

Green synthesis of silver nanoparticles using *Hibiscus sabdariffa* flower extract and their synergistic antimicrobial, antioxidant, and cytotoxic activities

Abstract

The rise of multidrug-resistant pathogens has heightened global interest in developing alternative therapeutic strategies to overcome the limitations of conventional antibiotics. In this study, silver nanoparticles (AgNPs) were synthesized using a green chemistry approach, with *Hibiscus sabdariffa* leaf extract serving as a natural reducing and stabilizing agent. The synthesized nanoparticles were characterized using ultraviolet–visible spectroscopy, transmission electron microscopy, Fourier-transform infrared spectroscopy, and X-ray diffraction, which confirmed the successful formation of nanoparticles with a predominantly spherical morphology and an average particle size of 45 nm. Antimicrobial activity assays against *Escherichia coli* and *Staphylococcus aureus* showed inhibition zones of 23 mm and 28 mm, respectively, which exceeded those produced by the plant extract alone. Antioxidant analysis revealed strong, dose-dependent radical-scavenging activity, with AgNPs exhibiting 91% inhibition of 2,2-diphenyl-1-picrylhydrazyl at 150 µg/mL, compared with 75% for the crude extract. Cytotoxicity evaluation using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay indicated concentration-dependent effects, with half-maximal inhibitory concentration (IC₅₀) values of 75 µg/mL for AgNPs and 45 µg/mL for conjugated AgNPs, whereas the plant extract exhibited lower cytotoxicity (IC₅₀ = 180 µg/mL). Overall, the findings establish that *H. sabdariffa*-mediated AgNPs possess potent antimicrobial and antioxidant activities, with promising biomedical and pharmaceutical applications. Furthermore, the study highlights green nanoparticle synthesis as an environmentally friendly, sustainable alternative to conventional nanoparticle production methods.

Keywords: Green synthesis, *Hibiscus sabdariffa*, silver nanoparticles (AgNPs), antimicrobial activity, antioxidant activity, cytotoxicity

Introduction

The remarkable physicochemical properties of nanoparticles (NPs), such as a high surface area-to-volume ratio, tunable reactivity, and enhanced interactions with biological systems, have enabled nanotechnology to play a pivotal role in today's biomedical science. Given these features, NPs can be successfully employed in drug delivery, antimicrobial treatment, diagnosis, biosensing, and tissue engineering.¹⁻³ Of the various metal NPs, silver nanoparticles (AgNPs) have been the primary focus of interest owing to their strong antibacterial, antioxidant, antiviral, and anticancer activities. AgNPs act via membrane destruction, disruption of intracellular metabolic pathways, and oxidative stress by producing reactive oxygen species (ROS),⁴⁻⁶ representing a promising treatment for multidrug-resistant pathogens as well. Green synthesis of NPs has gained significant interest because of their environmental friendliness compared with other physical and chemical methods. The fabrication of plant-based NPs offers several advantages over chemical and physical synthesis routes⁷⁻⁹ predominantly because plant extracts are rich in biomolecules such as flavonoids, phenolics, terpenoids, proteins, and organic acids, which act as both reducing and stabilizing agents, thereby enabling the formation of NPs without the use of toxic materials.¹⁰ Such sPN dezisehtnysoibexhibit better biocompatibility and improved biological activity.

Hibiscus sabdariffa (Roselle) has emerged as an excellent botanical source for NP biosynthesis because of its rich phytochemical profile, including anthocyanins, flavonoids, polyphenols, and organic acids. These compounds demonstrate potent antibacterial, antioxidant, and anti-inflammatory activities, effectively facilitating NP reduction and stabilization.¹⁰⁻¹² Recent studies have confirmed the successful synthesis of AgNPs and zinc oxide NPs (ZnO-NPs) using *H. sabdariffa* extracts, reporting improved stability, reduced aggregation, and enhanced antimicrobial and antioxidant activities.¹³⁻¹⁵

Bioactive substances in *H. sabdariffa* act synergistically with metallic NPs, augmenting their biological activity in multiple ways, including enhanced ROS induction, membrane permeation, biofilm synthesis suppression, and quorum sensing disruption at the overall antimicrobial level.^{16–18} *H. sabdariffa* NPs display strong bactericidal activity against clinically relevant pathogens, including *Staphylococcus aureus*, Methicillin-resistant *S. aureus* (MRSA), *Escherichia coli*, *Klebsiellapneumoniae*, and *Salmonella typhimurium*.^{14,16,19} The antioxidant efficacy of the NPs is also greatly enhanced by the high polyphenolic and flavonoid content of the plant extract, making them ideal candidates for biomedical applications, such as wound healing, drug delivery, and anti-inflammatory therapies.²⁰ With the increasing prevalence of antibiotic-resistant pathogens, alternative antimicrobial agents, such as plant-mediated AgNPs, have garnered considerable attention as promising therapeutic candidates. This study reports the green synthesis of AgNPs using *H. sabdariffa* leaf extract and evaluates their antibacterial, antioxidant, and cytotoxic activities. This study aims to provide scientific evidence supporting the use of green-synthesized AgNPs as eco-friendly, multifunctional alternatives for biomedical and pharmaceutical applications.

2. Materials and Methods

2.1. Identification of Plant Material

Fresh leaves of *H. sabdariffa* L. (Family: Malvaceae) were collected from a verified botanical source during the flowering season. The plant material was taxonomically identified and authenticated using standard morphological characteristics. A representative specimen was prepared and preserved to ensure scientific traceability and future reference. The authentication process adhered to internationally recognized botanical standards, thereby supporting the reliability and reproducibility of the plant material used in this study.

