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EVALUATION OF ANTIBACTERIAL AND TIME-KILL KINETICS ANALYSIS OF *ASPALATHUS LINEARIS* MEDIATED SELENIUM NANOPARTICLES AGAINST *STREPTOCOCCUS MUTAN*S AND *STAPHYLOCOCCUS AUREUS*

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Abstract

Aim

To study the antibacterial activity and time-kill kinetics of selenium nanoparticles (Se NPs) synthesized using *Aspalathus linearis* against *Streptococcus mutans* and *Staphylococcus aureus*, exploring their potential as alternative antimicrobial agents.

Background

Selenium-derived nanomaterials are desirable in nanomedicine due to their anticancer effectiveness, antioxidant capabilities, minimal cytotoxicity, and chemotherapeutic qualities. The antimicrobial properties of Se NPs present a compelling case for their use as a novel therapeutic agent against a variety of pathogens, especially in the context of rising antibiotic resistance.

Methods

Green synthesis of Se NPs using *A. linearis* was characterized by a UV visible spectrophotometer and the antibacterial activity of Se NPs was used against *S. mutans* and *S. aureus* using the agar well diffusion and time-kill kinetics method**.**

Result

The UV absorbance peak of *A.linearis* - Se nanoparticles observed at 320 nm. The inhibition zones increased with the concentration of Se NPs, reaching 26 mm for *S. mutans* and 27 mm for *S. aureus* at 100 µg/mL, compared to 20 mm in the control (Plant extract). The time-kill kinetics assay was measured at different time intervals 1, 2, 3, and 4 hours, showing a time and concentration-dependent reduction in bacterial growth. The bactericidal impact was greatest at the highest concentration, while the control showed no inhibition.

Conclusion

The green synthesis method enhances its applicability for treating bacterial infections in clinical backgrounds. This study validates the antibacterial activity of selenium nanoparticles synthesized using *A. linearis*, indicating their potential as antimicrobial agents that could address antibiotic resistance and support green synthesis in antimicrobial therapy.

Keywords: *Aspalathus linearis,* Selenium nanoparticles, Antibacterial, Time kill kinetics

INTRODUCTION

Aspalathus linearis (*A. linearis*), or rooibos, is a significant species of the Fabaceae family native to South Africa. The name "*Aspalathus*" is from the Greek '*Aspalathos*,' referring to a fragrant bush, while '*linearis*' describes the shape of its needle-like leaves. This erect shrub can grow up to 2 meters and has green, stalkless, stipule-free leaves on slender branches. It blooms small yellow flowers in spring to early summer, which develop into pods containing hard seeds. Both unfermented (green rooibos) and fermented forms are commercially valuable, widely consumed as herbal tea, and used in various extracts[1]. Rooibos tea is renowned for its health-promoting properties, containing bioactive phytochemicals, especially flavonoids like *Aspalathin*. Despite its consumption, its antioxidant activity and health benefits have been extensively documented only in the past two decades, contributing to its popularity as a health beverage [2]. Traditionally, rooibos tea remedies indigestion, heartburn, nausea, and nervous stress, and it stimulates hunger, showing promise for skin conditions[3].

Recent studies emphasize the antibacterial activity of *A. linearis*, expanding its medicinal applications. The bioactive compounds in rooibos tea, particularly flavonoids, have shown significant antimicrobial effects against bacterial pathogens, suggesting its potential as a natural antibacterial agent, especially against antibiotic-resistant bacteria. *Streptococcus mutans* (*S. mutans*) the main cause of dental caries, forms biofilms known as dental plaque, facilitated by extracellular polysaccharides synthesized by glycosyltransferase enzymes[4]. These polysaccharides help accumulate bacterial cells on tooth surfaces, creating a protective biofilm matrix[5]. *S. mutans* thrives in acidic oral environments[6], while *Staphylococcus aureus* (*S. aureus*), particularly methicillin-resistant *S. aureus* (MRSA), is linked to dental implant infections and forms biofilms resistant to common antimicrobial treatments[7,8]. Time-kill kinetics tests are essential to understanding microbe-antimicrobial interactions, illustrating whether antibiotics are bacteriostatic or bactericidal. Bacteria develop drug resistance through mechanisms like biofilm formation, drug efflux, enzyme-mediated drug inactivation, and alterations in drug target sites[9].

