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

SYNTHESIS, CHARACTERIZATION, AND ANTIBACTERIAL ACTIVITY OF QUERCETIN-CAPPED GOLD NANOPARTICLES AGAINST *ESCHERICHIA COLI, BACILLUS SUBTILIS,* AND *KLEBSIELLA PNEUMONIAE*

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ABSTRACT
In the face of rising antimicrobial resistance, this study explores the potential of quercetin-capped gold nanoparticles (Qu-AuNPs) as a novel antibacterial agent. Qu-AuNPs were synthesized using a green method and characterized using UV-visible spectroscopy, Zeta sizer analysis, and Fourier Transform Infrared (FTIR) spectroscopy. The UV-VIS spectrum showed a characteristic surface plasmon resonance band at 540 nm, confirming the formation of gold nanoparticles. Zeta sizer analysis revealed a mean hydrodynamic diameter of 29.92 nm, ideal for drug delivery applications. FTIR analysis confirmed the presence of quercetin on the nanoparticle surface, with characteristic peaks at 3333.45, 2128.60, 1638.36, and 682.45 cm^{-1}. The antibacterial activity of Qu-AuNPs was evaluated against Escherichia coli, Bacillus subtilis, and Klebsiella pneumoniae using the disc diffusion method. Qu-AuNPs demonstrated significant antibacterial activity against all three bacterial strains, with the highest efficacy observed against *B. subtilis*. The zone of inhibition increased with increasing Qu-AuNP concentration, reaching maximum values of 20 mm, 26 mm, and 18 mm for *E. coli*, *B. subtilis*, and *K. pneumoniae*, respectively, at 1000 µg/ml. These findings suggest that Qu-AuNPs hold promise as an effective antibacterial agent, potentially offering a new approach to combat antimicrobial resistance.

Keywords: *Quercitin, Antibacterial-Resistance, Gold Nanoparticles*

INTRODUCTION
Antimicrobial resistance (AMR) has become a significant global health issue, posing a serious threat to the effectiveness of traditional antibiotics and leading to an increasing prevalence of challenging infections. The World Health Organization (WHO) has classified AMR as one of the top ten global public health threats to humanity (Asghar *et al.,* 2024). The accelerated emergence of resistant strains is largely attributed to the misuse and overuse of antibiotics across human medicine, agriculture, and veterinary practices, highlighting the urgent need for the development of novel antimicrobial agents. Among the emerging strategies, nanotechnology-based approaches are gaining considerable attention due to their potential to circumvent conventional resistance mechanisms (Wani, 2024).
Nanotechnology, which involves the manipulation of matter at the nanoscale (typically between 1 and 100 nanometers), has catalyzed advancements across numerous scientific fields, including medicine. At this scale, materials exhibit unique physical, chemical, and biological properties distinct from their bulk forms (Mahajan *et al.,* 2024). Nanoparticles have been extensively utilized in drug delivery, imaging, diagnostics, and therapeutic applications, particularly in antimicrobial treatments where traditional antibiotics are increasingly ineffective due to rising resistance. Gold nanoparticles (AuNPs) are particularly promising in antimicrobial therapy because of their stability, straightforward synthesis, and ability to be functionalized with various bioactive molecules. Unlike other metal nanoparticles, AuNPs are relatively inert, exhibiting low intrinsic cytotoxicity, which makes them suitable for medical applications (Ghobashy *et al.,* 2024).

AuNPs can be synthesized in various morphologies, including spheres, rods, and stars, each with distinct properties that can be optimized for specific applications. The surface of AuNPs provides a versatile platform for attaching drugs, proteins, peptides, and other therapeutic molecules, enabling controlled release at the site of infection (Cordeiro *et al.,* 2024). Functionalization with antimicrobial agents can significantly enhance the antibacterial properties of AuNPs.

Flavonoids, a class of polyphenolic compounds prevalent in plants, are well-recognized for their diverse biological activities, including antimicrobial effects (Das *et al.,* 2024). Quercetin, a naturally occurring flavonoid found in a variety of fruits, vegetables, and grains, has been extensively studied for its wide-ranging biological activities, such as antioxidant, anti-inflammatory, antiviral, and antimicrobial properties (Singh *et al.,* 2024). Quercetin has demonstrated antimicrobial activity against a broad spectrum of pathogens, including bacteria, viruses, and fungi. Moreover, quercetin has been shown to enhance the effectiveness of conventional antibiotics, indicating its potential in addressing antibiotic resistance (Osman *et al.,* 2024). However, quercetin's therapeutic application is often constrained by its poor solubility and low bioavailability (Sangeetha, 2024). These limitations may be overcome by incorporating quercetin into nanoparticle-based delivery systems.

