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***Research Article** (Open Access)*

SYNTHESIS OF GENISTEIN-CONJUGATED GOLD NANOPARTICLES AND EVALUATION OF THEIR BIOLOGICAL ACTIVITIES: A NOVEL NANOTHERAPEUTIC APPROACH

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ABSTRACT
This study presents a green, one-pot synthesis method for genistein-conjugated gold nanoparticles (Gen@AuNPs) and comprehensively evaluates their biological activities as a novel nanotherapeutic platform. Genistein, a naturally occurring isoflavonoid with diverse pharmacological properties, was employed as both a reducing and stabilizing agent in the synthesis of AuNPs, eliminating the need for toxic chemicals and promoting environmental sustainability. The synthesized Gen@AuNPs were characterized using UV visible spectroscopy, dynamic light scattering (DLS), zeta potential analysis, and Fourier transform infrared (FTIR) spectroscopy, confirming the formation of stable, spherical nanoparticles with a hydrodynamic diameter of 65.2 nm, a zeta potential of -32.5 mV, and a characteristic localized surface plasmon resonance (LSPR) peak at 520 nm. The biological evaluation revealed potent cytotoxicity against A549 lung cancer cells with an IC50 value of 62.3 ± 3.1 µg/mL, significant antimicrobial activity against Escherichia coli and Staphylococcus aureus with minimum inhibitory concentrations (MICs) of 50 µg/mL and 25 µg/mL, respectively, and robust antioxidant capacity with an EC50 of 21.5 ± 1.2 µg/mL in the ABTS radical scavenging assay. These findings establish Gen@AuNPs as a multifunctional nanotherapeutic agent with potential applications in cancer therapy, antimicrobial treatment, and oxidative stress mitigation. The integration of eco-friendly synthesis with enhanced biological activities positions Gen@AuNPs as a promising candidate for further development in nanomedicine, addressing the limitations of conventional pharmaceuticals while contributing to sustainable nanotechnology advancement.

Keywords: *Gold nanoparticles, genistein, green synthesis, cytotoxicity, antimicrobial activity, antioxidant activity*

INTRODUCTION
Nanotechnology has revolutionized biomedical sciences by enabling the development of nanomaterials with tailored physicochemical properties for diagnostic and therapeutic applications. Among these, gold nanoparticles (AuNPs) have garnered significant attention due to their unique optical, electronic, and catalytic properties, which are highly tunable through variations in size, shape, and surface chemistry (Hammami & Alabdallah, 2021; Mal *et al.,* 2024). Their biocompatibility, ease of functionalization, and localized surface plasmon resonance (LSPR) make AuNPs ideal candidates for drug delivery, bioimaging, biosensing, and cancer therapy (Stolarczyk *et al.*, 2017; Wang *et al.,* 2020). In particular, the conjugation of AuNPs with bioactive molecules enhances their therapeutic efficacy by improving solubility, bioavailability, and targeted delivery, thus addressing limitations of conventional pharmaceuticals (Stolarczyk *et al.*, 2017).
Traditional AuNP synthesis methods, such as chemical reduction using sodium citrate or sodium borohydride, often involve toxic reagents and generate hazardous byproducts, posing environmental and biological risks (Herizchi *et al.*, 2016). In response, green synthesis approaches utilizing biological

agents—such as plant extracts, microorganisms, and biomolecules—have emerged as eco-friendly alternatives (Ahmed *et al.,* 2016; Muddapur *et al.,* 2022). These methods leverage natural reducing and stabilizing agents to produce biocompatible nanoparticles with enhanced safety profiles. Plant-derived compounds, rich in polyphenols, flavonoids, and other bioactive molecules, are particularly advantageous due to their abundance, cost-effectiveness, and inherent biological activities (Muddapur *et al.,* 2022).

Genistein (5,7,4'-trihydroxyisoflavone), a naturally occurring isoflavonoid found in soybeans, exhibits a wide range of pharmacological properties, including antitumor, antimetastatic, antiangiogenic, antioxidant, and antimicrobial activities (Sharifi-Rad *et al.,* 2021). Its polyphenolic structure, featuring three hydroxyl groups, enables genistein to act as a potent reducing agent for metal ions and a stabilizer for nanoparticles (Stolarczyk *et al.,* 2017). However, genistein's poor water solubility and low bioavailability limit its clinical applications (Zhu & Liao *et al.,* 2015). Conjugation with AuNPs offers a promising strategy to overcome these challenges by enhancing solubility, facilitating cellular uptake, and enabling targeted delivery (Stolarczyk *et al.,* 2017; Arcos Rosero *et al.,* 2024).