Combining herbal extract with nanoparticles enhances the antimicrobial potential of natural substances. Eco-friendly methods using microbes and plants provide simple, inexpensive ways to produce nanoparticles with significant biological effects[10]. Selenium (Se) is essential for selenoprotein function and is known for its antiviral, antibacterial, antifungal, antimutagenic, anticarcinogenic, and antiparasitic properties. Selenium-derived nanomaterials are desirable in nanomedicine due to their anticancer effectiveness, antioxidant capabilities, minimal cytotoxicity, and chemotherapeutic qualities[8,24]. They exhibit dose-dependent antimicrobial action against a broad spectrum of microbes[9]. Nanotechnology offers a novel approach to combating harmful microbes, with nanoparticles acting as bactericidal agents or carriers of therapeutic agents.

This study evaluates the time-kill kinetics and antibacterial activity of *A. linearis*-mediated selenium nanoparticles (Se NPs) against *S. mutans* and *S. aureus*, aiming to discover a natural approach to combat bacterial infections. The effectiveness of Se NPs in inhibiting bacterial growth is investigated, offering insights into their potential as alternative therapeutic agents against antibiotic-resistant strains of *S. mutans* and *S. aureus*.

METHODS

Preparation of Aspalathus linearis Extract and Synthesis of Se NPs

1 g of *A. linearis* was added to 100 mL of distilled water, heated to 50°C, and boiled for 10 minutes. The mixture was filtered through muslin cloth to obtain the extract which was then combined with a solution of 20 mM sodium selenite.

UV-visible Characterization of A. linearis mediated Se NPs

The synthesis of Se nanoparticles mediated by *A. linearis* using a double-beam UV-visible spectrophotometer. After 24 hours Se NPs solution was observed and then placed in a quartz cuvette to ensure accurate measurement. This process enabled the determination of the nanoparticle's optical characteristics, providing insights into their size, shape, and concentration based on their absorbance peaks. The use of a double-beam spectrophotometer ensured high precision and accuracy in the measurements, facilitating a detailed analysis of the synthesized selenium nanoparticle's properties.

Antibacterial Activity

The antibacterial activity of *A. linearis* – Se NPs against *S. mutans* and *S. aureus* was assessed using the agar well diffusion method. Mueller Hinton agar (MHA) plates were prepared, sterilized, and wells were created with a well-cutter. *S. mutans* and *S. aureus* were swabbed on the agar surface and incubated at 37°C for 24 hours. The cultures were treated with Se NPs at different concentrations (25, 50, 100 µg/mL). After incubation, the inhibition zones surrounding the wells were measured to evaluate antibacterial efficiency.

Time kill kinetics assay

The antibacterial activity of Se NPs synthesised from *A. linearis* was evaluated using a time-kill curve assay. *S. mutans* and *S. aureus* were cultured in Mueller Hinton Broth (MHB) and exposed to varying concentrations of Se NPs (25,50,100 µg/mL) and a standard antibiotic at 15 µg/mL in 96-well plates. Control groups without NPs were included for comparison. Different time intervals were taken at 1, 2, 3, and 4 hours using an ELISA plate reader to monitor viable bacterial populations. The results demonstrated the concentration and time-dependent antibacterial effects of Se NPs on *S. mutans* and *S. aureus*, indicating their potential as effective antimicrobial agents.

RESULTS

Synthesis of Se NPs

The synthesis of Se NPs using *A. linearis* was successfully achieved via a green synthesis method. A distinct colour change from orange to reddish brown in the solution indicated the reduction of sodium selenite and the formation of selenium nanoparticles. This visual change confirms the presence of Se NPs, aligning with previous studies on Se NP synthesis using plant extracts (Figure 1 A and 1 B).

