



In Vitro Evaluation of Neem Flower-mediated Selenium–curcumin Nano Formulation Incorporated into a Chitosan-based Formulation for Oral Applications

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ABSTRACT

Plant-mediated synthesis provides a green route for preparing selenium nanoparticles (SeNPs) using biological extracts as an alternative to conventional chemical synthesis. Neem flower extract contains phytochemicals that may participate in selenium precursor reduction and nanoparticle surface stabilization. Curcumin and chitosan are also of interest for experimental oral formulations because of their reported antioxidant, antimicrobial, and formulation-related properties. This study evaluated neem flower-mediated SeNPs and a selenium–curcumin nano formulation incorporated into a chitosan-based formulation, with emphasis on preliminary physicochemical characterization and in vitro biological activity. An aqueous neem flower extract was prepared and combined with sodium selenite to generate the SeNP preparation. Curcumin dissolved in dimethyl sulfoxide and diluted with water was combined with the SeNP preparation, and the resulting formulation was incorporated into a chitosan matrix. UV–visible spectroscopy, Fourier-transform infrared spectroscopy, and scanning electron microscopy were used for characterization. Antimicrobial activity was assessed against selected oral microorganisms, and preliminary biological evaluations, including DPPH radical-scavenging, brine shrimp, and zebrafish assays, were carried out. The synthesized nanomaterial showed an absorption at approximately 450 nm. The antimicrobial experiment demonstrated inhibition of the tested microorganisms, with larger inhibition zones at the highest tested sample volume and the largest zone reported for *Staphylococcus aureus*. Further investigation of neem-mediated SeNP and selenium–curcumin/chitosan formulations as experimental oral nanomaterials can be considered.

Keywords: Selenium nanoparticles; Green synthesis; Neem flower; Selenium–curcumin; Chitosan.

1. INTRODUCTION

Oral infectious diseases are associated with complex microbial communities and biofilm formation and remain important contributors to dental caries, periodontal disease, endodontic infection, and oral candidiasis. The persistence of microbial biofilms and the continuing need for effective antimicrobial strategies have encouraged investigation of alternative materials with distinct physicochemical and biological properties. Selenium nanoparticles (SeNPs) have received considerable attention because their biological behavior differs from that of bulk selenium and soluble selenium compounds. Their behavior is influenced by particle size, morphology, surface chemistry, and synthesis conditions. A recent review on SeNPs against potentially pathogenic oral microorganisms identified activity against organisms including *Candida albicans*,

Streptococcus mutans, *Enterococcus faecalis*, and other oral pathogens, while emphasizing that differences in concentration, physicochemical characteristics, and experimental conditions limit direct comparison among studies (Sans-Serramitjana *et al.* 2023).

Plant-mediated synthesis has emerged as an intensively investigated route for the preparation of SeNPs. Plant extracts contain phenolic compounds, flavonoids, proteins, sugars, and other metabolites that may contribute to precursor reduction and stabilization of the resulting nanoparticles. The approach can reduce reliance on some conventional chemical reducing systems and organic solvents. However, a synthesis should not be considered environmentally benign solely because a plant extract is used. Precursor consumption, temperature, stirring requirements, purification, washing, waste generation, scalability, pH, nanoparticle persistence, and environmental fate also contribute

to the overall environmental profile (Johnson *et al.* 2022; El-Saadony *et al.* 2026).

Neem (*Azadirachta indica*) is a widely available medicinal plant containing diverse phytochemicals. Another work has specifically investigated neem-mediated SeNP synthesis and has used complementary characterization methods, including UV-visible spectroscopy, FTIR, SEM, transmission electron microscopy (TEM), and X-ray diffraction (XRD), to examine the resulting material (Palayathan *et al.* 2026). The present study uses neem flower aqueous extract as the biological reaction medium. The aqueous extraction route is relevant to green-synthesis principles because water is used as the extraction medium and plant-derived constituents provide the biological component of the synthesis.

Curcumin, a polyphenolic compound obtained from *Curcuma longa*, has been extensively investigated for antioxidant and antimicrobial properties. Its limited aqueous solubility and physicochemical stability have encouraged the development of nanoformulations and polymer-associated delivery systems (Prasad *et al.* 2014). Ghoflchi *et al.* (2026) and Rajendran *et al.* (2026) reviewed selenium-curcumin nanoparticles and their conjugates. These systems remain an experimental area of research, and their biological behavior depends on composition, preparation route, particle characteristics, and dose.

