RESEARCH PAPER

Antibiofilm potential of quercetin mediated silver-selenium nanoparticles against skin infection causing Streptococcus pyogenes and Staphylococcus aureus

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Short title: Antibiofilm Activity of Quercetin-Mediated Ag–Se Nanoparticles Against Skin Pathogens.

ABSTRACT

Staphylococcus aureus and Streptococcus pyogenes are common skin infections that complicate the management of the infection due to biofilm formation that enhances resistance of the microbes to conventional antibiotics. The current paper intended to review and analyze the anti-biosis effects of quercetin-modified silver-selenium nanoparticles (Que-AgSeNPs) against these epidermidal pathogens. Quercetin is an antimicrobial and antioxidant with known antimicrobial and antioxidant properties and was utilized as a reducing and stabilizing agent in the preparation of Ag-Se nanoparticles by chemical synthesis. The properties of the synthesized nanoparticles were determined by UV-Visible spectroscopy, SEM, EDX, and FTIR. The UV-Vis analysis of Que-AgSeNPs indicates peaks around 270 nm and 410 nm are attributed to the plasmon resonance of selenium and silver nanoparticles, respectively. The SEM results indicates that size of the synthesis Que-AgSeNPs were found to be 37 -190 nm. The composition of the nanostructures was confirmed by EDX analysis to ascertain the elemental composition of silver and selenium. The FTIR spectra revealed that functional groups of quercetin stabilized the nanoparticles. The antimicrobial efficacy of Que-AgSeNPs against S. aureus and S. pyogenes was determined by the well diffusion procedure, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests. The nanoparticles were found to be highly antibacterial with evident areas of inhibition as well as low values of MIC and MBC of the two pathogens. Moreover, mixed biofilm assay was used to determine the antibiofilm efficacy which indicated that biofilm formation was significantly reduced in the presence of Que-AgSeNPs.

Keywords: Quercetin-mediated AgSeNPs, Antibiofilm activity, Staphylococcus aureus, Streptococcus pyogenes, Skin pathogens

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1. Introduction

Skin infections occupy one of the leading places in the world in terms of the number of infectious diseases, both mild and superficial, or dangerous and invasive diseases [1]. The skin plays a major role in defence against microbial attack, but the barrier of the skin is disturbed by the cuts, burns, surgical injuries or the immunocompromised condition leading to the colonization and infection of the microbes [2]. *Staphylococcus aureus* and *Streptococcal pyogenes* have been the most predominant bacteria in causing skin and soft tissue infections (SSTIs). The pathogens cause impetigo, cellulitis, erysipelas, folliculitis, and necrotizing fasciitis, which negatively affects morbidity and health care burden across the globe [3].

One of the great complications with skin infections is that these pathogens can develop biofilms on disturbed skin and on medical equipment [4]. Biofilms are organized multifaceted communities of microbial cells that are surrounded by an extracellular polymeric matrix which shields the bacteria against the host immune systems and antimicrobials. The infection of biofilm-related *S. aureus* and *S. pyogenes* is especially hard to eliminate, leading to chronicity, recurrence and delayed healing of the wounds. The existence of biofilms may enhance the antibiotic resistance of bacteria by up to

1000-fold, which renders the common methods of treatment ineffective and extends the disease process [5].

Topical and systemic antibiotics, such as b-lactams, macrolides, glycopeptides, and amino-glycosides, are currently used as the primary approach to the treatment of bacterial skin infections [6]. Even though these treatments are useful in acute infections, their widespread or persistent application has resulted in the development of multidrug-resistant (MDR) strains that include methicillin-resistant *Staphylococcus aureus* (MRSA). Moreover, antibiotics usually do not enter biofilms effectively and can be associated with some unwanted consequences, such as skin irritation, hypersensitivity reactions, normal skin dysbiosis, and systemic toxicity [7]. These constraints underscore the urgent requirement of alternative or complementary therapeutic measures that would be more effective against antibiofilm and have fewer side effects [8].

Over the last few years, phytochemical-inspired nanotechnology has become one of the potential solutions in biomedical studies and antimicrobial treatment [9]. Phytochemicals like flavonoids, polyphenols and terpenoids have antimicrobial, antioxidant, and anti-inflammatory properties inherent to them [10]. One of them is quercetin, a naturally occurring flavonoid present in fruits and vegetables, which was found to disrupt the cell membranes of bacteria, quorum sensing, and biofilm formation. The limited use of quercetin as a clinical agent, however, has been attributed to its low levels of solubility and bioavailability that could be improved greatly using nanoparticle-based delivery systems [11].

The metal-based nanoparticles, especially silver nanoparticles, are also famously reported to be having a broad-spectrum antimicrobial activity [12]. Selenium nanoparticles on the other hand have excellent biocompatibility, antioxidant capacity as well as lower toxicity than other metal nanoparticles [13]. In a way, bimetallic silver-selenium nanoparticles have been developed to provide synergized antimicrobial activities due to the combination of the high bactericidal effect of silver with the biological safety and redox-controlling ability of selenium [14]. Phytochemicals like quercetin are used to greenly synthesize such nanoparticles and also stabilize it, improve the biological activity of the nanoparticle and reduce the environmental and chemical risks caused by other methods used to synthesize the nanoparticle [15].

In this regard, the current research is directed at the production of silver-selenium nanoparticles through quercetin and the assessment of their antibiofilm and antimicrobial efficacy on *Streptococcus pyogenes* and *Staphylococcus aureus*, which cause skin infections. This phytochemical-inspired nanotherapeutic will address the weakness of the traditional antibiotics as it targets biofilm formation and bacterial viability simultaneously. The results of this research can be used in the formation of new, secure, and effective nanomaterials to control the biofilm-related skin infections.