Visual Observation

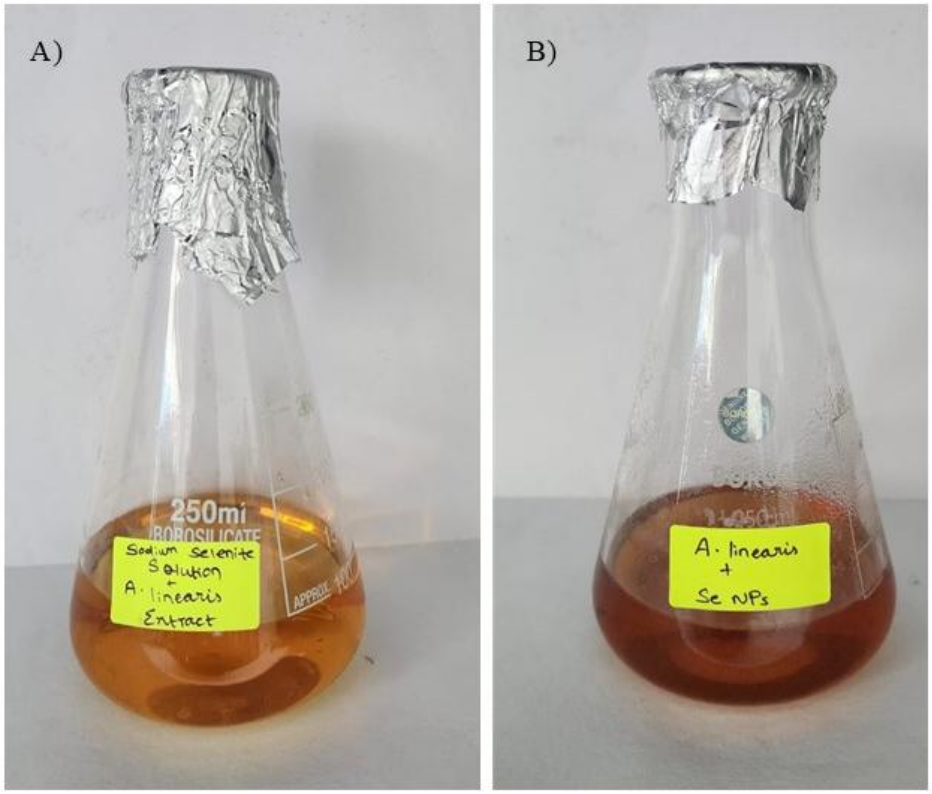

Figure 1: Formation of Se NPs using *A. linearis* extract, confirmed by colour change from pale orange to reddish-brown. A) Before 24 hours B) After 24 hours

UV-visible analysis of A. linearis mediated Se NPs

The UV-visible spectrophotometric analysis of *A. linearis-mediated* Se NPs revealed distinct absorbance peaks, confirming the successful synthesis of Se NPs. The absorbance spectrum showed a prominent peak around 320 nm at 24 hrs, characteristic of Se NPs. This peak indicates the presence of Se NPs and confirms their optical properties (Figure 2).