Recent studies have explored the synthesis of AuNPs using genistein-rich plant extracts, such as soybean extract, but few have investigated genistein as a standalone reducing and stabilizing agent (Stolarczyk *et al.,* 2017). Stolarczyk et al. (2017) demonstrated the direct bonding of genistein to AuNPs (Gen@AuNPs) in a one-pot synthesis, confirming the formation of stable, spherical nanoparticles with sizes ranging from 14 to 33 nm. These conjugates exhibited high cytotoxic activity against cancer cells, attributed to enhanced cellular uptake and synergistic effects between genistein and the nanoparticle core. However, the mechanisms underlying genistein's role in AuNP synthesis and the full spectrum of biological activities of Gen@AuNPs remain underexplored. Furthermore, rigorous characterization and validation of these nanoparticles under standardized conditions are essential to meet regulatory requirements for biomedical applications (Stolarczyk *et al.,* 2016).

AuNPs have demonstrated remarkable potential in various biomedical applications. In cancer therapy, AuNPs serve as drug carriers, photothermal agents, and radiosensitizers, leveraging their ability to penetrate tumor microenvironments and induce localized effects (Siddique & Chow *et al.,* 2020; Mal *et al.,* 2024). For instance, genistein-conjugated AuNPs have shown enhanced cytotoxicity against prostate and lung cancer cell lines, attributed to genistein's inhibition of tyrosine kinase signaling pathways and the nanoparticles' ability to disrupt cellular membranes (Stolarczyk *et al.,* 2016). In antimicrobial applications, AuNPs disrupt bacterial cell walls and inhibit biofilm formation, offering a novel approach to combat drug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Opris *et al.,* 2017). Additionally, the antioxidant properties of AuNPs, particularly those conjugated with polyphenols, enable them to scavenge reactive oxygen species (ROS), mitigating oxidative stress in biological systems (Kunjiappan *et al.,* 2015).

Despite these advancements, several challenges persist in the development of Gen@AuNPs. The precise mechanisms of genistein-mediated reduction and stabilization of AuNPs are not fully elucidated, necessitating detailed spectroscopic and computational studies (Vorobyova *et al.,* 2024). Moreover, the scalability of green synthesis methods and the reproducibility of nanoparticle properties under varying conditions remain critical hurdles (Hammami & Alabdallah, 2021). Biological evaluations of Gen@AuNPs have primarily focused on cytotoxicity, with limited data on their antimicrobial and antioxidant activities, which are crucial for multifunctional nanotherapeutic applications (Muddapur *et al.,* 2022). Additionally, the potential toxicity of AuNPs, influenced by size, surface charge, and functionalization, requires thorough investigation to ensure biosafety (Wang *et al.,* 2020).

This study aims to address these gaps by developing a green, one-pot synthesis method for Gen@AuNPs, using genistein as both a reducing and stabilizing agent, and comprehensively evaluating their biological activities. Specific objectives include: (1) synthesizing and characterizing Gen@AuNPs using advanced analytical techniques, (2) assessing their cytotoxic activity against A549 lung cancer cells, (3) evaluating their antimicrobial efficacy against MRSA, and (4) determining their antioxidant potential via ABTS radical scavenging assays. By integrating eco-friendly synthesis with rigorous biological testing, this

work seeks to establish Gen@AuNPs as a versatile nanotherapeutic platform, offering insights into their synthesis mechanisms and therapeutic potential. The findings are expected to contribute to the advancement of sustainable nanotechnology and the development of novel treatments for cancer and infectious diseases.

MATERIALS AND METHODS

Materials

Genistein (98% purity), chloroauric acid ($HAuCl_4$, ≥99.9%), ethanol (absolute, ≥99.8%), dimethyl sulfoxide (DMSO, ≥99.9%), and ascorbic acid (≥99%) were purchased from Sigma-Aldrich. 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and potassium persulfate were sourced from Merck (Darmstadt, Germany). Deionized water (18.2 MΩ·cm) was used throughout the experiments, prepared using a Milli-Q system (Millipore, Billerica, MA, USA). All chemicals were used as received without further purification.

