ORIGINAL ARTICLE

Biological fabrication and characterization of Silver nanoparticles using *Gymnemasylvestre* and its medical application

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Abstract

Background: The present study was aimed to green synthesis and characterization of silver nanoparticles (AgNPs) from *Gymnema sylvestre*, which was evaluated for their antimicrobial activity. **Materials and Methods:** The synthesized NPs were characterized by various analytical techniques such as ultraviolet–visible (UV–VIS) spectroscopy, Fourier transform-infrared (FT-IR), X-ray diffraction, dynamic light scattering (DLS), and transmission electron microscopy (TEM). It was confirmed through the UV–Vis spectrophotometer; corresponding peaks were identified at 424 nm. The green synthesized AgNPs were characterized by FT-IR studies to reveal the functional group attributed to the formation of AgNPs. Morphological size of AgNPs was 20 nm detect through characterization by DLS and TEM. **Results:** The green synthesized AgNPs showed vigorous antimicrobial activity against human pathogenic bacterial strains such as *Enterobacter cloacae, Staphylococcus, hemolytic Staphylococcus petrasii* subs. *pragensis Bacillus cereus,* and *Staphylococcus aureus.* **Conclusion:** These biosynthesized AgNPs were then used to demonstrate antimicrobial activity against a pathogen bacterium. The antibacterial activity of AgNP was clearly from the zone of inhibition. At concentrations (20 µg/ml–50µg/ml), the AgNP showed a clear zone of inhibition.

Key words: *Gymnema sylvestre,* antibacterial activity, silver nanoparticle

INTRODUCTION

Nanotechnology is the branch of science that deals with nanomaterials ranging about 1–100 nm nanoparticles (NP) play an important role in the field of science due to its size and shape. These particles were synthesized using metals such as silver, gold, zinc, and titanium based on their applications. The reduction of metal ions to NP can be achieved through various methods such as physical and chemical methods.[1] These methods were reported to be rapid, efficient, and effective. On the other hand, these methods were considered to be highly expensive and unsafe due to the usage of hazardous chemicals during synthesis.[2] To overcome this, biological method has been established for the synthesis of NP. Plants, algae, fungi, lichens, and bacteria were utilized for the bioconversion of metals to nanomaterials.[3] Bacteria mediated NP synthesis has been stated to be effective but considered to be undesirable due to the maintenance of the culture in aseptic laboratory conditions. Among various sources plants occupy a special place as reducing agent which is believed to be better capping agent.[4] This green synthesis is highly preferred than other conventional process since it supports affordable temperature, cost, and energy and does not involve the usage of any toxic compounds. Indian traditional plants with medicinal values are highly employed for synthesis of NP. *Gymnema sylvestre* belonging to family Apocynaceae was found to possess various biological roles.[5] Phytocompounds present in the plants remains as an essential factor for the biomedical applications such as antimicrobial

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activity, antioxidant, and anti-cancerous.[6] Apart from treating contagious infections, the plants extracts were also been reported to have beneficiary effects on non-contagious disease such as diabetics, cardiopathy, and constipation.[7] From ancient time, it has been reported that silver has several biotechnological and biomedicinal applications. Compared with silver ion, AgNP's were reported to be less reactive and a better alternative for chemically synthesized drugs especially for target-oriented drugs.[8]

MATERIALS AND METHODS

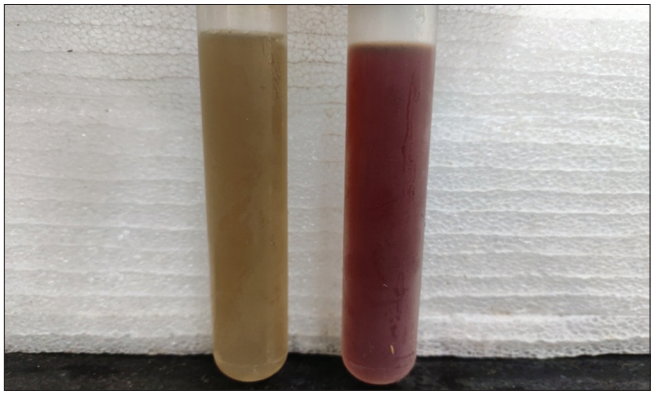
Silver nitrate (>99% pure) was purchased from Sigma-Aldrich, India. Nutrient broth, Muller Hinto Nagar, plate, was supplied by HiMedia, India. (Ciprofloxcin10 µL), The drug-resistant clinical strain *Enterobacter cloacae, Staphylococcus hemolytic, Staphylococcus petrasii* subs*. pragensis, Bacillus cereus,* and *Staphylococcus aureus,* which were acquired from Microbial Type culture, Chandigarh.