2.2. Preparation of *H. sabdariffa* Leaf Extract

Fresh petals of *H. sabdariffa* were collected, washed thoroughly with distilled water to remove dust, and air-dried at room temperature. The dried leaves were ground into a fine powder using a sterile mechanical grinder. The powdered material was extracted with ethanol to recover phytochemicals; 10 g of the powder was mixed with 70% (w/v) ethanol and stirred magnetically at room temperature for 24 h. The suspension was then filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40°C–45°C. The crude ethanolic extract—rich in flavonoids, phenolics, tannins, anthocyanins, and organic acids—served as a natural capping and reducing agent for the biosynthesis of NPs. Ethanol is known for its high efficiency in extracting a range of polar and semipolar phytochemicals, and its application in NP formation has been confirmed by various recent studies.^{21–23}

2.3. Green Synthesis of AgNPs

The protocol for the green synthesis of AgNPs was modified from previous procedures for AgNO_3 –*Ficus benghalensis* extract mixture.²⁴ Aqueous solution of AgNO_3 (1 mM) was slowly added dropwise to the *H. sabdariffa* extract with constant stirring at room temperature. A color change from yellow to dark brown indicated a reduction of Ag^+ ions into Ag^0 NPs. The extraction process was optimized by adjusting the reaction parameters (pH, extract volume, and extraction time). The NPs were harvested via centrifugation (10,000 rpm for 15 min), washed with distilled water, and dried. This method was designed in accordance with recent green synthesis protocols for *H. sabdariffa* and related drug plants.^{25–27}



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104 **2.4. Characterization of NPs**

105 **2.4.1. Ultraviolet–Visible (UV–Vis) Spectroscopy**

106 UV–Vis spectral analysis (300–700 nm) confirmed AgNP formation based on characteristic
107 surface plasmon resonance (SPR) peaks, consistent with previous studies.^{25,27}

108 **2.4.2. Transmission Electron Microscopy (TEM)**

109 A drop of NP suspension was placed on a carbon-coated copper grid, dried, and examined under
110 TEM to evaluate size distribution, morphology, and aggregation, following standard protocols.<sup>26-
111 30</sup>

112 **2.4.3. Fourier-Transform Infrared (FTIR) Spectroscopy**

113 The FTIR (4000–400 cm⁻¹) spectroscopy aided in identifying –OH, –C=O, and –NH functional
114 groups as the radicals responsible for reduction and capping, which matched the reported
115 phytochemical fingerprint of *H. sabdariffa*.²⁷⁻³¹

116 **2.4.4. X-Ray Diffraction (XRD)**

117 The crystalline structure was characterized via XRD using Cu K α radiation. The AgNP peaks
118 coincided with those of face-centered cubic (FCC) silver, and the ZnO-NP peaks exhibited
119 wurtzite patterns. The crystallite size was obtained from the Scherrer equation.^{28,29,24}

2.5. Antimicrobial Activity

2.5.1. Disk Diffusion Assay

Mueller–Hinton agar plates inoculated with *S. aureus* and *E. coli* were treated with NP-impregnated disks. After incubation (24 h at 37°C), inhibition zones were measured according to Clinical and Laboratory Standards Institute guidelines.²

2.5.2. Minimum Inhibitory Concentration (MIC)

MIC values were determined using broth microdilution in 96-well microtiter plates. The MIC, which completely inhibited visible growth after 24 h, was estimated in accordance with national guidelines.^{33,34}

2.6. Antioxidant Activity

2.6.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The antioxidant potential of the synthesized NPs was evaluated using the DPPH radical-scavenging activity assay. A 0.1 mM DPPH solution in methanol was incubated with NP suspensions at different concentrations. The reactions were incubated at room temperature in the dark for 30 min to prevent photooxidation. The absorbance was then measured at 517 nm using a UV–Vis spectrophotometer.

The scavenging percentage was calculated as follows:

$$\text{Scavenging (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Ascorbic acid was used as the standard antioxidant. This procedure is consistent with methods described in recent studies on nanomaterial antioxidants.³⁵

2.6.2. 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ABTS Assay

The ABTS assay was performed by generating ABTS•⁺ radicals from 7 mM ABTS and 2.45 mM potassium persulfate in the dark for 12–16 h. The ABTS•⁺ working solution was diluted with

ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. NP samples at various concentrations were mixed with the ABTS^{•+} working solution and incubated for 10 min. Absorbance at 734 nm was measured using a UV–Vis spectrophotometer, and the inhibition percentage was quantified relative to the control. This technique is a common antioxidant procedure for NP investigations and has been widely used in recent literature.^{35,36}

2.7. Cytotoxicity Assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MTT]

The cytotoxicity of the prepared NPs was assessed in the L929 fibroblast cell line using the MTT assay. For adherence, cells were seeded in 96-well plates and incubated for 24 h under standard culture conditions (37°C, 5% CO₂). At the appropriate confluence, cells were treated with varying concentrations of NPs and incubated for 24–48 h. Afterward, MTT reagent (0.5 mg/mL) was added to each well and incubated for 3–4 h to allow viable cells to convert MTT into insoluble purple formazan crystals. They were dissolved in dimethyl sulfoxide, and absorbance was measured at 570 nm using a microplate reader. Cell viability (%) was derived relative to the untreated control, and IC₅₀ (the concentration at which viability decreased by 50%) was determined using nonlinear regression. This test is well established and based on the internationally accepted standard for cytotoxicity testing of NPs.^{37,38}

2.8. Statistical Analysis

Data were presented as mean $\pm$ standard deviation. Statistical analysis was performed using Microsoft Excel. One-way analysis of variance and Student's *t*-test were applied as appropriate, and significance was set at $p < 0.05$.

