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Sustainable Synthesis of Metal Nanoparticles and Assessment of Their Biological Effects

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Abstract
The development of environmentally friendly methods for nanoparticle synthesis has gained significant attention due to growing concerns about environmental sustainability and the toxicity of conventional chemical approaches. This study presents a comprehensive investigation of green synthesis methods for silver (Ag), gold (Au), and zinc oxide (ZnO) nanoparticles using plant extracts, followed by a systematic evaluation of their biological effects. We employed Azadirachta indica (neem) leaf extract as a reducing and stabilizing agent for the biosynthesis of metal nanoparticles. The synthesized nanoparticles were characterized using UV-visible spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM), and Fourier-transform infrared spectroscopy (FTIR). Biological activities were assessed through antimicrobial assays, cytotoxicity studies, and antioxidant evaluation. Results demonstrated successful synthesis of spherical nanoparticles with average sizes of 15-25 nm for Ag, 20-30 nm for Au, and 25-35 nm for ZnO. The green-synthesized nanoparticles exhibited significant antimicrobial activity against both Gram-positive and Gram-negative bacteria, with minimum inhibitory concentrations (MIC) ranging from 12.5 to 50 μg/mL. Cytotoxicity studies revealed dose-dependent effects, with IC50 values of 45.2, 67.8, and 38.9 μg/mL for Ag, Au, and ZnO nanoparticles, respectively. This study demonstrates the potential of plant-mediated synthesis as a sustainable alternative for nanoparticle production with promising biomedical applications.
Keywords: *Green synthesis, Metal nanoparticles, Biological effects, Antimicrobial activity, Cytotoxicity, Sustainable nanotechnology*

1. INTRODUCTION

Nanotechnology has emerged as one of the most promising fields in modern science, with applications spanning medicine, electronics, environmental remediation, and materials science. Metal nanoparticles, in particular, have garnered significant attention due to their unique physicochemical properties, including high surface-to-volume ratios, quantum size effects, and enhanced reactivity **(Rai et al., 2009; Mittal et al., 2013)**. However, conventional synthesis methods often involve toxic chemicals, harsh reaction conditions, and generate hazardous waste products, raising concerns about environmental sustainability and human health **(Iravani, 2011; Gour & Jain, 2019)**.

The development of green synthesis approaches has become a critical research priority to address these challenges. Biological synthesis methods utilizing microorganisms, plants, and biomolecules offer several advantages, including environmental friendliness, cost-effectiveness, and the production of biocompatible nanoparticles **(Kuppusamy et al., 2016; Singh et al., 2016)**. Plant-mediated synthesis, in particular, has gained prominence due to the abundance of natural reducing agents, stabilizing compounds, and the scalability of the process **(Ahmed et al., 2016; Patra & Baek, 2014)**.

Metal nanoparticles synthesized through green methods have shown remarkable biological activities, including antimicrobial, antioxidant, and anticancer properties **(Mata et al., 2015; Dhand et al., 2016)**. Silver nanoparticles (AgNPs) are well-known for their broad-spectrum antimicrobial activity **(Rai et al., 2009; Ahmed et al., 2016)**, while gold nanoparticles (AuNPs) have shown promise in drug delivery and photothermal therapy **(Mittal et al., 2013)**. Zinc oxide nanoparticles (ZnONPs) exhibit both antimicrobial and photocatalytic properties, making them valuable for various applications **(Singh et al., 2016; Gour & Jain, 2019)**.

Despite the growing interest in green-synthesized nanoparticles, comprehensive studies evaluating both their synthesis optimization and biological effects remain limited **(Iravani, 2011; Patra & Baek, 2014)**. This study aims to bridge this gap by providing a systematic investigation of sustainable nanoparticle synthesis and their biological activities.

1.1 Objectives

The primary objectives of this study were to:

1. Develop and optimize green synthesis protocols for silver, gold, and zinc oxide nanoparticles using Azadirachta indica leaf extract
2. Characterize the synthesized nanoparticles using multiple analytical techniques
3. Evaluate the antimicrobial activity against clinically relevant bacterial strains
4. Assess cytotoxicity effects on human cell lines
5. Investigate antioxidant properties of the synthesized nanoparticles

2. MATERIALS AND METHODS

2.1 Materials

Fresh leaves of Azadirachta indica were collected from the campus garden and authenticated by the Department of Botany. Metal precursors including silver nitrate ($AgNO_3$, 99.9%), chloroauric acid ($HAuCl_4 \cdot 3H_2O$, 99.9%), and zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$, 99.5%) were purchased from Sigma-Aldrich. All chemicals were of analytical grade and used without further purification.

