Antibacterial Properties of Tannic Acid Functionalized Silver-Selenium Nanoparticles Towards Dental Pathogens

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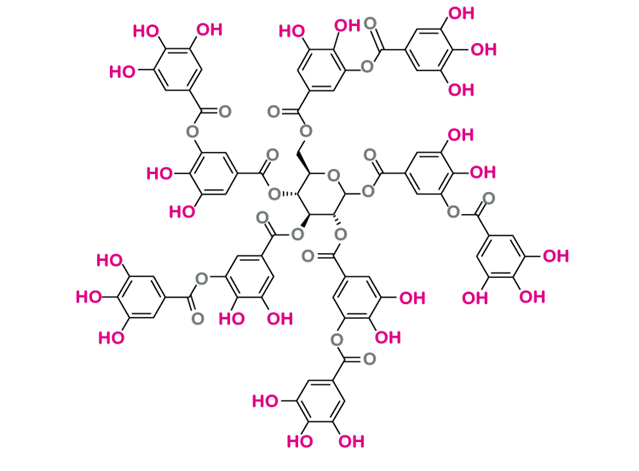
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**Abstract:** Oral infections are caused by bacterial and fungal pathogens, which leads to dental caries, periodontitis, and oral candidiasis, causing significant health concerns. The overuse of antibiotics may lead to side effects; therefore, plant-based nanomaterial synthesis has emerged as a promising alternative for combating drug-resistant dental pathogens. In this study the green synthesis of tannic acid-functionalized silver-selenium nanoparticles (TA-AgSeNPs) was successfully synthesised. The synthesised nanoparticles were analyzed using SEM, EDS, and FTIR techniques. The nanoparticles exhibited spherical and rod-like shape, with an average size ranging from 100 to 600 nm. EDS analysis confirmed the presence of selenium (Se) and silver (Ag) along with carbon and oxygen, indicating the high purity of the synthesized nanoparticles. The FTIR results indicate that the functional groups of tannic acid are attached on the surface of the AgSeNPs. The antimicrobial efficacy against *Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis*, *Escherichia coli*, and *Candida albicans* indicate that both tannic acid and TA-AgSeNPs are effective but TA-AgSeNPs demonstrating significantly higher efficacy than the tannic acid alone. Finally, this study suggests that TA-AgSeNPs might be good therapeutic drugs for the effective treatment of dental pathogens.

**Keywords:** Oral infections, Tannic acid, Silver-selenium nanoparticles, Antimicrobial resistance, Green synthesis.

# Introduction

Oral infections refer to infections that affect the tissues and structures within the mouth, such as the gums, teeth, tongue, and soft palate [(Ekom et al., 2021)](https://paperpile.com/c/C8Kx2f/6uV2). These infections can be caused by bacteria, viruses, fungi, and parasites[(Rajeshkumar & Lakshmi, 2021; Sivakumar et al., 2021)](https://paperpile.com/c/C8Kx2f/yYb2r+9tH9s). Poor dental care, health problems, injuries, and smoking are some of the main reasons people develop oral infections [(Burket, 1946)](https://paperpile.com/c/C8Kx2f/esbX). According to the World Health Organization (WHO), oral health remains a major issue, with 45% of the global population affected by dental biofilm and related microbial infections [(Ramsundar et al., 2023; Rieshy et al., 2023; Singh et al., 2023)](https://paperpile.com/c/C8Kx2f/nOMdF+ck3f9+Nceqm) . The dental pathogens include *Candida albicans, Streptococcus mutans, Escherichia coli, Enterococcus faecalis,* and *Staphylococcus aureus* [*(Chokkattu et al., 2023; Dharman et al., 2023; Govindaraj & Shanmugam, 2023)*](https://paperpile.com/c/C8Kx2f/dwMVR+hhnbQ+qh1Bv)*.* These microorganisms exhibit strong biofilm-formation, making infections persistent and highly resistant to conventional antibiotic treatments [(Bjarnsholt et al., 2014)](https://paperpile.com/c/C8Kx2f/oxJl). Although antibiotics are commonly used to treat dental caries and related infections, their overuse has contributed to the rapid rise of antimicrobial resistance [(Pace et al., 2005)](https://paperpile.com/c/C8Kx2f/YKil). As a result, existing antibiotics are becoming less effective against dental pathogens, prompting researchers to explore safer and more efficient alternatives that can eliminate these pathogens without causing harmful side effects [(Pavithra et al., 2023; Shenoy et al., 2023; Thomas & Jain, 2023)](https://paperpile.com/c/C8Kx2f/qHIfk+pUtXs+nxo6Y).The plant-based green synthesis of nanomaterials has gained more attention due to its cost-effectiveness, environmental friendliness, non-toxicity, rapid production, and high efficacy in treatment applications [(Husen, 2023)](https://paperpile.com/c/C8Kx2f/H0XL). Tannins, a class of plant-derived phenolic compounds, are known for their antioxidant, antimicrobial, antiviral, and anti-inflammatory properties [(Rajasekaran et al., 2021)](https://paperpile.com/c/C8Kx2f/NXtf). The chemical structure of tannic acid is shown in **Fig. 1**. Notably, metallic nanoparticles, such as gold (Au) and silver (Ag), have been widely explored for their therapeutic applications in treating cancer, infections, and other diseases [(Gandhi et al., 2021; Katyal et al., 2023; Priyadharshini et al., 2023)](https://paperpile.com/c/C8Kx2f/d4xW1+urF94+dMup5). Selenium, an essential nutrient for both humans and animals, has also been studied in the form of selenium nanoparticles due to their unique chemical properties, biocompatibility, and medical benefits [(Combs, 2012)](https://paperpile.com/c/C8Kx2f/l07n). AgNPs with the combination of SeNPs have shown enhanced biomedical properties. A previous study demonstrated that combining Rutin with selenium silver nanoparticles (Ag-SeNPs) enhanced antimicrobial activity, creating an effective nanodrug for treating dental pathogens[(Doshi et al., 2023; Lampl et al., 2023; Pandiyan et al., 2023)](https://paperpile.com/c/C8Kx2f/tTBPJ+hAEuQ+QjkbQ)