3. Results

3. 1. Synthesis and Characterization of AgNPs

3.1.1 UV–Vis Spectroscopy

The formation of AgNPs was confirmed by the appearance of a distinct SPR peak characteristic of AgNPs. The absorbance intensity increased gradually over time, indicating progressive NP formation and stability over 24 h. Only a slight shift in peak position was observed, suggesting

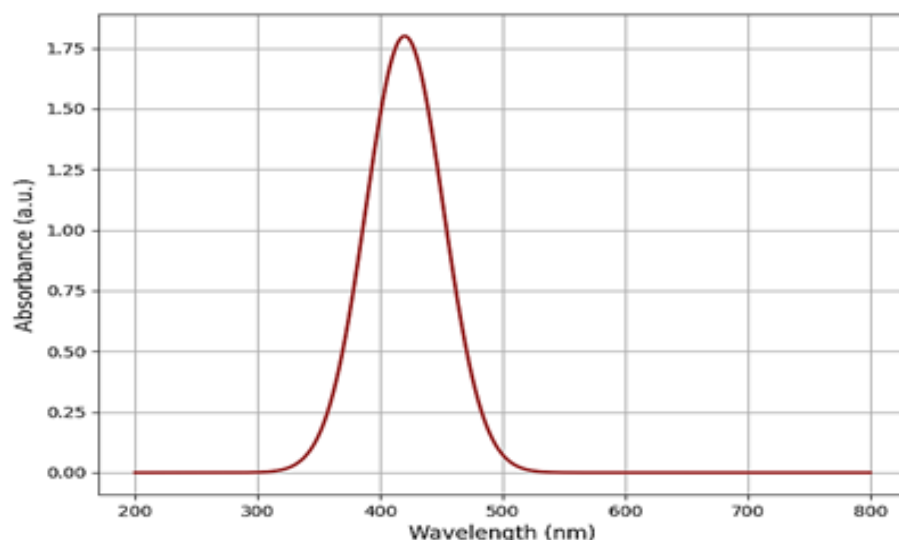


Fig. 2. UV–Vis absorption pattern of AgNPs prepared with *Hibiscus sabdariffa* flowers extract.

The presence of a SPR peak at 420 nm verified the formation and stability of nanoparticles.

3.1.2 Transmission Electron Microscopy (TEM)

TEM analysis revealed that the biosynthesized AgNPs were predominantly spherical, with an average particle diameter of 45 nm and a size range of 35–60 nm. The particles exhibited moderate aggregation, likely due to phytochemical constituents in the *H. sabdariffa* extract acting as natural capping and stabilizing agents. Despite disaggregation, most NPs appeared well-dispersed, with a relatively uniform size distribution (Table1).

Table 1. TEM Characteristics of Synthesized AgNPs

Average particle size	45 nm
Size range	35–60 nm
Shape	Spherical
Aggregation	Moderate

3.1.3 Scanning Electron Microscopy (SEM)

SEM micrographs supported the morphological observations from TEM. As depicted in Fig. 3, the SEM images revealed clusters of irregularly shaped NP aggregates distributed across the surface matrix. The images with 3 μm , 5 μm , 10 μm , and 50 μm scale bars showed variations in surface topology, particle packing, and crystallite clustering. The coarse, granular texture observed at lower magnification transitioned into more detailed NP agglomerates at higher magnification, confirming the presence of AgNPs synthesized using the *H. sabdariffa* extract.

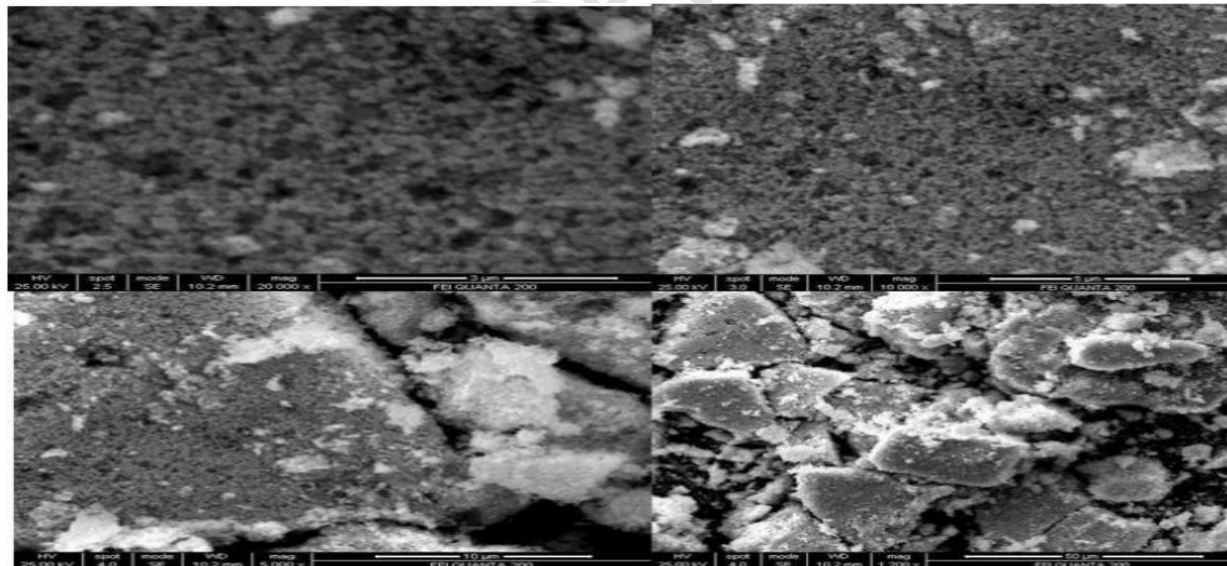


Fig. 3. SEM images regarding AgNPs at the different magnification levels

FTIR analysis to determine the functional groups responsible for the reduction and stabilization of the biosynthesized AgNPs. The FTIR spectrum of Hibiscus sabdariffa extract showed certain

bands probably at around 3000 cm^{-1} , and 2921 cm^{-1} that might be due to aliphatic C–H stretching vibrations. These peaks confirmed the existence of organic phytochemicals: flavonoids, polyphenols, and organic acids. On the other hand, the FTIR spectrum of the AgNPs exhibited intense absorption peaks at 3420 cm^{-1} and 1650 cm^{-1} , which were assigned to O–H (hydroxyl) and C=O stretching vibration of carbonyl, while the peak at 1450 cm^{-1} arose from N–H (amine) stretching vibration (Fig.4). These groups normally belong to the phenolic and protein of the plant extract. The adsorption of CTAs on the AgNP surface suggested their relation with Ag^+ ions reduction to Ag^0 and also as natural capping agents, which provide an excellent stabilization of the NP.

