Synthesis of *Gymnema Sylvestre* Based Silver Nanoparticle and its Antimicrobial and Antioxidant Activity Evalaution

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Abstract:

Background: The environmentally sustainable production of metallic nanoparticles through medicinal plants has attracted significant interest due to its eco-friendliness, cost efficiency, and biocompatibility. *Gymnema sylvestre,* a therapeutic herb abundant in flavonoids, phenolics, and saponins, exhibits many pharmacological attributes and acts as an effective reducing and stabilizing agent in the production of silver nanoparticles (AgNPs). The current research sought to produce silver nanoparticles mediated by *Gymnema sylvestre* and assess their physicochemical properties, antibacterial efficacy, and antioxidant capabilities.

Methods: Silver nanoparticles were produced by the reduction of silver nitrate solution utilizing the aqueous leaf extract of *Gymnema sylvestre*. The produced nanoparticles were analyzed using UV–Visible spectroscopy, Fourier-transform infrared spectroscopy, X-ray diffraction, dynamic light scattering, zeta potential measurement, scanning electron microscopy, and energy-dispersive X-ray spectroscopy. The antimicrobial efficacy was assessed against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa employing the agar well diffusion and minimum inhibitory concentration techniques. Antioxidant efficacy was assessed using DPPH and ABTS radical scavenging methods.

Results: UV-Visible spectroscopy revealed a distinct surface plasmon resonance peak at 432 nm, thereby validating the synthesis of silver nanoparticles. The produced AgNPs demonstrated an average particle size of 41.8 ± 3.2 nm, a polydispersity index of 0.231 ± 0.02, and a zeta potential of −31.6 ± 2.4 mV, suggesting satisfactory colloidal stability. XRD analysis validated the crystalline structure of face-centered cubic silver, whereas SEM imagery demonstrated primarily spherical nanoparticles exhibiting consistent morphology. The AgNPs exhibited considerable antibacterial efficacy, with inhibition zones measuring from 17.8 ± 0.8 to 25.4 ± 1.1 mm, and MIC values spanning 31.25 to 125 µg/mL. In antioxidant research, the produced AgNPs demonstrated a concentration-dependent ability to scavenge free radicals, with IC_{50} values of 38.6 ± 1.7 µg/mL (DPPH) and 42.3 ± 2.1 µg/mL (ABTS), surpassing the efficacy of the crude plant extract ($p < 0.05$).

Conclusion: Silver nanoparticles mediated by Gymnema sylvestre were effectively synthesized through a straightforward eco-friendly method and demonstrated favorable physicochemical properties, significant antimicrobial efficacy against both Gram-positive and Gram-negative bacteria, as well as impressive antioxidant

capabilities. These results indicate that the produced AgNPs may function as effective natural nanotherapeutic agents for antibacterial and antioxidant purposes, necessitating additional research for biomedical and pharmaceutical applications.
Keywords: *Gymnema sylvestre*; silver nanoparticles; green synthesis; antimicrobial activity; antioxidant activity; characterization; nanotechnology

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Introduction:

The swift rise of antimicrobial resistance (AMR) has transformed into a significant worldwide public health issue, diminishing the efficacy of traditional antibiotics and escalating the prevalence of challenging diseases [1]. The prevalent abuse of antimicrobial substances, together with microorganisms' capacity to evolve various resistance strategies, has heightened the quest for alternate treatment methods. Nanotechnology has surfaced as a viable approach to address microbial resistance, attributed to the distinctive physicochemical characteristics of nanoparticles, such as their elevated surface area-to-volume ratio, improved cellular contact, and extensive antibacterial efficacy [2, 3].