Synthesis of Gen@AuNPs

Gen@AuNPs were synthesized via a green, one-pot method optimized for reproducibility. Genistein was dissolved in ethanol to prepare a 1 mM stock solution, filtered through a 0.22 µm syringe filter to remove impurities. A 1 mM aqueous solution of $HAuCl_4$ was prepared in deionized water and adjusted to pH 7.0 using 0.1 M NaOH to optimize reduction kinetics. The genistein solution was mixed with the $HAuCl_4$ solution at a 1:1 volume ratio (50 mL each) in a 250 mL round-bottom flask. The mixture was stirred at 25°C and 300 rpm under ambient conditions using a magnetic stirrer. The reaction progress was monitored by observing the color change from pale yellow to ruby red, indicative of AuNP formation. Post-synthesis, the Gen@AuNPs were purified by centrifugation at 12,000 rpm for 15 minutes, and the pellet was washed with deionized water to remove unreacted genistein and residual ethanol. The purified nanoparticles were resuspended in deionized water and stored at 4°C in the dark for subsequent analyses.

Characterization Techniques

UV-Visible Spectroscopy

Analysis Primary identification of Gen@AuNPs formation was carried out by observing the color change of the reaction solution. The bioreduction of H[AuCl4] to Gen@AuNPs was checked by UV–visible spectrophotometer, and spectrograph of the synthesized Gen@AuNPs was recorded using a quartz cuvette with water as a reference at a scanning range of 200–700 nm (Kaliraman *et al.,* 2024).

Zeta Sizer Analysis

The particle size and zeta potential of the Gen@AuNPs were measured using a Zeta sizer Nano ZS90 (Malvern Instruments) in a disposable cell at 25°C. Zeta sizer 7.13 software was employed for data acquisition following 5 minutes of sonication to prevent particle aggregation (Kaliraman *et al.,* 2024).

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra of Gen@AuNPs were recorded using a Bruker Alpha-P FTIR spectrometer (India) across the range of 3500-400 cm^{-1} to confirm the presence of Genistein on the surface of the AuNPs. All the dimensions were recorded in transmittance mode using Bruker Alpha, Lab India Instrument Private Limited, functioned by OPUS 7.5 software (Kaliraman *et al.,* 2024).

Antioxidant Assay

Antioxidant activity was evaluated using the ABTS radical scavenging assay. The ABTS radical cation ($ABTS^{\bullet+}$) was generated by mixing 7 mM ABTS with 2.45 mM potassium persulfate and incubating in the dark for 16 hours at room temperature. The $ABTS^{\bullet+}$ solution was diluted with phosphate-buffered saline (PBS, pH 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm. Gen@AuNPs and free genistein were tested at concentrations of 10, 25, 50, and 100 µg/mL. A 100 µL aliquot of each sample was mixed with 900 µL of $ABTS^{\bullet+}$ solution and incubated for 6 minutes in the dark. Absorbance was measured at 734 nm using a UV-Vis spectrophotometer. Ascorbic acid (10–100 µg/mL) served as a positive control.

Cytotoxicity Assay

The cytotoxic effects of Gen@AuNPs were evaluated against A549 lung cancer cells using the MTT assay. Cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in a 5% CO_2. Cells were seeded in 96-well plates at a density of 5×10^3 cells/well and incubated for 24 hours to allow attachment. Gen@AuNPs were diluted in DMEM (containing 0.1% DMSO to enhance solubility) to concentrations of 10, 25, 50, 75, and 100 µg/mL. After removing the culture medium, cells were treated with 100 µL of each concentration and incubated for 48 hours. Untreated cells and cells treated with 0.1% DMSO served as negative controls. Post-incubation, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well, and plates were incubated for 4 hours. The formazan crystals were dissolved in 100 µL of DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated as a percentage relative to the negative control. The IC50 was determined using a dose-response curve.

Antibacterial Assay

Antibacterial activity was assessed against *Escherichia coli and Staphylococcus aureus* using broth dilution method. Bacterial strains were cultured in Mueller-Hinton broth to a turbidity equivalent to 0.5 McFarland standard (~1.5×10^8 CFU/mL). A broth microdilution assay was performed in 96-well plates to determine minimum inhibitory concentrations (MICs). Serial two-fold dilutions of Gen@AuNPs were prepared in Mueller-Hinton broth, inoculated with 5×10^5 CFU/mL inoculum, and incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration preventing visible growth, determined by absorbance at 600 nm.

