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

SYNTHESIS, *IN-VITRO* AND *IN-SILICO* EVALUATION OF QUERCETIN-SILVER NANOPARTICLES FOR ANTIBACTERIAL ACTIVITY

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ABSTRACT
The increasing prevalence of antibiotic-resistant bacteria necessitates novel antimicrobial strategies. This study investigates the synthesis, characterization, and antibacterial efficacy of Quercetin-capped Silver Nanoparticles (Q-AgNPs). Utilizing quercetin both as a reducing and capping agent in a green synthesis approach, Q-AgNPs were produced and characterized through UV-Vis spectroscopy, Zeta potential analysis, and Fourier-Transform Infrared Spectroscopy (FTIR). The synthesized nanoparticles exhibited significant antibacterial activity against *Escherichia coli, Bacillus subtilis, and Klebsiella pneumoniae*. Molecular docking studies further elucidated the interaction between quercetin and the enzyme Aminoglycoside-2"-phosphotransferase-IIa (APH2 "-IIa), with quercetin demonstrating a binding affinity of −8.5 kcal/mol. Hydrogen bonding and steric interactions were primarily observed with residues TYR87, GLY90, ARG92, ILE93, ALA195, and ASN196. *In-vitro* antibacterial assays using the disc diffusion method confirmed that Q-AgNPs possess potent antibacterial properties, highlighting their potential as a sustainable and effective alternative to conventional antibiotics. The combination of in-vitro and in-silico evaluations provides a comprehensive understanding of the antibacterial mechanisms of Q-AgNPs, paving the way for future therapeutic applications.

***Keywords**: Quercetin, Antibacterial-Resistance, Silver Nanoparticles, Molecular Docking*

INTRODUCTION

The global health crisis precipitated by the rise of antibiotic-resistant bacteria has become one of the most pressing challenges in modern medicine (World Health Organization, 2020). This phenomenon, exacerbated by the indiscriminate use of conventional antibiotics, has spurred an urgent need for innovative antimicrobial strategies (Ventola, 2015). In this context, nanotechnology has emerged as a promising field, offering novel approaches to combat bacterial infections that circumvent traditional resistance mechanisms (Wang *et al.,* 2017). Silver nanoparticles (AgNPs) have garnered significant attention in recent years due to their broad-spectrum antimicrobial properties (Rai *et al.,* 2009). The bactericidal action of AgNPs is attributed to multiple mechanisms, including disruption of bacterial cell membranes, generation of reactive oxygen species, and interference with DNA replication (Dakal *et al.,* 2016). However, the efficacy and stability of AgNPs can be further enhanced through the incorporation of bioactive compounds as capping agents (Keat *et al.,* 2015).

Quercetin, a naturally occurring flavonoid found in various fruits and vegetables, has been extensively studied for its antioxidant, anti-inflammatory, and antimicrobial properties (Anand David *et al.,* 2016). Its potential as a capping agent for AgNPs is particularly intriguing, as it may confer additional biological activities and improve the overall stability of the nanoparticles (Wang *et al.,* 2018). The combination of quercetin and AgNPs could potentially yield a synergistic antibacterial effect, thereby offering a more potent alternative to conventional antimicrobial agents (Patra & Baek, 2017). The synthesis of

nanoparticles using green methods has gained traction in recent years, owing to its eco-friendly nature and the potential to produce biocompatible materials (Iravani, 2011). Utilizing quercetin as both a reducing and capping agent in the synthesis of AgNPs aligns with these principles, potentially resulting in a more sustainable and biologically compatible product (Jain & Mehata, 2017). While the antimicrobial properties of both AgNPs and quercetin have been individually documented, the potential synergistic effects of quercetin-capped silver nanoparticles (Q-AgNPs) remain largely unexplored. Moreover, their molecular interactions with bacterial cellular components are not fully understood, necessitating a comprehensive approach that combines both *in-vitro* and *in-silico* evaluations (Lombardo *et al.,* 2019). In-vitro studies are crucial for assessing the practical antibacterial efficacy of Q-AgNPs against both Gram-positive and Gram-negative bacteria (Franci *et al.,* 2015). These experiments can provide valuable insights into the spectrum of activity, minimum inhibitory concentrations, and the kinetics of bacterial killing. Complementing these empirical observations with *in-silico* studies can offer a deeper understanding of the molecular mechanisms underlying the antibacterial activity of Q-AgNPs (Vázquez-Núñez *et al.,* 2020). Molecular docking simulations, for instance, can predict potential interactions between the nanoparticles and critical bacterial proteins, elucidating possible modes of action (Petchiammal *et al.,* 2019). The present study aims to bridge this knowledge gap by synthesizing Q-AgNPs using a green method and comprehensively evaluating their antibacterial potential through a combination of *in-vitro* and *in-silico* approaches.