Combining quercetin with gold nanoparticles offers a promising approach to overcoming the limitations of both components while enhancing their antimicrobial efficacy. Quercetin can serve as a reducing and stabilizing agent in the synthesis of AuNPs, resulting in quercetin-capped gold nanoparticles. This green synthesis method is environmentally friendly and eliminates the need for toxic chemicals, making it a more sustainable approach to nanoparticle production. Quercetin-capped AuNPs retain the antimicrobial properties of both quercetin and gold nanoparticles, potentially leading to synergistic effects that enhance overall efficacy. Additionally, the nanoparticle formulation can improve the solubility, stability, and bioavailability of quercetin, thereby increasing its effectiveness as an antimicrobial agent. The enhanced antibacterial activity of quercetin-loaded AuNPs could offer a valuable alternative to conventional antibiotics, particularly against resistant bacterial strains.

MATERIALS AND METHODS

Synthesis of Quercetin-Gold Nanoparticles

A total of 500 mg of quercetin was dissolved in 100 mL of a 0.001 M chloroauric acid solution. The mixture was then heated to 90°C for 15 minutes and subsequently allowed to cool to room temperature. The color change from pale yellow to wine red color indicated the formation of quercetin-gold nanoparticles (Qu-AuNPs). The synthesized nanoparticles were filtered twice using Whatman filter paper and stored at 4°C. The nanoparticles were then dispersed in deionized water at a concentration of 0.003 g/mL and subjected to sonication for 45 minutes. The resulting suspension was analyzed using Zeta sizer, UV-visible spectroscopy, and Fourier transform infrared (FTIR) spectroscopy.

Characterization of Quercetin-Gold Nanoparticles

UV-Visible Spectroscopy Analysis

Primary identification of Qu-AuNPs formation was carried out by observing the color change of the reaction solution. The bioreduction of H[AuCl4] to Qu-AuNPs was checked by UV–visible spectrophotometer, and spectrograph of the synthesized Qu-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 Qu-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 Qu-AuNPs were recorded using a Bruker Alpha-P FTIR spectrometer (India) across the range of 3500-400 cm^{-1} to confirm the presence of quercetin 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).

In-Vitro Antibacterial Activity

The antibacterial activity of 0.1 mM quercetin and the colloidal solution of Qu-AuNPs was evaluated against *Escherichia coli (E. coli)* ATCC-25922, *Bacillus subtilis (B. subtilis*) ATCC-6633, and *Klebsiella pneumoniae (K. pneumoniae)* ATCC-13885 using the disk diffusion method. Penicillin combined with streptomycin served as the positive control, while distilled water (DW) was used as the negative control (Kaliraman *et al.*, 2024).

RESULTS

Synthesis and characterization of Qu-AuNPs

The deployed method for synthesis was quite simple and holds great promise. It's comparatively efficient and nontoxic, thus making it better than other. The synthesis of Qu-AuNPs was confirmed primarily by the change in color of the reaction mixture from pale yellow to wine red color. The synthesized Qu-AuNPs were characterized by UV-VIS spectroscopy, FT-IR, and ZETA-sizer. UV-VIS spectrum is mostly adopted to confirm the synthesis and stability of NPs in aqueous solutions. The characteristic surface plasmon resonance (SPR) band in the UV-visible spectrum, indicated the formation of gold nanoparticle. The UV-VIS absorption spectrum of synthesized Qu-AuNPs with intense peak at 540 nm. Single peaks formed by the gold-quercetin nanoparticle conjugates observed in the UV–visible spectroscopic analyses confirmed the uniform size and shape of the nanoparticles.

Measurement of Particle Size by Zeta sizer

The precise dimensions of nanoparticles (NPs) are critical factor in their synthesis process. To determine the particle size distribution of the synthesized Quercitin gold nanoparticles (Qu-AuNPs), dynamic light scattering analysis was performed. The results revealed a mean hydrodynamic diameter of 29.92 nm for the AuNPs (Figure 1). This Z-average particle size falls within the optimal range typically observed for nanoparticles intended for drug delivery applications, suggesting that these AuNPs may be suitable candidates for potential therapeutic interventions.