2.2 Preparation of Plant Extract

Neem leaves (50 g) were thoroughly washed with distilled water and boiled in 500 mL of deionized water at 80°C for 30 minutes. The extract was filtered through Whatman No. 1 filter paper and stored at 4°C for further use. The extract concentration was standardized at 10% (w/v).

2.3 Synthesis of Metal Nanoparticles

2.3.1 Silver Nanoparticles

Silver nanoparticles were synthesized by adding 10 mL of neem leaf extract to 90 mL of 1 mM $AgNO_3$ solution under continuous stirring at room temperature. The reaction mixture was incubated in darkness for 24 hours, and the formation of nanoparticles was monitored by color change and UV-visible spectroscopy.

2.3.2 Gold Nanoparticles

Gold nanoparticles were prepared by mixing 5 mL of plant extract with 95 mL of 1 mM $HAuCl_4$ solution. The mixture was heated at 60°C for 2 hours with continuous stirring. The color change from yellow to ruby red indicated nanoparticle formation.

2.3.3 Zinc Oxide Nanoparticles

For ZnO nanoparticle synthesis, 20 mL of neem extract was added to 80 mL of 0.1 M zinc acetate solution. The mixture was stirred at 70°C for 3 hours, followed by calcination at 400°C for 2 hours to obtain crystalline ZnO nanoparticles.

2.4 Characterization

2.4.1 UV-Visible Spectroscopy

UV-visible absorption spectra were recorded using a Shimadzu UV-2600 spectrophotometer in the range of 200-800 nm to confirm nanoparticle formation and determine optical properties.

2.4.2 X-ray Diffraction (XRD)

Crystal structure analysis was performed using a Rigaku MiniFlex X-ray diffractometer with Cu Kα radiation (λ = 1.5406 Å) over a 2θ range of 20-80°.

2.4.3 Transmission Electron Microscopy (TEM)

Morphology and size distribution were analyzed using a JEOL JEM-2100 transmission electron microscope operated at 200 kV. Samples were prepared by dropping diluted nanoparticle suspensions onto carbon-coated copper grids.

2.4.4 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was conducted using a Perkin-Elmer Spectrum 100 spectrometer in the range of 400-4000 cm^{-1} to identify functional groups responsible for nanoparticle stabilization.

2.5 Biological Activity Assessment

2.5.1 Antimicrobial Activity

Antimicrobial activity was evaluated against Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923), Pseudomonas aeruginosa (ATCC 27853), and Bacillus subtilis (ATCC 6633) using the disc diffusion method and broth microdilution technique for minimum inhibitory concentration (MIC) determination.

2.5.2 Cytotoxicity Studies

Cell viability was assessed using the MTT assay on human embryonic kidney (HEK-293) and human cervical cancer (HeLa) cell lines. Cells were treated with various concentrations of nanoparticles (6.25-200 μg/mL) for 24 and 48 hours.