MATERIALS AND METHODS

In-silico Study

The *in-silico* analysis was performed utilizing Auto-dock Vina software (Eberhardt *et al.,* 2021). 3D Structure of Quercetin was retrieved from PubChem database with PubChem CID-5280343. The 3D structure was configured to the lowest energy conformation. For the purpose of investigating its antibacterial properties, quercetin was docked against the enzyme Aminoglycoside-2”-phosphotransferase-IIa (APH2 ”-IIa), with the Protein Data Bank (PDB) identifier 3HAV. This enzyme, produced by both Gram-positive and Gram-negative bacteria, deactivates aminoglycoside antibiotics (Young *et al.,* 2009). The docking study's validity was confirmed by docking the native ligand, streptomycin, at the active site of APH2 ”-IIa. The evaluation was conducted using the vina score, which reflects the stability of the ligand-receptor binding. The vina score represents the total energy derived from both external and internal ligand interactions. External interactions encompass the energy consistency of protein-ligand and cofactor-ligand interactions, while internal interactions account for energies dependent on the ligand's chemical structure, including torsional strain, sp^2-sp^2 steric, and electrostatic interactions.

Synthesis of Quercetin-Silver Nanoparticles

Quercetin-Silver Nanoparticles (Q-AgNPs) were prepared by reducing Silver Nitrate with Quercetin Dihydrate (Ajitha et al.,2014). 0.2 mM Querecitin dihydrate solution (Reagent-1) was prepared in methanol while 2mM AgNO3 solution (Reagent-2) was prepared in deionized water. Reagent-1 was dropwise mixed with Reagent-2 dropwise at 37°C on a magnetic stirrer (200 rpm), until colour change was observed from pale-yellow to dark-brown color. The resulting solution was cooled down and centrifuged at 20,000g for 20 minutes. The sedimented Q-AgNPs were washed with methanol and water 4 times repeatedly and air dried (Ajitha *et al.,* 2014).

Characterization of Quercetin-Silver Nanoparticles

UV-visible spectroscopy analysis

Primary identification of AgNPs formation was carried out by observing the color change of the reaction solution. The bioreduction of AgNO3 to Q-AgNPs was checked by UV–visible spectrophotometer, and

spectrograph of the synthesized Q-AgNPs was recorded using a quartz cuvette with water as a reference at a scanning range of 200–700 nm (Kubavat ***et al.,*** 2022).

Zeta sizer analysis

The size and zeta potential of the aliquots of AgNPs were experientially measured by Zeta sizer Nano ZS90, Malvern instruments in a disposable cell at 25°C using Zeta sizer 7.13 software after 5 min. of sonication to avoid aggregation of particles (Kubavat *et al.,* 2022).

Fourier-transform infrared (FTIR) analysis

The synthesized Q-AgNPs were analyzed by FTIR in the wavenumber frequency ranging from 3500 to 500 cm−1 for the functional groups present on it which are responsible for bioreduction of AgNO3. All the dimensions were recorded in transmittance mode using Bruker Alpha, Lab India Instrument Private Limited, functioned by OPUS 7.5 software (Ramteke *et al.,* 2013).

In-Vitro Antibacterial Activity

The antibacterial activity of the 0.1mM Quercetin and colloidal solution of Q-AgNPs was assessed against *Escherichia coli (E. coli)* (ATCC-25922), *Bacillus subtilis (B. subtilis)* (ATCC-6633), *Klebsella pneumonae (K. pneumonae)* (ATCC-13885) respectively, using disk-diffusion method. Penicillin + Streptomycin was used as the positive control, whereas DW as the negative control (Balouiri & Ibnsouda, 2016).