2. Materials and methods

2.1. Materials and microbial culture

Quercetin (Sigma-Aldrich with ≥95% purity) (**Fig. 1**), silver nitrate ($AgNO_3$) (Sigma-Aldrich with ≥99.0% purity), and selenium nitrate ($Se(NO_3)_2$) (Sigma-Aldrich ≥98% purity) were of analytical grade and were obtained without doing any further purification. The solutions were all prepared with the help of double-distilled water. Microbiological assays were done with Mueller-Hinton agar, nutrient broth, crystal violet and phosphate-buffered saline (PBS). *Streptococcus pyogenes* and *Staphylococcus aureus* bacterial strains were obtained in an authenticated microbial culture collection and grown on nutrient agar slants at 4 °C with periodical sub-culturing to maintain the bacteria [16].

2.2. Preparation of the quercetin-stabilized silver-selenium nanoparticles (Que-AgSeNPs)

Que-AgSeNPs are silver-selenium nanoparticles stabilized with quercetin (a reduction-based), green chemical methodology, in which quercetin served as both a reducing and stabilizing agent [17]. At the first, quercetin with 2mg/mL concentration was dissolved in sufficient quantity of aqueous solution by placing it in a flask with a small amount of heating and stirring until completely dissolved. Individually, solutions of 1M silver nitrate and 1M selenium nitrate were made in distilled water at a concentration [18]. Quercetin solution was made in the presence of a constant magnetic stirring rate the metal salt solutions were gradually added drop-by-drop to the quercetin solution. The reaction was kept under controlled temperature and pH to favour the nucleation and formation of nanoparticles [19]. The transformation of color of the reaction mixture slowly showed that Que-AgSeNPs were formed. The reaction was then completed whereby the suspension was centrifuged at high speed to retrieve the nanoparticles. Knowing that the unreacted ions and excess quercetin

remained unreacted, the pellet was rinsed with distilled water several times and dried to get powdered Que-AgSeNPs to be further analyzed [20].

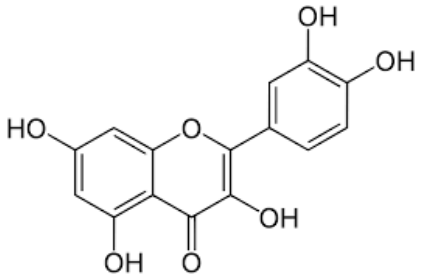

Figure 1. Structure of quercetin.

2.3. UV-visible spectroscopic analysis

UV-Visible spectroscopy (PerkinElmer Lambda 25) was used to determine the formation and optical properties of Que-AgSeNPs [21]. The scan of a nanoparticle suspension small aliquot was performed in the wavelength range of 200-800 nm in a UV-Vis spectrophotometer [22]. The emergence of typical absorption peaks that are associated with the surface plasmon resonance was evidence of the successful nanoparticle production and stability [23].

2.4. Energy-dispersive X-ray analysis and scanning electron microscopy (SEM-EDX)

Scanning electron microscopy (JEOL JSM-7600F Field Emission Scanning, Tokyo, Japan) was used to determine the surface morphology, the size of particles and the structures of Que-AgSe NPs [24]. Imaging of dried nanoparticle samples was done by mounted samples on aluminum stubs and sputtered with a thin conductive layer. Energy-dispersive X-ray spectroscopy (EDX) was used as an elemental analysis to analyze the composition of the silver and selenium incorporation into the SEM system [25].

2.5. Fourier transform infrared spectroscopy

The functional groups that are related to reducing and stabilizing nanoparticles by quercetin have been identified using the FTIR spectroscopy using Bruker tensor 27 FTIR Spectrometer (Billerica, MA, USA) [22]. Que-AgSeNPs were dried at 105 °C, and then analyzed between 4000-400 cm^{-1} [26]. The spectra obtained were compared to the one of pure quercetin to detect any changes in the locations of the peaks, which indicated the interaction of quercetin functional groups with metal ions in the formation of nanoparticles [27].

2.6. Assessment of antimicrobial activity using agar well diffusion method

The agar well diffusion assay was done on Que-AgSeNPs against *Streptococcus pyogenes* and *Staphylococcus aureus* to determine the antimicrobial activity effects. The bacterial suspensions were made and standardized in a turbidity. The bacterial cultures were uniformly inoculated on the sterile Mueller-Hinton agar plates using a sterile swab [28]. Uniformly diameter wells in the agar were punched and low and high concentrations of Que-AgSeNPs were added to each well with positive control. To determine the efficacy of antibacterial activity, plates were left at 37 °C to incubate them in 24 hours, and the diameter of the zone of inhibition around each well was then measured in millimeters [25].

2.7. Measuring minimum inhibitory concentration for fabricated Que-AgSeNPs

The lowest concentration of Que-AgSeNPs that inhibits was identified by the broth microdilution method. The nanoparticles were serially two-fold diluted in nutrient broth in sterile microtiter plates [20]. A bacterial suspension was standardized and introduced into each well which was then incubated at 37 °C during 24 hours. The minimum concentration of Que-AgSeNPs that exhibited no growth of bacteria was termed as the MIC [29].

2.8. Minimal bactericidal concentration

MIC wells that had no overt growth were sub-cultured on new nutrient agar plates at which the minimum bactericidal concentration (MBC) was established [30]. The plates were incubated at 37 °C and allowed to incubate after 24 hours. The lowest reading that gave total non-formation of the bacterial colonies was the MBC [31].

2.9. Mixed biofilm inhibition assay

Que-AgSeNPs were tested on a 96-well plate assay to determine its antibiofilm. Que-AgSeNPs were sub-culture at sub-MIC concentrations in 96-well plate containing nutrient broth and bacterial cultures inoculated and incubated under static conditions to facilitate the growth of biofilms [20]. The wells were then incubated and gently sprayed with PBS to clear off the planktonic cells then stained with crystal violet. Unbound stain was washed off and the bound dye was dissolved in the right solvent. The biofilm formation was determined by the absorbance of the plate at 570 nm with a microplate reader [29] and CLSM was performed to differentiate live and dead cells before and after treatment. For CLSM analysis, planktonic cells from the

Que-AgSeNPs-treated and control biofilms were removed and washed with PBS buffer. Then, the formed biofilm was stained with the LIVE/DEAD BacLight Bacterial Viability Kit (green-fluorescent SYTO 9 and red-fluorescent propidium iodide dyes) for 30 min in dark. Then, the live/dead cells proportion in the stained sample was visualized using CLSM system (Leica Microsystem-DMI8, Germany). Subsequently, the ratio of green to red fluorescence intensities were quantified using ImageJ software (National Institutes of Health, USA).