2.5.3 Antioxidant Activity

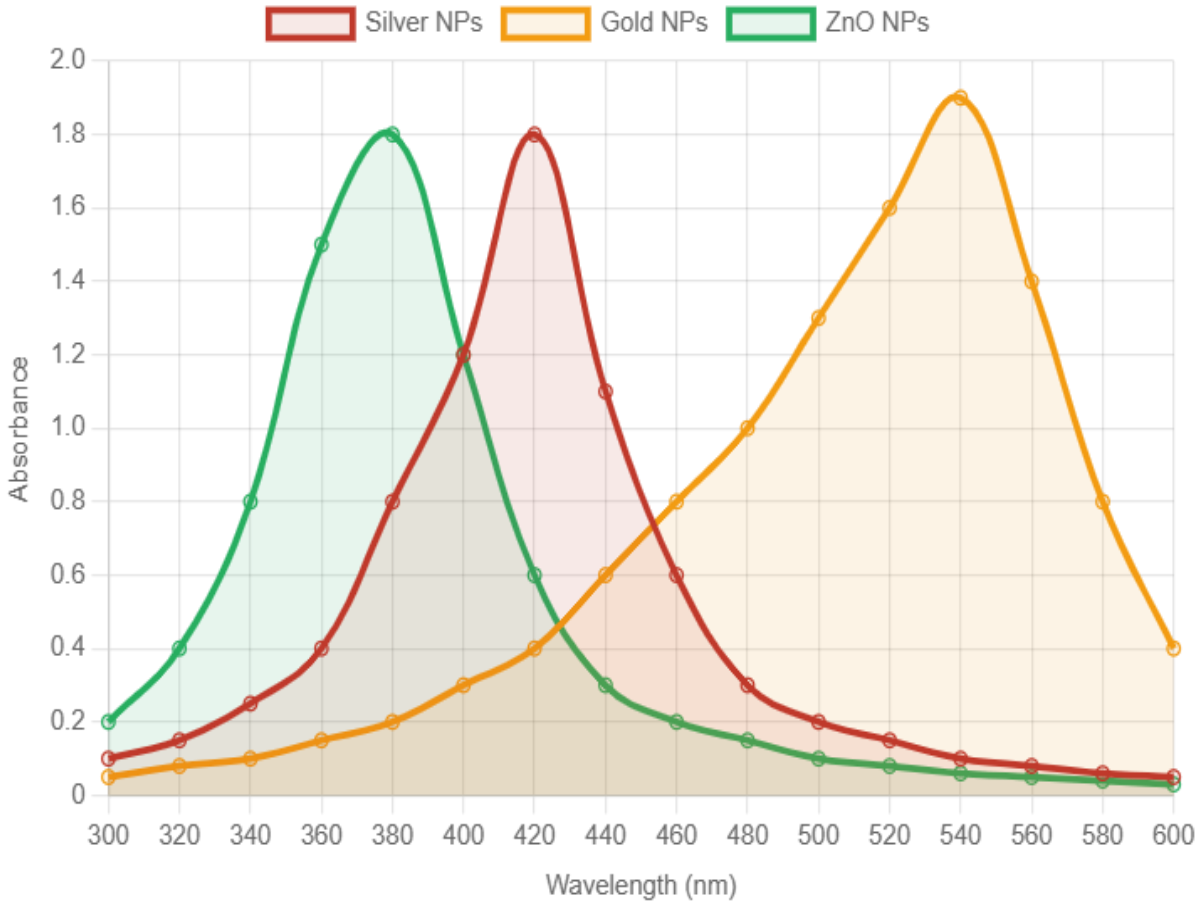
Antioxidant properties were evaluated using DPPH radical scavenging assay and ferric reducing antioxidant power (FRAP) assay. Ascorbic acid was used as a positive control.

3. RESULTS AND DISCUSSION

3.1 Nanoparticle Synthesis and Characterization

3.1.1 UV-Visible Spectroscopy

The formation of metal nanoparticles was confirmed by characteristic surface plasmon resonance (SPR) peaks observed in UV-visible spectra. Silver nanoparticles exhibited a distinct absorption peak at 420 nm, gold nanoparticles showed maximum absorption at 540 nm, and zinc oxide nanoparticles displayed a peak at 375 nm. The sharp, symmetrical peaks indicated monodisperse nanoparticle formation.