RESULTS

Molecular docking analysis

The binding site within the grid box was defined at coordinates X = −20.27 Å, Y = 8.05 Å, and Z = −16.46 Å, encompassing a cavity surrounded by 43 amino acids: Lys42, Arg55, Glu56, Lys106, Glu113, Lys118, Lys139, Lys142, Asp146, His190, Asp192, Phe193, Ser194, Asn196, Asn197, Asp210, Asp213, Asp218, Asp220, Asp222, Leu224, Cys225, Asp228, Ser230, Asp232, Asp233, Lys236, Arg240, Lys241, Lys244, Glu255, Arg256, Lys257, Glu259, Asn261, Asp262, Tyr264, Trp265, Asp268, Tyr272, Arg279, Lys284, Glu288. Molecular docking studies of quercetin with the Aminoglycoside-2"-phosphotransferase-IIa enzyme (APH(2")-IIa, PDB ID: 3HAV) revealed that quercetin forms hydrogen bonds and steric interactions, exhibiting an affinity value of −8.5 kcal/mol (Figure 1). These interactions suggest a low binding energy for streptomycin with the enzyme. Steric interactions involve the residues TYR87, GLY90, ARG92, ILE93, ALA195, ASN196, and ILE199. Hydrogen bonds, with bond distances of less than 3 Å, were observed with residues ARG92 and ASN196, indicating that closer hydrogen bond distances correlate with lower binding energies (Figure 1).

Synthesis and characterization of Q-AgNPs

The synthesized Q-AgNPs were characterized by UV-VIS spectroscopy, FT-IR, and ZETA-sizer. 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 color change (from pale yellow to dark brown) indicated the formation of AgNPs which was confirmed by UV-vis spectrum. UV-VIS spectrum is mostly adopted to confirm the synthesis and stability of NPs in aqueous solutions. Figure-2 presents the UV-VIS absorption spectrum of synthesized Q-AgNPs with intense peak at 420 nm.

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 Quercetin silver nanoparticles (Q-AgNPs), dynamic light

scattering analysis was performed. The results revealed a mean hydrodynamic diameter of 55.39 nm for the AgNPs (Figure 3). This Z-average particle size falls within the optimal range typically observed for nanoparticles intended for drug delivery applications, suggesting that these AgNPs may be suitable candidates for potential therapeutic interventions.

The FTIR (Fourier Transform Infrared) Analysis

The FTIR spectrum of Quercetin-Silver Nanoparticles presented with characteristic peaks at 3326.87 cm^{-1}, 2121.14 cm^{-1}, 1636.87 cm^{-1}, and 682.19 cm^{-1}. The peak at 3326.87 cm^{-1} corresponds to the O-H stretching vibration, which is typically found in hydroxyl groups (OH) (Agilent Technologies, n.d.). Quercetin is a flavonoid that contains multiple hydroxyl groups that are responsible for this peak. The presence of this peak suggests that the hydroxyl groups are still present in the quercetin structure after the formation of the nanoparticles. The 2121.14 cm^{-1} peak is generally associated with the stretching vibrations of triple bonds such as alkyne C≡C or nitrile C≡N groups (Agilent Technologies, n.d.). However, since Quercetin does not naturally contain such groups, this peak might indicate some interaction with the silver nanoparticles, or could be modifications introduced during the synthesis process. The peak at 1636.87 cm^{-1} is attributed to C=C stretching of the aromatic ring or C=O stretching vibrations of carbonyl groups, reflecting the presence of quercetin's aromatic rings and carbonyl functionalities (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 682.19 cm^{-1} represent aromatic C-H bending or other out-of-plane deformations. This peak indicates the retention of aromatic structures in quercetin post nanoparticle formation. These observations confirm that the primary structure of quercetin is preserved in the nanoparticle form, with some possible interactions or modifications due to the nanoparticle synthesis process.