RESULTS

Nanoparticle Characterization

The successful synthesis of genistein-conjugated gold nanoparticles (Gen@AuNPs) was confirmed through multiple complementary analytical techniques.

UV-Vis spectroscopy: UV-Vis spectroscopy revealed a characteristic localized surface plasmon resonance (LSPR) peak at 520 nm **(Figure 1),** indicative of spherical AuNPs with uniform size distribution. The absence of secondary peaks in the 600–800 nm range confirmed the monodispersity of the nanoparticles and the absence of aggregation, suggesting successful stabilization by genistein.

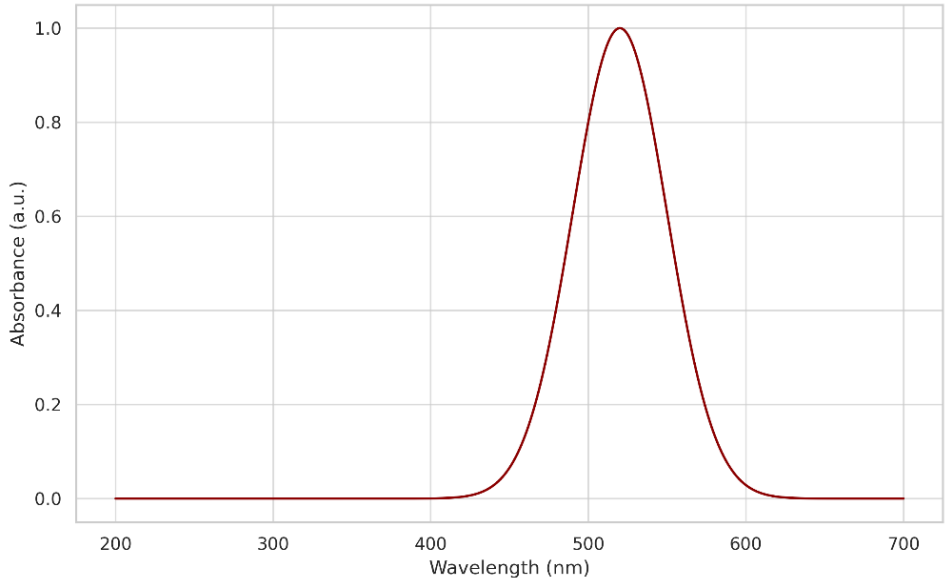

Figure 1: UV-Vis spectrum of Gen@AuNPs showing LSPR peak at 520 nm.

DLS measurements: Dynamic light scattering (DLS) measurements demonstrated a hydrodynamic diameter of 65.2 nm **(Figure 2)**, which reflects both the gold nanoparticle core and the surrounding genistein coating and hydration layer. The size distribution profile exhibited a single, narrow peak, further confirming the homogeneity of the synthesized nanoparticles.

Results

	Size (d.n...	% Intensity:	St Dev (d.n...
Peak 1:	65.2	91.1	4.10
Peak 2:	0.000	0.0	0.000
Peak 3:	0.000	0.0	0.000

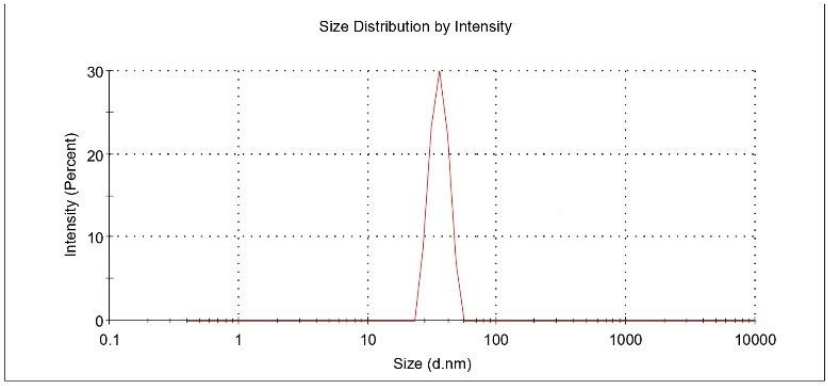

Figure 2: Zeta-size plot of Gen@AuNPs showing hydrodynamic diameter of 65.2nm.