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**Fig. 1.** Structure of tannic acid

In this study, tannic acid-functionalized silver-selenium nanoparticles (TA-AgSeNPs) were synthesized and evaluated for their antimicrobial properties against dental pathogens. The synthesized TA-AgSeNPs were further characterized using standard analytical techniques, including UV–visible spectroscopy (UV–vis), Scanning Electron Microscopy (SEM), Energy-Dispersive X-ray spectroscopy (EDX), and Fourier Transform Infrared Spectroscopy (FTIR), to confirm their surface morphology, crystalline nature, and functional groups.

# Materials and methods

## Chemicals

Tannic acid was purchased from Tokyo Chemical Industry, Japan. Nutrient broth, Sabouraud Dextrose Agar (SDA), and Mueller Hinton Agar (MHA) were obtained from Himedia (Mumbai, India). Silver nitrate (AgNO3) was procured from Finar Chemicals (Ahmedabad, India). Sodium selenite (Na2SeO3), Sodium hydroxide (NaOH), and dimethyl sulfoxide (DMSO) were procured from Merck Life Science (Mumbai, India). Deionized water obtained from Indion Lab-Q Water Maker was used for nanomaterial synthesis studies. All other chemicals and reagents used in this study were of analytical grade.

## Synthesis of Tannic acid-functionalized silver-selenium nanoparticles

TA-AgSeNPs was synthesized by mixing 750 µM tannic acid with 250 µM AgNO3 and 250 µM Na2SeO3 in deionized water at pH 8 and 30 ˚C. The formation of TA-AgSeNPs was confirmed by turning the reaction solution from yellow to dark brown in color and full wavelength scanning (270 nm λ max and 410 nm λ max for SeNPs and AgNPs) using UV–visible spectroscopy (Jasco V- 730). The synthesized TA-AgSeNPs were centrifuged at 8000 rpm for 5 min at 4 ˚C. Finally, the obtained pellet was dried and stored in an airtight container at 25 ˚C for further studies [(Selvaraj et al., 2024)](https://paperpile.com/c/C8Kx2f/huzu).

## Physicochemical characterization

The size, shape, and composition of the synthesized TA-AgSeNPs were analyzed by SEM. FTIR was used to identify the functional groups present, ensuring successful tannic acid functionalization and nanoparticle stabilization [(Shukla & Iravani, 2018)](https://paperpile.com/c/C8Kx2f/7LC0). EDX analysis determined the elemental composition, verifying the presence of silver (Ag), selenium (Se), and oxygen (O) in the synthesized nanoparticles, further confirming their purity and chemical integrity [(“Eradication of Dental Pathogens Using Flavonoid Rutin Mediated Silver-Selenium Nanoparticles,” 2023)](https://paperpile.com/c/C8Kx2f/CRK0)**.**