2.10. Statistical analysis

The obtained experimental data were expressed as mean ± standard deviation (SD) of triplicate experiment settings.

3. Results

3.1. UV-visible spectroscopic of Que-AgSeNPs

The formation of quercetin-stabilized silver-selenium nanoparticles (Que-AgSeNPs) was first observed by the visual change in the color of the reaction solution that showed a reduction of both metal ions and the formation of nanoparticles [32]. The realization of successful synthesis and optical stability of the Que-AgSeNPs was confirmed by the UV-Visible spectroscopic analysis and a characteristic absorption band in the expected range of silver-selenium based nanoparticles [33]. The sharpness and strength of the absorption peak indicated that the particles were formed uniformly and that quercetin is an effective stabilizer [22].

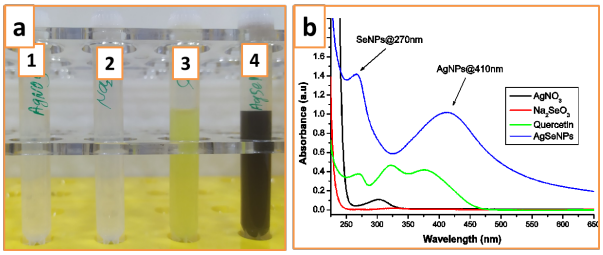

Fig. 2. (a) Synthesis of Que-AgSeNPs and (b) UV-vis spectrum of Que-AgSeNPs.

The UV–visible absorption spectra of $AgNO_3$, Na_2SeO_3, quercetin, and the synthesized Que-AgSeNPs were recorded in the range of 200–650 nm to confirm the formation of AgSe nanoparticles and their optical properties. **Fig. 2**ab shows the synthesis of AgSeNPs using flavonoid quercetin ad their respective UV-vis spectrum. The spectrum of silver nitrate ($AgNO_3$) shows a strong absorption band in the UV region below 250 nm, which corresponds to the electronic transitions of Ag^+ ions in solution. Similarly, sodium selenite (Na_2SeO_3) exhibits an intense absorption peak in the deep UV region (<240 nm) due to charge transfer transitions associated with selenium oxyanions. The quercetin spectrum displays characteristic absorption bands between 250–380 nm, attributed to its flavonoid structure. These bands arise from π–π* transitions within the aromatic rings and n–π* transitions associated with the carbonyl and hydroxyl functional groups. The peaks around ~260–270 nm (Band II) and ~350–370 nm (Band I) are typical of flavonol compounds and confirm the presence of quercetin as a reducing and stabilizing agent in the synthesis process [34].

After the reaction, the UV–Vis spectrum of the synthesized Que-AgSeNPs (blue curve) shows two distinct absorption features [35]. A shoulder peak appears around ~270 nm, which can be attributed to the selenium nanoparticle (SeNPs) component and possible ligand to metal charge transfer interactions involving quercetin-bound selenium species [36]. More importantly, a broad and prominent absorption band is observed around ~410 nm, corresponding to the surface plasmon resonance (SPR) of silver nanoparticles (AgNPs) [37]. The presence of this SPR band confirms the successful reduction of Ag^+ ions and formation of metallic silver within the AgSe nanostructure [38].

3.2. SEM-EDX of Que-AgSeNPs

The analysis of scanning electron microscopy (SEM) showed that the Que-AgSeNPs synthesized mainly had spherical to quasi-spherical geometry with a relatively uniform distribution and low agglomeration. **Fig. 3**a illustrates the SEM image of Que-AgSeNPs. **Fig. 3**b shows the EDX micrograph of Que-AgSeNPs. The particle size was observed to be at the nanometer level which meant that there was control of nucleation and growth during the synthesis process. The SEM results indicates that size of the synthesis Que-AgSeNPs were found to be 37 -190 nm. The elemental composition of the nanoparticles was determined using energy-dispersive X-ray (EDX) analysis and the presence of strong signals of silver and selenium with traces of carbon and oxygen can be explained by the capping of quercetin on the surface of the nanoparticles [39].

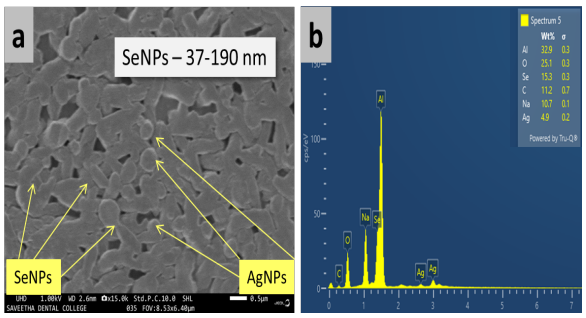

Fig. 3. (a) SEM image of Que-AgSeNPs and (b) EDX micrograph of Que-AgSeNPs.

The surface morphology of the synthesized nanoparticles was examined using scanning electron microscopy. The SEM micrograph (Fig. 3a) reveals the formation of aggregated nanoparticle clusters with irregular and quasi-spherical morphology distributed across the surface. The particles appear relatively compact and closely packed, indicating strong interactions between the synthesized components. The particle size was estimated to be in the range of 37–190 nm, confirming the formation of nanoparticles within the nanometer scale. The observed morphology suggests that selenium nanoparticles (SeNPs) form the primary matrix, while silver nanoparticles (AgNPs) are distributed over the surface or embedded within the selenium structure [40].