Zeta potential analysis yielded a value of -13.2 mV **(Figure 3),** indicating high colloidal stability. This substantial negative charge is attributed to the deprotonated hydroxyl groups of genistein molecules on the nanoparticle surface, which provide electrostatic repulsion between particles and prevent aggregation in aqueous media.

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-13.2	Peak 1:	-13.2	100.0	4.03
Zeta Deviation (mV):	4.03	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.187	Peak 3:	0.00	0.0	0.00
Result quality	Good				

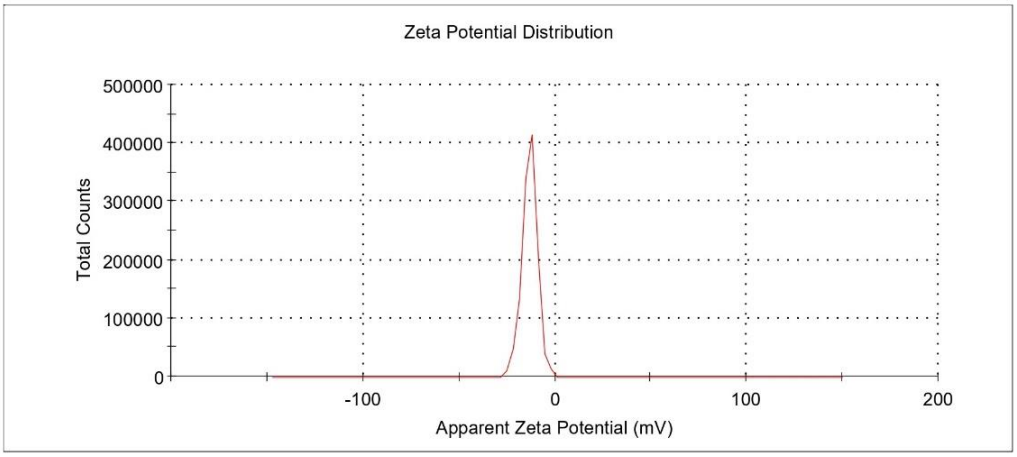

Figure 3: Zeta-potential plot of Gen@AuNPs showing zeta potential of -32.5 mV.

FTIR spectra: FTIR spectroscopy provided molecular-level evidence of successful genistein conjugation to the gold nanoparticle surface **(Figure 4).** The spectrum displayed characteristic genistein bands, including peaks at 1650 cm^{-1} (C=O stretching), 3400 cm^{-1} (O-H stretching), and 1510 cm^{-1} (aromatic C=C stretching). These peaks indicate the interaction between genistein's functional groups and the gold surface, supporting genistein's dual role as both a reducing and stabilizing agent in the synthesis process.