## Antibacterial activity assessment

Clinical isolates of *S. aureus*, *S. mutans*, *E. faecalis*, *E. coli* and *C. albicans* were collected from Green Lab, The antimicrobial activity of TA-AgSeNPs was evaluated against dental pathogens using the Kirby-Bauer disk diffusion method [(Schwalbe et al., 2007)](https://paperpile.com/c/C8Kx2f/rPpS). The bacterial cultures were maintained on a nutrient medium, while the fungal culture was maintained on a Sabouraud Dextrose medium for 24 hours. Overnight cultures were swabbed onto the MHA and SDA plates using a sterile cotton swab, three wells were created using a well cutter and filled with appropriate concentration of tannic acid, Tan-AgSeNPs and antibiotics cephalexin for *S.aureus*, amikacin for *S. mutans* , gentamicin for *E. faecalis*, chloramphenicol for *E. coli*, and fluconazole for *C. albicans* were filled separately and allowed to diffuse at room temperature for 2 h, then the cultured plates were incubated at 37◦ C for 24 hours, and the zones of inhibition were measured using a HiAntibiotic ZoneScaleTM (Himedia, India) and the results were expressed as the mean value of the triplicate experiment [(Rohatgi et al., 2023)](https://paperpile.com/c/C8Kx2f/F4kJ).

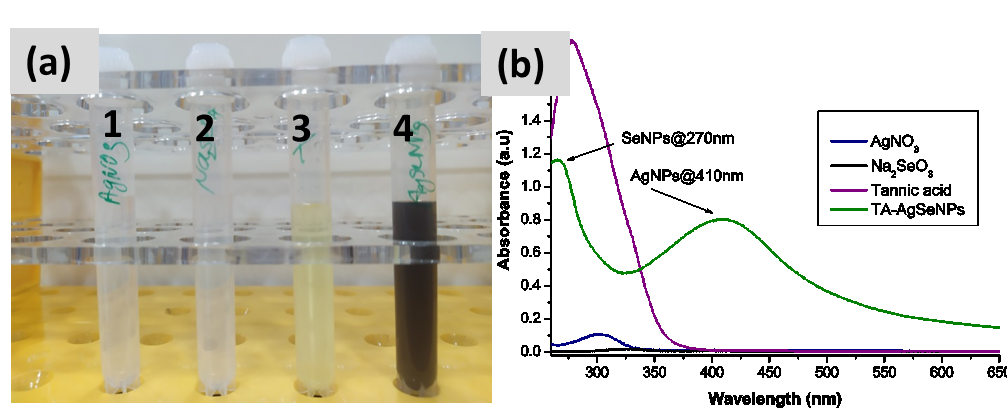
# Statistical analysis

All the experiments were performed in triplicate, and the results were expressed as mean with standard deviation values. Origin software was used for data analysis and graphical representation.

# Results and discussion

## Synthesis of TA-AgSeNPs

Generally, metallic nanoparticles are very small (1 to 100 nm) in size, such as gold (AuNPs), silver (AgNPs), selenium (SeNPs), and copper (CuNPs). They have versatile properties, making them useful in medicine, sensors, catalysis, and environmental protection. In this study, a combined bimetallic SeNPs and AgNPs were biosynthesized using tannic acid by simple approach. As shown in **Fig. 2a**, their formation was confirmed by a color change from light yellow to deep brown. In **Fig. 2b**, UV-Vis spectrum confirms the successful synthesis of TA-AgSeNPs by showing distinct absorption peaks. The spectrum of tannic acid exhibits a strong peak at around 270 nm. The synthesized TA-AgSeNPs shows a broad peak at approximately 410 nm, corresponding to the surface plasmon resonance (SPR) of AgNPs, while the peak at 270 nm confirms the presence of SeNPs [(Gunti et al., 2019)](https://paperpile.com/c/C8Kx2f/X3UM)**.** The SPR indicates successful nanoparticle synthesis, with tannic acid acting as a reducing and stabilizing agent.