Chitosan is a naturally derived amino polysaccharide with film-forming, mucoadhesive, and antimicrobial properties. Its antimicrobial activity is influenced by molecular weight, degree of deacetylation, pH, concentration, and the physicochemical characteristics of the formulation (Cakici and Cakici, 2024; Nasaj *et al.* 2024). So, chitosan-based systems have been investigated for oral and endodontic applications, although their performance varies among formulations and test methods.

Despite the growing literature on plant-mediated SeNPs, selenium-curcumin systems, chitosan-based materials, and the combination of neem flower-mediated SeNPs with curcumin and a chitosan matrix for experimental oral applications remains insufficiently characterized. The present study therefore investigated a neem flower-mediated SeNP preparation, a selenium-curcumin nanoformulation, and its incorporation into a chitosan-based formulation. Antimicrobial activity against selected oral microorganisms, antioxidant activity using the DPPH assay, and preliminary biological responses using brine shrimp and zebrafish models were evaluated. The study was designed as an initial laboratory investigation and does not establish clinical effectiveness, wound-healing activity, or long-term safety.

2. MATERIALS AND METHODS

2.1 Study Setting and Materials

The study was conducted in the Nano-biomedicine Laboratory of Saveetha Dental College and Hospital, Chennai, India. The chemicals used for the experimental procedures were obtained from Sigma-Aldrich. Sodium selenite was used as the selenium precursor. Curcumin, dimethyl sulfoxide (DMSO), chitosan, glacial acetic acid, and analytical reagents were used. Oral microbial cultures were obtained from Saveetha Dental College and Hospital. Neem flowers were collected from the herbal garden and local surroundings associated with Saveetha Dental College and Hospital, Chennai.

2.2 Preparation of Neem Flower Aqueous Extract

Neem flowers were washed under running tap water to remove visible surface contaminants and shade-dried for 5–6 days. The dried flowers were ground into a fine powder (Fig. 1). Powdered neem flower (2 g) was mixed with 200 mL of distilled water and heated at 50–60 °C for 10–15 minutes using a heating mantle. The resulting extract was filtered through Whatman No. 1 filter paper and used for nanoparticle synthesis.



Fig. 1: Dried and ground neem flowers used for aqueous extract preparation

2.3 Green Synthesis of Neem Flower-mediated Selenium Nanoparticles

Selenium nanoparticles were synthesized using sodium selenite as the selenium precursor and neem flower aqueous extract as the biological reaction medium (Fig. 2). Briefly, 30 mM sodium selenite was dissolved in 50 mL of distilled water. Separately, 50 mL of the filtered neem flower extract was prepared. The plant extract was added gradually to the sodium selenite solution under continuous stirring. The mixture was

allowed to react to form the SeNP preparation. The available experimental record did not specify the exact reaction time, reaction temperature, pH, or stirring speed.

2.4 Preparation of Selenium–curcumin Nanoformulation

Curcumin (0.1 g) was dissolved in 5 mL of DMSO. After complete dissolution, 45 mL of distilled water was added to obtain a total volume of 50 mL. Separately prepared neem flower-mediated SeNP solution (50 mL) was combined with the 50 mL curcumin solution under continuous stirring to obtain the selenium–curcumin nanoformulation.

2.5 Preparation of the Chitosan-based Formulation

Chitosan (0.5 g) was dissolved in 49 mL of distilled water containing 1 mL of glacial acetic acid. The solution was stirred at 700 rpm for 24 hours. Subsequently, 50 mL of the chitosan solution was combined with 50 mL of the selenium–curcumin nanoformulation and stirred to facilitate incorporation. Because final pH, appearance, and stability were not assessed, the product is referred to as a chitosan-based formulation rather than as a fully characterized mouthwash.

2.6 Purification and Storage

The reaction mixture was centrifuged at 8000 rpm for 10 minutes. The recovered nanoparticle fraction was washed twice with double-distilled water and stored in sealed Eppendorf tubes at 4 °C until further analysis.

2.7 Physicochemical Characterization

2.7.1 UV–visible Spectroscopy

UV–visible spectroscopy was performed using a double-beam spectrophotometer (ESICO). Absorbance was recorded over the reported range of 250–450 nm to monitor the optical characteristics associated with nanoparticle formation.

2.7.2 Fourier-transform Infrared Spectroscopy

The FTIR spectroscopy was used to evaluate functional groups associated with plant-derived surface constituents and the nanoparticle preparation. The spectrum was interpreted qualitatively with reference to characteristic functional-group regions. FTIR was not used as a stand-alone method for establishing elemental selenium.