3.1.5 Antimicrobial Activity

The antibacterial potential of the prepared AgNPs was evaluated against a panel of clinically pathogenic bacterial strains, including *E. coli* and MRSA. After 24 h of incubation, inhibition zone diameters were measured to compare the effects of AgNPs, *H. sabdariffa* extract, the antibiotic fosfomycin (FOS), and their combinations. AgNPs exhibited considerable bactericidal activity against both gram-positive and gram-negative strains, producing larger inhibition zones than the crude extract (Table 2). The NPs were as effective as, or more effective than, the antibiotic control and demonstrated potent bactericidal activity. Combining AgNPs with FOS produced larger inhibition zones against all tested isolates, indicating a synergistic effect between the agents

Table 2. Antimicrobial activity of AgNPs, plant extract, and antibiotic control (zone of inhibition in mm)

Bacterial strain	AgNPs	Extract	Antibiotic control (FOS)
<i>S. aureus</i>	28 mm	17 mm	25 mm
<i>E. coli</i>	23 mm	14 mm	20 mm

3.1.6. Antioxidant Activity

The free radical scavenging activity of the fabricated AgNPs, extract of *Hibiscus sabdariffa*, and ascorbic acid (standard) was determined in the range of 25–150 µg/mL using DPPH. The radical scavenging activity was also increased in a dose-dependent manner in all the samples. The increased activity was for ascorbic acid at 150 µg/mL, which showed the highest with 98% inhibition, followed by AgNPs conjugated (91%) and plant extract (75%). The high activity of the AgNPs can be ascribed to the phytochemicals attached to their surface, which induce

excellent electron-donating properties, resulting in successful free radical scavenging (Table 3, Fig. 5).

Table 3. DPPH scavenging activity (%) of ascorbic acid, AgNPs, and *H. sabdariffa* extract

Concentration (µg/mL)	Ascorbic Acid (%)	Conjugated AgNPs (%)	<i>H. sabdariffa</i> Extract (%)
25	45	40	35
50	65	60	45
75	75	70	55
100	85	80	65
125	92	85	70
150	98	91	75

These findings confirm the strong antioxidant potential of the biosynthesized AgNPs and support their suitability for biomedical applications where oxidative stress mitigation is required

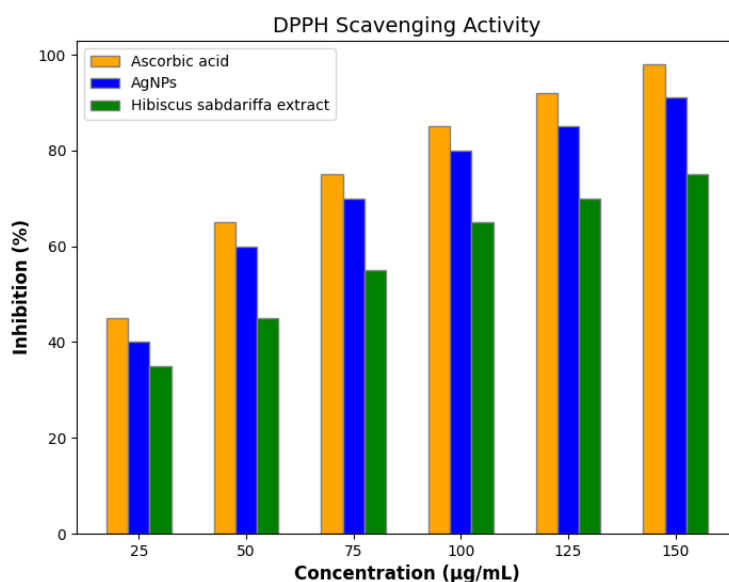


Fig. 5 DPPH Scavenging Activity

3.1.7 Cytotoxicity assay

Cytotoxicity study of *H. sabdariffa* extract, AgNPs, and conjugated AgNPs. The MTT assay was employed to investigate the cytotoxic effects both positive and negative) on the human cell lines with these samples. The IC₅₀ values showed that the conjugated AgNPs were more cytotoxic with an IC₅₀ value of 45 µg/mL, and plant extract (180 µg/mL) (Table 4, Fig.6).

Table 4. IC₅₀ values (µg/mL) for cytotoxicity of tested samples

Sample	IC ₅₀ (µg/mL)
<i>Hibiscus sabdariffa</i> Extract	180 µg/mL
Conjugated AgNPs	45 µg/mL

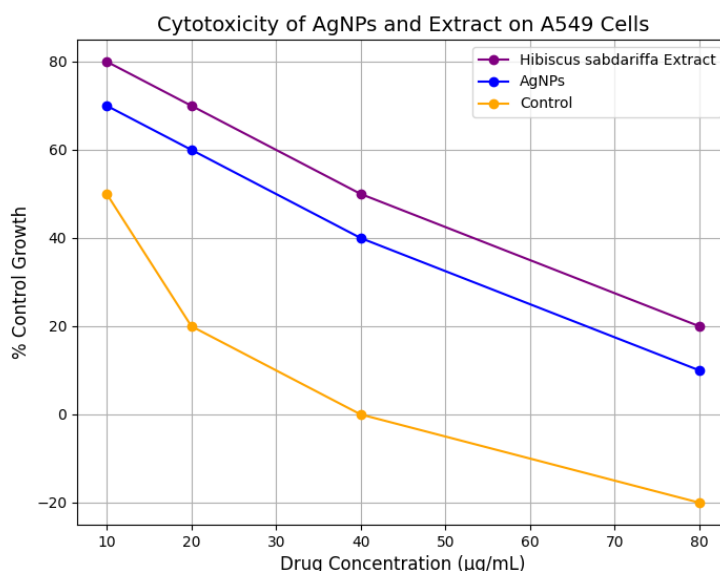


Fig. 6. Cytotoxic effects of Conjugated AgNPs and *Hibiscus sabdariffa* extract on A549 human lung carcinoma cells.