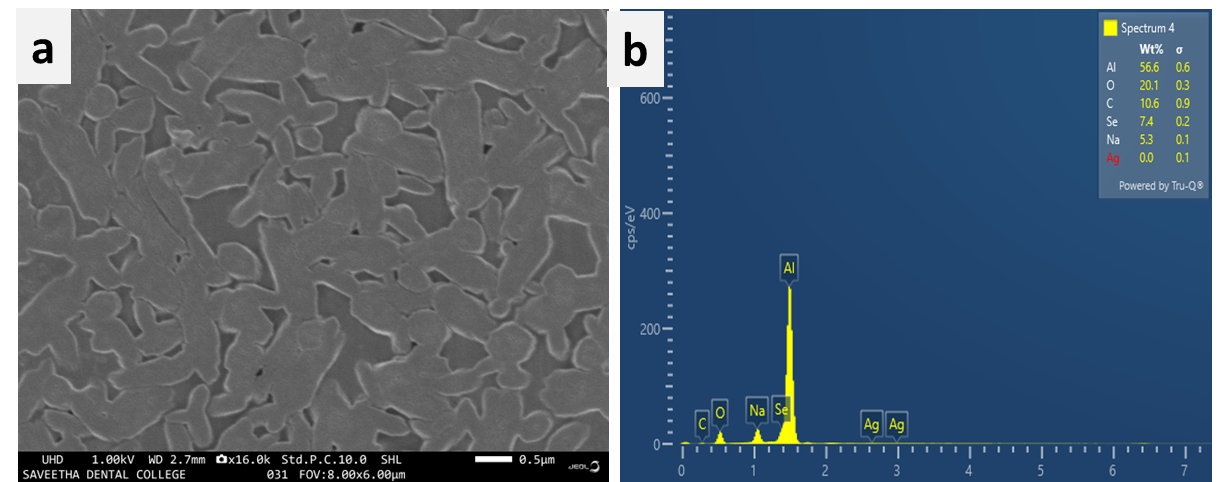


**Fig. 2.** (a) Synthesis and (b) UV-vis analysis of TA-AgSeNPs.

(1. Silver nitrate (AgNO3), 2. Sodium selenite (Na₂SeO₃), 3. Tannic acid, 4 TA-AgSeNPs)

## Characterization of TA-AgSeNPs

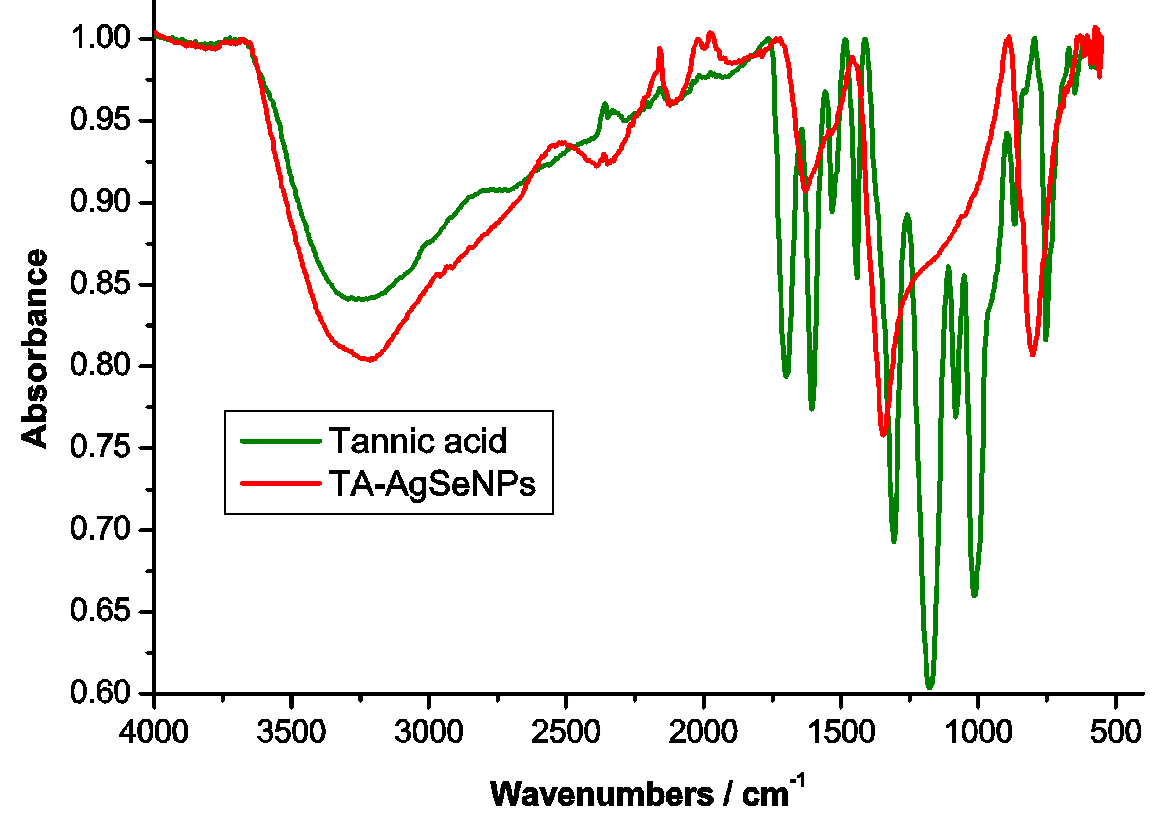
SEM is used for nanoparticle characterization as it provides high-resolution imaging to analyze surface morphology, particle size, and distribution coupled with Energy Dispersive X-ray Spectroscopy (EDX), as shown in **Fig. 3a and 3b**. The SEM image reveals that the synthesized nanoparticles exhibit distinct shapes, with AgNPs appearing spherical and SeNPs displaying a cylindrical morphology, with a size range of 100 to 600 nm [(Ezhuthupurakkal et al., 2017)](https://paperpile.com/c/C8Kx2f/tIuK). Additionally, EDX confirms the elemental composition of the synthesized nanomaterials, to verify the presence of silver (Ag) and selenium (Se), along with other stabilizing elements from tannic acid. EDX results shows the characteristic peaks were appeared at 0.3 keV for carbon (C; 10.6 wt%), 0.52 keV for oxygen (O; 20.1 wt%), 1.05 keV for sodium (Na; 5.3 wt%), 1.37 keV for selenium (Se; 7.4 wt%), 1.48 keV for aluminum (Al; 56.6 wt%) and 2.7 keV and 3.0 keV for silver (Ag; 0.0 wt%), as indicated in **Fig. 3b**. The combined SEM-EDX analysis validates the successful synthesis of TA-AgSeNPs, highlighting their well-defined morphology and purity of elements, which are essential for their biomedical and antimicrobial applications.



