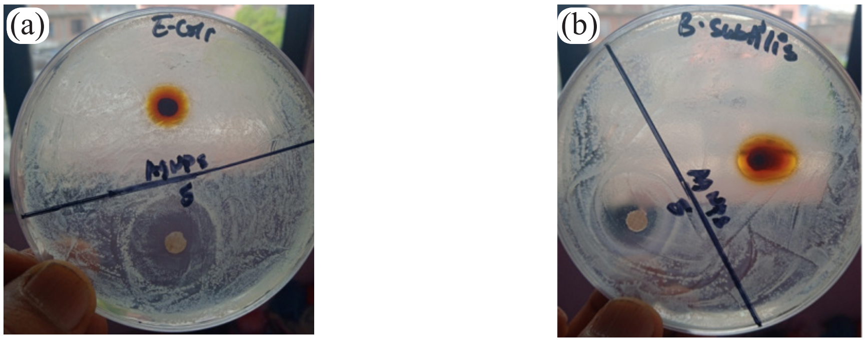

Fig7. Antibacterial activity shown by green synthesized AgNPs on microorganisms; (a) Escherichia coli (gram-negative bacteria), (b) Bacillus subtilis(gram-positive bacteria

Conclusions

The present study illustrates the synthesis of silver nanoparticles by green synthesis method using leaf extract of ginger (*Zingiber officinale)* and chemical reduction method using aqueous solution of sodium borohydride. A comparative analysis of the as-synthesized AgNPs was carried out in terms of both characterization and applications. Out of these two methods, the green synthesis method of silver nanoparticle synthesis was found simple, convenient and eco-friendly. Characterizations of the synthesized silver nanoparticles from ginger leaf and chemical reduction has been successfully done using- UV-Vis, FTIR, and XRD techniques. UV-Vis spectroscopy study evidenced the formation of silver nanoparticles. FTIR analysis shows the possible functional groups of the phytochemicals in the extract. Investigation of the microbial effect of the synthesized silver nanoparticles by green synthesis method exhibited better performance towards the antibacterial effect compared to those synthesized by chemical reduction method. All in all, green synthesis method of nanoparticles synthesis can be an alternative method for the synthesis of nanoparticles.

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