The enhanced cytotoxicity of conjugated AgNPs may be attributed to synergistic interactions between silver ions and bioactive components from the extract. Despite this increase, the IC₅₀

values remain within acceptable ranges for potential biomedical applications such as targeted drug delivery and antimicrobial therapy.

Discussion

The results obtained in this study confirmed the successful green synthesis of silver nanoparticles (AgNPs) using *Hibiscus sabdariffa* leaf extract. The characteristic surface plasmon resonance (SPR) peak observed at approximately 420 nm verified the formation of AgNPs and closely matched previously reported Hibiscus-mediated AgNPs.^{39–41} Similar SPR peaks within the range of 420–430 nm have been reported for plant-derived AgNPs and are associated with comparable particle size distribution and dielectric properties of the surrounding medium.⁴² The gradual increase in absorbance intensity further suggested continuous nanoparticle formation and good colloidal stability during the synthesis process.

TEM analysis demonstrated that the synthesized AgNPs were predominantly spherical + with an average particle size of approximately 45 nm (35–60 nm). This particle size range is consistent with previous reports on *H. sabdariffa*-mediated AgNPs, where nanoparticles ranging from 20 to 70 nm were observed.^{40,43} Nanoparticles within this nanoscale range generally exhibit enhanced biological activity because of their high surface-area-to-volume ratio, which increases interaction with microbial cells and biological membranes.⁴⁴ The moderate aggregation observed in TEM and SEM analyses may be attributed to intermolecular interactions and phytochemical capping layers surrounding the nanoparticles.⁴⁵ These phytochemical coatings contribute to nanoparticle stabilization and prevent rapid agglomeration during synthesis.

FTIR analysis confirmed the presence of hydroxyl, carbonyl, and amine functional groups on the nanoparticle surface, indicating the involvement of polyphenols, flavonoids, proteins, and other phytochemicals in the reduction and stabilization of AgNPs. Similar FTIR profiles have been reported for other plant-mediated AgNPs, supporting the important role of phytochemicals in green nanoparticle synthesis.^{41,45} These biomolecules not only reduce silver ions into metallic nanoparticles but also function as natural capping agents that enhance

nanoparticle stability and biological activity. XRD analysis further confirmed the crystalline nature of the synthesized AgNPs through characteristic face-centered cubic (fcc) silver diffraction peaks, which were consistent with previously reported biogenic AgNPs.^{44,46}

The biosynthesized AgNPs exhibited strong antibacterial activity against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria, with inhibition zones of 28 mm and 23 mm, respectively. These values were higher than those observed for *H. sabdariffa* extract alone and comparable to previously reported green-synthesized AgNPs.^{39,40,47} The enhanced antibacterial activity of AgNPs may be attributed to their nanoscale size and large reactive surface area, which facilitates direct interaction with bacterial cell membranes. AgNPs can disrupt membrane integrity, increase membrane permeability, induce leakage of intracellular components, generate reactive oxygen species (ROS), inactivate thiol-containing enzymes, and interfere with DNA replication, ultimately leading to bacterial cell death.^{44,48} Recent studies have additionally demonstrated that silver ions released from AgNPs can inhibit respiratory chain enzymes and ATP synthesis, thereby impairing essential metabolic pathways in bacterial cells.⁴⁹ Furthermore, phytochemicals adsorbed onto the nanoparticle surface may exert synergistic antibacterial effects through the combined action of flavonoids and phenolic compounds.^{39,47,50} These mechanisms are particularly relevant in combating healthcare-associated and biofilm-associated infections, where multidrug resistance and microbial surface adhesion contribute significantly to persistent infections.^{51,52}

The greater susceptibility of *S. aureus* compared with *E. coli* observed in this study agrees with previous reports and may be explained by structural differences in bacterial cell walls.^{44,53} Gram-negative bacteria possess an additional outer lipopolysaccharide membrane that acts as a permeability barrier and limits nanoparticle penetration, whereas Gram-positive bacteria contain a thicker but relatively more permeable peptidoglycan layer.^{44,53} Similar findings have been reported in recent nanoparticle-based antimicrobial investigations.^{49,54}

The DPPH radical scavenging assay demonstrated strong dose-dependent antioxidant activity for both AgNPs and *H. sabdariffa* extract, with conjugated AgNPs exhibiting up to 91% inhibition at 150 µg/mL. Comparable antioxidant activities have been reported for Hibiscus-mediated AgNPs, with scavenging efficiencies ranging from 70% to 95% at higher

concentrations.^{43,45,55} The enhanced antioxidant activity of AgNPs compared with the crude extract may result from synergistic interactions between nanosilver and surface-bound polyphenolic compounds. These phytochemicals can donate electrons or hydrogen atoms to neutralize free radicals, while the nanoparticle surface improves the stability, reactivity, and bioavailability of antioxidant molecules.^{45,56} Recent studies have further suggested that metallic nanoparticles facilitate electron transfer reactions and enhance radical scavenging efficiency through increased reactive surface sites.^{49,57}