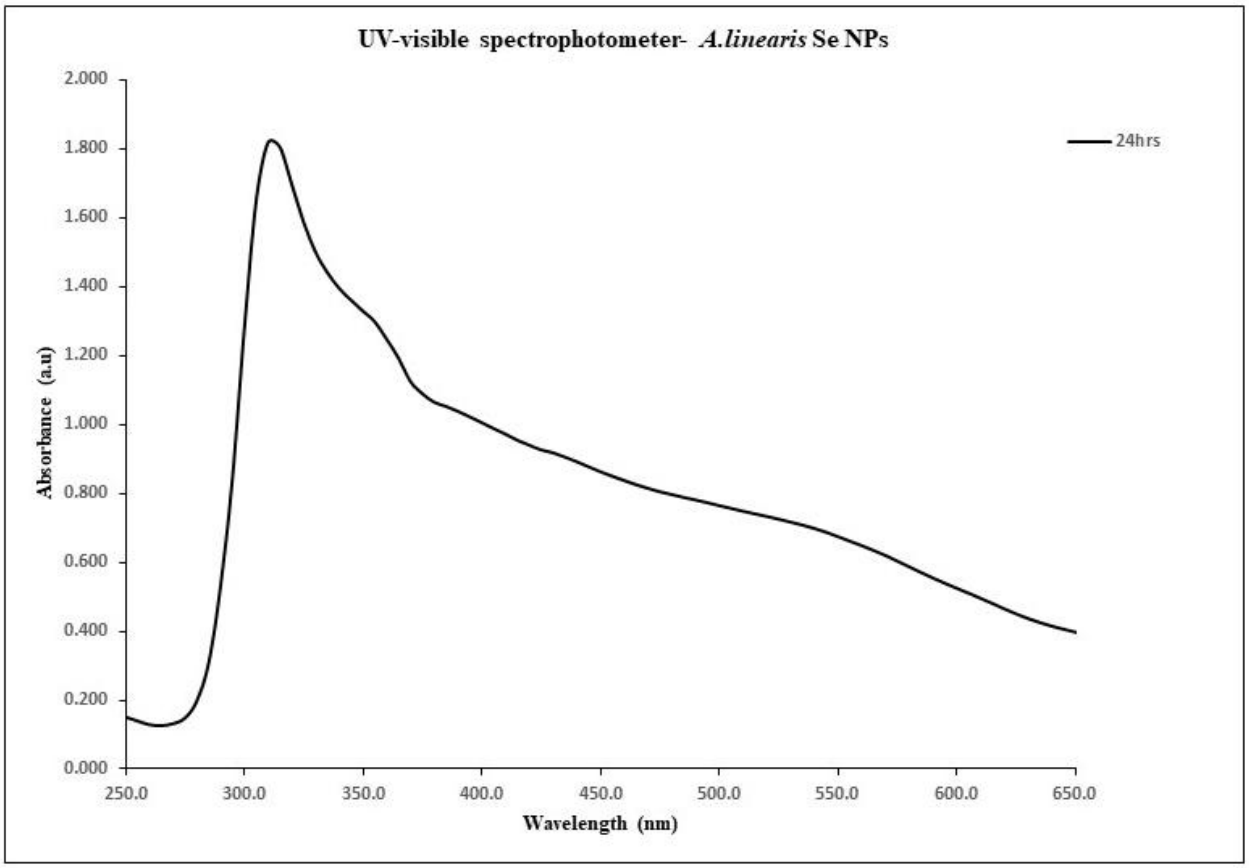

Figure 2: UV-visible spectrophotometric analysis of *A. linearis* mediated Se NPs

Antibacterial Activity

Antibacterial activity assessment of Se NPs *A. linearis* revealed pronounced effectiveness against both *S. mutans* and *S. aureus*, as determined by the agar well diffusion method. The results indicated notable inhibition zones at varying concentrations: 24 mm at 25 µg/mL, 25 mm at 50 µg/mL, 26 mm at 100 µg/mL for *S. mutans* and 24 mm at 25 µg/mL, 25 mm at 50 µg/mL, 27 mm for *S. aureus* at 100 µg/mL (Figure 3A and 3B). Control groups (*A. linearis* extract) exhibited baseline inhibition zones of 20 mm for both strains, affirming the inherent antimicrobial activity of the medium. These findings highlight the promising antibacterial potential of *A. linearis* Se NPs, suggesting comparability with conventional antibiotics (Figure 3C).