Silver nanoparticles (AgNPs) have garnered significant scientific attention among different metallic nanoparticles due to their strong antibacterial, antioxidant, anti-inflammatory, and wound-healing capabilities. Silver nanoparticles demonstrate their antimicrobial properties via various mechanisms, such as compromising the bacterial cell membrane, producing reactive oxygen species (ROS), obstructing DNA replication, and inhibiting crucial cellular enzymes [4]. In contrast to traditional antibiotics, these diverse mechanisms of action diminish the probability of microbial resistance, rendering AgNPs appealing options for pharmaceutical and biological uses [5].

Traditional techniques for producing silver nanoparticles frequently utilize toxic substances, require substantial energy, and operate under ecologically detrimental conditions. Conversely, the utilization of plant extracts for green synthesis has garnered considerable interest as an environmentally benign, cost-effective, and sustainable option. Phytochemicals sourced from plants, including flavonoids, phenolic acids, terpenoids, alkaloids, glycosides, and proteins, serve as natural reducing and stabilizing agents, promoting nanoparticle synthesis while reducing environmental toxicity. Furthermore, nanoparticles influenced by phytochemicals frequently demonstrate improved biological efficacy owing to the synergistic interaction of bioactive plant compounds on their surfaces [5, 6].

Gymnema sylvestre (Retz.) R.Br. ex Sm., a member of the Apocynaceae family, is a significant medicinal herb prevalent in India, Southeast Asia, and tropical areas. Historically referred to as "Gurmar" or the "sugar annihilator," this plant has been widely utilized in Ayurvedic medicine for the treatment of diabetes mellitus. Besides its recognized antidiabetic effects, G. sylvestre has antibacterial, antioxidant, anti-inflammatory, hepatoprotective, immunomodulatory, and anticancer characteristics. The pharmacological effects are mainly ascribed to its abundant phytochemical constituents, such as gymnemic acids, gymnemagenin, flavonoids, saponins, tannins, and phenolic compounds, which also function as effective reducing and capping agents in nanoparticle synthesis [7, 8].

Numerous studies have shown the effective green synthesis of silver nanoparticles through medicinal plant extracts; nonetheless, systematic research on Gymnema sylvestre-mediated AgNPs, including thorough physicochemical characterization and concurrent assessment of antimicrobial and antioxidant properties, is scarce. Comprehending the correlation between nanoparticle attributes and their biological efficacy is crucial for the advancement of safe and effective nanotherapeutic agents [9].

Consequently, this research was conducted to produce silver nanoparticles utilizing the aqueous leaf extract of Gymnema sylvestre via an eco-friendly synthesis method. The produced nanoparticles were analyzed using UV–Visible spectroscopy, FTIR, XRD, dynamic light scattering (DLS), zeta potential assessment, SEM, and EDX. Additionally, their antibacterial efficacy against certain Gram-positive and Gram-negative bacterial strains, as well as their antioxidant capacity assessed using DPPH and ABTS radical scavenging assays, were analyzed. The results of this research could offer significant understanding for the creation of eco-friendly silver nanoparticle systems with prospective pharmacological and biological uses [10, 11].

Material and Methods:

Materials:

Fresh leaves of *Gymnema sylvestre* were collected from a local medicinal plant garden and authenticated by a qualified taxonomist. Silver nitrate ($AgNO_3$, ≥99.9% purity) was procured from Sigma-Aldrich (USA). Mueller–Hinton agar (MHA), Mueller–Hinton broth (MHB), nutrient agar, nutrient broth, and analytical-grade chemicals including methanol, ethanol, hydrochloric acid, sodium hydroxide, potassium bromide, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)), potassium persulfate, and ascorbic acid were obtained from HiMedia Laboratories (India). All reagents used in the study were of analytical grade, and double-distilled water was used throughout the experiments.

Methods:

Preparation of *Gymnema sylvestre* Leaf Extract:

Gymnema sylvestre leaves were cleaned by first immersing them in distilled water and then running them under running water to get rid of any dust or other contaminants. After being let to dry in the shade for 10–14 days at room temperature, the leaves were ground into a powder using a mechanical grinder. Twenty minutes at 70 degrees Celsius were spent boiling about twenty grams of powdered leaves in two hundred milliliters of double-distilled water. Before being stored at 4°C until later use, the extract was cooled and filtered using Whatman No. 1 filter paper [12].