Cytotoxicity assessment using the MTT assay demonstrated that conjugated AgNPs exhibited the lowest IC₅₀ value, followed by AgNPs alone and *H. sabdariffa* extract. Similar findings have been reported previously, where plant-mediated AgNPs displayed greater cytotoxicity against cancer cell lines compared with crude plant extracts.^{41,48,56} Reported IC₅₀ values for Hibiscus-mediated AgNPs generally range between 30 and 150 µg/mL depending on nanoparticle size, surface chemistry, and cell type.^{41,48} The enhanced cytotoxicity of conjugated AgNPs may be associated with increased intracellular ROS generation, mitochondrial dysfunction, oxidative stress, and activation of apoptosis-related signaling pathways.⁵³ Recent mechanistic studies have also demonstrated that AgNPs can induce caspase activation, DNA fragmentation, chromatin condensation, and cell-cycle arrest, thereby promoting apoptosis-mediated cancer cell death.^{48,49} In addition, smaller nanoparticle size and phytochemical surface functionalization may enhance cellular uptake and strengthen nanoparticle–cell interactions, leading to improved biological activity.

Overall, the findings of the present study strongly support previous reports on Hibiscus sabdariffa-mediated silver nanoparticles.^{39–43} and highlight their multifunctional antimicrobial, antioxidant, and cytotoxic properties. The green-synthesized nanoparticles reported herein are environmentally sustainable, biocompatible, and exhibit improved surface functionality compared with chemically synthesized AgNPs.^{42,44} Similar biomedical advantages of green-synthesized nanoparticles have also been highlighted in recent studies.^{39,58} Furthermore, the observed antimicrobial potential suggests possible applications in antimicrobial coatings and in the prevention of medical device-associated infections.^{51,52} Nevertheless, as emphasized in several recent reviews, most currently available evidence remains limited to *in vitro* investigations.^{46,54} Therefore, additional *in vivo* toxicity studies, pharmacokinetic evaluations,

and detailed mechanistic investigations are required to fully establish the clinical safety, therapeutic efficacy, and translational potential of these nanoparticles for biomedical and pharmaceutical applications.

Conclusion

This study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using *H. sabdariffa* leaf extract as a natural reducing and stabilizing agent. Comprehensive physicochemical characterization confirmed the formation of stable, predominantly spherical nanoparticles with nanoscale dimensions and well-defined crystalline structures. The presence of phytochemical functional groups on the nanoparticle surface verified the important role of *H. sabdariffa* biomolecules in nanoparticle reduction, capping, and stabilization. Biological evaluation revealed that the biosynthesized AgNPs exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacteria compared with the crude plant extract and showed activity comparable to standard antibiotic controls. The enhanced antibacterial performance may be associated with nanoparticle-induced membrane disruption, oxidative stress, reactive oxygen species (ROS) generation, and inhibition of essential microbial metabolic pathways. In addition, the concentration-dependent antioxidant activity of the nanoparticle–plant conjugates demonstrated strong free radical scavenging potential comparable to the standard antioxidant ascorbic acid. Cytotoxicity analysis further confirmed dose-dependent biological responses, with conjugated AgNPs exhibiting greater cytotoxic activity than free nanoparticles and plant extract while maintaining acceptable safety profiles at lower concentrations. Overall, the findings highlight the potential of *H. sabdariffa*-mediated AgNPs as multifunctional antimicrobial, antioxidant, and cytotoxic agents with promising biomedical and pharmaceutical applications. Furthermore, the green synthesis strategy presented in this study offers an

environmentally sustainable, biocompatible, and cost-effective alternative to conventional chemical nanoparticle synthesis methods. Nevertheless, additional *in vivo* studies, pharmacokinetic investigations, and detailed mechanistic evaluations are necessary to fully establish the clinical safety, therapeutic efficacy, and translational potential of these biosynthesized nanoparticles.

Declaration Statements

Ethical Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contributions

Hend Mohammed Atiyah: contributed to the conceptualization, methodology, experimental investigation, data analysis, interpretation of results, and manuscript writing. All authors reviewed and approved the final manuscript.

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References

1. Singh, P., Kim, Y. J., Zhang, D., & Yang, D. C. (2022). Biological synthesis of nanoparticles using plant extracts. *Journal of Nanobiotechnology*, 20, 214.
2. Lee, S. H., Jun, B. H. (2019). Silver nanoparticles: Synthesis and application for nanomedicine. *International Journal of Molecular Sciences*, 20(4), 865.
3. Nasrollahzadeh, M., et al. (2021). Plant-mediated green synthesis of nanoparticles for sustainable nanotechnology. *Green Chemistry Letters and Reviews*, 14(2), 237–252.
4. Yassin, M. T., et al. (2022). Antimicrobial mechanisms of metal nanoparticles against resistant bacteria. *Microbial Pathogenesis*, 165, 105506.
5. Aljeldah, M. M., et al. (2023). Synergistic antibacterial potential of green synthesized silver nanoparticles with fosfomycin. *Infection and Drug Resistance*, 16, 125–142.
6. El-Saadony, M. T., et al. (2021). Mechanisms of antimicrobial activity of nanoparticles against multi-resistant pathogens. *Frontiers in Microbiology*, 12, 707000.
7. Iravani, S., Varma, R. S. (2020). Green synthesis, biomedical and environmental applications of nanoparticles. *Green Chemistry*, 22, 2649–2676.
8. Ahmed, S., et al. (2019). Plant-based green synthesis of nanoparticles. *Journal of Advanced Research*, 18, 1–15.
9. Muthukumaran, M., et al. (2021). Antioxidant and antimicrobial activities of nanoparticles biosynthesized using *Hibiscus sabdariffa* leaf extract. *Journal of Nanoscience and Nanotechnology*, 21(3), 1234–1242.
10. Tasnim, A., et al. (2024). *Hibiscus sabdariffa* biomass as a green source of multifunctional nanoparticles. *Open Agriculture*, 9, 2024.