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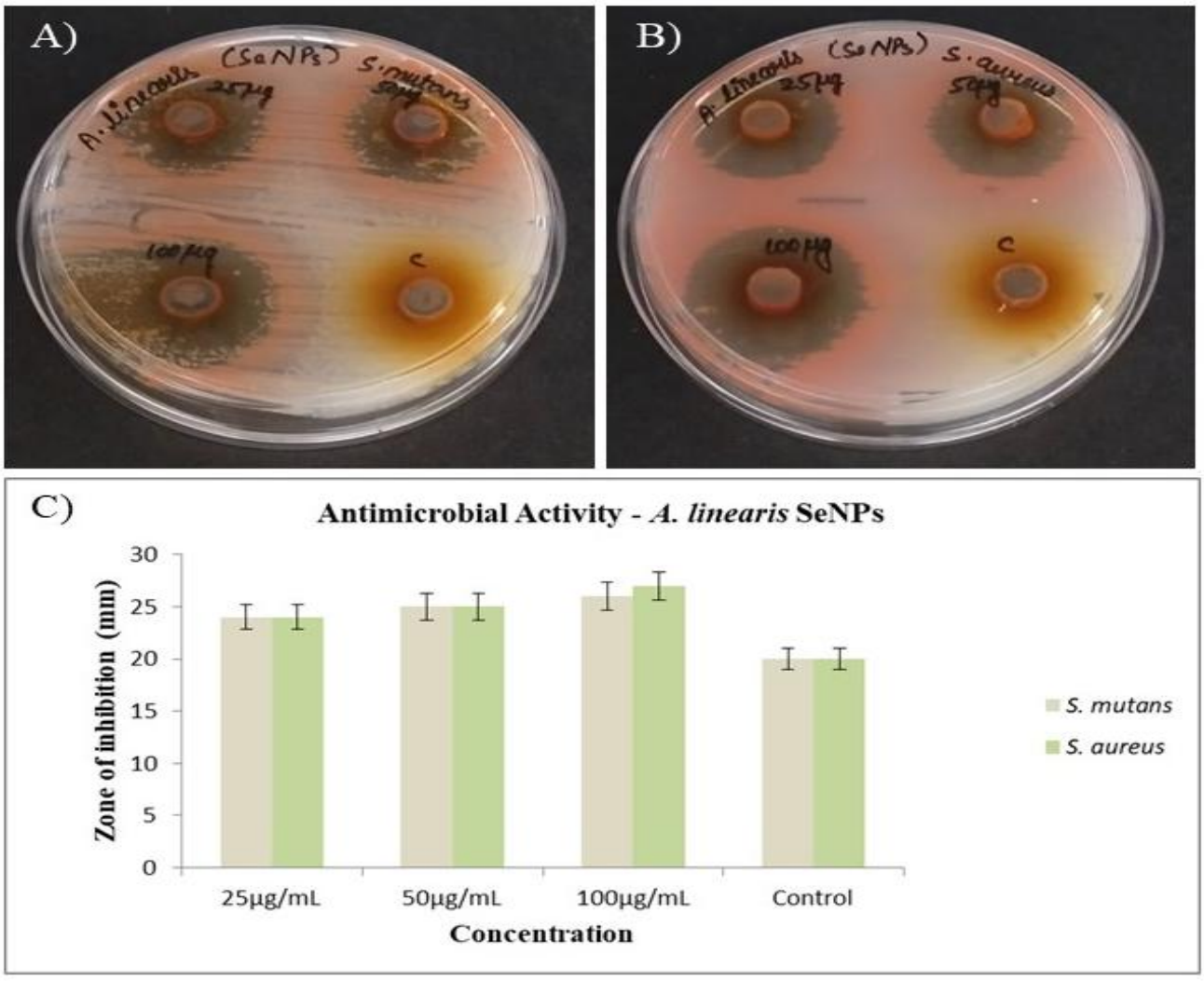

Figure 3: A) and **B)** show the zones of inhibition at different concentrations of the *A. linearis* against *S. mutans* and *S. aureus*. **C)** Graphical representation of the antibacterial activity of *A. linearis*-mediated Se NPs against *S. mutans* and *S. aureus*.

