RESEARCH ARTICLE

INTERNATIONAL RESEARCH JOURNAL OF MULTIDISCIPLINARY TECHNOVATION

Asian Research Association

DOI: 10.54392/irjmt25617

Antimicrobial Activity of Selenium Nanoparticles-*Syzygium aromaticum* against Oral Pathogens

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DOI: https://doi.org/10.54392/irjmt25617

Received: 18-01-2025; Revised: 30-10-2025; Accepted: 17-11-2025; Published: 30-11-2025

Abstract: Oral microbiota plays an essential role in maintaining oral health. However, when pathogenic bacteria colonize the oral cavity and integrate into the biofilm, diseases such as gingivitis, dental caries, periodontitis, and peri-implantitis may occur. In this study, the antimicrobial, antibiofilm, antioxidant activities, and toxicity of selenium nanoparticles (SeNPs) were examined. SeNPs were synthesized using a green method with *Syzygium aromaticum* extract, which enhanced their stability and antimicrobial effect. Characterization using UV–Visible Spectroscopy, Fourier Transform Infrared Spectroscopy, and Scanning Electron Microscopy confirmed the formation of stable, spherical SeNPs with sizes ranging from 20-80 nm. The antimicrobial assays revealed that Candida albicans exhibited the largest zone of inhibition, indicating strong antifungal activity of the SeNPs, while antibacterial effects were also significant against oral pathogens at 100 µg/mL concentration. In addition, SeNPs demonstrated antibiofilm potential, effectively reducing biofilm formation at (100 µg/mL) higher concentrations, which showed sensitive to Enterococcus faecalis. Toxicity evaluation using zebrafish embryos confirmed their biocompatibility at therapeutic concentrations, with minimal adverse effects. These findings suggest that SeNPs hold promise as a natural, effective and biocompatible agent for oral healthcare applications, particularly in the management of oral infections and biofilm-associated diseases.

Keywords: Antimicrobial Activity, Selenium Nanoparticles, *Syzygium aromaticum*, Time-Kill Curve, Toxicity

1. Introduction

Selenium nanoparticles (SeNPs) possess unique antimicrobial activity and low toxicity levels and have received a lot of attention for their potential use in biomedical applications. Plant used for synthesis of NPs has benefits such as improved biocompatibility and stability of NPs [1]. NPs help in improving targeting, reducing toxicity, increasing bio-activity and supply many ways to change the release profile of the capsulized moiety [2]. Metal inorganic NPs such as silver, gold, selenium, titanium and zinc occupy a prominent position among various NPs due to the uniqueness of nanomorphology [3]. An essential element of the organism Selenocysteine (Sec) is integrated into selenoproteins, the most important substance in the active core of its enzymatic activity [4]. Since many SeNPs have oxidoreductase activity, they maintain the redox balance of the body. The therapeutic window of Se is narrow and the toxicity range is low, whereas SeNPs are less toxic. SeNPs have been studied for therapeutic efficacy in various oxidative stress and infectious diseases including kidney disease, diabetes, cancer and arthritis. SeNPs possess many advantageous features to act as in targeted drug delivery system [5]. Present study identifies whether the size of SeNPs has variations to be impounded on pharma dynamic and actions on broad range of inflammatory and oxidative stress-associated diseases. This study analyzes the ways SeNPs can enhance the pharmacokinetics and pharmacodynamics of selenoproteins and the impact on therapeutic possibilities [6]. It can be realized that SeNPs when the size and various properties are adjusted properly, can be

used optimally for improving drug percolation and enabled release and action, making it a useful tool for the treatment of diseases, however, is not well understood. Methods based on nanomedicine envision ways to tackle the intricate problems related to traditional medication forms and dosing dosages with increased safety is one of nanomedicine's benefits [7].

SeNPs have superior biological activity and bioavailability when compared to both organic and inorganic Se compounds. However, SeNPs limited cellular absorption is their main drawback [8]. Targeted ligands have been conjugated on the outside of the NPs in an attempt to solve this problem this provides a helpful platform for anticancer treatment. It has been demonstrated that the addition of amphoteric ligands like polyethylene glycol, significantly aids in the formation of NPs [9]. Since bacterial separation and cultivation require methods, expertise and specialized equipment, plant extracts are even more economical than employing bacteria or fungi. The procedure may produce high-quality SeNPs with minimal solvent consumption, no product waste and no specialist equipment. For this reason, these methods are considered one-step, inexpensive and safe for the environment [10]. A few studies recently highlighted the significance of plant-based SeNPs, their ease of synthesis and their potential for biological applications [11]. Additionally, the therapeutic characteristics of SeNPs may be enhanced if they are prepared to utilize medicinal herbs.

Syzygium aromaticum also known as clove contains bioactive compounds that have documented antimicrobial effects that can enhance the preparation of stable SeNPs [12]. S. aromaticum is a tree of the Myrtaceae family that produces fragrant cloves, which are used as spices [13]. The anticancer, anti-diabetic, anti-inflammatory, antinociceptive, antibacterial, antifungal and antithrombotic properties and wide range of pharmacological properties, making it a promising medication candidate for a variety of conditions. These biological catalysts are essential for the environment because they convert the harmful oxyanions found there into elemental selenium, which is non-toxic [14]. This research aims to prepare SeNPs from selenium ions using the green method by clove extract as a reducing and capping agent and then to explore the structural and functional characteristics of the obtained SeNPs [15].

When used in food preservation, especially in meat processing, clove is preferred to chemical preservatives because of its excellent antioxidant and microbial activity [16]. Compared with other aromatic herbs including cinnamon, oregano, thyme, mint and others, Clove exhibits very high antibacterial, antiviral, antifungal and anticarcinogenic properties. This huge efficiency is explained by the high concentration of bioactive compounds with antioxidant activity [17]. These compounds are very important in case of prevention of various diseases related to degeneration of the body. Therefore, the practice and applications of clove are inseparable because it is documented finding its use in medicine, industries and in food processing while being established as a natural preservative and therapeutic ingredient [18].

Clove essential oil has been traditionally employed for therapeutic purposes especially for burns and wounds and oral pain relief. It is widely prescribed for the treatment of tooth infections as well as tooth decay diseases [19]. Outside the medical field, clove essential oil has various uses in the industrial fields as a fragrance in perfumes, being used in soaps as well as being used in preparing specimens for histological assessment. In ancient Indian and Chinese practices cloves have been used as warm and stimulating adjuncts to other medicines [20].