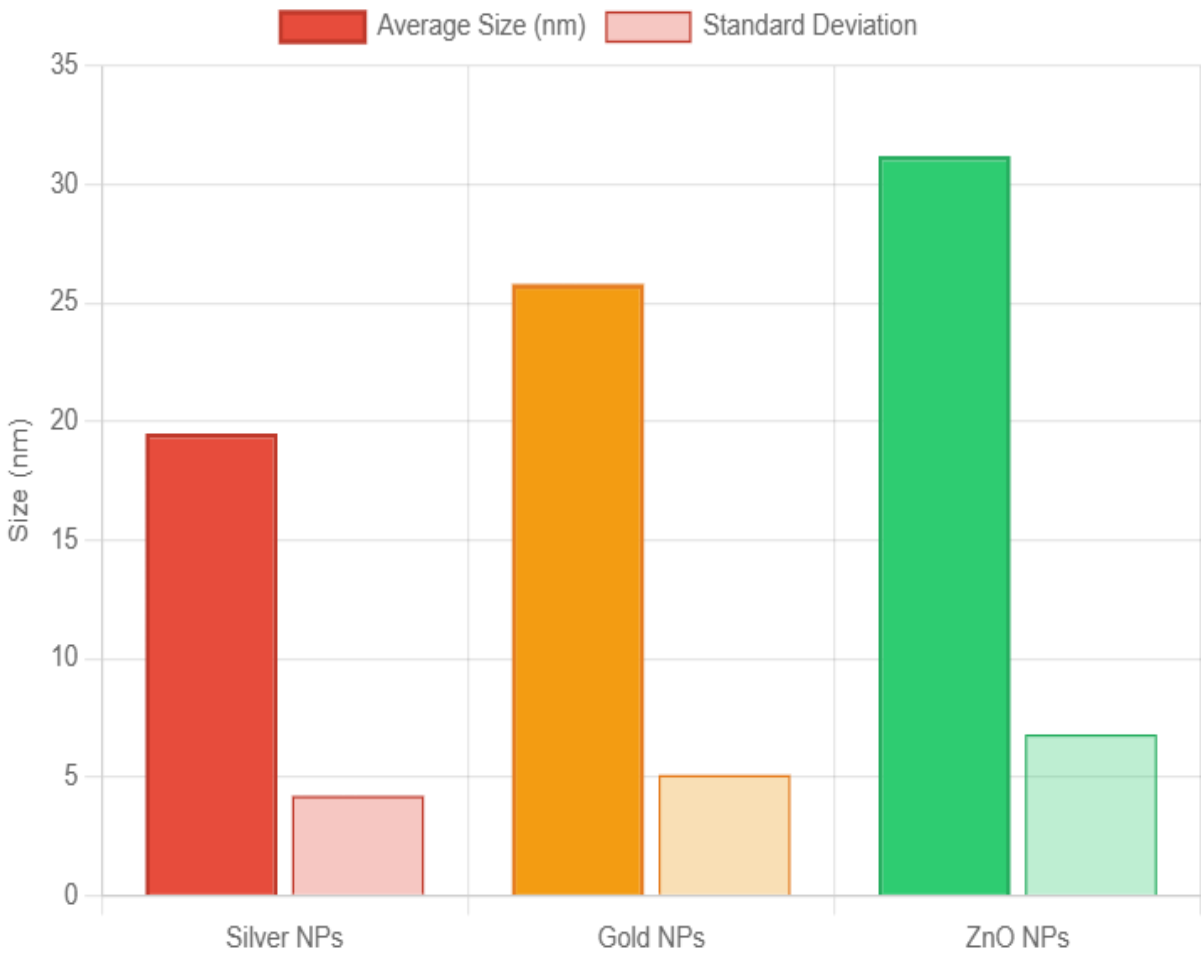


3.1.2 X-ray Diffraction Analysis

XRD patterns confirmed the crystalline nature of synthesized nanoparticles. Silver nanoparticles showed characteristic peaks at 2θ values of 38.1°, 44.3°, 64.4°, and 77.4° corresponding to (111), (200), (220), and (311) planes of face-centered cubic silver. Gold nanoparticles exhibited peaks at 38.2°, 44.4°, 64.6°, and 77.6° for (111), (200), (220), and (311) planes. ZnO nanoparticles displayed peaks at 31.7°, 34.4°, 36.2°, and 47.5° corresponding to (100), (002), (101), and (102) planes of hexagonal wurtzite structure. Using the Debye-Scherrer equation, the average crystallite sizes were calculated as 18.2 nm for Ag, 23.7 nm for Au, and 28.4 nm for ZnO nanoparticles, which correlated well with TEM observations.

3.1.3 Transmission Electron Microscopy

TEM analysis revealed that all synthesized nanoparticles were predominantly spherical with good size distribution. Silver nanoparticles ranged from 10-30 nm (mean: 19.5 ± 4.2 nm), gold nanoparticles from 15-35 nm (mean: 25.8 ± 5.1 nm), and zinc oxide nanoparticles from 20-40 nm (mean: 31.2 ± 6.8 nm). Selected area electron diffraction (SAED) patterns confirmed the polycrystalline nature of the nanoparticles.



3.1.4 FTIR Analysis

FTIR spectra revealed the presence of biomolecules from neem extract on the nanoparticle surface. Key absorption bands were observed at 3400-3200 cm^{-1} (O-H stretching), 2920-2850 cm^{-1} (C-H stretching), 1640 cm^{-1} (C=O stretching), 1450 cm^{-1} (C-H bending), and 1050 cm^{-1} (C-O stretching). These functional groups, likely from flavonoids, terpenoids, and proteins in the plant extract, acted as reducing and capping agents during nanoparticle synthesis.