The aggregation observed in the image may be attributed to interparticle interactions and stabilization by quercetin molecules, which act as both reducing and capping agents during the green synthesis process. The elemental composition of the synthesized nanoparticles was analyzed using energy dispersive X-ray spectroscopy (EDX) (Fig. 3 b). The EDX spectrum confirms the presence of the main elements associated with the synthesized nanocomposite. Characteristic peaks corresponding to silver (Ag) and selenium (Se) are clearly observed, confirming the formation of AgSe nanoparticles [41]. The elemental composition obtained from the EDX analysis shows approximately Al (32.9 wt%), O (25.1 wt%), Se (15.3 wt%), C (11.2 wt%), Na (10.7 wt%), and Ag (4.9 wt%). The presence of selenium and silver peaks verifies the successful incorporation of both elements into the nanostructure. The carbon and oxygen signals may originate from the organic molecules of quercetin, which act as stabilizing agents on the nanoparticle surface. The sodium signal may be attributed to the precursor sodium selenite (Na_2SeO_3) used during synthesis, while the aluminum peak likely arises from the aluminum sample holder used during SEM analysis [42].

3.3. FTIR spectroscopy of Que-AgSeNPs

FTIR spectroscopy was used to discover the functional groups which play a role in the formation and stabilization of the nanoparticles mention in **Fig 4.** After comparing the FTIR spectrum of Que-AgSeNPs with pure quercetin, it was observed that there were evident changes in shifts and intensities of distinct peaks at hydroxyl (-OH) and carbonyl (C=O) groups [43]. These spectral variations tell of the participation of quercetin functional groups to the reduction of metal ions and the resultant stabilization of the nanoparticles [44].

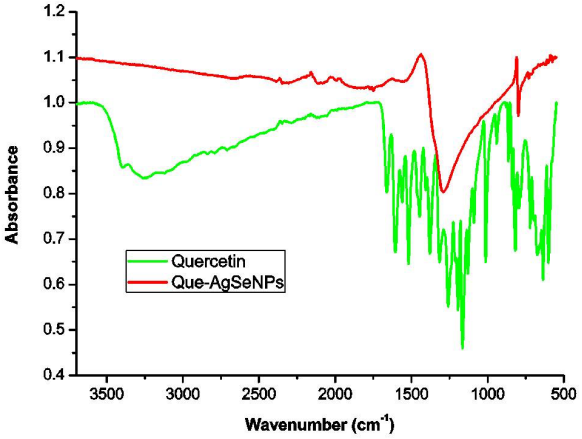

Fig. 4. FTIR spectra of quercetin-AgSeNPs.

3.4. Antimicrobial activity of Que-AgSeNPs through well diffusion method

Que-AgSeNPs antibacterial activity was also determined by agar well diffusion technique against *Streptococcus pyogenes* and *Staphylococcus aureus* demonstrate in **Fig 5** [45]. Both bacterial strains demonstrated clear and concentration-dependent zones of inhibition, which proved the antibacterial activity of the synthesized nanoparticles [46]. A relatively bigger zone of inhibition was found with *Staphylococcus aureus* as it was more susceptible and *Streptococcus pyogenes* showed moderate sensitivity to Que-AgSeNP treatment [47].

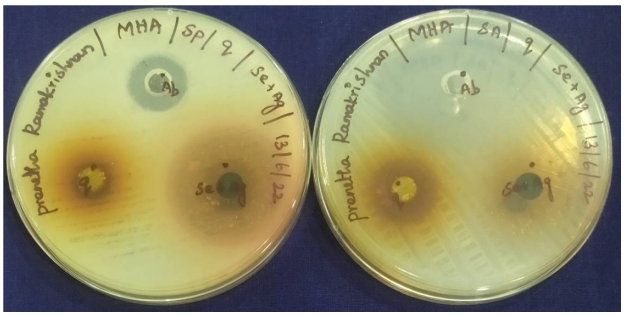

Fig. 5. Antimicrobial activity of QUE-AgSeNPs against skin pathogens.

Zone of inhibition (ZOI) study. Q – quercetin. MHA - Mueller Hinton Agar. 1. *Streptococcus pyogenes*, 2. *Staphylococcus*, Ab – Antibiotic s (Penicillin for SA / Cephalexin for SA).

3.5. Antimicrobial activity of Que-AgSeNPs through MIC

Que-AgSeNPs exhibited good growth inhibition with low concentration of both strains of bacteria. The MIC of *S. aureus* was also less than that of *S. pyogenes*, indicating strain dependent antibacterial action. These results show that the Que-AgSeNPs can prevent bacterial growth effectively at low levels [48]. **Fig. 6** shows the MIC of Que-AgSeNPs against *S. aureus* and *S. pyogenes.*

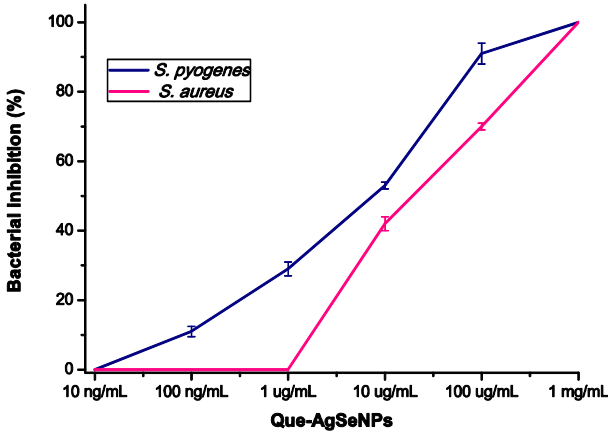

Fig. 6. Minimal inhibitory concentration (MIC) test of QUE-AgSeNPs against skin pathogens.