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

## Fourier Transform Infrared Spectroscopy spectrum

FTIR spectrum comparing tannic acid (green) and TA-AgSeNPs (red). The peaks in the FTIR spectra correspond to different functional groups present in the samples. The shift in peak positions and intensity variations between tannic acid and TA-AgSeNPs indicate potential interactions between tannic acid and silver selenium nanoparticles(Saadh et al., 2024). Specifically, the broad peak around 3200–3400 cm⁻¹ corresponds to –OH stretching vibrations, suggesting hydrogen bonding, which is slightly shifted in TA-AgSeNPs, indicating nanoparticle functionalization(Almatrafi et al., 2024). The peaks around 1600–1700 cm⁻¹ represent C=O stretching from tannic acid’s carboxyl and ester groups, which moves to the nanoparticle spectrum, which confirms the nanoparticle stabilization. These spectral modifications confirm the successful capping and reduction of AgSeNPs by tannic acid, demonstrating the formation of a stable nanoparticle system with potential enhanced bioactivity.



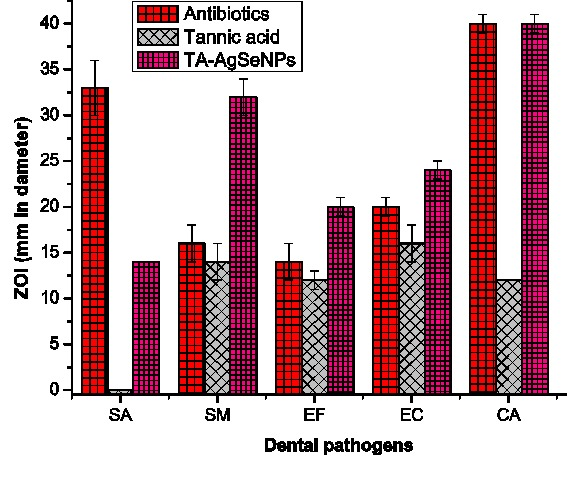
**Fig. 4.** FTIR spectra of tannic acid and TA-AgSeNPs.