Biflorin, 5,7-dihydroxy-2-methylchromone-8-C-β-D-glucopyranoside, orsellinic acid glucoside, myricetin, rhamnocitrin, gallic acid, oleanolic acid, ellagic acid and flavonoid triglycosides present in S. aromaticum have been found to exhibit high virulence Ethanol soluble of the plant has demonstrated great antioxidant activity and has been found to possess moderate hepatoprotective activity against the paracetamol intoxicated liver [21]. It may involve membrane stabilization, reducing intracellular enzymatic inhibition and maintaining serum transaminase homeostasis by encouraging new hepatic tissue formation and microbial defense against *Pseudomonas aeruginosa*, *Escherichia coli* and recovery from microbial infections [19]. As other in vitro investigations the antimicrobial efficacy of S. aromaticum versus of a various of bacterial species showing its significant effectiveness against B. subtillis other than being more effectful than extract of other medical plants [22]. It has also shown considerable activity against a range of antibiotic-resistant bacteria inclining Salmonella typhi and others like Bacillus sp., and Serratia marcescens [23]. Interactions between ethanolic extracts of S. aromaticum and antibiotics have been synergistic, which has better efficacy as compared to aqueous extract against S. aureus. Also, S. aromaticum is reported to possess antifungal activity [24]. Eugenol and clove oil exhibited effectiveness against different type of fungi such as yeasts, filamentous fungi and human pathogenic fungi, supporting the viewpoint on the multiple microbial activity of the compounds obtain from clove [25]. Effectiveness of the different natural bioactive molecules that includes thymol, eugenol, carvacrol and cinnamaldehyde on the E. coli and it was established that eugenol was the least effective while carvacrol and thymol together with cinnamaldehyde and eugenol revealed additive effectivity [26]. This work also seeks to determine the ability of the synthesized SeNPs to inhibit conventional oral pathogens to assess their suitability as possible alternatives to conventional antimicrobial agents in dental treatment. Through this study, clove used for synthesis of SeNPs will be evaluated as a

potential against oral pathogens due to the emergence of antibiotic resistance and would ascertain novel antiseptic solutions originating from plant bio-resources for management and enhancement of oral infections and health. Table 1 shows different clove used in oral health.

2. Materials and Methods

SeNPs were synthesized employing a biological, nontoxic, easily available extract of clove as a reducing and stabilizing agent. The fresh clove buds were washed, dried and pulverized to obtain fine powder, which when boiled in distilled water and the liquid so obtained filtered to get the water extract. Selenium dioxide, sodium hydroxide and double distilled water are the precursors used for the synthesis procedure.

2.1 Collection of Plant Materials

The collection of S. aromaticum was collected in Chennai, Tamil Nadu, India. The University of Madras Centre for Advanced Studies in Botany in Chennai, India, validated the sample. Small pieces of S. aromaticum were cut up, cleaned three times with tap water and then allowed to air dry for three days in a shady spot. They were crushed into a fine powder and stored for later use once the S. aromaticum flowers had completely dried. Next, 300 mL of double-distilled H2O was mixed with 25 g of S. aromaticum flower powder and heated to 60°C for 20 min. After that, the extract was filtered through muslin cloth and then further purified by the use of 125 mm diameter Whatman filter paper. Before being used again, the filtered solution was kept at 4° C [27].

2.2 Preparation of Selenium Selenite Solution

Selenium selenite solution is prepared by dissolving selenium dioxide in water to form selenious acid, then adding sodium hydroxide to adjust the pH to 7-14. A 5mM selenium selenite solution was made by dissolving it in 300 mL of distilled water.

2.3 Synthesis of Selenium Nanoparticle

SeNPs were synthesized by mixing 90 mL of a selenium solution (5 mM) with 10 mL of S. aromaticum extract and stirring the mixture at 35° C. The reaction mixture degree of acidity was regularly measured and noted. Following that, the reaction mixtures were incubated for three hours at 40° C and 300 rpm on a revolving shaker in a dark room. The reaction mixture was incubated for 72 h at room temperature after the color was visually assessed. Following the incubation period, the reaction mixture showed a complete color shift, indicating that the NPs production was effective. UV spectroscopy, FTIR and SEM with EDAX were used to analyze the SeNPs. Initially, synthesis was identified in the 200-800 nm region using UV-visible spectrophotometry. The precise functional groups on the SeNPs were effectively determined by the FTIR analysis at 4000-400 cm-1. Using SEM with EDAX, the morphology and purity were assessed [28]. Figure 1 shows the overall methodology of SeNPs.

2.4 Antimicrobial Activity

Antimicrobial activity is important in making discoveries and in confirming of efficacy of existing treatments. The agar-positive diffusion method was used to evaluate the antibacterial activity of plant SeNPs. Prepare Mueller Hinton agar plates and sterilize them by autoclaving at 121° C for 15 to 20 min. After sterilization, cover the sterile petri dish with a culture medium and allow to cool to room temperature. Removal of bacteria (Streptococcus mutans, Lactobacilli sp., Staphylococcus aureus and Candida albicans) was spread evenly on the agar plates using sterile cotton swabs. Make a 9 mm diameter agar plate using sterile polystyrene lids. Different concentrations of SeNPs (25, 50 and 100 µg/mL) were then added to the wells. For fungal cultures, plates were incubated at 37° C for 24 and 48 h. The inhibition zone around the well was used to measure the antibody response. Use a ruler to measure the area of the inhibition zone, then record it in millimeters (mm) and use it to calculate the zone of inhibition [29]. All antimicrobial assays were performed in triplicate (n=3) and data are presented as mean ± standard deviation.

2.5 Time-Kill Curve

The time-kill curve was used to evaluate the bactericidal qualities of SeNPs, the association between the growth patterns of *Streptococcus mutans, Lactobacilli sp., Staphylococcus aureus and Candida albicans* towards SeNPs was examined. In this test, pathogens were cultured in Mueller Hinton broth containing different concentrations of SeNPs in a concentration range 100, 250, 500 and 1000 µg/ml and their growth was measured at different time intervals. Hence, preliminary growth curves were developed to get standard growth values. Following five hours in antibiotic free Mueller Hinton broth the pathogens moved out from an early steady state to the mid-log phase of the cell division cycle. 0.5 McFarland of each pathogen was separately grown in sterile phosphate-buffered saline (PBS), from cultures grown on Mueller Hinton agar for 18-20 h at 37° C. The remaining 30 µL of this inoculum was further diluted in 15 mL of Mueller Hinton broth warmed to 37°C that did not contain bacteria. The suspension was also plate into a 96-well ELISA plate subsequently, the resulting suspension was mixed with an equal volume of PBS containing 0.05 % Tween 20 and 1 % normal goat serum. To each well, 10 µL of SeNPs synthesized using S.