Collection of Sample and Preparation of Leaf Extract

Leaves of *G. sylvestre* were collected from Acharya N G Ranga Agricultural University, Tirupati, Andhra Pradesh, India. Fresh and healthy leaves weighing 5 g were cut into fine pieces and washed with distilled water and boiled with 100 mL of double distilled water for 15 min at 70°C. Boiled mixture was filtered using Whatman No. 1 filter paper. The filtrate was stored in a cool and dry place.[9]

Synthesis of AgNP Using Plant Extracts

Aqueous solution of 1 mM $AgNO_3$ was prepared and used for the synthesis of AgNPs. 10 mL of aqueous leaf extract was added to 5 mL of 1 mM $AgNO_3$ solution in a 50-mL flask and observed at sun light condition for about 15 min the solution turned dark brown in color. The synthesis of AgNPs was primarily detected by observing the solution for color change from yellow to dark brown.[10]



Synthesis AgNP

Characterization of GS-AgNPs

In this study, the color changing ability reaction combination was well-thought-out as the pilot event to detect the NP synthesis. Fourier transform-infrared (FT-IR) spectrum analysis was used by FT-IR-ALPHA interferometer instrument and the scanning range between 500 and 4000 cm^{-1}. Horiba NPs analyzer was employed to regulate the exact average size and Zeta potential values of the synthesized NP. The average size of bio fabricated AgNP-GS was determined by X-Ray diffraction (XRD)-6000-Shimadzu Analytical, India. The size, shape, agglomeration outline, and scattering nature of NP were elucidated by Transmission Electron Microscope (TEM TEM- FEIQuanta, 200 FEGHRSEM equipped).

Antibacterial Activity of AgNP

Pathogenic bacterial strain was used to evaluate the antibacterial activities. The disc diffusion assay was executed to screen the antibacterial activity of AgNP. Muller Hinton agar was prepared and 50 µL fresh bacterial cultures were spread on the

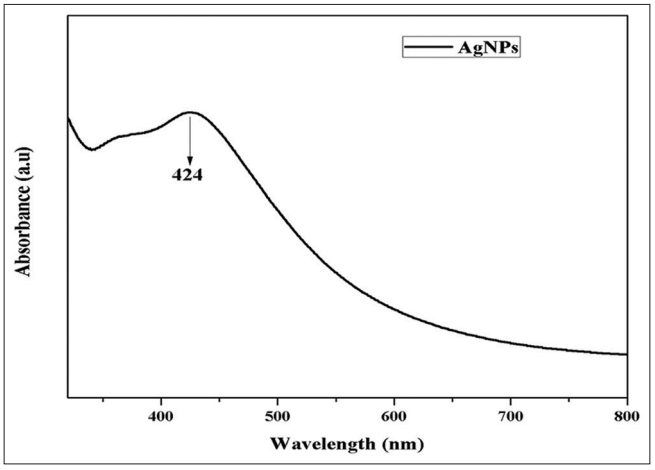


Figure 1: Ultraviolet–visible spectra of synthesized silver nanoparticles (AgNPs) from *Gymnema sylvestre*. The strong absorption spectrum of AgNPs exhibited at 424 nm

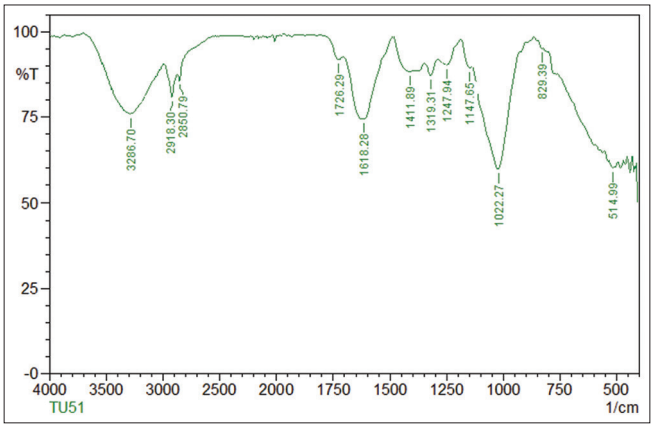


Figure 2: Fourier transform-infrared analysis of leaf extract of *Gymnema sylvestre*

agar plate. The plates were incubated at 37°C for 24 h and the zone of inhibition was measured in terms of the millimeter.[11]