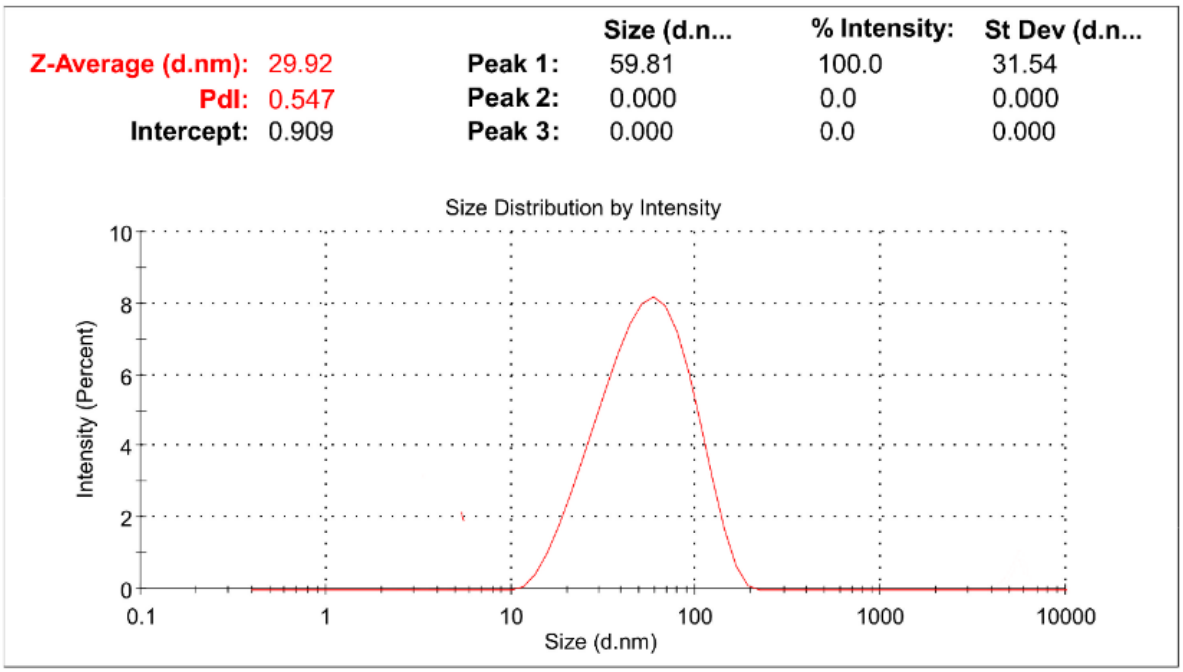

Figure 1: Particle size of synthesized Q-AuNPs.

The FTIR (Fourier Transform Infrared) Analysis

The FTIR spectrum of Quercetin-Gold Nanoparticles (Qu-AuNPs) revealed several characteristic peaks, indicating the presence of specific functional groups. The broad peak at 3333.45 cm^{-1} corresponds to the O-H stretching vibrations, suggesting the presence of hydroxyl groups, which are likely involved in the capping and stabilization of the nanoparticles(Agilent Technologies, n.d.). The peak at 2128.60 cm^{-1} can be attributed to the C≡C stretching vibrations, indicating the presence of alkyne groups (Agilent Technologies, n.d.). The strong peak at 1638.36 cm^{-1} is associated with the C=O stretching vibrations, which are characteristic of carbonyl groups, possibly from the quercetin structure (Agilent Technologies, n.d.). Quercetin contains both aromatic rings and carbonyl groups (in its ketone and ester forms), indicating that the fundamental structure of quercetin is retained in the nanoparticle form. Lastly, the peak at 682.45 cm^{-1} corresponds to the C-H bending vibrations, indicating the presence of aromatic rings or other out-of-plane deformations. These findings confirm the successful synthesis of Qu-AuNPs, with quercetin acting as both a reducing and stabilizing agent, and highlight the various functional groups involved in the nanoparticle formation process.