The antibacterial activity of the synthesized quercetin-mediated silver–selenium nanoparticles (Que-AgSeNPs) was evaluated using the minimal inhibitory concentration (MIC) assay against two Gram-positive bacterial strains, *S. pyogenes* and *S. aureus*. The results demonstrate a concentration-dependent antibacterial effect of the synthesized nanoparticles. As shown in the figure, the inhibitory activity increased progressively with increasing nanoparticle concentration. For *S. pyogenes*, a gradual increase in inhibition was observed from the lowest tested concentration, indicating that the bacterium is highly susceptible to Que-AgSeNPs. The inhibition percentage increased steadily and reached its maximum at the highest nanoparticle concentration, suggesting strong antibacterial activity [49].

In contrast, *S. aureus* exhibited lower sensitivity at the initial concentrations, with minimal inhibition observed at the lower doses. However, a significant increase in bacterial inhibition occurred at higher nanoparticle concentrations, indicating that the antibacterial effect becomes more pronounced as the dosage increases. At the highest concentration tested, both bacterial strains showed comparable levels of inhibition, demonstrating the broad-spectrum antibacterial potential of the synthesized nanoparticles [50].

The enhanced antibacterial activity of Que-AgSeNPs may be attributed to the synergistic effect of silver and selenium nanoparticles combined with the bioactive properties of quercetin. Silver nanoparticles are known to disrupt bacterial cell membranes, generate reactive oxygen species (ROS), and interfere with cellular metabolic pathways, while selenium nanoparticles can induce oxidative stress and damage bacterial proteins and DNA. Quercetin may further enhance this activity by facilitating nanoparticle stabilization and contributing additional antimicrobial effects [50].

3.6. Antimicrobial activity of Que-AgSe NPs through MBC

Studies on minimum bactericidal concentration (MBC) also supported the bactericidal capability of Que-AgSeNPs. The total inhibition of the growth of bacterial colonies was detected at the levels slightly above the MIC values. Like the MIC results, the *S. aureus* had lower values of MBC than *S. pyogenes*, which implies that it is more susceptible to bactericidal activity [51].

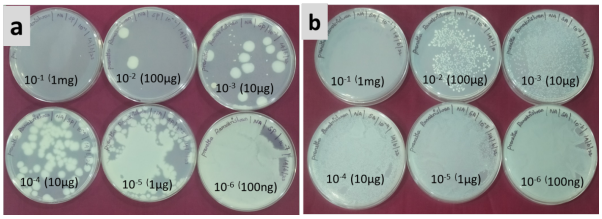

Fig. 7. Minimal bactericidal concentration (MBC) test for Que-AgSeNPs against

skin infection causing (a) *Streptococcus pyogenes* and (b) *Staphylococcus aureus.*

3.7. Antibiofilm assay of Que-AgSeNPs

Que-AgSeNPs were evaluated based on a microtiter plate biofilm assay to determine their antibiofilm activity. Sub-MIC concentration of Que-AgSeNPs showed a great reduction in biofilm formation in both the bacterial strains as compared to untreated controls. Biofilm formation was more inhibited in *S. aureus* implying that Que-AgSeNPs would disrupt the bacterial adhesion and initial stage of biofilm formation [52]. As shown in **Fig 8**AB, the CLSM results reveal that complete deadness of mixed *S. aureus* and *S. pyogenes* biofilm was found at 2.0xMIC of Que-AgSeNPs after 48 h.

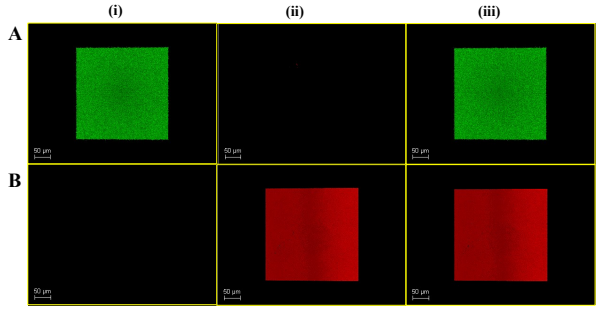

Figure 8. CLSM studies. (A) Control (7-days developed) and (B) 2xMIC Que-AgSeNPs-treated mixed *S. aureus* and *S. pyogenes* biofilm after 24 h. (i) Live cells, (ii) Dead cells, and (iii) Live/dead combined.

4. Discussion

The current work tested the antibiofilm and antibacterial activity of silver selenium nanoparticles mediated by quercetin on *Streptococcus pyogenes* and *Staphylococcus aureus* as one of the primary causes of skin and soft tissue infections. The nanoparticles synthesis and their characteristics should be confirmed by UV-Visible spectroscopy, SEM-EDX, and FTIR and then they should be tested in terms of agar well diffusion, MIC, MBC, and antibiofilm.

The synthesis of nanoparticles by UV-Visible spectroscopy was confirmed by the characteristic surface plasmon resonance (SPR) maximum of silver nanoparticles. The same was observed in other earlier nanoparticle synthesis research findings that resulted in UV-Vis peaks at 400-450 nm to indicate the successful formation of AgNPs because of collective oscillation of electrons on the surface of the nanoparticle. The application of such spectral confirmation is common to green-synthesized nanoparticles and is associated with nanoparticle formation and stability. Similar UV-Vis validation was also observed in literature that synthesized plant-mediated silver nanoparticles where typical peaks were observed that serve as indicators of reduction of silver ions into nanoscale particles [53].

SEM analysis in the current study has shown that the formed particles are of nanoscale morphology and EDX analysis showed that the elements within the particles were silver and selenium which proved that the nanocomposites were formed. The same morphological findings have been established on biosynthesized silver nanoparticles, which are normally spherical with a diameter of between 40-90 nm and have a high antibacterial activity on *S. aureus* [53]. Selenium also in combination with silver can also be an added benefit as it could work synergistically with antimicrobial effects by enhancing reactive oxygen species formation and bacterial membrane perturbation.

FTIR analysis revealed the presence of quercetin functional groups, and this proved that quercetin was a reducing and stabilizing agent in the production of nanoparticles. Flavonoids like quercetin have hydroxyl and carbonyl functionalities that aid in reducing metal ions as well as stabilization of nanoparticles by capping interactions. Other researchers have also been able to prove that quercetin can be used as a reducing and capping molecule in the production of nanoparticles as a stabilizer of silver nanoparticles and enhancing the biological functions of the latter [54].