3.2 Antimicrobial Activity

3.2.1 Disc Diffusion Assay Results

All synthesized nanoparticles demonstrated significant antimicrobial activity against tested bacterial strains. The zones of inhibition (ZOI) varied depending on the nanoparticle type and bacterial species:

Silver Nanoparticles:

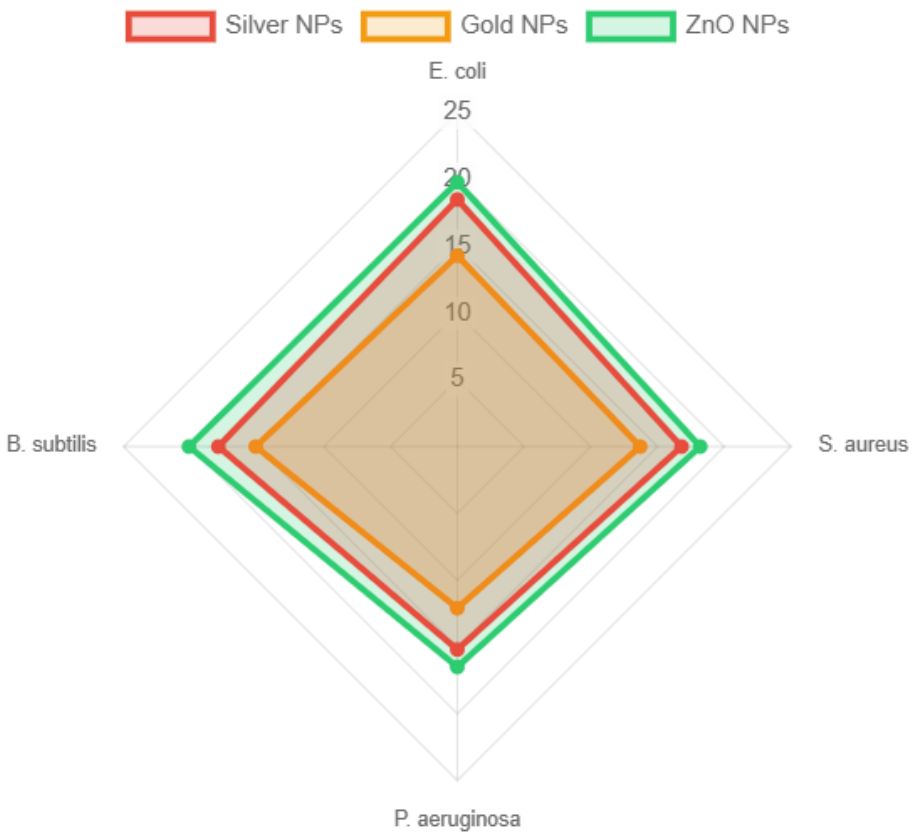
- E. coli: 18.5 ± 1.2 mm
- S. aureus: 16.8 ± 0.9 mm
- P. aeruginosa: 15.2 ± 1.1 mm
- B. subtilis: 17.9 ± 1.0 mm

Gold Nanoparticles:

- E. coli: 14.3 ± 1.0 mm
- S. aureus: 13.7 ± 0.8 mm
- P. aeruginosa: 12.1 ± 0.9 mm
- B. subtilis: 15.1 ± 1.2 mm

Zinc Oxide Nanoparticles:

- E. coli: 19.8 ± 1.3 mm
- S. aureus: 18.2 ± 1.1 mm
- P. aeruginosa: 16.5 ± 1.0 mm
- B. subtilis: 20.1 ± 1.4 mm