2.7.3 Scanning Electron Microscopy

Scanning electron microscopy was used to examine the morphology of the selenium–

curcumin/chitosan formulation. The images were evaluated qualitatively at different magnifications. The scale bars are presented only to indicate image magnification and are not interpreted as particle size.

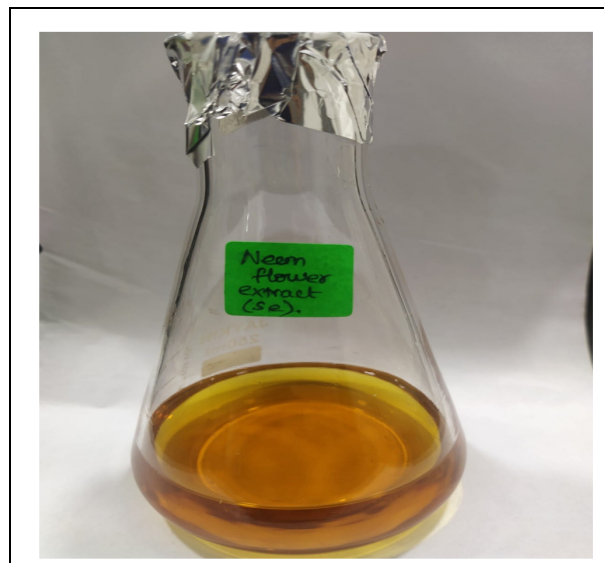


Fig. 2: Preparation of neem flower-mediated selenium nanoparticles

2.7.4 Energy-dispersive X-ray Spectroscopy

An EDX spectrum was included in the original submission; however, the spectrum showed carbon, oxygen, potassium, chlorine, and approximately 10.1% silver by weight, with no detected selenium. Silver was not part of the stated experimental synthesis, which used sodium selenite as the selenium precursor. The spectrum was therefore considered not traceable to the intended SeNP sample and has been excluded from the revised Results. Because the correct elemental dataset was not retained, no elemental composition is reported here. Accordingly, the revised manuscript does not use EDX to claim quantitative selenium composition.

2.7.5 Complementary Characterization

XRD, TEM, and DLS data were not available. Therefore, no claims are made regarding crystalline phase, primary particle size, hydrodynamic diameter, polydispersity, or nanoscale morphology that would require these measurements.

2.8 Microbial Culture and Antimicrobial Assay

Fresh cultures of *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Candida albicans* were prepared according to the experimental protocol. For the bacterial assay, cultures of *E. faecalis*, *S. aureus*, and *S. mutans* were inoculated into sterile Hi-veg broth and incubated for 18 hours in an orbital shaker at 120–150 rpm. Mueller–Hinton agar plates were prepared, and 5-mm wells were punched

using sterile tips. The specified volumes of the biosynthesized SeNP preparation (25, 50, and 100 μL) were introduced into the wells together with the antimicrobial positive control. Plates were incubated at 37 $^{\circ}\text{C}$ for 24 hours, after which zones of inhibition were recorded. The antimicrobial activity was expressed as the zone of inhibition for the experimental groups of 25, 50, and 100 μL and a standard control.

2.9 DPPH Antioxidant Assay

Antioxidant activity was evaluated using the DPPH free-radical scavenging assay. Samples were mixed with 1 mL of 0.1 mM DPPH in methanol and 450 μL of 50 mM Tris-HCl buffer (pH 7.4), followed by incubation for 30 minutes. Absorbance was measured at 517 nm. Butylated hydroxytoluene was used as the reference antioxidant control. Percentage inhibition was calculated as follows:

$$\text{DPPH inhibition (\%)} = [(A^c - A^s) / A^c] \times 100$$

where A^c is the absorbance of the control and A^s is the absorbance of the test sample.

2.10 Brine Shrimp Assay

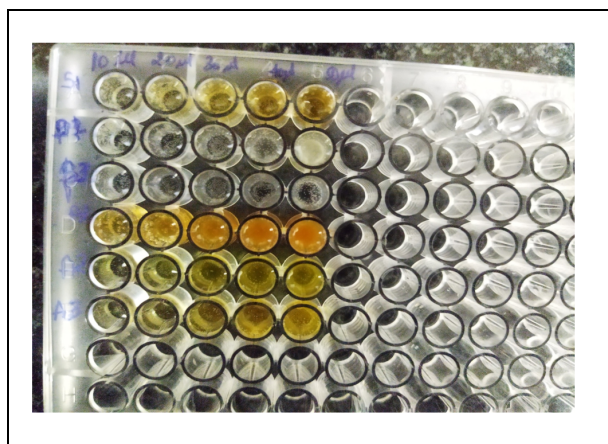


Fig. 3: Brine shrimp assay used for preliminary biological-response assessment

Preliminary biological response was assessed using a brine shrimp lethality assay (Fig. 3). Two grams of iodine-free salt was dissolved in 200 mL of distilled water. A 12-well plate was divided into wells containing 6–8 mL of saline solution, and 10 nauplii were transferred to each well. The selenium–curcumin nanoformulation was added at the experimental doses of 5, 10, 20, 40, and 80 μL . Following 24 hours of exposure, live and dead nauplii were counted. Percentage mortality was calculated as follows:

$$\text{Mortality (\%)} = [\text{Number of dead nauplii} / \text{Total number of nauplii}] \times 100$$

The brine shrimp assay was performed using 10 nauplii per well. The selenium–curcumin nanoformulation was added at experimental doses of 5, 10, 20, 40, and 80 μL . Following 24 hours of exposure, live and dead nauplii were counted. Percentage mortality was calculated as follows:

2.11 Zebrafish Assay

Zebrafish embryos were transferred at the early blastula stage, with 10 embryos reported per Petri plate (Fig. 4 and Fig. 5). Embryos were exposed for 24 hours to selenium nanoparticle concentrations of 5, 10, 15, 20, and 25 $\mu\text{g/mL}$. A control group was maintained under the same conditions. Embryonic development was monitored microscopically.

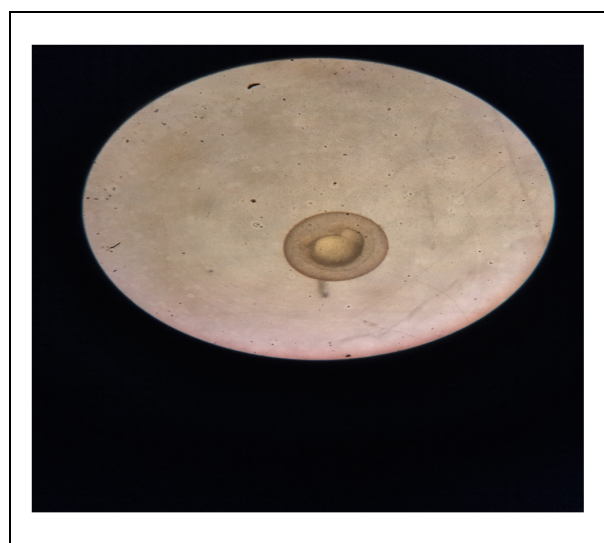


Fig. 4: Representative zebrafish embryo image

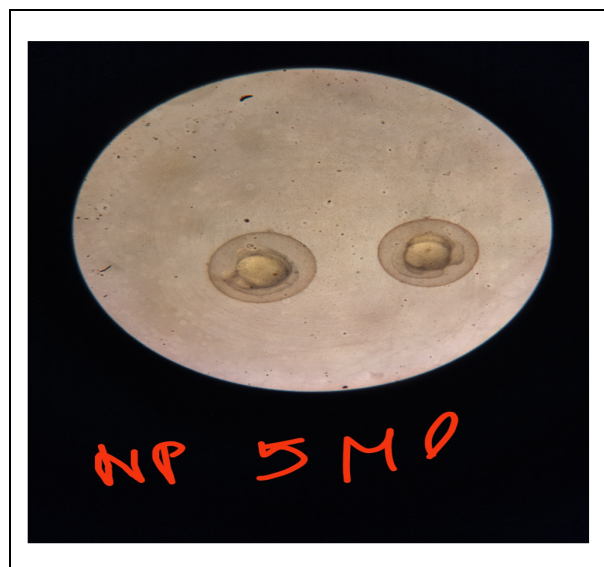


Fig. 5: Representative zebrafish embryo image

2.12 Statistical Analysis

Statistical analysis was descriptive because the available experimental record did not provide sufficient replicate-level data or a statistical analysis plan to support inferential testing. The reported findings are therefore interpreted as preliminary observations.

3. RESULTS

3.1 Visual Observation and UV–visible Spectroscopy

The synthesized reaction mixture was visually documented following preparation of the neem flower-mediated selenium nanoparticle formulation. The available UV–visible spectrum showed an absorption feature centered at approximately 450 nm (Fig. 6). The absorption feature is considered an optical characteristic consistent with nanoparticle formation but is not treated as independent proof of elemental selenium because UV–visible spectroscopy cannot establish elemental composition by itself.