Green Synthesis of Silver Nanoparticles:

The process of creating silver nanoparticles involved combining a 1:9 (v/v) ratio of water-based leaf extract with a 1 mM solution of silver nitrate ($AgNO_t$). For four hours in complete darkness, the reaction mixture was whisked constantly. Surface plasmon resonance caused a discernible shift in hue from light yellow to dark brown, indicating the formation of silver nanoparticles. Centrifugation was used at 15,000 rpm for 20 minutes to collect the generated nanoparticles. They were then washed three times with distilled water to eliminate any unreacted components. Finally, they were dried at 50°C before being characterized [13].

UV–Visible Spectroscopic Analysis:

The establishment of silver nanoparticles was corroborated utilizing a UV–Visible spectrophotometer by surveying the reaction mixture within the wavelength interval of 300–700 nm. The unique surface plasmon resonance peak of silver nanoparticles was observed [14].

Particle Size, Polydispersity Index, and Zeta Potential:

Zetasizer Nano ZS (Malvern Instruments, UK) dynamic light scattering (DLS) was used to measure the zeta potential, average particle size, and polydispersity index (PDI) of the produced silver nanoparticles. Prior to analysis, samples were diluted using double-distilled water, and each measurement was taken three times at a temperature of 25 ± 1°C [15].

Fourier Transform Infrared (FTIR) Analysis:

FTIR spectroscopy was conducted to ascertain the functional groups that facilitate the reduction and stabilization of silver nanoparticles. The spectra of the botanical extract and manufactured AgNPs were obtained within the range of 4000–400 cm^{-1} with the potassium bromide pellet technique [16].

X-Ray Diffraction (XRD) Analysis:

The crystalline configuration of the produced silver nanoparticles was examined utilizing an X-ray diffractometer with Cu-Kα radiation (λ = 1.5406 Å). Diffraction patterns were captured at a scanning interval of 20°–80° (2θ) [17].

Scanning Electron Microscopy (SEM) and Energy-Dispersive X-Ray (EDX) Analysis:

Scanning electron microscopy was used to analyze the made silver nanoparticles' surface morphology. Utilizing energy-dispersive X-ray (EDX) spectroscopy in conjunction with the scanning electron microscope apparatus, the purity and elemental makeup of the nanoparticles were ascertained [18].

Antimicrobial Activity:

Staphylococcus aureus (ATCC 25923), Bacillus subtilis (ATCC 6633), Escherichia coli (ATCC 25922), and Pseudomonas aeruginosa (ATCC 27853) were used in the agar well diffusion method to assess the antibacterial activity of the silver nanoparticles that were manufactured. On Mueller-Hinton agar plates, bacterial samples were distributed after being adjusted to 0.5 McFarland standard. The plates were incubated at 37°C for 24 hours after being filled with wells measuring 6 mm and varying amounts of AgNP suspension. The inhibition zones' diameters were measured in millimeters. Using the broth microdilution method in accordance with CLSI recommendations, the minimum inhibitory concentration (MIC) was ascertained [19].

Antioxidant Activity:

Through the use of DPPH and ABTS radical scavenging assays, the antioxidant activity of the

produced silver nanoparticles was assessed. Under dark conditions, different quantities of AgNPs (10-100 µg/mL) were incubated with their corresponding radical solutions. We used a UV-Visible spectrophotometer to measure the absorbance, and then we determined the IC_{50} values and percentage radical scavenging activity. The reference standard was ascorbic acid [20].

Statistical Analysis:

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using GraphPad Prism Version 10.0. Comparisons among groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A p-value < 0.05 was considered statistically significant.