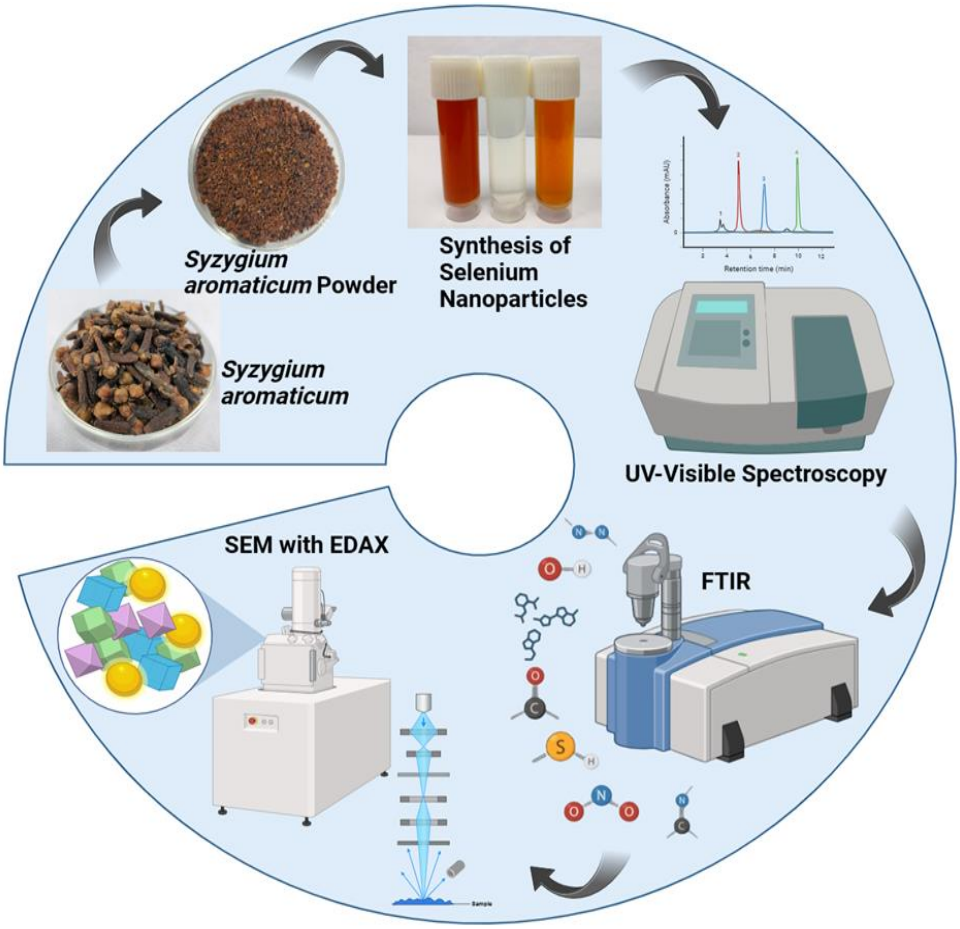

Figure 1. Selenium nanoparticles synthesised by *Syzygium aromaticum* Characterization and antimicrobial activity against oral pathogens.

Aromaticum at five different SeNPs concentrations were assayed and a no SeNPs control was used. IC50 values for growth inhibition were determined in all the tested circumstances [30].

2.6 Protein Leakage Analysis

Protein leakage analysis is a method used to assess the antimicrobial properties of NPs and other substances. It can help determine the potential uses of these substances, such as in medical device coatings and wound care. The integrity of bacterial cells was evaluated by measuring the release of protein of bacteria into the supernatant. Different quantities of S. aromaticum- SeNPs (25, 50 and 100 µL) were added to three bacterial suspensions (Streptococcus mutans, Lactobacilli sp., and Staphylococcus aureus) with a volume of 10 mL each. The standard was ampicillin, while the positive controls were bacterial suspensions [31].

2.7 Anti-biofilm Assay

The term antibiofilm is defined as the measure effectively a particular agent inhibits biofilm formation by microorganisms such as bacteria and fungus or reduces the existing biofilms. Biofilms are complex partnerships of bacterial cells in a protected micro-environment that is embedded within a rugged secreted material attached to bi-tensile surfaces and are anathema to many of the broad-spectrum antimicrobial agents [17]. Biofilm development is a major problem in medicine and technology due to chronic infection, equipment colonization and diminished sensitivity to antimicrobial agents. It has been proved that natural extracts, metal nanomaterials and synthetic chemistries possess the antibacterial films capacity. The SeNPs synthesized with plant extracts like S. aromaticum, show good anti-biofilm potential. Such NPs can effectively transverse the biofilm matrix, weaken or destroy the organization of microbes and either eliminate or slow the growth of these microbes. This activity is particularly important against bacteria that form biofilms such as Streptococcus mutans, Lactobacilli sp., Staphylococcus aureus and Candida albicans which are common in medical and industrial cases. The inoculation of sterile 96-well plates with 1 × 10^6 cells/ml bacterial suspension and culture it for 24 h. Add various concentrations of SeNPs and incubate for 4 h at 37° C. Remove media and wash wells to eliminate non-adherent bacteria. Apply crystal violet to wells for staining and wash off excess after staining. For quantification, dissolve retained crystal violet in ethanol and measure absorbance at 595 nm [32].

2.8 Brine Shrimp Lethality Assay

The brine shrimp lethality assay is a convenient, efficient bioassay that is frequently used to determine the cytotoxicity or biological efficacy of chemical, plant and other NPs. A feature of this method is the use of test organisms in the form of Artemia salina (brine shrimp) nauplii, which are rather easily cultured and sensitive to toxic substances. Nauplii are exposed to varying concentrations of the test substance at varying time intervals, often 24-48 h and the deaths of nauplii are then determined by the number of dead nauplii at the different concentrations. The assay gives an initial determination of a compound's cytotoxicity and indeed may be used in initial screening for possible anticancer, antimicrobial or pesticidal applications. If a compound has a high toxicity rate to brine shrimp, then it likely possesses bioactivity and should be further tested in another biological assay. There are several benefits associated with the assay, it is relatively simple to perform, needs little equipment and gives consistent results. The brine shrimp lethality assay is a simple and inexpensive method for assessing toxicity. It is used to test the toxicity of many substances including heavy metals, pesticides, drugs and natural plants [33].

2.8.1 Saltwater Preparation

Dissolve 2 g of non-iodized salt in 200 mL of distilled water. Add ten to twelve mL of saline to a six-well ELISA plate. Ten nauplii (5, 10, 20, 40 and 80 µg/mL) were slowly added to each well. Then, SeNPs were added depending on the concentration level and allow the plate to sit for 24 h. Examine the ELISA plate after one day, record the number of viable nauplii and calculate the results using the formula below,

Number of viable nauplii= Number of dead nauplii/number of dead nauplii + number of live nauplii×100 (1)

2.8.2 Embryonic Toxicology Study on SeNPs using Zebrafish

In culture plates with 0.2 mL of culture water, viable embryos were deposited. Each well received 0.1 mL of SeNPs produced by S. aromaticum extract at different concentrations (10, 5, 20, 40 and 80 µg/mL). Three duplicates of the experiment were used, with embryos in the culture medium serving as the control group. An incubator that was set at 26° C was used to hold the plates. After fertilization, the development of embryos and zebrafish larvae was monitored at different times. The hatching rate and mortality rate were determined at 12 h intervals according to the number of live embryos. NPs have been shown in the laboratory to cause malformations in embryos. Mortality data were used to calculate LC50 values by probit analysis. Survival results are presented as mean ± SD, and statistical significance between treatment groups was evaluated using one-way ANOVA ($p < 0.05$) [34].