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## Antimicrobial activities of TA-AgSeNPs

The antimicrobial activity of tannic acid (1 mg) and TA-AgSeNPs (500 µg) was assessed against dental pathogens, including *S. aureus, S. mutans, E. faecalis, E. coli,* and *C. albicans*, using the Kirby-Bauer disk diffusion method in **Fig. 4**. The standard antibiotics (cephalexin, amikacin, gentamicin, chloramphenicol, and fluconazole) effectively inhibited the tested pathogens. The zone of inhibition (ZOI) of TA-AgSeNPs recorded as follows: 16 ± 1.0 mm, 23 ± 2.0 mm, 14 ± 0.5 mm, 16 ± 1.0 mm and 14 ± 0.5 mm for *S. aureus, S. mutans, E. faecalis, E. coli,* and *C. albicans*; whereas, tannic acid inhibits 11 ± 1.0 mm, 12 ± 1.5 mm, 11 ± 1.0 mm, 11 ± 1.0 mm and 14 ± 0.5 mm, respectively in **Fig. 5**. These results indicate that both tannic acid and TA-AgSeNPs exhibit antimicrobial properties against the tested pathogens, with TA-AgSeNPs demonstrating significantly higher efficacy. According to literature, nanocomposites alter bacterial surface properties, membrane permeability, and cellular behavior, generating oxidative stress via hydrogen peroxide formation. The primary mechanism involves reactive oxygen species (ROS) diffusion, which leads to bacterial inhibition and cell death [(Sun et al., 2020)](https://paperpile.com/c/C8Kx2f/i9QV). The strong interaction between nanocomposites and microbial cells enhances antimicrobial activity. Thus, TA-AgSeNPs show an effective antimicrobial agent against dental pathogens [(Abdel Maksoud et al., 2022)](https://paperpile.com/c/C8Kx2f/FpaP).**Fig. 4.** Antimicrobial activity of TA-AgSeNPs against dental pathogens.

Zone of inhibition (ZOI) study (1. Staphylococcus aureus, 2. Streptococcus mutans, 3. Enterococcus faecalis, 4. Escherichia coli and 5. Candida albicans ) (T – Tannic acid)



**Fig. 4.** Comparative analysis of TA-AgSeNPs antimicrobial efficacy. (1. Candida albicans, 2. Streptococcus mutans, 3. Escherichia coli,4. Enterococcus faecalis and 5. Staphylococcus aureus)

# Discussion

A green synthesis method was used effectively for the synthesis of tannic acid-functionalized silver-selenium nanoparticles (TA-AgSeNPs) demonstrating their potential for biomedical applications [(Janani et al., 2021; Kachhara et al., 2021; Subramanian et al., 2023)](https://paperpile.com/c/C8Kx2f/LdAPI+TocBr+wlK6o). The UV-Vis spectroscopy results confirmed the formation of TA-AgSeNPs, with distinct surface plasmon resonance peaks indicative of nanoparticle synthesis. SEM and EDX analyses further validated the morphological and elemental composition, revealing well-defined spherical AgNPs and cylindrical SeNPs with uniform distribution. FTIR spectroscopy confirmed the successful functionalization and stabilization of the nanoparticles by tannic acid. The antimicrobial evaluation showed that TA-AgSeNPs exhibited significantly enhanced activity against dental pathogens, including *S. aureus, S. mutans, E. faecalis, E. coli*, and *C. albicans*, compared to tannic acid alone. Various studies conclude that silver selenium nanoparticles possess antimicrobial effects against many bacteria and fungi. For example, Hinaz et al. 2024 reported that Myr-AgSeNPs demonstrated strong anticandidal activity against *C. albicans*, with a 40±0 mm ZOI, significantly higher than myricetin (13±0.5 mm) [(Hinaz et al., 2024)](https://paperpile.com/c/C8Kx2f/OEna)**.** Mostafa et al. (2023) reported that *Orobanche aegyptiaca* extract enabled the eco-friendly synthesis of bimetallic Ag-Se NPs, showing strong antimicrobial and anti-biofilm activity against *S. aureus, E. coli, P. aeruginosa,* and *C. albicans* [(“Promising Antimicrobial and Antibiofilm Activities of Orobanche Aegyptiaca Extract-Mediated Bimetallic Silver-Selenium Nanoparticles Synthesis: Effect of UV-Exposure, Bacterial Membrane Leakage Reaction Mechanism, and Kinetic Study,” 2023)](https://paperpile.com/c/C8Kx2f/5prZ). Similarly, Miere et al. (2022) synthesized AgNPs and SeNPs using *Stellaria media* (L.), demonstrating greater antimicrobial effectiveness against *S. aureu*s [(Miere et al., 2022)](https://paperpile.com/c/C8Kx2f/ABv0).

# Conclusion

Overall, this study highlights the potential of eco-friendly, phytochemical-mediated nanoparticle synthesis as an alternative to conventional chemical methods. The antimicrobial properties of TA-AgSeNPs against dental pathogens suggest their suitability for further exploration in clinical and pharmaceutical sectors. Future research should focus on *in vivo* studies.

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