RESULTS AND DISCUSSION

Characterization of AgNPs NP Using *Gymnema sylvestre* leaf extract

The biological synthesis of AgNPs from *G. sylvestre* was initially conformed by the color change of the reaction mixture from colorless to brown color indicated the synthesis of AgNPs preliminarily. Then, the synthesized NP were exhibits as strongest Ultraviolet (UV) absorbance peak at 424 nm [Figure 1].

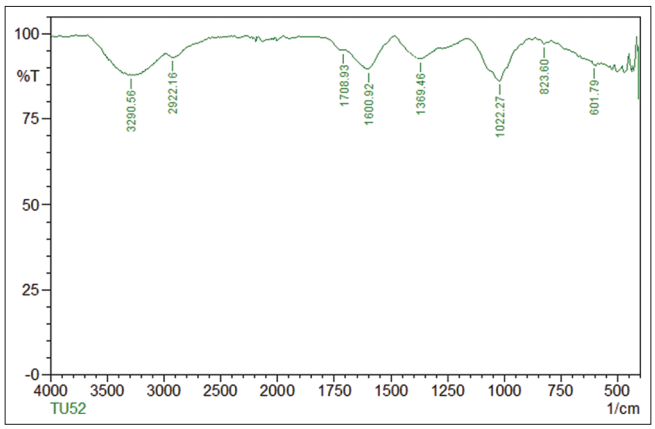


Figure 3: Fourier transform-infrared analysis of silver nanoparticles synthesized using the leaf extract of *Gymnema sylvestre*

Moreover, FTIR analysis was carried out to determine the possible biomolecules in leaf extract of A. *G. sylvestre* that was responsible for capping that lead to the efficient stabilization of the AgNPs. The bands observed at 3286, 2918, 1726, 1618, and 1022 cm^{-1} on the FTIR spectrum of *G. sylvestre* extract, as shown in Figure 2 and bands at 3286, 2918, 1726, 1618, and 1022 cm^{-1} on the FTIR spectrum of the synthesized AgNPs from *G. sylvestre* [Figure 3]. The sharp bands at wavelength 3286 cm^{-1} corresponding to –N–H, –C–N, and –C=C– functional groups. These functional groups are also present in the studies. The variation in the absorption bands observed on both spectra of the FTIR might be responsible for the reduction of silver ions, formation, and stabilization of the synthesized NP from A. *G. sylvestre* leaves. The vibrational bands corresponding to bonds such as–N–H, –C=C–, and –C–N had being reported to be peculiar with flavonoids, terpenoids, and protein compound. This could signify that the extract has flavonoids, terpenoids, and protein base compounds.[12]

Moreover, the particle size of the synthesized NP was analyzed by Zeta potential and particle size analysis. The particle size and Zeta potential analysis revealed that 25.4 nm average size [Figure 4] and −9.9 mv Zeta potential value[13]

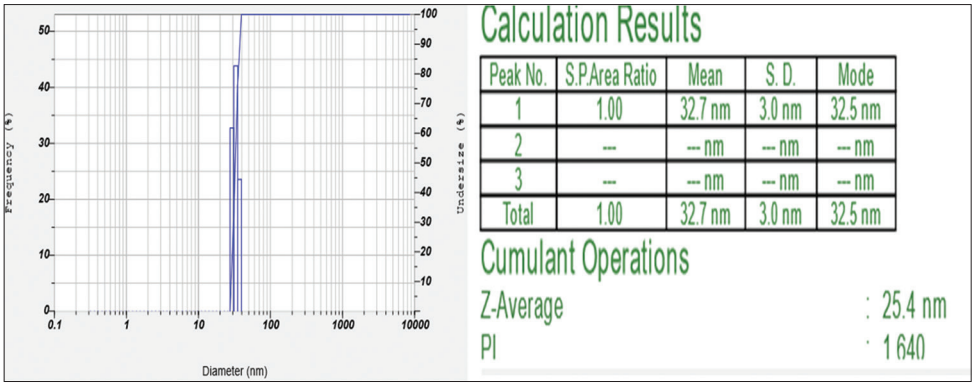


Figure 4: Particle size analysis of silver nanoparticles synthesized using the leaf extract of *Gymnema sylvestre*