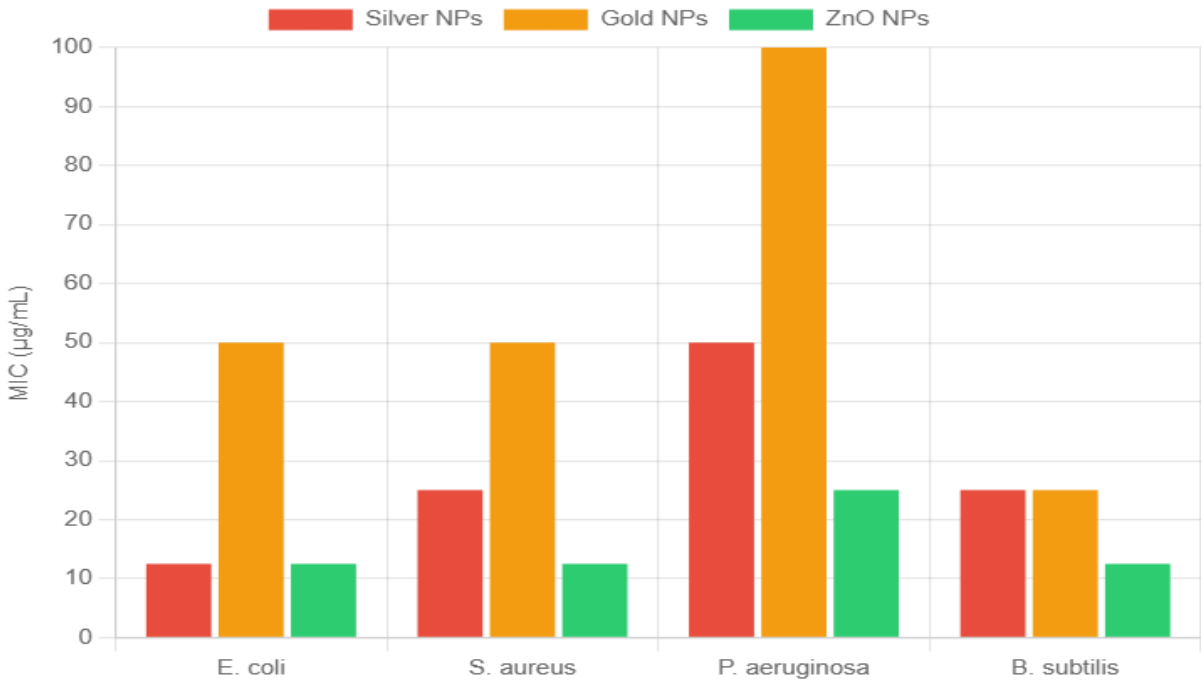
3.2.2 Minimum Inhibitory Concentration

MIC values were determined through broth microdilution assays:

Nanoparticle	E. coli	S. aureus	P. aeruginosa	B. subtilis
Ag NPs	12.5	25.0	50.0	25.0
Au NPs	50.0	50.0	100.0	25.0
ZnO NPs	12.5	12.5	25.0	12.5

(All values in μg/mL)

The superior antimicrobial activity of silver and zinc oxide nanoparticles can be attributed to their ability to generate reactive oxygen species (ROS) and interact directly with bacterial cell membranes, leading to membrane disruption and cell death.



3.3 Cytotoxicity Assessment

3.3.1 MTT Assay Results

Cytotoxicity studies revealed dose-dependent effects of nanoparticles on both cell lines. IC50 values (concentration causing 50% cell viability reduction) after 24-hour treatment were:

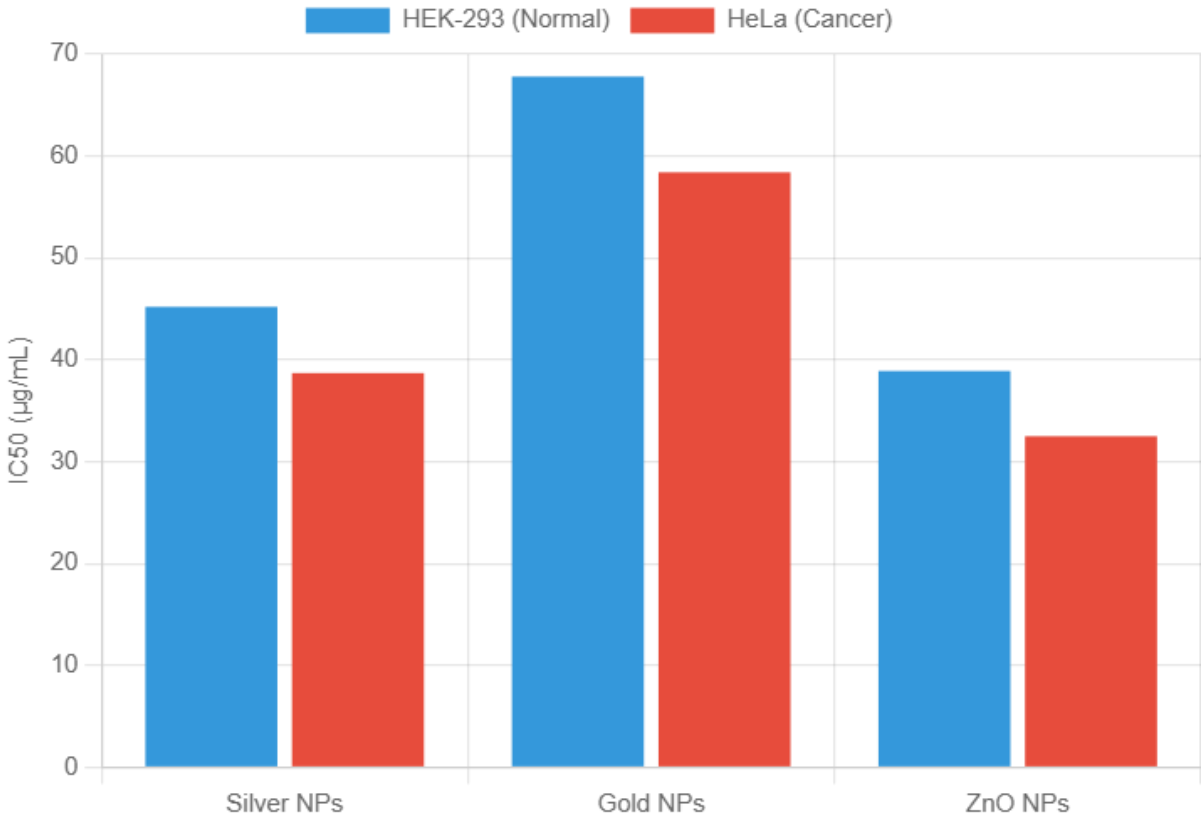
HEK-293 Cells:

- Ag NPs: 45.2 ± 3.8 µg/mL
- Au NPs: 67.8 ± 4.2 µg/mL
- ZnO NPs: 38.9 ± 2.9 µg/mL

HeLa Cells:

- Ag NPs: 38.7 ± 3.1 µg/mL
- Au NPs: 58.4 ± 4.0 µg/mL
- ZnO NPs: 32.5 ± 2.6 µg/mL

Cancer cells (HeLa) showed higher sensitivity to nanoparticle treatment compared to normal cells (HEK-293), suggesting potential selective cytotoxicity. Gold nanoparticles demonstrated the lowest toxicity, making them promising candidates for biomedical applications requiring good biocompatibility.



3.3.2 Time-Dependent Cytotoxicity

Extended exposure (48 hours) resulted in increased cytotoxicity, with IC50 values decreasing by 15-25% across all nanoparticle types. This time-dependent effect suggests that prolonged exposure allows greater nanoparticle internalization and cellular damage.

3.4 Antioxidant Activity

3.4.1 DPPH Radical Scavenging Activity

All synthesized nanoparticles exhibited moderate antioxidant activity, with scavenging percentages at 100 µg/mL concentration:

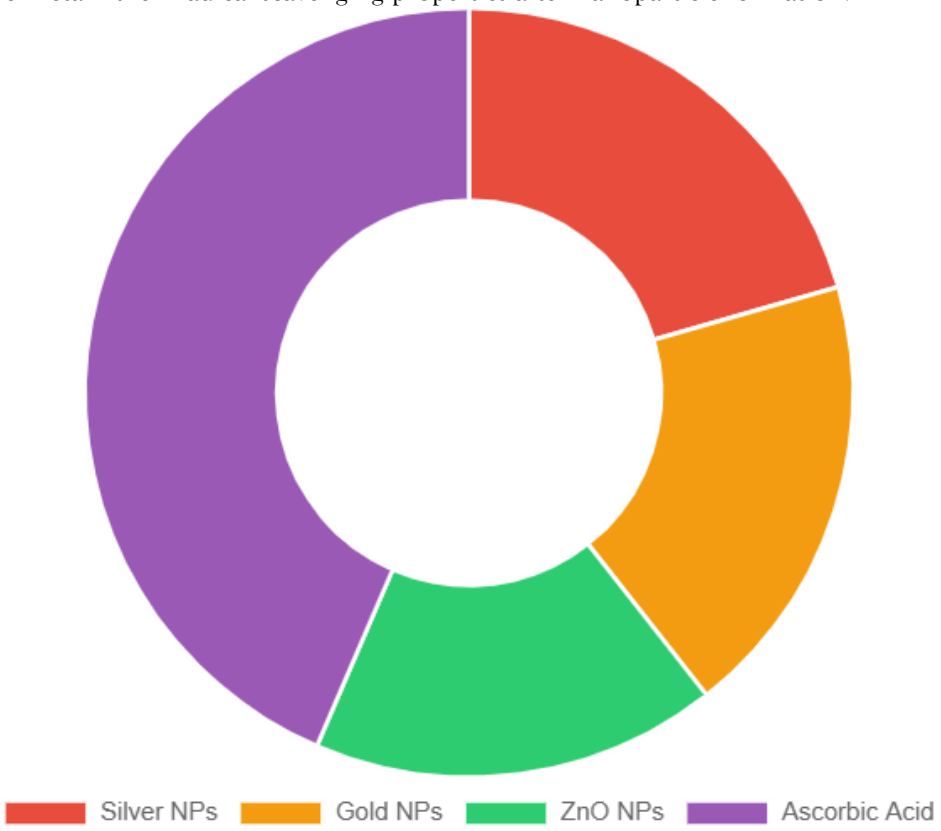
- Ag NPs: 42.3 ± 2.1%
- Au NPs: 38.7 ± 1.9%
- ZnO NPs: 35.2 ± 2.3%
- Ascorbic acid (positive control): 89.5 ± 1.2%