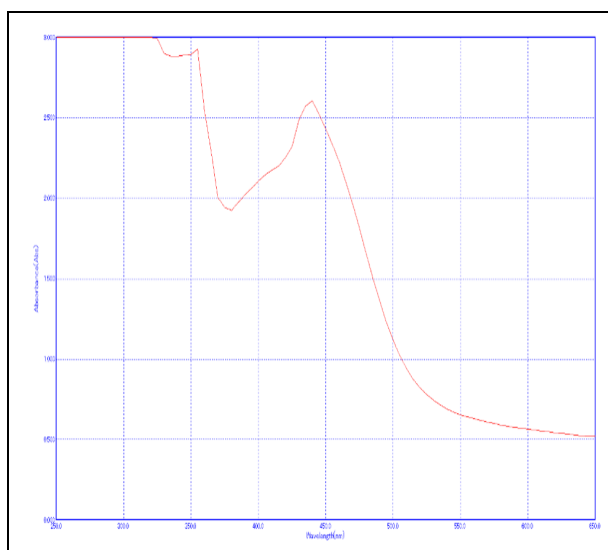


Fig. 6: UV–visible spectrum of neem flower-mediated SeNPs

3.2 FTIR Spectroscopy

The FTIR spectrum displayed several absorption peaks attributable to functional groups associated with the plant-derived material (Fig. 7). The broad absorption region in the higher-wavenumber range was compatible with hydroxyl- and/or amino-containing constituents, while additional peaks were observed across the fingerprint region. Exact peak assignments were not made because the available data were insufficient for definitive chemical-bond attribution.

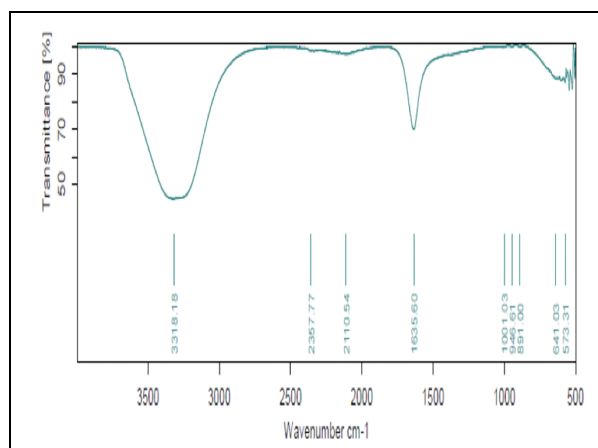


Fig. 7: FTIR spectrum of the synthesized selenium nanoparticle

3.3 Antimicrobial Activity

The SeNP preparation produced measurable zones of inhibition against the microorganisms tested (*Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Candida albicans*; Fig. 8). The largest experimental inhibition zones were observed with the 100 μ L sample volume, whereas the 25 μ L group showed smaller zones. Among the tested microorganisms, *S. aureus* showed the largest zone. The result indicates greater inhibition with increasing volume of the tested preparation under the specific agar-well conditions used.

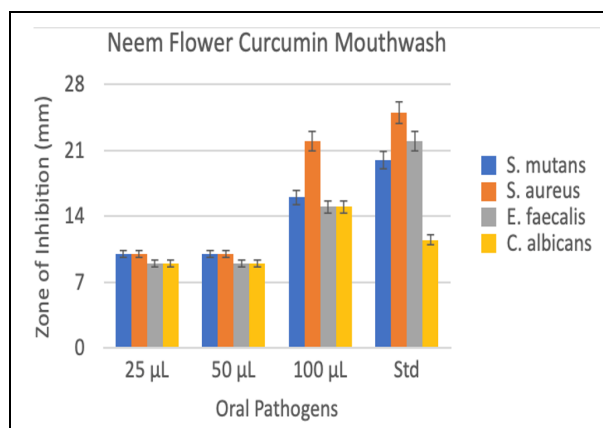


Fig. 8: Antimicrobial activity of the SeNP preparation against *S. mutans*, *S. aureus*, *E. faecalis*, and *C. albicans*

3.4 Antioxidant Activity

The DPPH assay was performed as described in the experimental protocol. Quantitative DPPH results and a corresponding assay image were not available for inclusion; therefore, the antioxidant findings are not quantitatively interpreted.

3.5 Preliminary Biological-response Assays

The brine shrimp exposure to the selenium–curcumin nanoformulation and zebrafish exposure to SeNPs provided preliminary biological-response observations. Representative brine shrimp and zebrafish images are presented as documentation of the experimental models rather than as quantitative evidence of biocompatibility or safety.