2.9 Statistical Analysis

All experiments were carried out in triplicates and data are expressed as mean ± standard error of mean.

3. Results and Discussion

SeNPs were synthesized using S. aromaticum extract as a reducing agent and the appearance of UV visible absorption peaks corresponding to SeNPs was evident. SEM micrographs supported the spherical nature of the particles with average size of 50-100 nm. This FTIR analysis further validated that the bioactive compounds of clove extract were adsorbed on SeNPs surface, which might improve the stability. The outcomes of the antimicrobial tests indicated that the SeNPs are active against the oral pathogens S. mutans and C. albicans as well as the biofilm-associated bacteria, though the bactericidal effects at comparatively low concentrations make SeNPs a prime candidate for oral care products.

3.1 UV-Visible Spectroscopy

UV-Vis spectrum analysis observed S. aromaticum based SeNPs exhibit a strong absorbance band around 286 nm as shown in Figure 2. Wavelength in the spectrum shows strong increase in absorbance due to high particles density and quantum confinement typical for the NPs and nanostructures respectively. These effects stem from the small size of the particles by indicating that the electronic transitions reported here are not for bulk selenium. The tremendous value in the UV region points to the fact that SeNPs have been synthesized with the requisite optical properties. This smooth decline shows that there is no aggregation of the NPs and stable; aggregated particles of irregular size distribution manifest broad and shifted peaks in the visible region [35].

3.2 Fourier Transform Infrared Spectroscopy

FTIR measures the wavelengths at which absorption occurs. Figure 3 shows FTIR spectrum of SeNPs with characteristic peaks at 3247, 1691, 1347, 1017 and 417 cm-1 which corresponds to functional groups like -OH, C=O and C-O which are responsible for stabilization and reduction of Se to form SeNPs. The highest intensity located at around 3247 cm-1 is attributed to the O-H stretching mode.

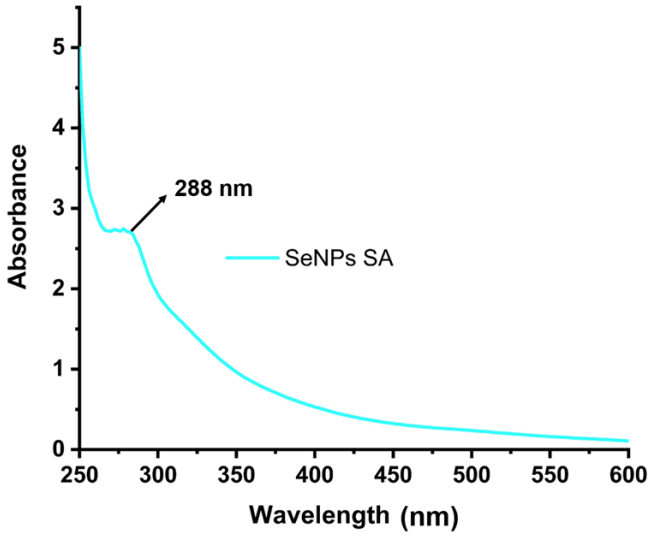

Figure 2. UV-Vis spectrum analysis of *Syzygium aromaticum* based SeNPs

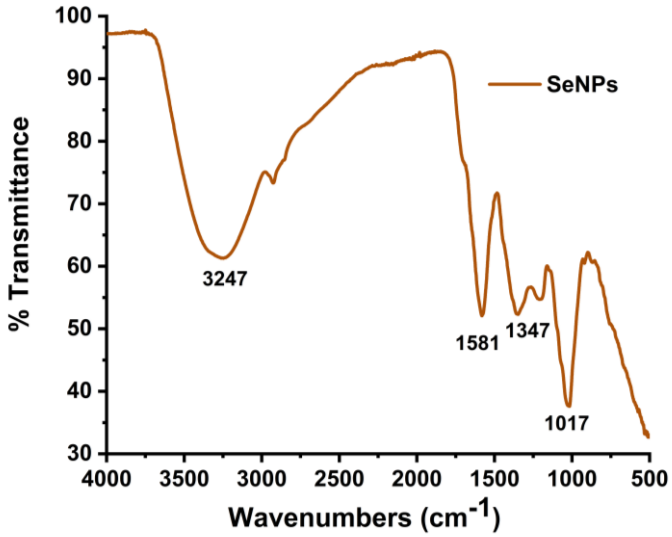

Figure 3. FTIR spectrum of *Syzygium aromaticum* used for synthesis of Selenium Nanoparticles

Hydroxyls are typical for Phenolic Compounds, Alcohols, or Carboxylic Acids; it is read from 3429 cm-1. S. aromaticum contains polyphenols, specifically eugenol which might reduce and stabilize the SeNPs [36]. This peak further establishes the engagement of hydrogen bonding and water molecules which surround the NPs.

The sharp small peak around 2922 cm-1 is generally assigned to aliphatic C-H stretching in the environments of -CH2 and -CH_3 species. These vibrations may be due to long chain hydrocarbons or organic compounds in the S. aromaticum extract that could be involved in nanoparticle stabilization. The peak at 1591 cm-1 is assigned to the C=C stretching of aromatic rings in phenolic compounds. This suggests that there are aromatic bioactive molecules such as eugenol or other flavonoids that are involved in the reduction and stabilization of SeNPs. Interestingly, it may also include peak for amide I vibration indicating interaction of protein with the NPs at the peak [37]. At 1347 cm-1 there is the next peak, which refers to C-N stretching or bending vibrations characteristic for amines or amides. Such peaks could represent the reaction between proteins, enzymes and nitrogen containing species which does exist in the S. aromaticum extract. These interactions may contribute to the stability of nanoparticles in solution and may possibly work as capping agents. The FTIR spectrum offers powerful confirmation of the interaction of S. aromaticum bioactive compounds in the synthesis of SeNPs. The hydroxyl and phenolic groups (3247 cm-1, 1017 cm-1) reduce selenium salts into NPs through reductive deposition. Aromatic compounds at 1591 cm-1 and nitrogen containing compounds at 1347 cm-1 may have a crucial role in stabilization and avoiding aggregation. The appearance of unique Se-O vibration band (at 417

cm-1) is blatantly positive evidence for the formation of SeNPs [38].