Time kill kinetics

The time-kill curve assay showed that *A. linearis* - Se NPs effectively inhibited the growth of *S. mutans* and *S. aureus* bacteria in a way that depended on concentration and time. Time kill curve can help to evaluate the efficiency of antimicrobial properties. Se NPs- *A. linearis* shows concentration-dependent time killing and exhibits antimicrobial properties. It leads to a more distinct reduction in bacterial count overtime. Several times, the concentration of *A. linearis* - Se NPs (25 µg/mL, 50 µg/mL, and 100 µg/mL) made a significant distinction in the amount of *S. aureus* and *S. mutans* (**Figure 4A and 5B**). In particular, when the concentration reached the highest level at 100 µg/mL, there was a significant decrease in the number of *S. mutans* colonies, which shows that *A. linearis*- Se NPs rapidly destroyed the bacteria. it shows that, unlike *S. mutans*, the type *S. aureus* had a strong ability to kill bacteria. Small particles of *A. linearis*-Se NPs caused a significant decrease in the number of *S. aureus* colonies at all doses when compared to the control group.

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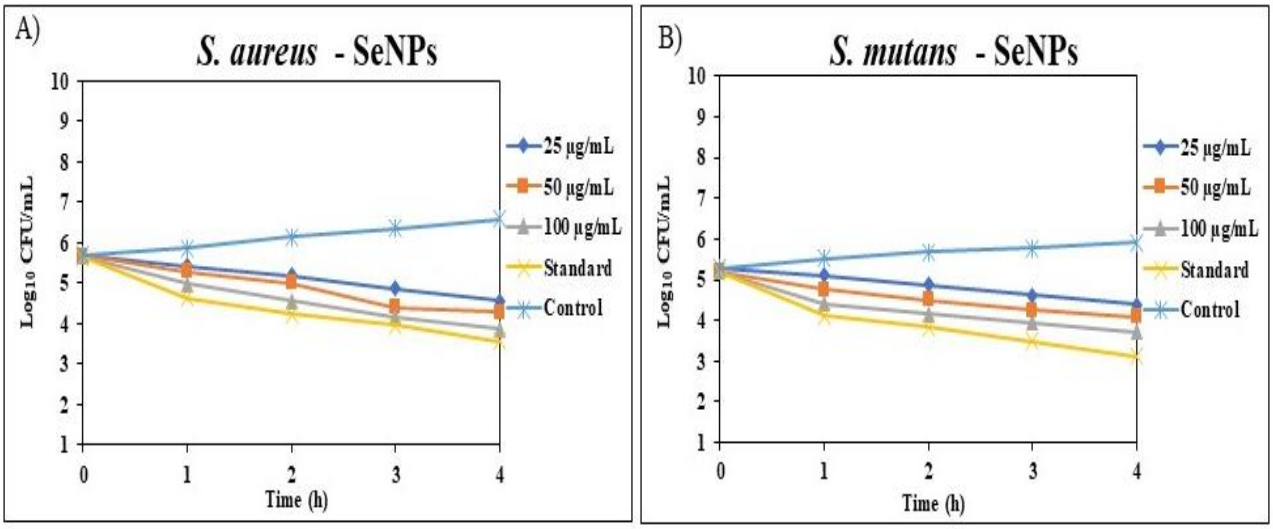

Figure 4: **A)** and **B)** Time kill kinetics assay of *A. linearis* mediated Se NPs against *S. mutans* and *S. aureus*.