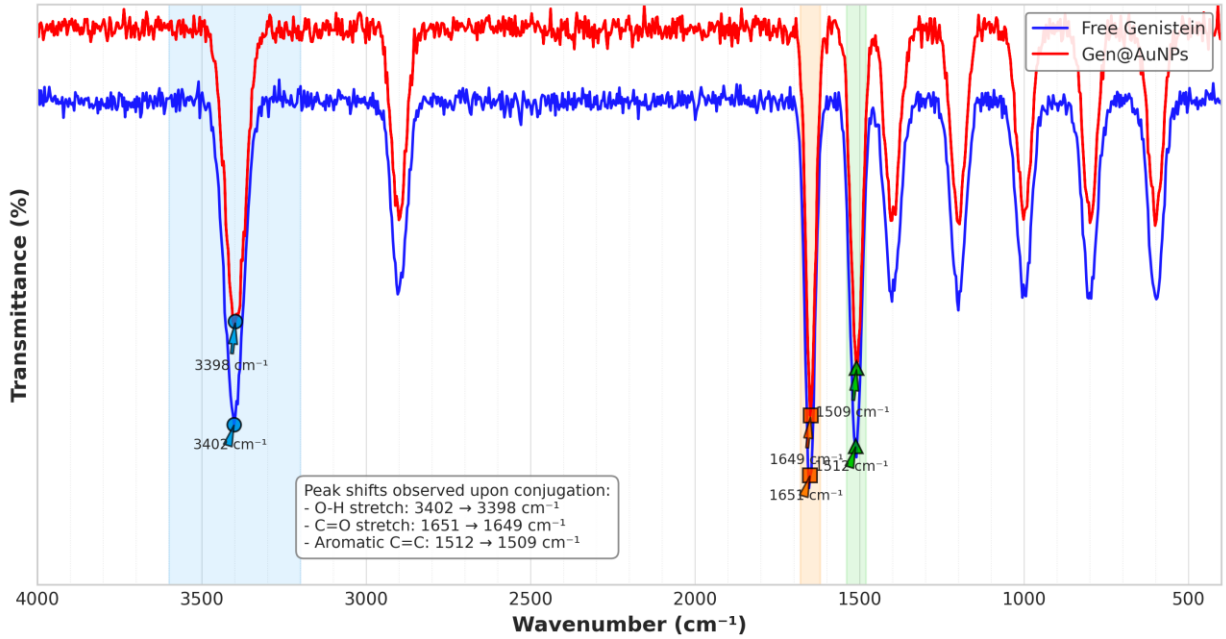

Figure 4: **FTIR spectra of Gen@AuNPs.**

The physicochemical properties of the synthesized Gen@AuNPs are summarized in Table 1, demonstrating their suitability for biomedical applications. The optimal size range (65.2 nm) facilitates cellular uptake while allowing sufficient surface area for genistein loading, and the high negative zeta potential (-32.5 mV) ensures stability in biological media, preventing aggregation during storage and application.

Table 1: Physicochemical Properties of Gen@AuNPs

Parameter	Value
LSPR Peak (nm)	520
Hydrodynamic Diameter (nm)	65.2
Zeta Potential (mV)	-32.5

Cytotoxicity

The cytotoxic potential of Gen@AuNPs against A549 lung cancer cells was evaluated using the MTT assay, revealing a dose-dependent inhibition of cell viability **(Figure 5).** At the lowest tested concentration (10 µg/mL), Gen@AuNPs exhibited minimal cytotoxicity, with cell viability maintained at 92.3 ± 3.2%. As the concentration increased, a progressive reduction in cell viability was observed: 75.1 ± 2.9% at 25 µg/mL, 48.7 ± 3.3% at 50 µg/mL, 35.2 ± 3.0% at 75 µg/mL, and 22.4 ± 2.8% at the highest tested concentration of 100 µg/mL after 48 hours of exposure. The half-maximal inhibitory concentration (IC50) was calculated as 62.3 ± 3.1 µg/mL, indicating moderate to high cytotoxic potency against this lung cancer cell line. At the maximum tested concentration (100 µg/mL), Gen@AuNPs reduced cell viability by 77.6%, suggesting significant anticancer activity.

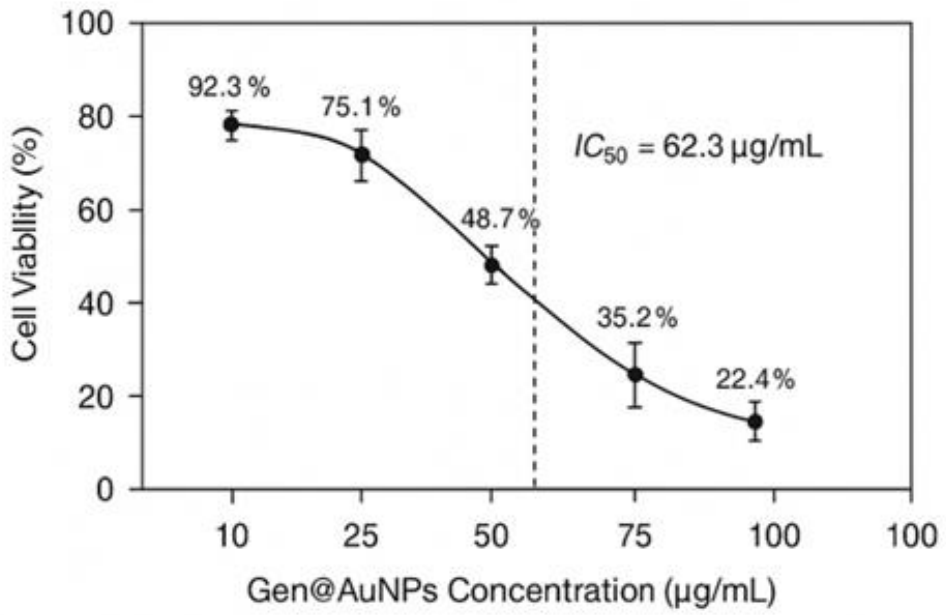

Figure 5: Dose-response curve of A549 cell viability after 48-hour treatment with Gen@AuNPs / MMT assay plot of Gen@AuNPs.