3.3 Scanning Electron Microscopy with Energy Dispersive X-Ray Analysis

The SEM image provides the morphology of SeNPs, which indicates that the spherical particles are distributed on the rough surface thereby ascertaining the synthesis of the NPs. The EDAX spectrum in Figure 4(a) reveals elemental characterization of the peaks related to selenium (Se), oxygen (O), carbon (C), potassium (K), sodium (Na), Platinum (Pt) and chlorine (Cl), which indicated that the synthesized SeNPs contained with other elements originated from the clove extract. The SEM image also shows the surface morphology of the SeNPs synthesized employing S. aromaticum extract as represented in the Figure 4(b). The synthesized NPs have an average size between 70-100 nm with irregular and aggregated structure which points to the presence of a stabilizing organic matrix originating from the plant extract. This aggregation might be because the capping agents (such as phenolics, flavonoids or proteins) help to avoid excessive particle coalescence yet prevent the stability. The enlarged SEM image also reveals that the surface of the synthesized NPs is not smooth and includes porous characteristics; therefore, the NPs should possess a higher surface area [39]. This morphological characteristic is of particular importance for such applications as drug delivery, catalysis and environmental cleanup since surface processes are critical for these systems.

Selenium signals represent the energy levels about 1.4 keV and 11.2 keV which are characteristic for selenium in an elemental state The presence of a very intense oxygen peak indicates that oxygen-containing chemical groups (for example hydroxyl and carbonyl) from extract containing plants participate in capping and stabilization of SeNPs. These groups probably lie at the nanoparticle surface, to maintain structure and reactivity.

The carbon peak points towards the organic moiety of S. aromaticum extract adsorbed on the nanoparticle surface. It is noteworthy that these organic compounds including polyphenols, terpenoids and flavonoids which are present in tea act as reducing as well as capping agents in the synthesis process. The carbon peak shows the Alkanes from the S. aromaticum extract attached to the nanoparticle surface.

Some of these organic compounds include polyphenol, terpenoids and flavonoids for example they function as reductants and capping agents during synthesis. The slight humps of potassium and sodium may be due to the inorganic impurities or the inorganic salts or minerals inherent with the plant extract [40].

In this work, SeNPs were synthesized with S. aromaticum (clove) extract, the same as Abd-Elraoof et al., which has a number of bioactive compounds proven to have antimicrobial activity. UV-Vis spectroscopy was then used to establish the synthesis of SeNPs. The UV-Vis spectra showed maximum absorbance at 650 nm, as reported in earlier work and other maxima were observed at 280 nm, confirming the formation of NPs. Among SeNPs, the most significant material is that which possesses highly efficient antimicrobial action accompanied by low toxicity, which makes these NPs suitable for application in the biomedical field. Benefits of plant used synthesis of NPs, especially the one derived from S. aromaticum include the combination of biocompatibility in addition to stability.

3.4 Antimicrobial Activity

The synthesized SeNPs under the influence of S.aromaticum had efficient antimicrobial efficacy against oral bacteria, which might be beneficial for treating complications of oral microbial infections. The anti-microbial activity against oral bacteria through the zone of inhibition on petri plates for oral infection treatment was shown in Figure 5