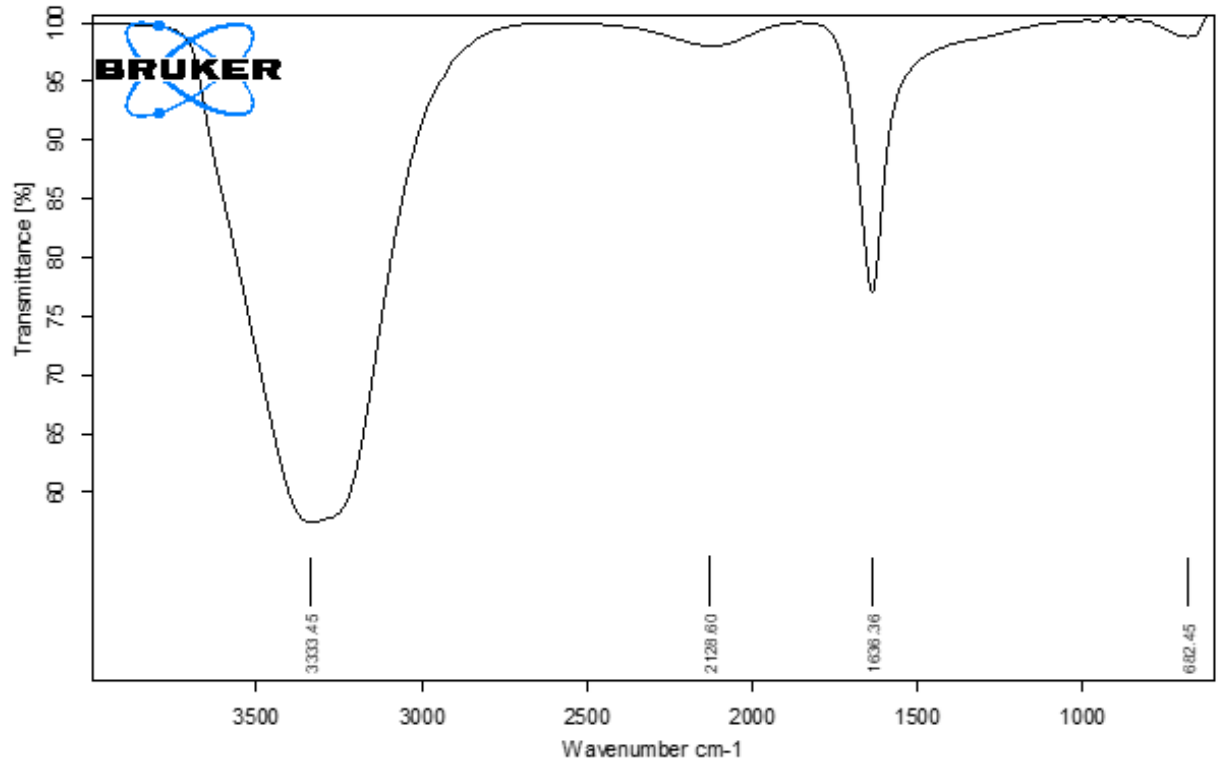

Figure 2: FTIR transmittance spectrum of synthesized Qu-AuNPs with observed peak intensities at 3333.45 cm^{-1},2128.60 cm^{-1}, 1638.36 $cm^{-1}$and 682.45 cm^{-1}.

Antibacterial Activity Assessment Using Disc Diffusion Method

The antibacterial activity of Quercetin-gold nanoparticles (Qu-AuNPs) was evaluated against *Escherichia coli (E. coli), Bacillus subtilis (B. subtilis), and Klebsiella pneumoniae (K. pneumoniae)* utilizing the disc diffusion method. The assay involved the application of four distinct concentrations of Qu-AuNPs, as detailed in Table 1. The resultant zones of inhibition were measured to determine the efficacy of Qu-AuNPs at varying concentrations. The findings reveal critical insights into the potential therapeutic applications of Qu-AuNPs in combating bacterial infections.

Table 1: Zone of Inhibition for Different Concentrations of Qu-AuNPs

Concentration (µg/ml)	Zone of inhibition (mm)		
	E. coli	*B.subtillis*	*K.pneumonae*
1000	20	26	18
500	19	24	16
250	15	17	13
125	14	12	12
Control (Penicillin 60 µg/ml+ Streptomycin 100µg/ml)	22	28	23

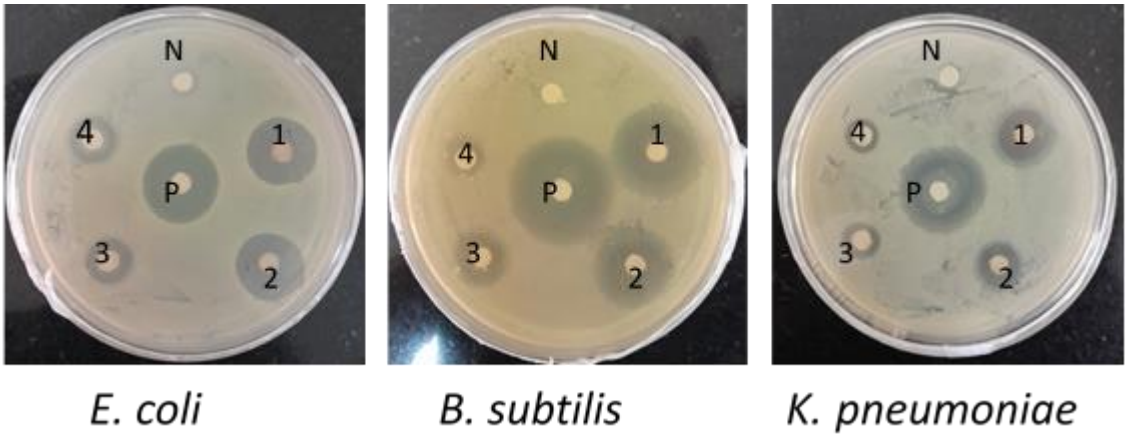

Figure 3: Disk diffusion plates of *E. coli, B. subtilis, and K. pneumoniae, against synthesized Q-AgNPs,* with concentrations, 1(1000 µg/ml), 2(500 µg/ml), 3(250 µg/ml), and 4(125 µg/ml), along with Positive control (Penicillin 60 µg/ml+ Streptomycin 100µg/ml) and normal saline as negative control.