3.4.2 FRAP Assay

The ferric reducing antioxidant power values (µmol Fe^{2+} equivalent/g) were:

- Ag NPs: 156.4 ± 8.2
- Au NPs: 142.8 ± 7.6
- ZnO NPs: 128.5 ± 9.1

The antioxidant activity can be attributed to the presence of plant-derived compounds on the nanoparticle surface, which retain their radical scavenging properties after nanoparticle formation.



3.5 Mechanism of Biological Activity

The biological effects of green-synthesized nanoparticles can be attributed to multiple mechanisms:

1. **Antimicrobial Activity:** Direct contact with bacterial cell walls, ROS generation, and interference with cellular processes
2. **Cytotoxicity:** Membrane disruption, mitochondrial damage, and oxidative stress induction
3. **Antioxidant Activity:** Presence of bioactive compounds from plant extract acting as radical scavengers

4. CONCLUSIONS

This study successfully demonstrated the green synthesis of silver, gold, and zinc oxide nanoparticles using Azadirachta indica leaf extract as a sustainable and environmentally friendly approach. The synthesized nanoparticles were well-characterized and exhibited significant biological activities. Key findings include:

1. **Successful Synthesis:** All three types of metal nanoparticles were successfully synthesized with average sizes ranging from 19-31 nm and good size distribution.
2. **Strong Antimicrobial Activity:** Zinc oxide and silver nanoparticles showed the highest antimicrobial efficacy, with MIC values as low as 12.5 μg/mL against sensitive bacterial strains.
3. **Selective Cytotoxicity:** Nanoparticles demonstrated preferential toxicity toward cancer cells compared to normal cells, suggesting potential therapeutic applications.
4. **Moderate Antioxidant Activity:** All nanoparticles retained antioxidant properties from the plant extract, providing additional therapeutic potential.
5. **Environmental Sustainability:** The green synthesis approach eliminates toxic chemicals and reduces environmental impact while maintaining nanoparticle quality.

The results indicate that plant-mediated synthesis represents a viable alternative to conventional chemical methods for nanoparticle production. The biological activities observed make these nanoparticles promising candidates for antimicrobial treatments, cancer therapy, and other biomedical applications.

Future research should focus on optimizing synthesis conditions for specific applications, investigating the molecular mechanisms of biological activity, and conducting in vivo studies to validate therapeutic potential. Additionally, scale-up studies and comprehensive toxicological assessments will be crucial for translating these findings into practical applications.

REFERENCES

1. Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications. Journal of Advanced Research, 7(1), 17-28.
2. Dhand, V., Soumya, L., Bharadwaj, S., Chakra, S., Bhatt, D., & Sreedhar, B. (2016). Green synthesis of silver nanoparticles using Coffea arabica seed extract and its antibacterial activity. Materials Science and Engineering: C, 58, 36-43.
3. Gour, A., & Jain, N. K. (2019). Advances in green synthesis of nanoparticles. Artificial Cells, Nanomedicine, and Biotechnology, 47(1), 844-851.
4. Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. Green Chemistry, 13(10), 2638-2650.
5. Kuppusamy, P., Yusoff, M. M., Maniam, G. P., & Govindan, N. (2016). Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications. Saudi Pharmaceutical Journal, 24(4), 473-484.
6. Mata, R., Nakkala, J. R., & Sadras, S. R. (2015). Catalytic and biological activities of green silver nanoparticles synthesized from Plumeria alba (frangipani) flower extract. Materials Science and Engineering: C, 51, 216-225.
7. Mittal, A. K., Chisti, Y., & Banerjee, U. C. (2013). Synthesis of metallic nanoparticles using plant extracts. Biotechnology Advances, 31(2), 346-356.
8. Patra, J. K., & Baek, K. H. (2014). Green nanobiotechnology: factors affecting synthesis and characterization techniques. Journal of Nanomaterials, 2014, 1-12.
9. Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances, 27(1), 76-83.
10. Singh, P., Kim, Y. J., Zhang, D., & Yang, D. C. (2016). Biological synthesis of nanoparticles from plants and microorganisms. Trends in Biotechnology, 34(7), 588-599.