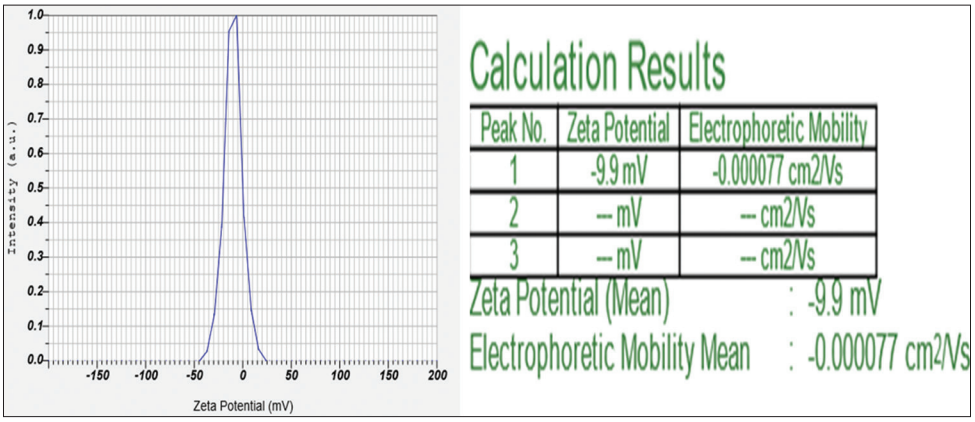


Figure 5: Zeta potential analysis of silver nanoparticles synthesized using the leaf extract of *Gymnema sylvestre*

[Figure 5] of the synthesized NP. The synthesis of AgNPs using the leaf extract of *G. sylvestre*. From the results, it can be concluded that the synthesized NP are stable.[14]

The crystal nature-based structure is often crucial conformation of AgNP which are determined by XRD [Figure 6]. The XRD patterns of Ag nanocomposite material face-centered cubic which indicates the well-indexed XRD peaks corresponding to the planes (111), (200), (220), (311), and (222). These results indicate that the products consisted of pure phases. Furthermore, the diffraction peaks were more intensive and narrower, implying a good crystalline nature of Ag nanocomposite products.[15]

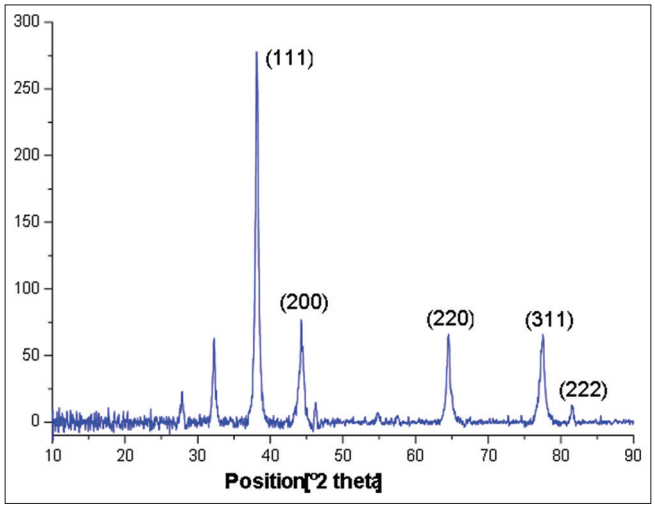


Figure 6: X-ray diffraction analysis of silver nanoparticles synthesized using the leaf extract of *Gymnema sylvestre*

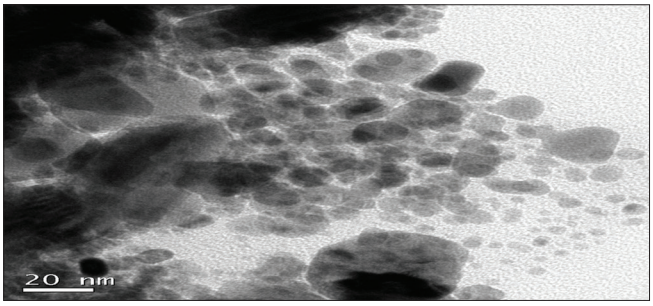


Figure 7: Transmission electron microscopy analysis of silver nanoparticles synthesized using the leaf extract of *Gymnema sylvestre*