Discussion

The quercetin-capped gold nanoparticles represent a significant advancement in the field of nanomedicine and antimicrobial therapy. The green synthesis method is not only efficient but also environmentally friendly. Here, Quercetin acts as both a reducing and stabilizing agent in nanoparticle formation. The characteristic surface plasmon resonance band at 540 nm, aligns with previous studies on AuNPs (Fong & Yung, 2013). The observed single peak suggests uniformity in size and shape of the synthesized Qu-AuNPs, which is crucial for consistent biological activity. The mean hydrodynamic diameter of 29.92 nm is the optimal range for nanomaterials intended for drug delivery applications. Such, size range allows for enhanced cellular uptake and improved biodistribution compared to larger particles, potentially leading to more effectiveness (Cabral *et al.*, 2024). The successful capping of gold nanoparticles with quercetin is crucial as it not only stabilizes the nanoparticles but also contributes to their biological activity. The retention of quercetin's fundamental structure on the nanoparticle surface suggests that its inherent antibacterial properties are preserved in the Qu-AuNP formulation.

The antibacterial activity results are particularly promising, demonstrating the broad-spectrum efficacy of Qu-AuNPs against both Gram-positive (*B. subtilis*) and Gram-negative (*E. coli and K. pneumoniae*) bacteria. The observed dose-dependent increase in the zone of inhibition indicates that the antibacterial effect is directly related to the concentration of Qu-AuNPs. The superior activity against *B. subtilis*

(maximum zone of inhibition: 26 mm) compared to *E. coli* (20 mm) and *K. pneumoniae* (18 mm) at 1000 µg/ml suggests that Qu-AuNPs may be particularly effective against Gram-positive bacteria. This differential activity could be attributed to variations in cell wall structure between Gram-positive and Gram-negative bacteria, with the thicker peptidoglycan layer of Gram-positive bacteria potentially facilitating greater nanoparticle interaction and penetration. The antibacterial efficacy of Qu-AuNPs can be attributed to several potential mechanisms. Firstly, the small size of the nanoparticles may enable them to penetrate bacterial cell membranes more easily, disrupting cellular functions. Secondly, the release of gold ions from the nanoparticle surface could interfere with bacterial proteins and enzymes, leading to cell death. Lastly, the quercetin molecules on the nanoparticle surface may contribute additional antibacterial effects through their known mechanisms, such as inhibition of DNA gyrase and disruption of cell membrane integrity. It is noteworthy that while the Qu-AuNPs showed significant antibacterial activity, their efficacy was slightly lower than the positive control (penicillin + streptomycin) for all tested bacteria. This observation suggests that while Qu-AuNPs may not completely replace conventional antibiotics, they could serve as a valuable adjunct therapy, potentially allowing for lower antibiotic doses and reduced risk of resistance development. The findings of this study have important implications for addressing the global challenge of antimicrobial resistance. The broad-spectrum activity of Qu-AuNPs, combined with their potential for overcoming traditional resistance mechanisms, positions them as a promising alternative or complementary approach to conventional antibiotics. Moreover, the green synthesis method employed in this study offers a sustainable and environmentally friendly approach to nanoparticle production, addressing growing concerns about the environmental impact of nanomaterial synthesis. Future research directions should focus on elucidating the exact mechanisms of antibacterial action of Qu-AuNPs, investigating potential synergistic effects with conventional antibiotics, and evaluating their efficacy against a wider range of pathogenic bacteria, including antibiotic-resistant strains. Additionally, *in vivo* studies will be crucial to assess the biocompatibility, pharmacokinetics, and therapeutic efficacy of Qu-AuNPs in animal models before progressing to clinical trials. In conclusion, this study demonstrates the successful synthesis and characterization of quercetin-capped gold nanoparticles with significant antibacterial activity against both Gram-positive and Gram-negative bacteria. These findings pave the way for the development of novel nanoparticle-based antimicrobial therapies that could play a crucial role in combating the growing threat of antimicrobial resistance.

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