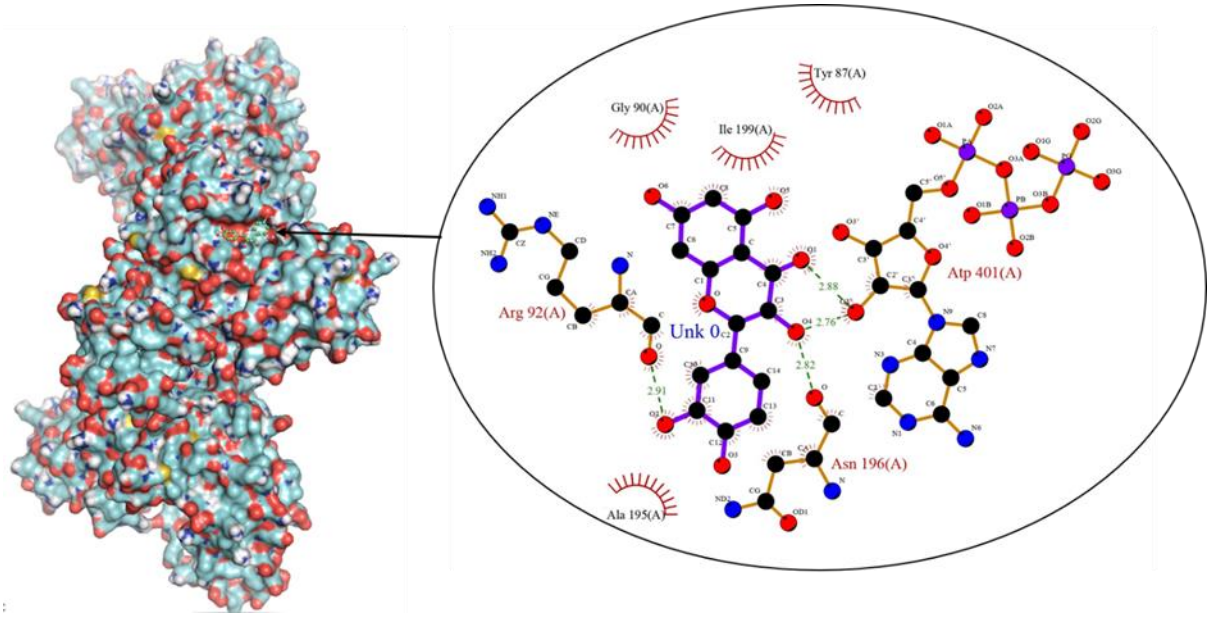

Figure 1. The molecular docking interaction between Quercetin and amino acid residues in the active site of APH(2'')-IIa.

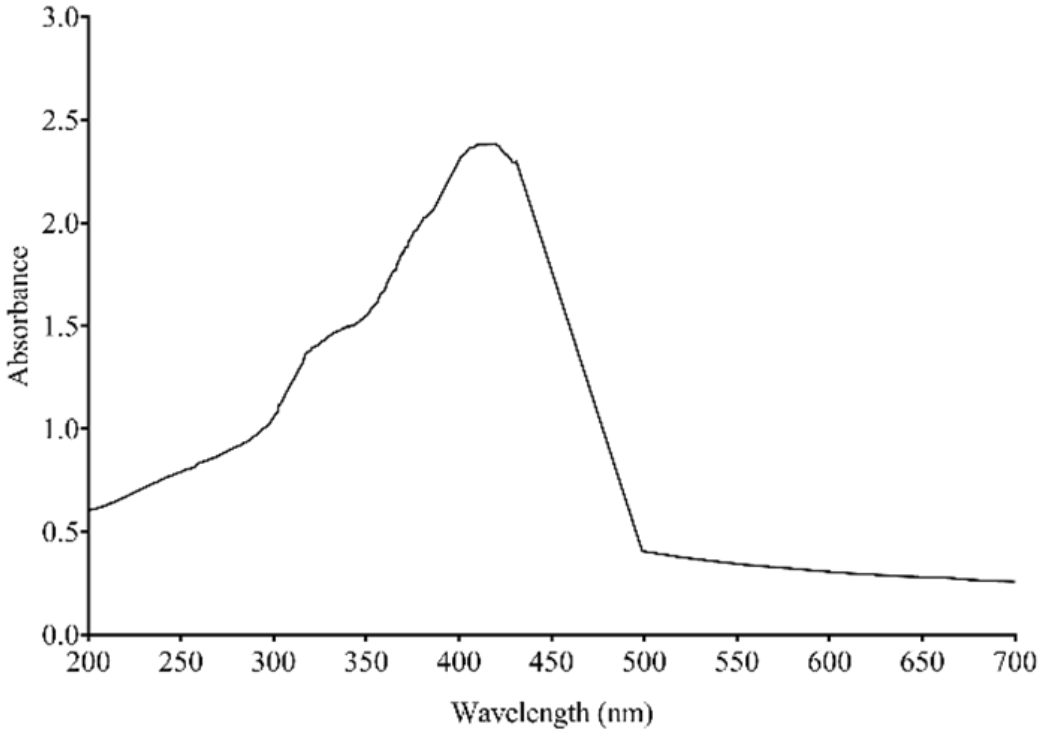

Figure 2: UV-VIS absorption spectrum of synthesized Q-AgNPs with observed peak intensity at 420 nm.