Results:

Visual Observation and Green Synthesis of Silver Nanoparticles:

Silver ions were reduced to silver nanoparticles using the water-based Gymnema sylvestre leaf extract. Incubation with a 1 mM silver nitrate solution caused a gradual shift in the reaction mixture's color from pale yellow to dark brown. This change was caused by the creation of silver nanoparticles by surface plasmon resonance. The control solution, which only included silver nitrate, did not exhibit any color change.

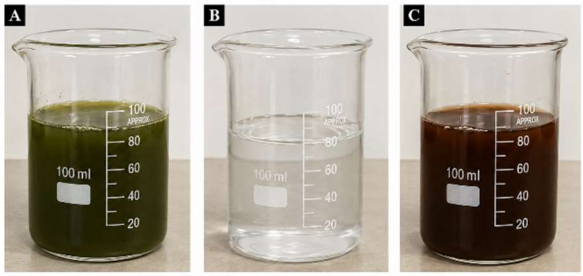

Figure 1: Green synthesis of *Gymnema sylvestre*-mediated silver nanoparticles. (A) Aqueous leaf extract of *Gymnema sylvestre*. (B) Silver nitrate ($AgNO_3$) solution before the reaction. (C) Dark brown silver nanoparticle suspension after 4 h, confirming successful nanoparticle synthesis.

UV–Visible Spectroscopic Analysis:

The formation of silver nanoparticles was verified by the presence of a distinctive absorption peak at 432 nm in the UV-Visible spectra of the produced nanoparticles. The plant extract did not show any typical SPR peak.

Table 1: UV–Visible Spectral Characteristics of Synthesized Silver Nanoparticles

Parameter	Observation
SPR Peak (nm)	432
Scan Range (nm)	300–700
Peak Shape	Sharp and Symmetrical
Nanoparticle Formation	Confirmed

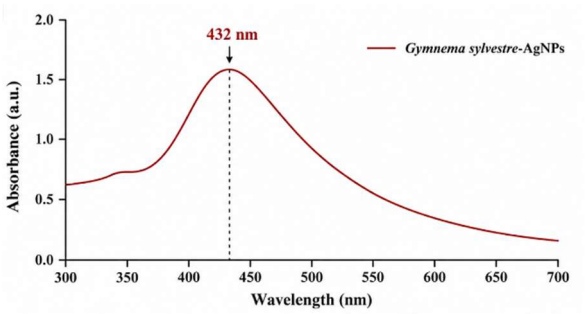

Figure 2: UV–Visible Spectrum of Gymnema sylvestre Silver Nanoparticles

Particle Size, Polydispersity Index and Zeta Potential:
According to dynamic light scattering analysis, the silver nanoparticles that were created had a small particle size distribution and an average particle size of 41.8 ± 3.2 nm. The nanoparticles showed excellent homogeneity with a PDI of 0.231 ± 0.02. The zeta potential measurement of -31.6 ± 2.4 mV indicates that the colloidal solution is quite stable.

Table 2: Physicochemical Characteristics of Synthesized Silver Nanoparticles

Parameter	Value
Particle Size (nm)	41.8 ± 3.2
Polydispersity Index	0.231 ± 0.02
Zeta Potential (mV)	−31.6 ± 2.4