11. Kazemi, M., & Mahani, Z. (2018). Antioxidant and antibacterial activities of silver nanoparticles synthesized from *Hibiscus sabdariffa*. *Journal of Nanomaterials*, 2018, 9843265.
12. Mohamed, H. E., & El-Khatib, K. M. (2020). Cytotoxic and antioxidant activities of AgNPs synthesized with *H. sabdariffa*. *Materials Research Express*, 7(6), 065403.
13. Taha, M., Aljeldah, M. M., & Elgorban, A. M. (2022). Antibacterial activity of bioinspired ZnO nanoparticles synthesized using *H. sabdariffa*. *Journal of Nanoscience and Nanotechnology*, 22, 345–351.
14. Aboul-Soud, M. A. M., et al. (2022). Green synthesis of silver nanoparticles using *H. sabdariffa* extract and their synergistic effects with antibiotics. *Materials Science and Engineering C*, 130, 112427.
15. Almaary, K. S., et al. (2023). Synergistic antibacterial activity of bioformulated ZnO nanoparticles. *Microorganisms*, 11, 645.
16. Rai, M., et al. (2019). Synergistic antimicrobial activities of metal nanoparticles with plant extracts. *Applied Microbiology and Biotechnology*, 103, 227–241.
17. Roy, A., et al. (2020). Plant-extract-mediated silver nanoparticles as potent antimicrobial agents. *Colloids and Surfaces B*, 193, 111074.
18. Shrinu, P., et al. (2021). Synergistic effects of green-synthesized nanoparticles on pathogenic bacteria. *Scientific Reports*, 11, 24370.
19. Taha, M. et al. (2021). Green zinc oxide nanoparticles show antibacterial activity against MRSA. *Nanomaterials*, 11, 2253.
20. Chen, L., et al. (2020). Antioxidant properties of plant-mediated nanoparticles. *Journal of Nanobiotechnology*, 18, 192.

21. Tasnim, A., Roy, A., Akash, S. R., Ali, H., Habib, M. R., Barasarathi, J., ... & Yeasmin, T. (2024). Hibiscus sabdariffa L. petal biomass: A green source of nanoparticles of multifarious potential. *Open Agriculture*, 9(1), 20220332.
22. Ahmed, S., et al. (2019). Plant-based green synthesis of metallic nanoparticles. *J. Advanced Research*, 18, 1–15.
23. Sunny, S. (2025). *Role of surface termination in charge storage mechanism of 2D metal carbide* (Doctoral dissertation, Université de Toulouse).
24. Balouiri, M., et al. (2016). Methods for evaluating antimicrobial activity. *Journal of Pharmaceutical Analysis*, 6(2).
25. Huq, M. A. (2020). Green synthesis of silver nanoparticles using *Pseudoduganellaeburnea* MAHUQ-39 and their antimicrobial mechanisms investigation against drug resistant human pathogens. *International journal of molecular sciences*, 21(4), 1510.
26. Mohamad, N. R., Abdul Gani, S. S., Abdul Wahab, R., & Darham, W. (2022). Natural antioxidant from Hibiscus sabdariffa extract: Assessments on extraction yield, antioxidant capacity and total polyphenol content of different polarities of solvent extracts. *Journal of Smart Science and Technology*, 2(2), 52-60.
27. Eker, F., Duman, H., Akdaşçı, E., Witkowska, A. M., Bechelany, M., & Karav, S. (2024). Silver nanoparticles in therapeutics and beyond: A review of mechanism insights and applications. *Nanomaterials*, 14(20), 1618.
28. Zannat, M., Jahan, I., Rahaman, M. A., Yeasmin, N. J., & Angon, P. B. (2024). Potentiality of Metal Nanoparticles in Precision and Sustainable Agriculture.

29. Keshari, A. K., Srivastava, R., Singh, P., Yadav, V. B., & Nath, G. (2020). Antioxidant and antibacterial activity of silver nanoparticles synthesized by *Cestrum nocturnum*. *Journal of Ayurveda and integrative medicine*, 11(1), 37-44.
30. Hussein, H., Ibrahim, S. S., & Khairy, S. A. (2025). Green synthesis of ZnO nanoparticles using *Hibiscus sabdariffa* L: Rapid Pb²⁺ ion removal, photocatalytic degradation of methylene blue, and biomedical applications. *Journal of Water Process Engineering*, 69, 106649.
31. Tasnim, A., Roy, A., Akash, S. R., Ali, H., Habib, M. R., Barasarathi, J., ... & Yeasmin, T. (2024). *Hibiscus sabdariffa* L. petal biomass: A green source of nanoparticles of multifarious potential. *Open Agriculture*, 9(1), 20220332.
32. Mohammed Atiyah, M., Shnawa Jasim, H., & Mohammed Atiyah, H. (2022). Phytochemical screening and antibacterial activities of aqueous and alcoholic extracts of *Averrhoa bilimbi* leaf against bacteria isolated from oral cavity. *Archives of Razi Institute*, 77(2), 923-928.
33. Al-Otibi, F. O., Yassin, M. T., Al-Askar, A. A., & Maniah, K. (2023). Green biofabrication of silver nanoparticles of potential synergistic activity with antibacterial and antifungal agents against some nosocomial pathogens. *Microorganisms*, 11(4), 945.
34. Gaur, P., Hada, V., Rath, R. S., Mohanty, A., Singh, P., & Rukadikar, A. (2023). Interpretation of antimicrobial susceptibility testing using European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) breakpoints: analysis of agreement. *Cureus*, 15(3).