The agar well diffusion method of evaluating antibacterial activity showed there was significant inhibition zone against both *S. aureus* and *S. pyogenes*. These results match the previous research that found good antibacterial properties of biosynthesized silver nanoparticles against *Staphylococcus aureus* with inhibition zone of about 10-21 mm at different concentrations of nanoparticles. It is primarily believed that the antibacterial effect is the result of nanoparticle interaction with the cell membranes, the cell wall disruption, and release of silver ions that disrupt cellular metabolic pathways.

Another confirmation of the bacteriostatic and bactericidal effect of the synthesized nanoparticles was the MIC and MBC results. The values of MIC are usually the lowest with nanoparticles because they have a high

surface area and increased ability to penetrate bacterial membranes. The earlier findings on nanoparticles have also been associated with high antimicrobial activity at low dosages, which implies that they can be used as substitutes to traditional antibiotics. Specifically, silver nanoparticle preparations have been demonstrated to inhibit methicillin resistant *S. aureus* at very low MIC values, and as such, they exhibit a high antibacterial potential [55].

The main results of the study consist of the high antibiofilm potential of the produced nanoparticles. The formation of biofilms is a significant virulence attribute of *S. aureus* and Streptococcus species that leads to chronic infections and resistance to antibiotics. The synergistic action of silver, selenium and quercetin could explain the antibiofilm action in this study. Quercetin as such has been reported to disrupt bacterial adhesion, quorum sensing and gene expression in biofilm formation. Moreover, nanoparticle-based preparations have also been reported to interfere with biofilm structures and reduce bacterial survival in biofilms.

It has also been observed in the previous researches that quercetin has augmented antibiofilm outcomes at the nanoparticles. As an illustration, nanoparticles decorated with quercetin were demonstrated to prevent bacterial growth and biofilm formation by modifying the expression of genes that are related to virulence and disrupting the process of bacterial adhesion [56]. Likewise, nanocomposites of silver-quercetin have been said to have a major role in disrupting the structure of biofilms and compromising the viability of bacteria in developed biofilms [57].

The increased activity of the current study could be explained by the joint antimicrobial action of silver, selenium, and quercetin. Silver nanoparticles have been known to induce membrane damage and oxidative stress, selenium nanoparticles have been known to generate reactive oxygen species and disrupt cellular metabolism, and quercetin has been known to prevent bacterial adhesion and the gene expression of biofilms. This synergistic activity probably leads to the powerful antibacterial and antibiofilm activity against skin infection pathogens.

In general, experimental results in the current paper indicate that silver-selenium nanoparticles mediated by quercetin have a potential application as an antimicrobial approach to biofilm forming pathogens like *Streptococcus pyogenes* and *Staphylococcus aureus*. The nanoparticles of metallic particles in combination with the natural flavonoids can provide a new and efficient way of developing antimicrobial agents that are directed to treating skin infections and overcoming resistance to antibiotics.

5. Conclusions

Finally, this study showed how it is possible to greenly synthesize quercetin-stabilized nanoparticles of silver-selenium with the defined physicochemical properties. The addition of silver, selenium, and quercetin led to the stabilization of nanoparticles with the increased functionality associated with biology, as it was confirmed with the help of spectroscopic and microscopic analysis. In addition, it was found that Que-AgSeNPs had strong antibacterial and antibiofilm effects on *Streptococcus pyogenes* and *Staphylococcus aureus*, which suggests that they could serve as alternative antibiotics. The above synergetic outcomes indicate that these nanoparticles have potential further use in biomedical and pharmaceutical settings, especially in fighting biofilm-related infections and antibiotic-resistant pathogens.

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References

1. Nibret, G., M.A. Messelu, and A. Dagne, *Clinical Application of Benzathine Penicillin G as Secondary Prophylaxis for Patient with Acute Rheumatic Fever and Rheumatic Heart Disease: A Comprehensive Review.* International Journal of Cardiology Congenital Heart Disease, 2026: p. 100661.
2. Anshory, M., et al., *Dermatological manifestation of HIV infection: systematic review and meta-analysis.* BMC infectious diseases, 2025. **25**(1): p. 1446.
3. Paulson, D., *A Comprehensive Review of Bacterial Skin Infections: Clinical Insights and Life-Threatening Outcomes.* 2025.
4. Kumari, M., et al., *Nanotherapeutics for skin and soft tissue infections*, in *Applications of Nanotherapeutics and Nanotheranostics in Managing Infectious Diseases*. 2025, Elsevier. p. 135-156.
5. Pujol Vallverdú, R.M., et al., *Special Conditions, Symptoms, and Syndromes*, in

Dermatology Cyberderm WebBook. 2025, Springer. p. 633-686.