3.6 SEM Morphology

The SEM images of the selenium–curcumin/chitosan formulation showed heterogeneous surface features with irregular structures and areas of aggregation. Images were available at scale bars corresponding to approximately 10 μm , 1 μm , 0.5 μm , and 100 μm (Fig. 9–12). These images provide qualitative information regarding surface morphology.

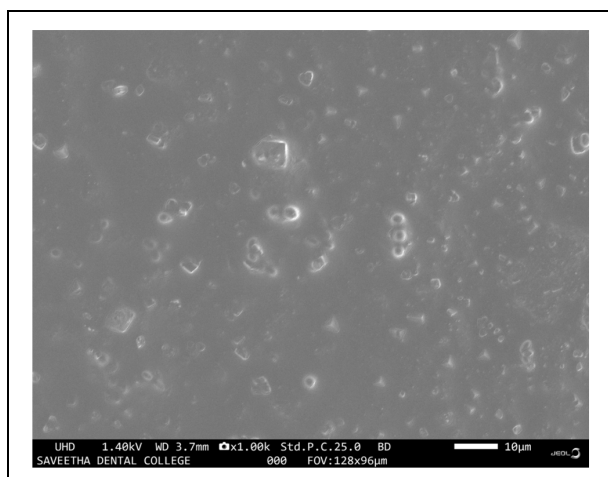


Fig. 9: SEM image of the selenium–curcumin/chitosan formulation; scale bar approximately 10 μm

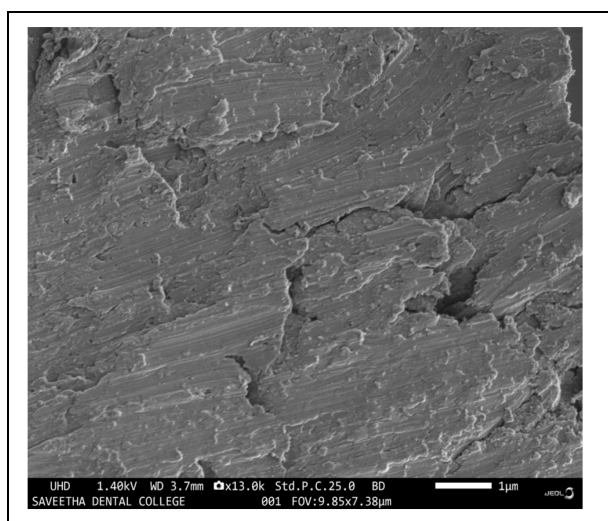


Fig. 10: SEM image of the selenium–curcumin/chitosan formulation; scale bar approximately 1 μm

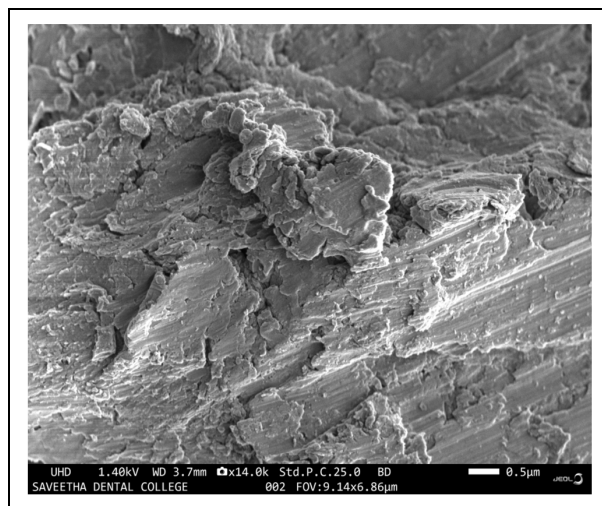


Fig. 11: SEM image of the selenium–curcumin/chitosan formulation; scale bar approximately 0.5 μm

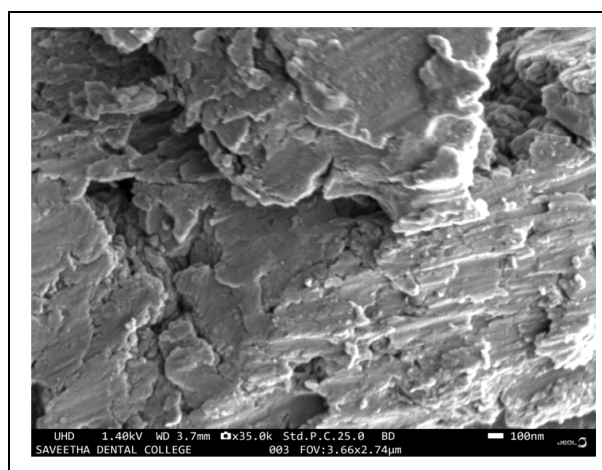


Fig. 12: SEM image of the selenium–curcumin/chitosan formulation; scale bar approximately 100 μm