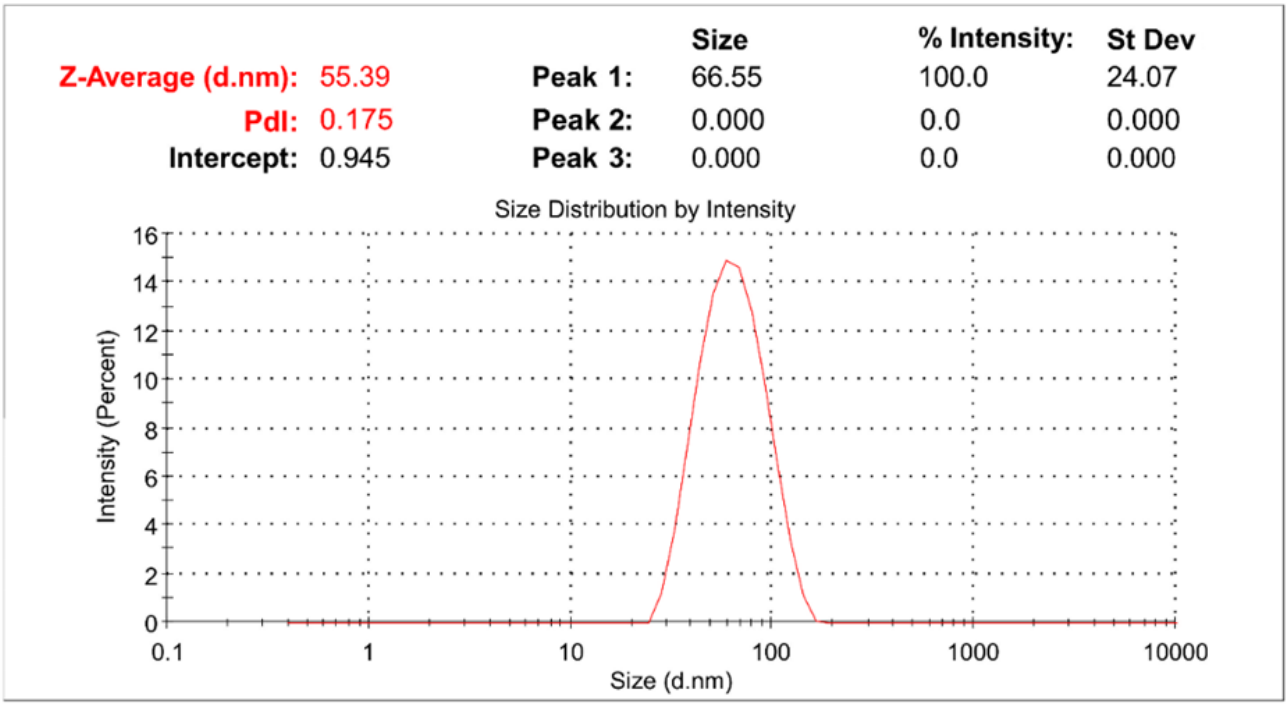

Figure 3: Particle size of synthesized Q-AgNPs.