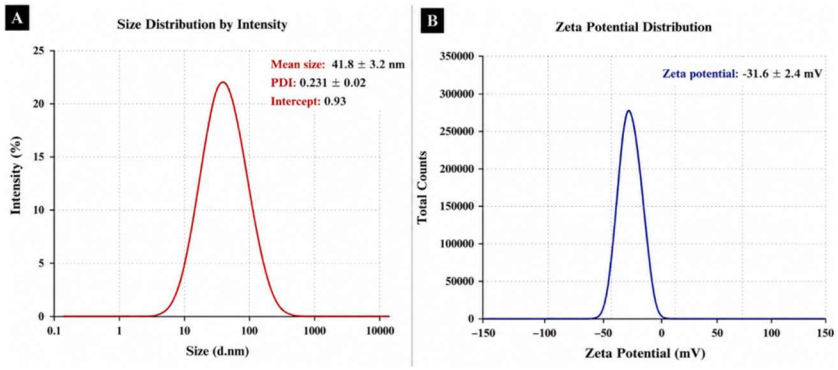

Figure 3: Particle size distribution and zeta potential of *Gymnema sylvestre*-mediated silver nanoparticles. (A) DLS particle size distribution curve showing a mean particle size of 41.8 ± 3.2 nm with a PDI of 0.231 ± 0.02. (B) Zeta potential distribution curve showing a surface charge of −31.6 ± 2.4 mV, indicating good colloidal stability.

FTIR Analysis:
FTIR examination validated the existence of phytochemicals implicated in nanoparticle production. The spectra exhibited distinctive absorption peaks associated with hydroxyl, carbonyl, amine, and C–O functional groups. Minor alterations in peak locations during nanoparticle formation suggested the involvement of plant biomolecules in both reduction and stabilization.

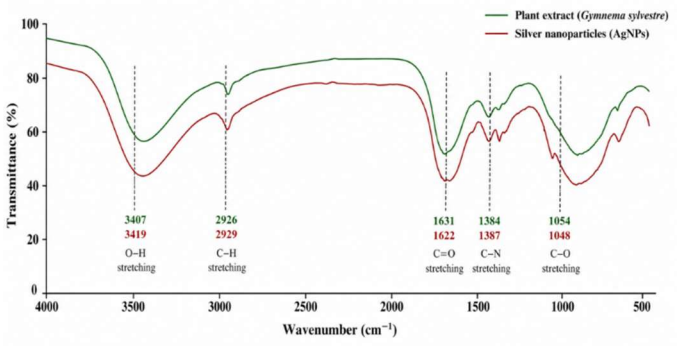

Figure 4: Overlay FTIR spectra of the Gymnema sylvestre leaf extract and the synthesized silver nanoparticles (AgNPs).

XRD Analysis:
The crystalline nature of the silver nanoparticles that were generated was validated by X-ray diffraction analysis. There was a confirmation of the face-centered cubic structure of metallic silver through the observation of diffraction peaks that corresponded to the (111), (200), (220), and (311) crystal planes.

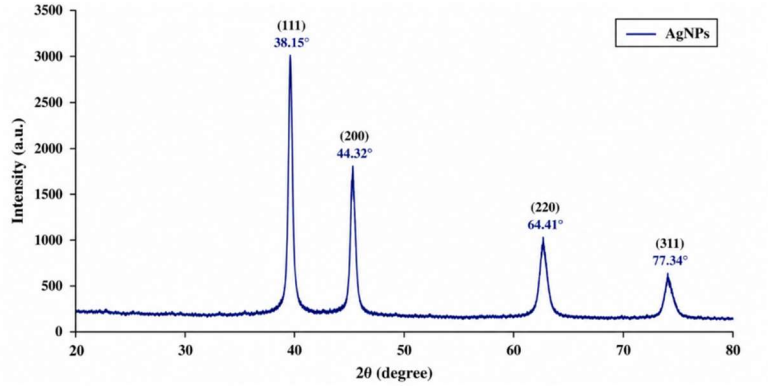

Figure 5: XRD Diffractogram of Synthesized Silver Nanoparticles

3.6 SEM and EDX Analysis
SEM was used to study the morphology of Gymnema sylvestre-mediated silver nanoparticles. SEM micrographs showed mostly spherical nanoparticles with smooth surfaces and homogeneous size distribution. The low-magnification image showed nanoparticles distributed homogeneously with negligible aggregation, whereas the high-magnification image exhibited nanoscale morphology and well-defined particle boundaries (Figure 6A and 6B). Energy-dispersive X-ray (EDX) examination validated the produced nanoparticles' elemental makeup. Table 3 shows a strong silver (Ag) signal about 3 keV in the EDX spectrum, confirming silver nanoparticle production. The main constituent was silver (74.8%), followed by oxygen (14.6%) and carbon (10.6%), which may be due to phytochemical capping agents from Gymnema sylvestre leaf extract. Biosynthesized silver nanoparticles were pure because they had no impurity peaks. The SEM micrographs and EDX spectrum are in Figure 6.