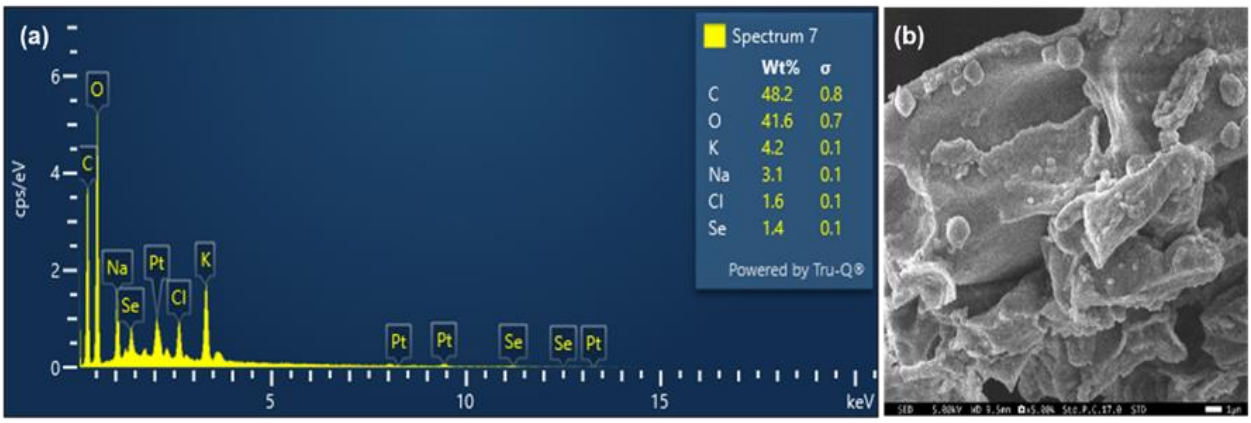

Figure 4. (a) EDAX and (b) SEM spectrum of *Syzygium aromaticum*-SeNPs

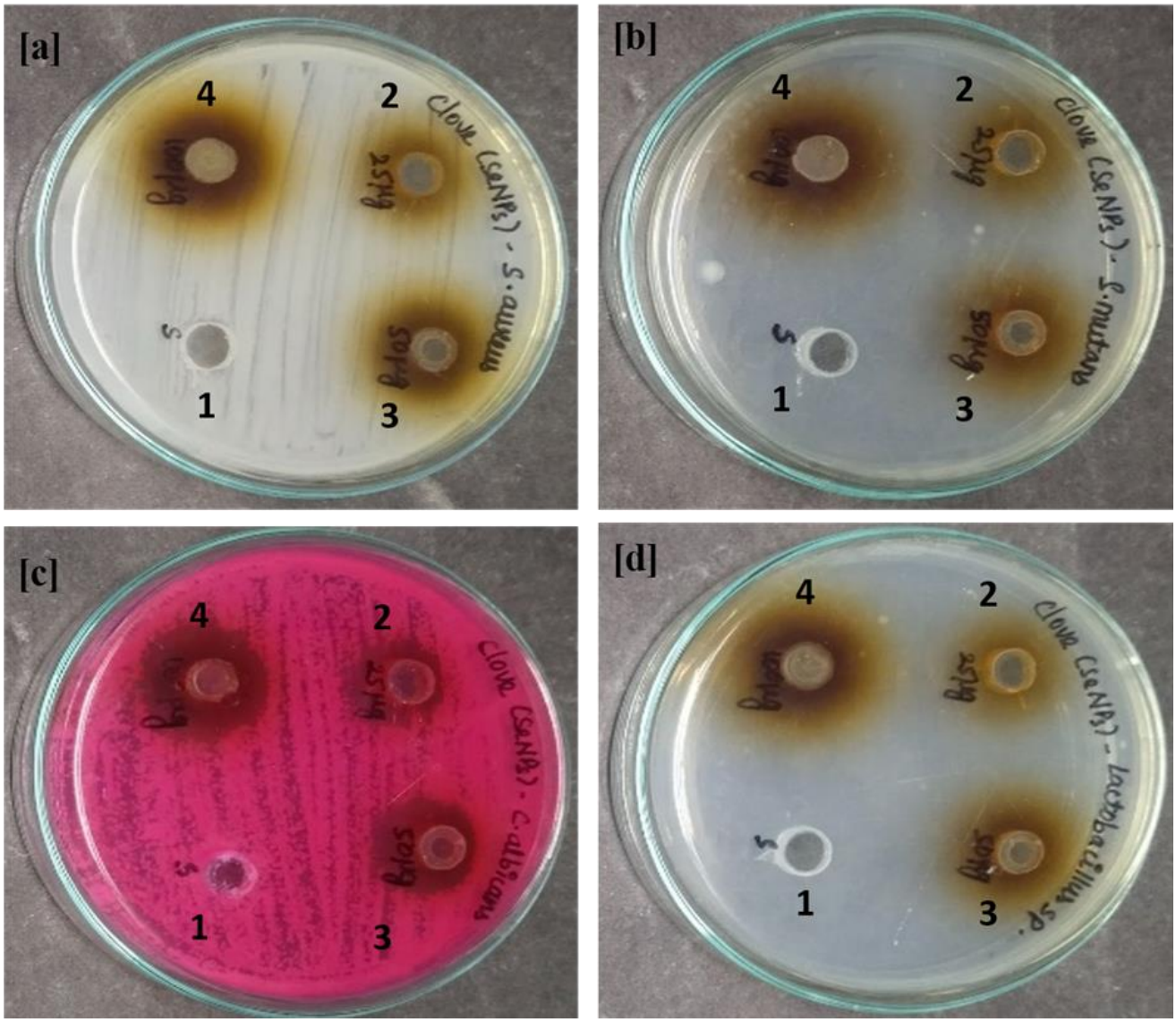

Figure 5. Antimicrobial activity against oral bacteria through the inhibition zone on petri plates for oral infection treatment, (a) Streptococcus mutans, (b) Lactobacillus acidophilus, (c) Candida albicans and (d) Staphylococcus aureus ; (1) Control, (2) 25 µg/mL, (3) 50 µg/mL and (4) 100 µg/mL

Oral germs inhibition activity of SeNPs proved by the zone of inhibition visible in the petri plates [41]. The inhibition zone of the control wells may contain plain S. aromaticum extract and will have smaller or no inhibition zones as compared to the SeNPs treated samples. This enhancement results because the plant bioactive compounds and selenium work in concert by affecting bacterial actions. In Figure 5[a-d], shows the inhibition zone of SeNPs treatment for Streptococcus mutans, Lactobacillus acidophilus, Candida albicans and Staphylococcus aureus pathogens. The present investigation proves that using S. aromaticum to enhance the properties of SeNPs could be beneficial in preventing and treating oral infection-causing agents, including dental caries, gingivitis and periodontitis. Its activity against C. albicans also points toward the potential for fighting oral thrush and other fungal diseases. The result obtained from the inhibition zones in the antimicrobial activity study shows that there is a significant antibacterial and antifungal potential of SeNPs. Due to their potential to generate large inhibition sectors regarding main oral pathogens, they are prospective for the manufacture of antimicrobial dental items such as mouthwash, toothpaste or topical gels. Combination of Se and plant derived compounds will underpin both biocompatibility and high efficacy [41–43].

Figure 6 shows the antimicrobial activity of SeNPs against selected oral pathogens as measured by the zone of inhibition assay. A concentration-dependent increase in inhibition was observed, with C. albicans showing the largest zone of inhibition (20.8 ± 0.4 mm). Lactobacillus sp. exhibited moderate inhibition (14.3 ± 0.5 mm), whereas S. mutans and S. aureus showed relatively smaller zones, ranging from 8.6 ± 0.3 mm to 9.8 ± 0.4 mm at 100 µg/mL concentration. Control wells showed no inhibition. These results demonstrate that SeNPs possess significant antifungal activity against C. albicans, along with measurable antibacterial effects against tested bacterial strains.

C. albicans and Lactobacillus sp. in Figure 7 reveals that SeNPs possess an increased antimicrobial activity with an increased concentration. The control group does not show a decrease in the numbers of the cold and fluorescence CFU during the entire experiment time of five hours. Nevertheless, the treatment which constituted 25 µg/mL exhibited a gentle but constant reduction of influence on the microbial count. At 50 and 100 µg/mL the effect is considerably more profound and reaches the level of a standard treatment within 5 h. The current study reveals that SeNPs possess the potential to retard the growth of Lactobacillus sp. and the enhancement of its concentration provided higher efficiency [44].

This implies that the NPs are able to target bacterial pathogens through disruption of the metabolic and structural forms. Similar trends can also be seen in the S. mutans graph, where higher concentrations of SeNPs exhibit more pronounced antimicrobial activity. The control group that received no treatment also does not have its CF count reduced, meaning that there is normal microbial growth. 25 µg/mL decreases microbial yield mildly and 50 µg/mL and 100 µg/mL treatments significantly reduce microbial yield over time. The 100 µg/mL SeNPs group is equally potent as the standard, underscoring the antimicrobial potential of the SeNPs [45].

The bioactive compounds of clove oil eugenol and flavonoids are important for not only the stabilization of the NPs produced but also responsible for significant antimicrobial activity [46]. Synthesis and characterization of SeNPs using Selenium of S. aromaticum together with assessment of the antimicrobial properties of the NPs on oral pathogens are the major objectives of this study.

The continued appearance of antibiotic resistance makes it important to embrace different approaches to controling oral infections. The findings of this study are expected to reveal the possible use of green synthesized SeNPs as a viable solution in check-pointing oral pathogens. With an understanding of the interaction between selenium and plant-derived bioactive compounds, this research moves to uncover new strategies to help with antibiotic resistance, which has become a significant threat to oral health [43].

3.5 Protein Leakage Analysis Assay

A protein leakage analysis assay is a method used to assess the antimicrobial activity of NPs. It is used to study the interaction of NPs with microorganisms. The later protein leakage assay for S. aromaticum–coordinated SeNPs explain its contribution to the microbial membrane permeability and alters it at different concentrations (25, 50 and 100 µg/mL) than that of standard and control groups. The optical density (OD) at a given wavelength, of the sample is determined and is proportional to the amount of protein released from microbial cells. These results suggest that increased protein leakage shows potential for the alteration of cell membrane structure due to the NPs.

The data obtained from the experiments confirm that microbial protein leakage depends on the nanoparticle concentration as well as the type of microbial strain [47].

Figure 8 shows the protein leakage analysis of SeNPs against oral pathogens. At 100 µg/mL the highest leakage was recorded with Lactobacillus sp. (0.41 ± 0.02) and S. aureus (0.40 ± 0.02) showing greater sensitivity compared to S. mutans (0.38 ± 0.01) and C. albicans (0.37 ± 0.01). The standard antibiotic exhibited comparable OD values (0.42-0.44), whereas the control group remained low (0.29-0.32). These results confirm that SeNPs cause dose-dependent disruption of microbial membranes, with Lactobacillus sp. and S. aureus being more susceptible.