6. Ali, F.Z., et al., *Exogenous Methicillin-Resistant Staphylococcus aureus Endophthalmitis is Caused by Multidrug-Resistant Lineages that are Associated with Poor Outcomes.* Ocular Immunology and Inflammation, 2025. **33**(3): p. 446-456.
7. Mohammed, R., et al., *Public health implications of multidrug-resistant and methicillin-resistant Staphylococcus aureus in retail oysters.* Scientific Reports, 2025. **15**(1): p. 4496.
8. Dos Santos, I.R., et al., *First record of methicillin-resistant Staphylococcus aureus (MRSA) isolated from the Meia Ponte River and effluent in Brazil: An analysis of 1198 isolates.* New Microbes and New Infections, 2025. **66**: p. 101603.
9. Dawane, V., et al., *Biomimetic Route-Assisted Synthesis of Nanomaterials: Characterizations and Their Applications*, in *Handbook of Green and Sustainable Nanotechnology: Fundamentals, Developments and Applications*. 2023, Springer. p. 3-21.
10. Rao, R.B., et al., *Screening the Antibiotic Activity of Cave Actinobacteria Against Multidrug-Resistant Strains of Pseudomonas aeruginosa and Methicillin-Resistant Staphylococcus aureus.* International Journal of Microbiology, 2026. **2026**(1): p. 9984546.
11. Akinfenwa, A.O. and A.A. Hussein, *Phyto-metallic nanoparticles: Biosynthesis, mechanism, therapeutics, and cytotoxicity*, in *Toxicity of Nanoparticles-Recent Advances and New Perspectives*. 2023, IntechOpen.
12. Mikhailova, E.O., *Green silver nanoparticles: an antibacterial mechanism.* Antibiotics, 2024. **14**(1): p. 5.
13. Anjum, S., et al., *Selenium nanoparticles in cancer therapy: unveiling cytotoxic mechanisms and therapeutic potential.* Cancer Reports, 2025. **8**(6): p. e70210.
14. Wang, X., et al., *Fate and physiological effects of foliar selenium nanoparticles in wheat.* ACS nano, 2025. **19**(23): p. 21792-21806.
15. Varlamova, E.G., *Selenium-containing compounds, selenium nanoparticles and selenoproteins in the prevention and treatment of lung cancer.* Journal of Trace Elements in Medicine and Biology, 2025. **88**: p. 127620.
16. Mmbando, G.S. and M. Wilson Salaja, *The potential of Streptococcus pyogenes and Escherichia coli bacteriocins in synergistic control of Staphylococcus aureus.* Preparative Biochemistry & Biotechnology, 2025. **55**(7): p. 846-854.
17. El-Behery, R.R., E.-S.R. El-Sayed, and G.S. El-Sayyad, *Gamma rays-assisted bacterial synthesis of bimetallic silver-selenium nanoparticles: powerful antimicrobial, antibiofilm, antioxidant, and photocatalytic activities.* BMC microbiology, 2023. **23**(1): p. 224.
18. Attia, M.S., et al., *Promising antagonistic effect of bimetallic silver-selenium nanoparticles against Ralstonia solanacearum-causing wilt disease in eggplant (Solanum melongena L.).* Physiological and Molecular Plant Pathology, 2024. **133**: p. 102369.
19. Reddy, J.R.S.M., et al., *Eradication of dental pathogens using flavonoid rutin mediated silver-selenium nanoparticles.* Inorganic Chemistry Communications, 2023. **157**: p. 111391.
20. Khormi, M.A., et al., *Unwrapping the phytofabrication of bimetallic silver–selenium nanoparticles: Antibacterial, Anti-virulence (Targeting magA and toxA genes), anti-diabetic, antioxidant, anti-ovarian, and anti-prostate cancer activities.* Green Processing and Synthesis, 2025. **14**(1): p. 20250087.
21. Singh, R.K., et al., *Green synthesis of silver nanoparticles using neem and turmeric extract and its antimicrobial activity of plant mediated silver nanoparticles.* Journal of Oral Biology and Craniofacial Research, 2025. **15**(2): p. 395-401.
22. Hinaz, F., L.S. Venkatesan, and R. Duraisamy. *Myricetin-capped biosynthesis of silver-selenium nanoparticles and its anticandidal activities*. in *Journal of Physics: Conference Series*. 2024. IOP Publishing.
23. Ali, I.A.M. and A.B. Ahmed, *Recent Advances in Green Synthesis, Chemical-Physics Characterizations, and Biological Adeptness of*

Selenium and Silver Nanoparticles for Medical Application. 2025.

24. Elakraa, A.A., et al., *Retracted Article: Cefotaxime incorporated bimetallic silver-selenium nanoparticles: promising antimicrobial synergism, antibiofilm activity, and bacterial membrane leakage reaction mechanism.* RSC advances, 2022. **12**(41): p. 26603-26619.
25. Unal, İ., *Green synthesis of multi-walled carbon nanotube-reinforced hydroxyapatite doped with silver and silver-core selenium-shell nanoparticles: synthesis, characterization, and biological activity.* Nanomaterials, 2025. **15**(3): p. 179.
26. Adefisan-Adeoye, A.O., et al., *Modulatory effects of bisdemethoxycurcumin-conjugated silver/selenium nanoparticles on 7, 12-dimethylbenz (a) anthracene-induced mammary tumorigenesis.* Cancer Nanotechnology, 2025. **16**(1): p. 19.
27. Hassan, M.G., et al., *Biogenic selenium nanoparticles and selenium/chitosan-Nanoconjugate biosynthesized by Streptomyces parvulus MAR4 with antimicrobial and anticancer potential.* BMC microbiology, 2024. **24**(1): p. 21.
28. Aktepe, N., A. Baran, and C. Keskin, *Cytotoxic and antimicrobial analysis of biosynthesized selenium nanoparticles from Solanum tuberosum peels.* International Journal of Agriculture Environment and Food Sciences, 2025. **9**(2): p. 559-569.
29. Soliman, M.K. and S.S. Salem, *Uncovering the potential of biofabricated Ananas comosus peel selenium nanoparticles for antibacterial, antibiofilm, suppression of virulence genes (can and LuxS), anticancer, and antioxidant properties.* BMC biotechnology, 2025. **25**(1): p. 51.
30. Ganesan, L., et al., *Pentatropis Capensis as a Novel Bioresource for Selenium Nanoparticle Synthesis: Insights into Biocompatibility, Cytotoxicity and in Vitro Toxicity Test on Zebrafish.* Applied Biochemistry and Biotechnology, 2025: p. 1-30.
31. Selim, S., et al., *Novel royal jelly-mediated green synthesis of selenium nanoparticles and their multifunctional biological activities.* Nanotechnology Reviews, 2025. **14**(1): p. 20250242.
32. Sivabalan, K., et al., *Synergistic effect of silver nanoparticles and titanium nanotubes loaded chitosan hydrogels for biomedical applications.* International Journal of Biological Macromolecules, 2025: p. 148460.
33. Hussain, A., *Plasmonic photothermal effect on cytotoxicity of biogenic nanostructure synthesized through Litchi chinensis Sonn.* Inorganic and Nano-Metal Chemistry, 2023. **53**(10): p. 1137-1149.
34. Tanvir, F., et al., *Synthesis of iron oxide nanoparticles, characterization, uses as nanozyme and future prospects.* Bioinspired, Biomimetic and Nanobiomaterials, 2023. **11**(4): p. 156-167.
35. Madheslu, M., et al., *Engineering optimization of silver nanoparticle synthesis using Clitoria ternatea leaf extract: Response surface methodology approach and biocompatibility assessment.* Chemical Physics Impact, 2026: p. 100995.
36. Penzel, S., et al., *A novel turbidity compensation method for water measurements by UV/Vis and fluorescence spectroscopy.* Measurement, 2025. **239**: p. 115447.
37. Singh, A.V., et al., *Investigating tattoo pigments composition with UV-Vis and FT-IR spectroscopy supported by chemometric modelling.* Current Analytical Chemistry, 2025. **21**(8): p. 988-1004.
38. Krishnan, P.H., et al., *Optimization of La and Ce mixed metal oxide hybrid for enhanced photoresponse in UV–Visible photosensing applications.* Journal of Materials Science: Materials in Electronics, 2025. **36**(22): p. 1377.
39. Uluer, M.E., et al., *Evaluation of advanced SEM-EDX tools for classification of complex particles in respirable dust.* Journal of Hazardous Materials, 2025: p. 139732.
40. Bork, M., et al., *Characterization of microplastics from antifouling coatings released under controlled conditions with an automated SEM-EDX particle analysis method.* Environmental Pollution, 2025: p. 127251.