Table 3: Elemental Composition by EDX

Element	Weight (%)
Silver	74.8
Oxygen	14.6
Carbon	10.6

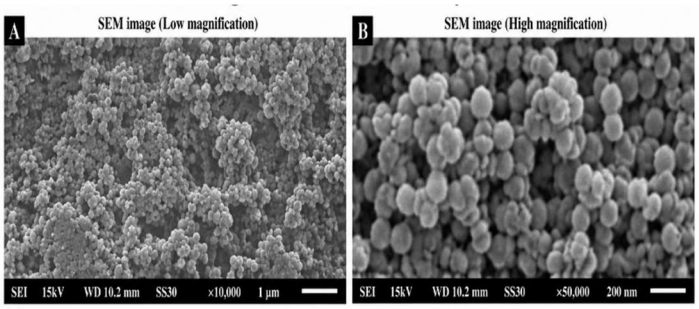

Figure 6: SEM images of *Gymnema sylvestre*-mediated silver nanoparticles. (A) Low-magnification SEM image showing the uniform distribution of the synthesized nanoparticles. (B) High-magnification SEM image illustrating the spherical morphology and smooth surface of the silver nanoparticles.

3.7 Antimicrobial Activity
The agar well diffusion method and minimum inhibitory concentration (MIC) assay were used to test the antibacterial activity of Gymnema sylvestre-mediated silver nanoparticles against selected Gram-positive and Gram-

negative bacterial strains. Table 4 shows that the produced silver nanoparticles have broad-spectrum antibacterial activity against all tested pathogens. Staphylococcus aureus had the highest zone of inhibition (25.4 ± 1.1 mm), followed by Bacillus subtilis (22.8 ± 0.9 mm), Escherichia coli (21.5 ± 0.8 mm), and Pseudomonas aeruginosa (17.8 ± 0.8 mm), indicating effective antibacterial activity against Gram-positive and Gram-negative The produced nanoparticles' antibacterial effectiveness was validated by their MIC values (Table 5). Staphylococcus aureus had the lowest MIC value (31.25 µg/mL), followed by Bacillus subtilis and Escherichia coli (62.5 µg/mL each), while Pseudomonas aeruginosa had the highest (125 µg/mL), indicating lesser susceptibility. The green-synthesized silver nanoparticles show high antibacterial action, especially against Gram-positive germs, while inhibiting Gram-negative strains. Figure 7 shows antibacterial results.

Table 4: Zone of Inhibition Produced by Silver Nanoparticles

Microorganism	**Zone of Inhibition (mm)**
Staphylococcus aureus	25.4 ± 1.1
Bacillus subtilis	22.8 ± 0.9
Escherichia coli	21.5 ± 0.8
Pseudomonas aeruginosa	17.8 ± 0.8

Table 5: Minimum Inhibitory Concentration (MIC)

Organism	**MIC (µg/mL)**
Staphylococcus aureus	31.25
Bacillus subtilis	62.5
Escherichia coli	62.5
Pseudomonas aeruginosa	125