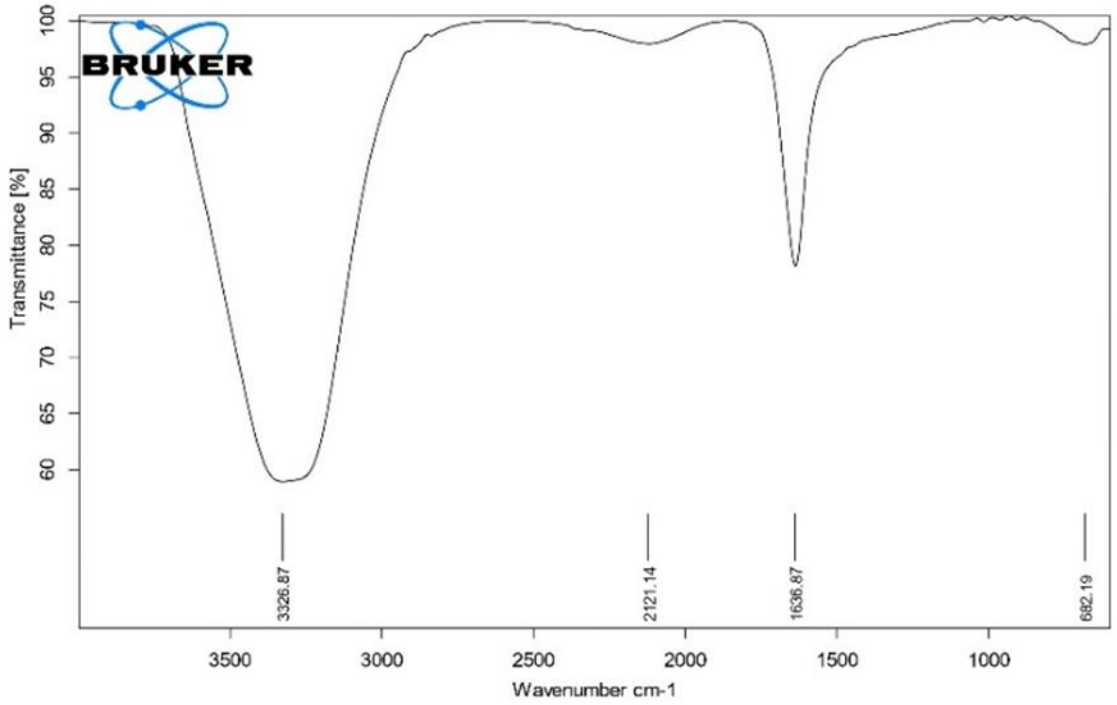

Figure 4: FTIR transmittance spectrum of synthesized Q-AgNPs with observed peak intensities at 3326.87 cm^{-1}, 2121.14 cm^{-1}, 1636.87 cm^{-1}, and 682.19 cm^{-1}.

Antibacterial Activity Assessment Using Disc Diffusion Method

The antibacterial activity of Quercetin-silver nanoparticles (Q-AgNPs) 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 Q-AgNPs, as detailed in Table 1. The resultant zones of inhibition were measured to determine the efficacy of Q-AgNPs at varying concentrations. The findings reveal critical insights into the potential therapeutic applications of Q-AgNPs in combating bacterial infections.

Table 1: Zone of Inhibition for Different Concentrations of Q-AgNPs

Concentration (µg/ml)	Zone of inhibition (mm)		
	E. coli	*B. subtillis*	*K. pneumonae*
1000	21	28	25
500	16	26	23
250	15	20	17
125	14	22	14
Control (Penicillin 60 µg/ml+ Streptomycin 100µg/ml)	28	26	26

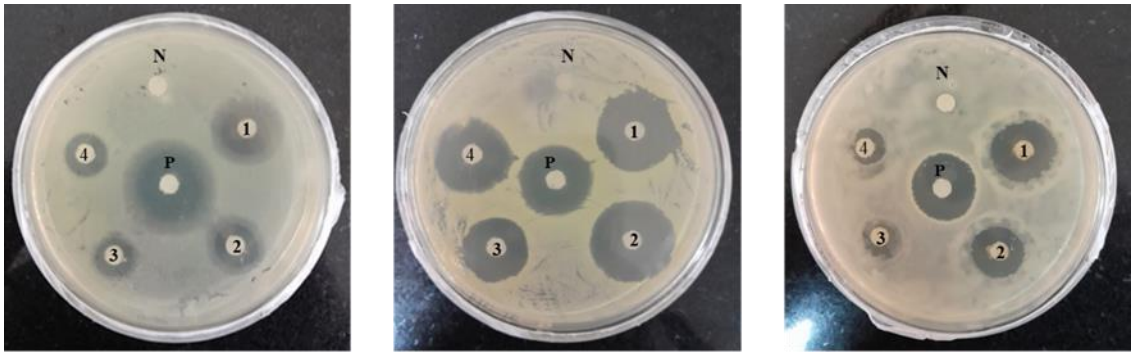

Figure 5: Disk diffusion plates of *E. coli, B. subtilis, and K. pneumoniae, against synthesized Q-AgNPs, with concentrations,* 1(1000 µg/ml), 2(500 µg/ml), 3(250 µg/ml), and 4(125 µg/ml), along with Positive control (Penicillin 60 µg/ml+ Streptomycin 100µg/ml) and normal saline as negative control.