41. Orilisi, G., et al., *Time-Effect Comparative Evaluation of Three Remineralizing Agents on Artificial Enamel Lesions: A SEM-EDX In Vitro Study.* Journal of clinical medicine, 2025. **14**(20): p. 7389.
42. Guendouz, A., et al., *New benzimidazole derivatives as efficient organic inhibitors of mild steel corrosion in hydrochloric acid medium: electrochemical, SEM/EDX, MC, and DFT studies.* Journal of Molecular Structure, 2025. **1321**: p. 139901.
43. Bunaciu, A.A., V.D. Hoang, and H.Y. Aboul-Enein, *Fourier transform infrared spectroscopy used in drug excipients compatibility studies.* Applied Spectroscopy Reviews, 2025. **60**(5): p. 385-403.
44. Shukla, U., *Fourier transform infrared spectroscopy: A power full method for creating fingerprint of molecules of nanomaterials.* Journal of Molecular Structure, 2025. **1322**: p. 140454.
45. Pushpanathan, S., et al., *Caffeic acid functionalized silver nanoparticles: a bionanoformulation and its assessment of cell cycle and in vitro cytotoxicity.* Next Nanotechnology, 2025. **7**: p. 100105.
46. Gwada, C.A., et al., *Phytochemical-assisted synthesis, optimization, and characterization of silver nanoparticles for antimicrobial activity.* RSC advances, 2025. **15**(18): p. 14170-14181.
47. Cai, Y., et al., *Self-assembling lauroylated antimicrobial peptide with superior antimicrobial activity, stability, and selectivity.* ACS Applied Materials & Interfaces, 2025. **17**(9): p. 13646-13659.
48. Radocchia, G., et al., *Carvacrol and thymol, a synergistic antimicrobial activity against bacterial and candida species.* MicrobiologyOpen, 2025. **14**(5): p. e70089.
49. Gunasekar, B., et al., *Biogenic synthesis of silver nanoparticles using Parthenium hysterophorus floral extract and their multifaceted biomedical applications.* Plant Nano Biology, 2025. **12**: p. 100148.
50. Long, N., et al., *Antimicrobial Activity and Metabolomic Analysis of Linalool Against Pathogenic Bacteria Methicillin-Resistant Staphylococcus aureus.* Infection and Drug Resistance, 2025: p. 731-744.
51. Schiavone, M.L., et al., *Green Chemistry Within the Circular Bioeconomy to Harness Chestnut Burr Extract's Synergistic Antimicrobial Activity Against Helicobacter pylori.* Molecules, 2025. **30**(2): p. 324.
52. Jeon, Y.-N., et al., *Bioactive molecules of microalgae Haematococcus pluvialis–mediated synthesized silver nanoparticles: Antioxidant, antimicrobial, antibiofilm, hemolysis assay, and anticancer.* Bioinorganic Chemistry and Applications, 2025. **2025**(1): p. 8876478.
53. Singh, J., et al., *Antibacterial, anti-biofilm and anti-virulence activity of biosynthesized silver nanoparticles against drug-resistant Staphylococcus aureus.* Veterinary Research Communications, 2025. **49**(6): p. 345.
54. Erci, F., *Antibacterial and antibiofilm activity of carboxymethyl cellulose stabilized silver nanoparticles synthesized using quercetin and their effects on soil respiration and enzymes.* Geomicrobiology Journal, 2022. **39**(8): p. 679-688.
55. González-Garibay, A.S., et al., *Efficacy of Stenocereus queretaroensis-Derived Silver Nanoparticles Against Methicillin-Resistant Staphylococcus aureus Biofilms: Synthesis and Antibiofilm Activity.* Inorganics, 2025. **13**(6): p. 178.
56. Habibi, A., M. Pakpour Roudsari, and S.M. ZiaZiabari, *Quercetin-conjugated magnetic nanoparticles inhibit Staphylococcus aureus growth and biofilm formation via downregulation of Coa and Hla genes.* AMB Express, 2025. **15**(1): p. 107.
57. Yu, L., et al., *The anti-biofilm effect of silver-nanoparticle-decorated quercetin nanoparticles on a multi-drug resistant Escherichia coli strain isolated from a dairy cow with mastitis.* PeerJ, 2018. **6**: p. e5711.