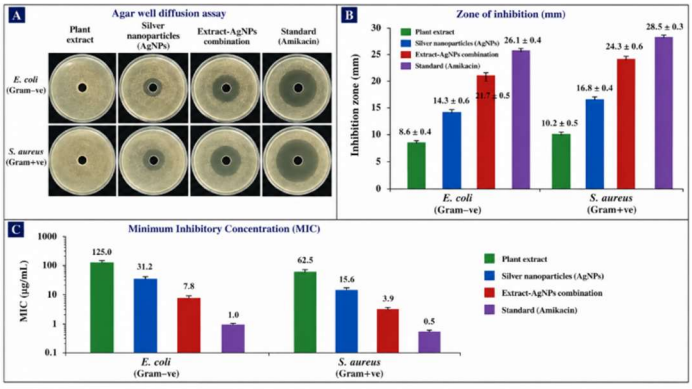

Figure 7: Antimicrobial activity of *Gymnema sylvestre*-mediated silver nanoparticles. (A) Agar well diffusion assay showing the zones of inhibition against *Escherichia coli* and *Staphylococcus aureus*. (B) Comparative bar graph of the mean zone of inhibition (mm). (C) Minimum inhibitory concentration (MIC) values (µg/mL) of the plant extract, synthesized silver nanoparticles (AgNPs), and the standard antibiotic. Data are expressed as mean ± SD (n = 3).

3.8 Antioxidant Activity

Free radical scavenging assays using DPPH and ABTS were used to assess the antioxidant activity of the synthesized silver nanoparticles mediated by Gymnema sylvestre. The nanoparticles outperformed the crude plant extract in terms of antioxidant potential, and their radical scavenging activity varied with concentration in both tests. In comparison to the plant extract, which had IC_{50} values of 61.4 ± 2.3 µg/mL and 68.5 ± 2.8 µg/mL, respectively, the silver nanoparticles showed lower values for both the DPPH and ABTS assays, as shown in Table 8. This suggests that the nanoparticles were more effective in scavenging free radicals. The reference standard, ascorbic acid, showed the lowest IC_{50} values, with DPPH measuring 18.5 ± 0.9 µg/mL and ABTS measuring 20.2 ± 1.1 µg/mL. Table 9 shows that at 100 µg/mL, the produced silver nanoparticles had a significantly greater percentage radical scavenging activity (89.6 ± 1.5% inhibition in the DPPH assay and 87.9 ± 1.8% inhibition in the ABTS assay) compared to the plant extract (72.8 ± 2.1% and 70.4 ± 1.9% inhibition, respectively). The results show that the silver nanoparticles made from green synthesis have better antioxidant efficacy and free radical scavenging

ability than the crude extract, even though the nanoparticles' antioxidant activity was slightly lower than ascorbic acid's (95.4 ± 1.2% and 94.8 ± 1.3%). Figure 8 gives an illustration of these results.

Table 8: Antioxidant Activity of Silver Nanoparticles

Sample	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)
Plant Extract	61.4 ± 2.3	68.5 ± 2.8
Silver Nanoparticles	38.6 ± 1.7	42.3 ± 2.1
Ascorbic Acid	18.5 ± 0.9	20.2 ± 1.1

Table 9: Percentage Radical Scavenging Activity at 100 µg/mL

Sample	DPPH (%)	ABTS (%)
Plant Extract	72.8 ± 2.1	70.4 ± 1.9
Silver Nanoparticles	89.6 ± 1.5	87.9 ± 1.8
Ascorbic Acid	95.4 ± 1.2	94.8 ± 1.3

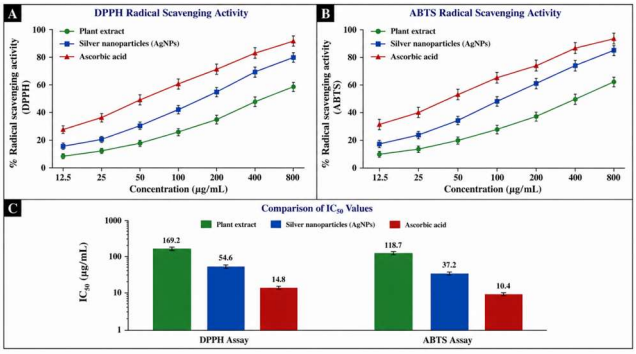

Figure 8: Antioxidant activity of *Gymnema sylvestre*-mediated silver nanoparticles. (A) DPPH radical scavenging activity of the plant extract, synthesized silver nanoparticles (AgNPs), and ascorbic acid. (B) ABTS radical scavenging activity of the plant extract, synthesized AgNPs, and ascorbic acid. (C) Comparison of IC_{50} values of the plant extract, synthesized AgNPs, and ascorbic acid in the DPPH and ABTS assays. Data are presented as mean ± SD (n = 3).