As shown in Figure 7, demonstrates the surface and shape with size morphology of Ag were characterized from the microscopical studies of TEM. This study evident that AgNPs were spherical was poly-dispersed. The measured average size was 20 nm, occasional cluster of the AgNPs has been observed. These characterization studies are all scientifically evident that presents in NP are AgNPs.[16]

Antibacterial Activity of AgNP-GS

Antibacterial activity was performed by disc diffusion method as reported in with some alterations. The GS-AgNPs were inspect for antibacterial activity using four varied pathogenic bacteria such as *E. cloacae,* (MG763134) *S. hemolytic* (MG744417). *S. petrasii* subs. *pragensis* (MG970131), *B. cereus* (MH393374), and *S. aureus,* (MH431700) which were acquired from Microbial Type culture, Chandigarh. On the Muller Hinton agar plate, overnight grown subculture was spread and GS-AgNPs were disk diffusion different concentrations (20, 30, and 40.50 µg) and then the plates were incubated at room temperature, followed by zone of inhibition studies to estimate the antibacterial activity of GS-AgNPs which was additionally compared with standard control.[17] Similar results have been documented earlier where MICs of *Gymnema sylvestre*-AgNPs against some bacterial strains were 32 µg/ml, while for ESβL-producing *E. coli*, it ranged from 32 to 64 µg/ml [Figure 8].[18]

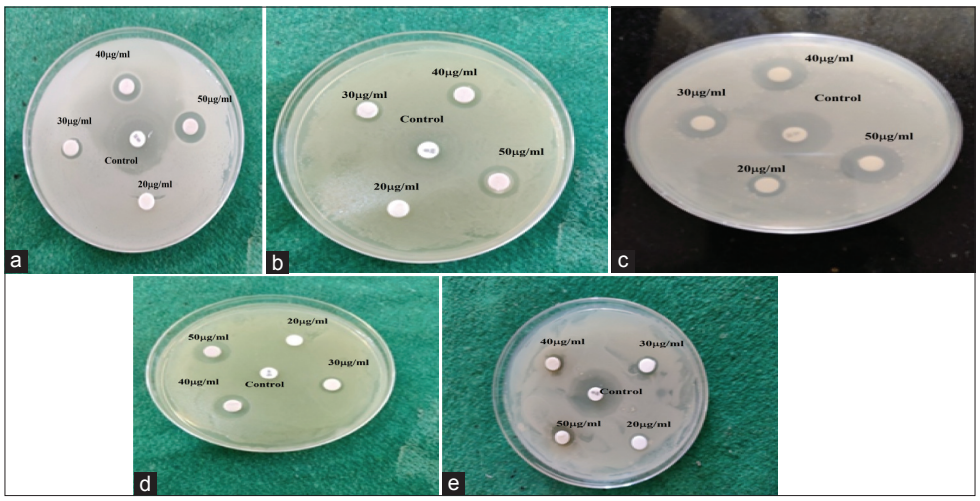


Figure 8: Antibacterial activity Ag nanoparticles using *Gymnemasylvestre* in different concentration (a) *Enterobacter cloacae*, (b) *Hemolytic staphylococcus*, (c) *Staphylococcus petrasii* subs. *pragensis*, (d) *Bacillus cereus*, (e) *Staphylococcus aureus*

		Zone of inhibition in mm				
S. no.	**Test organism**	**50 µg/ml**	**40 µg/ml**	**30 µg/ml**	**20 µg/ml**	**Control**
1	*Enterobacter cloacae*	13 mm	12 mm	8 mm	-	16 mm
2	*Staphylococcus hemolytic*	13 mm	12 mm	8 mm	-	
3	*Staphylococcus petrasii* subs. *pragensis*	15 mm	13 mm	10 mm	8 mm	18 mm
4	*Bacillus cereus*	12 mm	11 mm	-	-	15 mm
5	*Staphylococcus aureus*	12 mm	11 mm	-	-	19 mm

CONCLUSION

We described a work in simple and green synthesis of AgNP using the aqueous extract of *G. sylvestre*. The formation of AgNP was confirmed by UV-visible spectroscopy. The TEM images showed that the particles were mostly spherical. These biosynthesized AgNPs were then used to demonstrate antimicrobial activity against a pathogen bacterium. The antibacterial activity of AgNP was clearly from the zone of inhibition. At concentrations (20 µg/ml to 50 µg/ml), the AgNP showed a clear zone of inhibition.

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