The data indicates that Q-AgNPs exhibit significant antibacterial properties, as evidenced by the zones of inhibition observed. At a concentration of 1000 µg/ml, the zones of inhibition for *E. coli, B. subtilis, and K. pneumoniae* were 21 mm, 28 mm, and 25 mm, respectively. As the concentration of Q-AgNPs decreased, a corresponding reduction in the zones of inhibition was noted, demonstrating a dose-dependent antibacterial activity. These results provide a foundational understanding of the antibacterial potential of Q-AgNPs, suggesting their applicability in therapeutic contexts where bacterial infections pose a significant challenge. Further research is warranted to explore the mechanistic aspects of Q-AgNPs' antibacterial action and their potential integration into clinical practices.

DISCUSSION

The synthesis and characterization of quercetin-silver nanoparticles (Q-AgNPs) as investigated in this study demonstrate significant advancements in the field of nanotechnology and its application in antimicrobial therapy. Our results indicate that the use of quercetin as both a reducing and capping agent in the synthesis of silver nanoparticles yields stable and biologically active nanoparticles with potent antibacterial properties. This dual functionality of quercetin not only simplifies the synthesis process but also enhances the bioactivity of the resultant nanoparticles. The Fourier Transform Infrared (FTIR) analysis confirmed the presence of functional groups associated with quercetin in the Q-AgNPs. The characteristic peaks at 3326.87 cm^{-1}, 2121.14 cm^{-1}, 1636.87 cm^{-1}, and 682.19 cm^{-1} correspond to O-H stretching, triple bond stretching, C=C and C=O stretching, and aromatic C-H bending, respectively (Agilent Technologies, n.d.). The retention of these peaks indicates that the core structure of quercetin is preserved in the nanoparticles, suggesting that quercetin effectively stabilizes the AgNPs through its hydroxyl and carbonyl groups. This structural integrity is crucial for the bioactivity and stability of Q-AgNPs. The antibacterial activity of Q-AgNPs was evaluated against three bacterial strains: *Escherichia coli (E. coli), Bacillus subtilis (B. subtilis), and Klebsiella pneumoniae (K. pneumoniae).* The results from the disc diffusion method indicate a clear dose-dependent inhibition of bacterial growth. At the highest concentration of 1000 µg/ml, Q-AgNPs exhibited significant zones of inhibition for all tested bacteria, with *B. subtilis* showing the largest inhibition zone of 28 mm. This suggests that Q-AgNPs are particularly effective against Gram-positive bacteria, which is consistent with previous studies highlighting the heightened sensitivity of Gram-positive bacteria to nanoparticle-induced stress due to their thicker peptidoglycan layers (Sheng *et al.*, 2022). The observed antibacterial activity can be attributed to several mechanisms. The primary mode of action is likely the disruption of bacterial cell membranes by the Q-AgNPs, leading to increased permeability and eventual cell death. Additionally, the

generation of reactive oxygen species (ROS) by Q-AgNPs can induce oxidative stress in bacterial cells, further contributing to their antibacterial efficacy. The binding of silver ions to thiol groups in bacterial proteins and enzymes can also disrupt critical cellular functions, culminating in bacterial cell death .

The findings from this study underscore the potential of Q-AgNPs as a versatile and effective antimicrobial agent. The ease of synthesis and the robust antibacterial properties of Q-AgNPs make them a promising candidate for further development and application in clinical settings. Future research should focus on elucidating the detailed molecular mechanisms underlying the antibacterial activity of Q-AgNPs through advanced in-vitro and in-silico studies. Additionally, exploring the cytotoxicity and biocompatibility of Q-AgNPs will be essential for assessing their safety and therapeutic potential.

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