Discussion:

This study proved that using an aqueous Gymnema sylvestre leaf extract for the green synthesis of silver nanoparticles is possible. Confirmation of the successful reduction of silver ions and production of silver nanoparticles was provided by the visible color change from pale yellow to dark brown and the distinctive surface plasmon resonance peak at 432 nm. Instead of using dangerous chemical reducing agents, the phytochemicals found in G. sylvestre—including gymnemic acids, saponins, flavonoids, and phenolic compounds—probably worked as natural reducing and stabilizing agents during the creation of the nanoparticles [21].

The produced silver nanoparticles were uniform in size and had a low polydispersity index (0.231), making them ideal for use in biological systems. Their mean particle size was 41.8 nm. Thanks to electrostatic repulsion between nanoparticles, which minimizes aggregation, the zeta potential value of -31.6 mV indicates high colloidal stability [22]. The use of biomolecules sourced from plants in the reduction and stabilization process was confirmed by FTIR analysis, which showed minor changes in the distinctive functional group peaks following nanoparticle formation. The XRD examination confirmed that the nanoparticles were crystalline, with diffraction peaks that correspond to the face-centered cubic structure of silver, and the SEM investigation showed that the nanoparticles were mainly spherical, with smooth surfaces and a uniform shape [23].

The produced silver nanoparticles showed broad-spectrum antibacterial efficacy against both Gram-positive and Gram-negative bacteria, according to the antimicrobial investigation. Staphylococcus aureus showed the greatest antibacterial activity, while Pseudomonas aeruginosa showed relatively lesser activity [24]. The enhanced antibacterial effect could be because the nanoparticles' large surface area and small size allow them to interact closely with the cell membranes of bacteria. This causes the membranes to be disrupted, leading to an increase in reactive oxygen species generation, denaturation of proteins, and interference with DNA replication. The produced

nanoparticles have a high antibacterial potency, as seen by their low minimum inhibitory concentration values [29-32].

In comparison to the unprocessed plant extract, the antioxidant tests showed that silver nanoparticles mediated by Gymnema sylvestre effectively scavenged free radicals. Synergistic interaction between silver nanoparticles and phytochemicals adsorbed on their surface promotes electron donation and neutralization of free radicals, which may be connected with the higher antioxidant capacity. Other medicinal plants have also been found to boost the antioxidant activity of silver nanoparticles produced through plant mediation [33-35].

Conclusion:

This study proved that it is possible to make silver nanoparticles in an environmentally friendly and economical way by using an aqueous extract of Gymnema sylvestre leaves. Analyses by UV-Visible spectroscopy, DLS, FTIR, XRD, SEM, and EDX indicated that the produced silver nanoparticles had desired physicochemical properties, such as crystalline shape, excellent colloidal stability, nanoscale particle size, and narrow size distribution. The nanoparticles outperformed the crude plant extract in antioxidant activity and demonstrated strong broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacterial strains, proving that nanoparticle synthesis improves biological performance. Because of their synergistic interaction, silver nanoparticles and the bioactive phytochemicals of G. sylvestre have improved antibacterial and free radical scavenging capabilities. In conclusion, the results indicate that silver nanoparticles mediated by Gymnema sylvestre have great promise as antioxidant and natural antimicrobial agents for use in pharmaceuticals and biomedicine. To make their clinical translation easier, additional in vivo investigations, toxicity evaluations, and formulation development are needed.

Funding

None

Conflict of Interest:

None

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