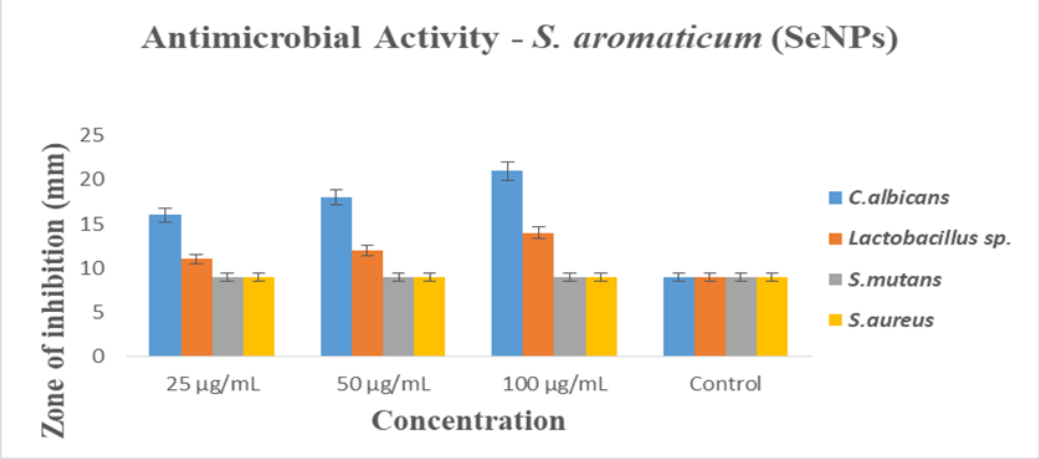

Figure 6. Anti-microbial efficacy against various oral pathogenic microorganisms

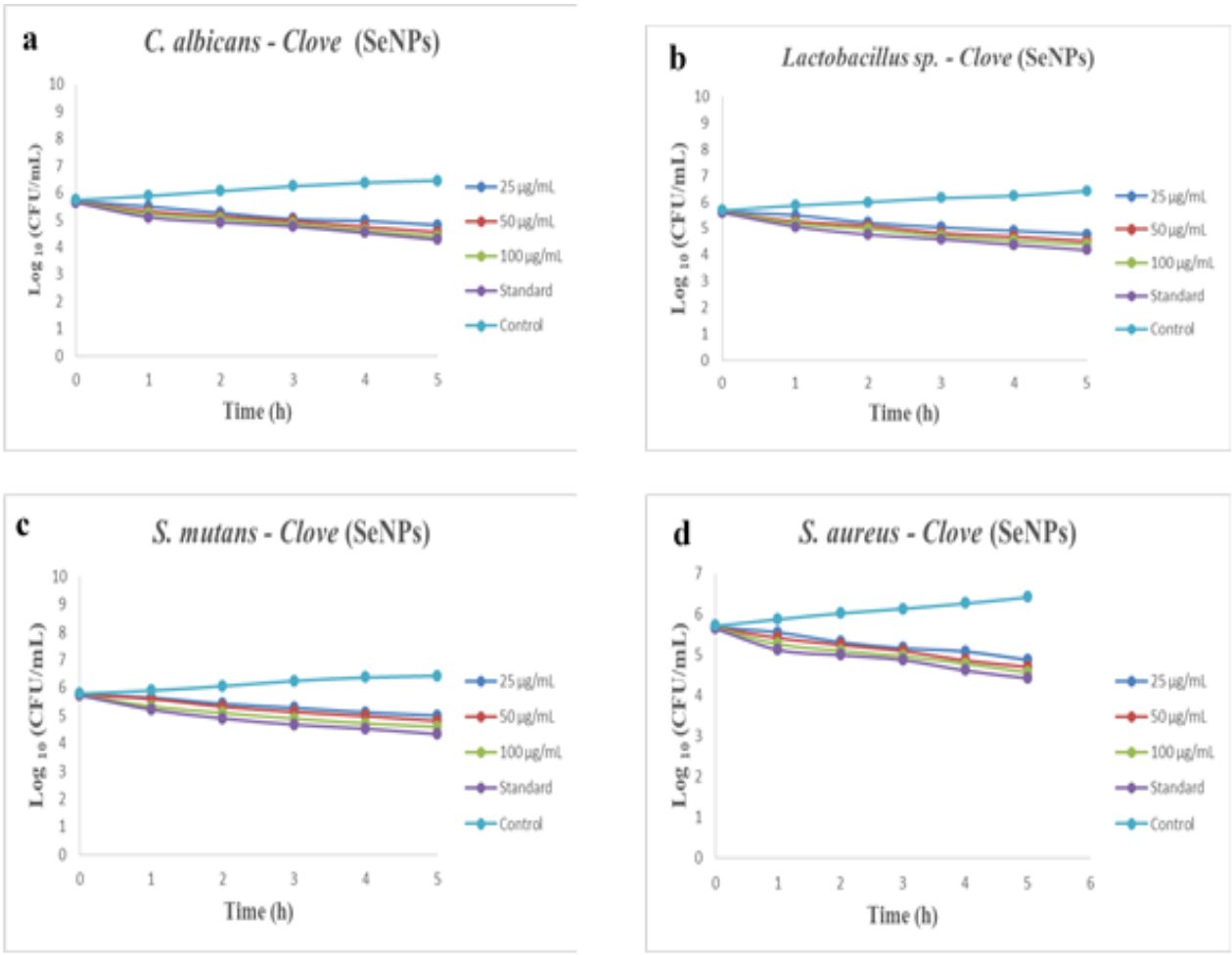

Figure 7. Anti-microbial efficacy against various oral pathogenic microorganisms **(a)** C. albicans, **(b),** Lactobacillus sp., **(c)** S. mutans and **(d)** S. aureus.