4. DISCUSSION

The present study investigated a neem flower-mediated route for preparing selenium nanoparticles and examined a selenium–curcumin formulation incorporated into a chitosan-based matrix for potential oral applications. The available findings include an optical absorption feature at approximately 450 nm, qualitative FTIR evidence of plant-associated functional groups, qualitative SEM observations, and antimicrobial activity against the tested microorganisms. DPPH, brine shrimp, and zebrafish assays were also performed, but quantitative datasets were not available for full interpretation. The discussion therefore focuses on findings directly supported by the available experimental evidence.

Plant-mediated synthesis is increasingly investigated as a route for producing SeNPs because plant metabolites can contribute to precursor reduction

and surface stabilization. Reviews of plant-mediated SeNP synthesis describe phenolics, flavonoids, proteins, and other metabolites as potential contributors to nanoparticle formation and capping (Johnson *et al.* 2022; El-Saadony *et al.* 2026). The present study used an aqueous neem flower extract rather than an organic plant-extraction procedure. This is relevant to green synthesis because water served as the extraction medium and the biological component of the reaction was derived from plant material. Heating, stirring, precursor use, centrifugation, repeated washing, waste generation, and downstream nanoparticle handling all contribute to the resource and environmental burden of the process. Recent reviews similarly emphasize the need for standardized synthesis conditions, reproducibility, and assessment of long-term environmental and biological effects (El-Saadony *et al.* 2026).

The antimicrobial experiment showed inhibition of the tested microorganisms, with the highest experimental sample volume producing the largest zones and *S. aureus* showing the largest reported zone. The finding should be interpreted as formulation-specific evidence rather than as evidence that all SeNPs exhibit a uniform antimicrobial effect.

The experimental groups were evaluated using 25, 50, and 100 μL volumes of the nanoparticle preparation. Because the corresponding stock concentration was not available, these volumes cannot be converted reliably into $\mu\text{g/mL}$ or mg/mL . Consequently, the observed increase in inhibition zone with sample volume should not be described as a validated dose-response relationship. Zone-of-inhibition assays also do not establish minimum inhibitory concentration, minimum bactericidal concentration, antibiofilm activity, or clinical efficacy.

Curcumin was incorporated into the selenium formulation because of its established interest as a polyphenolic antioxidant and antimicrobial compound. Its poor aqueous solubility is an important formulation consideration, and nanoscale or polymer-associated systems have been investigated to improve dispersion and delivery (Prasad *et al.* 2014). Recent literature specifically addressing selenium–curcumin nanoparticles indicates that the combination remains an active research area and that the biological behavior of these materials depends on formulation and physicochemical characteristics (Ghoflchi *et al.* 2026; Rajendran *et al.* 2026). In the present study, curcumin was dissolved in DMSO and subsequently diluted with water before combination with the SeNP preparation. Because quantitative physicochemical analysis of the final selenium–curcumin formulation was not available, claims regarding chemical conjugation, encapsulation efficiency, or synergistic activity are not made.

Chitosan was used as the matrix component because it is a biodegradable polysaccharide with reported mucoadhesive and antimicrobial properties. Recent oral literature supports interest in chitosan and chitosan nanoparticles for antimicrobial applications, while emphasizing that activity depends on formulation characteristics and test method (Cakici and Cakici, 2024; Nasaj *et al.* 2024). The present experiment demonstrated incorporation of the selenium–curcumin preparation into a chitosan solution, but no formal synergy analysis was performed. Any enhancement observed after incorporation would require direct comparison of the individual components and combination treatments using a predefined synergy model.

The original study proposed reactive oxygen species generation, microbial membrane disruption, and improved interaction with bacterial surfaces as possible mechanisms of antimicrobial activity. These mechanisms are plausible based on the broader literature but were not directly measured. No intracellular ROS assay, membrane-permeability assay, leakage analysis, electron microscopy of treated bacteria, or molecular target assessment was reported. The observed inhibition zones should therefore be distinguished from mechanistic hypotheses.