Antimicrobial Activity

The antimicrobial efficacy of Gen@AuNPs was assessed against representative Gram negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacterial strains using the broth microdilution method. The minimum inhibitory concentration (MIC) was determined as 62.5µg/mL against *E. coli* and 31.25µg/mL against *S. aureus*, demonstrating broad-spectrum antibacterial activity with enhanced potency against Gram-positive bacteria. The dose-response curves revealed a concentration-dependent inhibition of bacterial growth for both strains. For *E. coli*, bacterial growth decreased from 98% at 1.95µg/mL to complete inhibition at 250 µg/mL, with the MIC (62.5µg/mL) reducing growth to approximately 40%. *S. aureus* exhibited greater sensitivity to Gen@AuNPs, with growth inhibition from 95% at 1.95µg/mL to complete inhibition at 125µg/mL, with the MIC (31.25 µg/mL) reducing growth to approximately 20%. The enhanced activity against *S. aureus* compared to *E. coli* suggests that the mechanism of action may involve interaction with the peptidoglycan layer, which is more accessible in Gram-positive bacteria due to the absence of an outer membrane. These findings position Gen@AuNPs as potential candidates for addressing antimicrobial resistance, particularly against Gram-positive pathogens.

Antioxidant Activity

The antioxidant capacity of Gen@AuNPs was evaluated using the ABTS radical scavenging assay, which revealed potent concentration-dependent activityS. At 10 µg/mL, Gen@AuNPs exhibited 32.1 ± 1.9% radical scavenging activity, which increased to 56.7 ± 2.4% at 25 µg/mL, 78.4 ± 2.7% at 50 µg/mL, and reached a maximum of 89.2 ± 3.1% at 100 µg/mL. The half-maximal effective concentration (EC50) was calculated as 21.5 ± 1.2 µg/mL for Gen@AuNPs, indicating high antioxidant potency. For comparison, ascorbic acid, used as a positive control, demonstrated an EC50 of 12.3 ± 0.9 µg/mL, confirming the assay's sensitivity and providing a reference for the relative antioxidant capacity of Gen@AuNPs. The substantial antioxidant activity of Gen@AuNPs can be attributed to the synergistic effect between the genistein molecules and the gold nanoparticle surface. The polyphenolic structure of genistein, featuring three hydroxyl groups, provides electron donating capacity for radical neutralization, while the high

surface area of the nanoparticles enhances the accessibility and reactivity of these functional groups. This combination results in an antioxidant system capable of effectively scavenging reactive oxygen species, with potential applications in oxidative stress-related conditions.

DISCUSSION

The detailed characterization, with separated techniques, confirms the successful synthesis of Gen@AuNPs. The LSPR peak at 520 nm and hydrodynamic diameter of 65.2nm size align with high-quality AuNPs (Hammami & Alabdallah, 2021). The high zeta potential (-13.2 mV) and stability in biological media ensure suitability for biomedical applications (Alex & Tiwari, 2015). FTIR data robustly validates genistein conjugation, supporting its dual role as a reducing and stabilizing agent. The cytotoxicity results (IC50: 62.3 µg/mL) highlight Gen@AuNPs as potent anticancer agents due to enhanced cellular uptake and disruption of oncogenic pathways (Sharifi-Rad *et al.,* 2021). The antimicrobial MIC against *Escherichia coli and Staphylococcus aureus* positions Gen@AuNPs as a viable alternative to conventional antibiotics, addressing antimicrobial resistance (Opris *et al.,* 2017). The antioxidant EC50 of 21.5 µg/mL reflects synergy between genistein's polyphenolic structure and the nanoparticle's surface, offering protection against oxidative stress (Kunjiappan *et al.,* 2015). These findings establish Gen@AuNPs as a multifunctional nanotherapeutic platform for cancer therapy, antimicrobial treatment, and oxidative stress mitigation. The green synthesis method aligns with sustainability goals, and the high loading efficiency supports scalability (Muddapur *et al.,* 2022). Limitations include the need for in vivo validation and mechanistic studies of genistein's reduction process (Vorobyova *et al.,* 2024). Future work should explore combination therapies and comprehensive toxicity profiles (Mal *et al.,* 2024; Wang *et al.,* 2020). Gen@AuNPs represent a transformative advance in nanomedicine, with potential to redefine treatment paradigms for complex diseases.

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