35. Li, C. W., Li, L. L., Chen, S., Zhang, J. X., & Lu, W. L. (2020). Antioxidant nanotherapies for the treatment of inflammatory diseases. *Frontiers in bioengineering and biotechnology*, 8, 200.
36. George, J., Edwards, D., Pun, S., & Williams, D. (2022). Evaluation of antioxidant capacity (ABTS and CUPRAC) and total phenolic content (Folin-Ciocalteu) assays of selected fruit, vegetables, and spices. *International Journal of Food Science*, 2022(1), 2581470.
37. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*. 1983;65(1–2):55–63.
38. Stockert, J. C., Blázquez-Castro, A., Cañete, M., Horobin, R. W., & Villanueva, Á. (2012). MTT assay for cell viability: Intracellular localization of the formazan product is in lipid droplets. *Acta histochemica*, 114(8), 785-796.
39. Aljeldah, M. M., Yassin, M. T., Mostafa, A. A. F., & Aboul-Soud, M. A. (2023). Synergistic antibacterial potential of greenly synthesized silver nanoparticles with fosfomycin against some nosocomial bacterial pathogens. *Infection and Drug Resistance*, 125-142.
40. Tasnim, A., Roy, A., Akash, S. R., Ali, H., Habib, M. R., Barasarathi, J., ... & Yeasmin, T. (2024). Hibiscus sabdariffa L. petal biomass: A green source of nanoparticles of multifarious potential. *Open Agriculture*, 9(1), 20220332.
41. Ahirwar, B., Ahirwar, D., Jain, R., Agrawal, B., Sahu, P., Sakure, K., & Badwaik, H. (2023). Biofabricated green synthesized hibiscus silver nanoparticles potentiates antibacterial and cytotoxicity in human lung cancer cells.

42. Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. *Green chemistry*, 13(10), 2638-2650.
43. Maeah, R. K., Hasoon, B. A. A. H., Abdalwahab, A. I., Al-Azawi, K. F. F., & Hameedi, W. B. A. (2020). Biosynthesis of silver nanoparticles using Hibiscus sabdariffa and their biological application. *EurAsian Journal of Biosciences*, 14(2), 3377-3383.
44. Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology advances*, 27(1), 76-83.
45. Mittal, A. K., Chisti, Y., & Banerjee, U. C. (2013). Synthesis of metallic nanoparticles using plant extracts. *Biotechnology advances*, 31(2), 346-356.
46. Kareem, E. A. A., Sultan, A. E., & Oraibi, H. M. (2023). Synthesis and characterization of silver nanoparticles: A review. *Ibn AL-Haitham Journal for Pure and Applied Sciences*, 36(3), 177-200.
47. Fayaz, A. M., Balaji, K., Girilal, M., Yadav, R., Kalaichelvan, P. T., & Venketesan, R. (2010). Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria. *Nanomedicine: Nanotechnology, Biology and Medicine*, 6(1), 103-109.
48. Hwang IS, Lee J, Hwang JH, Kim KJ, Lee DG. Silver nanoparticles induce apoptotic cell death in *Candida albicans* through the increase of hydroxyl radicals. *The FEBS Journal*. 2012;279(7):1327–1338.
49. Eker F, Duman H, Akdaşçı E, Witkowska AM, Bechelany M, Karav S. Silver nanoparticles in therapeutics and beyond: A review of mechanism insights and applications. *Nanomaterials*. 2024;14(20):1618.

50. Atiyah MM, Merlin T, Vijayan S. Antifungal Potential of *Sophora flavescens* Root Extract: HR-LCMS Analysis and Molecular Docking of Bioactive Compounds Against Nosocomial Fungal Pathogens. *J Pure Appl Microbiol.* 2025;19(3):1854-1867. doi: 10.22207/JPAM.19.3.10.
51. Asokan S, Pandey RK, Jalil MA, Abd Alhussen SK, Yousif SI, Abbas RK, Vijayan S, Rajeswary D, Jacob T, Atiyah MM. Biofilm associated infections on medical devices: pathogenesis, diagnostic challenges, and control strategies. *The Microbe.* 2026;100712.
52. Asokan S, Banerjee N, Saleem M, Atiyah HM, Pandey RK, Abbas RK, Yousif SI, Radhamanalan G, Parashar A, Gowtham B, Balaji VK. Healthcare associated infections (HAI): insights into epidemiology, microbiology, and diagnostics. *Diagnostic Microbiology and Infectious Disease.* 2026;117376.
53. Mousavi B, Tafvizi F, Zaker Bostanabad S. Green synthesis of silver nanoparticles using *Artemisia turcomanica* leaf extract and study of anti-cancer effect and apoptosis induction on gastric cancer cell line (AGS). *Artificial Cells, Nanomedicine, and Biotechnology.* 2018;46(S1):499–510.
54. Tran QH, Le AT. Silver nanoparticles: Synthesis, properties, toxicology, applications and perspectives. *Advances in Natural Sciences: Nanoscience and Nanotechnology.* 2013;4(3):033001.
55. Kotakadi, V. S., Gaddam, S. A., Rao, Y. S., Prasad, T. N. V. K. V., Reddy, A. V., & Gopal, D. S. (2014). Biofabrication of silver nanoparticles using *Andrographis paniculata*. *European Journal of Medicinal Chemistry*, 73, 135-140.
56. Huang H, Yang X. Synthesis of polysaccharide-stabilized gold and silver nanoparticles: A green method. *Carbohydrate Research.* 2004;339(15):2627–2631.

- 591 57. Chen L, et al. Antioxidant properties of plant-mediated nanoparticles. *Journal of*
592 *Nanobiotechnology*. 2020;18:192.
- 593 58. Asokan S, Sony S, Jacob T, Jisha MS, Vijayan S. Biogenic ZnO nanoparticle-coated
594 endotracheal tubes: A study on their biocompatibility and antibacterial activity. *Next*
595 *Nanotechnology*. 2025;8:100274.

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