Discussion

The primary advantage of this study was the use of *A. linearis*, a plant known for its rich antioxidant properties, as a reducing agent in the synthesis of Se NPs. This green synthesis method is environmentally friendly and avoids toxic chemicals, making it a sustainable approach. The UV-visible spectrophotometric analysis of *A. linearis-mediated* selenium nanoparticles (Se NPs) demonstrated a prominent absorbance peak around 320 nm, consistent with previous studies on Se NP synthesis. Such absorbance peaks in the UV region are characteristic of Se NPs and indicate successful synthesis, as supported by similar findings in the literature, where peaks in the UV range confirm the optical properties of Se NPs. This characteristic absorbance further validates the formation of Se NPs, consistent with the distinct UV absorbance peaks reported in various nanoparticle synthesis studies [11,12]. The time-kill assay employed in this study provides dynamic information on the bactericidal kinetics of Se NPs, offering a more comprehensive understanding of their antimicrobial efficacy over time compared to static methods such as the agar well diffusion assay. The antibacterial activity of *A. linearis* Se NPs demonstrated significant zones of inhibition against *S. mutans* and *S. aureus*, with inhibition zones increasing in a concentration-dependent manner. These results align with previous literature that emphasizes the potent antimicrobial properties of Se NPs [13]. Furthermore, the comparable efficacy of *A. linearis* Se NPs to standard antibiotics highlights their potential as alternative antimicrobial agents, particularly in combating antibiotic-resistant bacterial strains. These findings correlated with the previous literature on the application of Se NPs in clinical settings for the treatment of bacterial infections [14,15]. Additionally, time-kill assay results indicate that Se NPs synthesized from *A. linearis* exhibit significant antibacterial activity against both *S. mutans* and *S. aureus*. The Log_{10} CFU/mL measurements demonstrated a clear time and concentration-dependent reduction in bacterial growth, with the highest concentration (100 µg/mL) showing the greatest bactericidal effect. These results align with previous literature that emphasizes the antimicrobial properties of Se NPs[16,17,23]. The observed antibacterial activity of *A. linearis* Se NPs against *S. mutans*, a primary causative agent of dental caries. A previous study demonstrated the efficacy of Se NPs in reducing *S. mutans* biofilm formation and acid production[18,19]. Moreover, the strong inhibition of *S. aureus* corroborates the findings of Sonkusre et.al., 2015, who highlighted the potential of Se NPs as an alternative to conventional antibiotics[20,21,22]. However, this study has certain limitations. The *in vitro* conditions of the time-kill assay may not accurately replicate the *in vivo* environment, potentially impacting the relevance of the results to clinical applications. Additionally, the research focuses solely on two bacterial strains, necessitating further studies to assess the efficacy of *A. linearis* Se NPs against a broader spectrum of pathogens. Despite these limitations, the study's findings are noteworthy as they highlight the potential of *A. linearis* Se NPs as effective antimicrobial agents. The natural, plant-based synthesis method presents an innovative approach to nanoparticle production while also enhancing the antibacterial activity of the resulting nanoparticles, thereby supporting their potential use in treating bacterial infections.

Conclusion

The antibacterial activity and time-kill kinetics of Se NPs synthesized using *A. linearis* against *S. mutans* and *S. aureus*. The findings demonstrated significant antibacterial effects, with inhibition zones increasing in a concentration-dependent manner, confirming the potent antimicrobial properties of Se NPs. The time-kill kinetics assay further revealed a clear time and concentration-dependent reduction in bacterial growth, indicating the efficacy of Se NPs over time. The usage of *A. linearis* mediated Se NPs offers a sustainable and environmentally friendly approach, avoiding the use of hazardous chemicals. The comparable efficacy of *A. linearis* Se NPs to standard antibiotics their potential as alternative treatments, particularly in the face of rising antibiotic resistance. Despite some limitations, such as the need for further in vivo studies and broader pathogen testing, the results support the application of Se NPs synthesized from *A. linearis* in clinical settings for the treatment of bacterial infections. Overall, this study contributes to the green-synthesized nanoparticles and their applications in antimicrobial therapy, providing promising for future studies and potential clinical use.

CONFLICT OF INTEREST

The authors declare that no conflict of interest would prejudice the impartiality of this scientific work.

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