The DPPH assay provides a chemical measure of radical-scavenging activity and is useful as a preliminary antioxidant screen. Quantitative DPPH data were not available for analysis. Similarly, the brine shrimp and zebrafish assays can provide preliminary biological information but do not establish human oral safety. Selenium nanoparticle toxicology is strongly influenced by dose, particle characteristics, exposure route, and biological model. Reviews of repeated selenium exposure and SeNP toxicity have identified uncertainty regarding dose-response relationships and long-term effects (Ryabova *et al.* 2024; Hadrup and Ravn-Haren, 2023).

The EDX spectrum showed C, O, Ag, K, and Cl, including approximately 10.1 wt% Ag, while selenium was not detected. Silver was not part of the stated synthesis, which used sodium selenite as the precursor. Because the spectrum could not be confidently linked to the intended SeNP sample, it was excluded from the Results and is not used to claim quantitative elemental composition.

The SEM images provide qualitative evidence of heterogeneous surface morphology and aggregation at the imaged scales, but the available document does not provide a validated particle-size distribution. This distinction is important because an image scale bar represents the spatial scale of the image and does not by itself establish particle diameter. Recent green SeNP studies commonly combine SEM or TEM with XRD, EDX, DLS, and other complementary techniques to

establish morphology, elemental composition, crystallinity, and dispersion characteristics (Palayathan *et al.* 2026; Assad *et al.* 2025). The present study should therefore be considered preliminary from a physicochemical characterization perspective.

The study also has limitations related to formulation characterization. Although the chitosan-based formulation was prepared using a defined mass and volume of chitosan, water, acetic acid, and selenium–curcumin formulation, final pH, appearance, viscosity, storage stability, and shelf-life measurements were not available. For this reason, the formulation is described as a chitosan-based experimental formulation rather than as a validated mouthwash. Such characterization would be necessary before evaluating its suitability for routine oral use.

From an environmental-nanotechnology perspective, the aqueous neem extraction and plant-mediated reduction route provides a reasonable basis for further study of a lower-solvent synthesis strategy. Nevertheless, environmental claims should remain proportional to the evidence. Future studies should evaluate reaction yield, energy requirements, precursor utilization, washing demand, residual selenium species, nanoparticle stability, degradation, and environmental fate. Green synthesis is best considered a process-design objective rather than a guarantee of environmental safety (El-Saadony *et al.* 2026; Assad *et al.* 2025). Recent plant-mediated nanoparticle studies involving other material systems, including calcium oxide, gold, and phytochemical-capped silver nanoparticles, further illustrate the breadth of phytochemical-assisted nanomaterial synthesis (Srinivasan *et al.* 2026; Shafiee *et al.* 2026; Subhalakshmi *et al.* 2026). Plant-assisted synthesis has also been reported for zinc oxide nanoparticles using *Cleome gynandra* extract, with subsequent evaluation of cytotoxic activity in A549 cells (Velmani *et al.* 2024).

Overall, the available data indicate that the neem flower-mediated selenium formulation warrants further investigation for experimental oral antimicrobial applications. The findings should be interpreted as preliminary because definitive elemental characterization, complementary structural and size measurements, replicate-level numerical data for several biological assays, and formal statistical analysis were not available. Future work should address these gaps using verified SeNP samples and standardized characterization and biological-testing protocols before any translational or clinical claims are considered.

5. LIMITATIONS

The principal limitations are the incomplete characterization and biological datasets. The EDX spectrum available for the study contained silver and did

not demonstrate selenium; XRD, TEM, and DLS datasets were not available. Quantitative SEM particle-size analysis could therefore not be established. The experimental record also lacked replicate-level numerical results, statistical outputs, and sufficient methodological details for some biological assays, including reaction conditions, exact sample concentrations, replicate numbers, and complete control descriptions. These limitations restrict quantitative interpretation and should be considered when evaluating the findings.

5. CONCLUSION

The present study investigated neem flower-mediated selenium nanoparticles and a selenium–curcumin formulation incorporated into a chitosan-based matrix for experimental oral applications. The findings demonstrate an optical absorption feature associated with the synthesized material, qualitative FTIR and SEM characteristics, and antimicrobial activity against microorganisms. The aqueous neem-mediated synthesis provides a basis for further exploration of plant-assisted SeNP production within a green-nanotechnology framework.

The conclusions of this study are intentionally limited to the available experimental evidence. The study does not establish crystalline phase, definitive particle-size distribution, elemental composition from EDX, clinical efficacy, wound-healing activity, prolonged oral safety, or therapeutic effectiveness. Comprehensive physicochemical characterization, complete quantitative biological datasets, formulation stability assessment, standardized antimicrobial testing, and appropriately controlled mechanistic and biological studies are required before further translational evaluation.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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