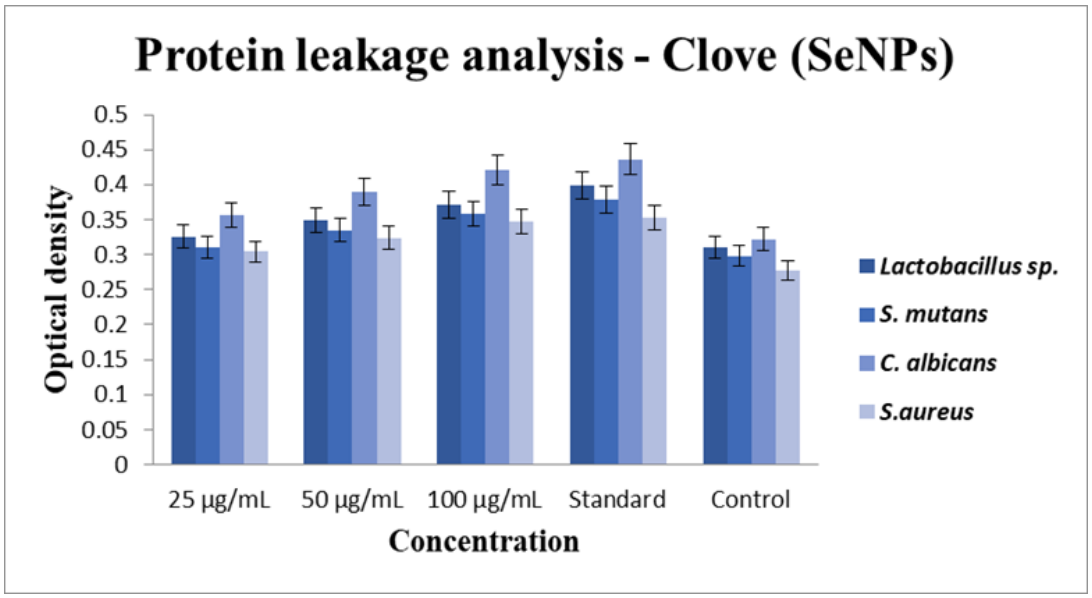

Figure 8. The protein leakage assay of synthesised by *Syzygium aromaticum* to selenium nanoparticles.

3.6 Antibiofilm Assay

The antibiofilm activity of SeNPs was evaluated against E. faecalis, Pseudomonas sp., E. coli and S. aureus at different concentrations (25, 50 and 100 µg/mL) (Figure 9). The results showed a dose-dependent reduction in biofilm mass for all tested microorganisms.

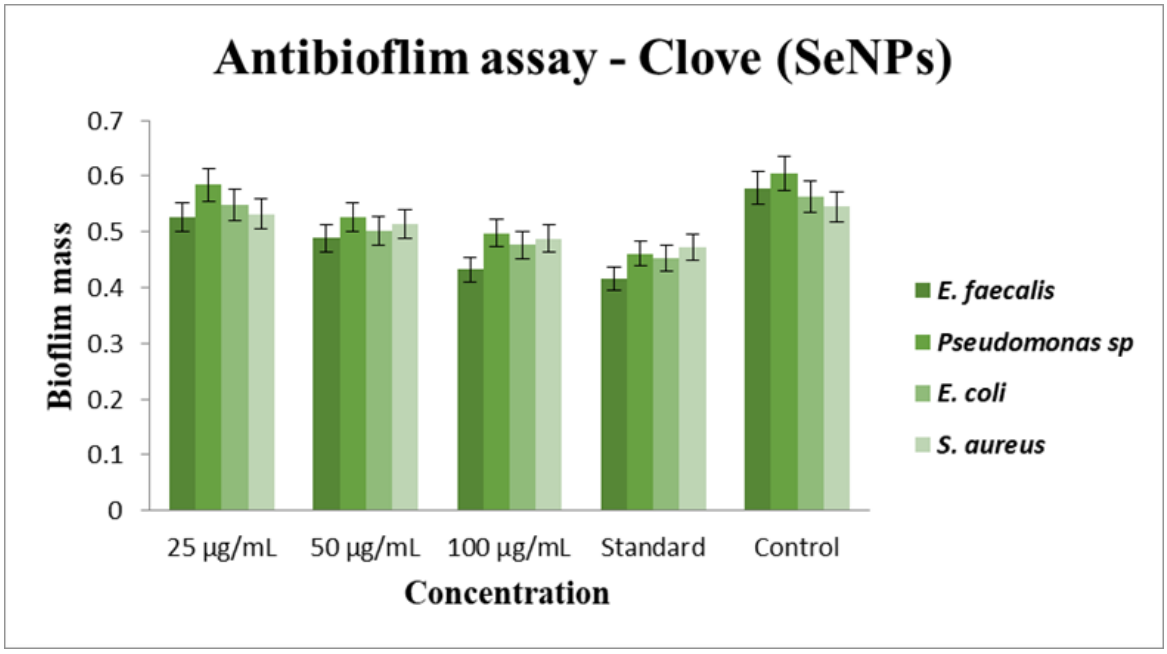

Figure 9. Antibiofilm Assay of Selenium nanoparticles

At 25 µg/mL, a moderate decrease in biofilm formation was observed compared to the control, whereas 50 µg/mL demonstrated a more pronounced inhibition. The highest concentration (100 µg/mL) exhibited the most significant antibiofilm activity, comparable to the standard, indicating effective biofilm disruption. Among the tested strains, E. faecalis showed greater sensitivity to SeNPs, with the largest reduction in biofilm mass, while S. aureus displayed slightly higher resistance. The control group maintained the highest biofilm biomass, confirming that inhibition was due to the SeNPs treatment. These findings highlight the potential of SeNPs to suppress biofilm formation of oral pathogens effectively.

3.7 Toxicology Study

The toxicity studies of S. aromaticum containing SeNPs in zebrafish are outlined to establish its safety for its therapeutic uses. SeNPs have properties such as antimicrobial and antioxidant; nevertheless, biocompatibility and toxicity must be evaluated to get a better understanding for its safe usage in any field, especially biomedical and pharmaceutical applications. Zebrafish make a good model for toxicogenic studies because of genetic similarities to humans, opaque embryos and short generation time through which the toxicity impacts can be observed in real-time. In the toxicology assay determining the toxicity effect of SeNPs, zebrafish embryos were brought in contact with different concentrations of SeNPs. Some of the observations that were taken to check toxicity were the provisions such as the survival rate, hatching rate, morphological abnormalities and heart rate. This work gives information on the flow and safer range of S. aromaticum in assisting SeNPs and investigating their antimicrobial profile and promising uses with recommendations for comprehensive toxicological studies [45].

Affection, lethargy, decrease in activity, dull coloration or sluggishness may be noticed in larvae with toxicosis. This graph shows that zebrafish development under the influence of SeNPs is brought in a detailed manner. However, if the embryos display some degree of teratogenic effects, with curved spines or pigmentation that is irregular, or hatch in a delayed manner, then the SeNPs carry toxic effects at certain concentrations. On the other hand, the toxicity to cells may be undetectable or marginal at lower concentration or when the nanoparticles are well encapsulated, thus supporting the biocompatibility of the green synthesized SeNPs [48].

Figure 10 shows the embryos of zebrafish related to the toxicity test for SeNPs at different concentrations. The graph shows the hatching rate and viability rate at different NPs concentrations. Relative hatchability shows the number of embryos from each concentration of SeNPs that hatched successfully out of the non-damaged ones. At a lower concentration (5 µg/mL) the hatching rate was found comparable to the control group, which suggests a very low level of toxicity in this concentration. Such findings indicate that the NPs, at low doses, make them compatible with embryonic development and have low toxicity effects. The hatching rate starts to reduce at an Intermediate Concentration level of 10 µg/mL to 20 µg/mL which could be regarded as slightly toxic. This might be a sign that nanoparticles disrupt typical developmental procedures, which may be induced by oxidative tension or other cytocellular shifts. Concentration At 40 and 60 µg/mL these concentration exhibits considerably lower hatching rates implying higher toxicity.

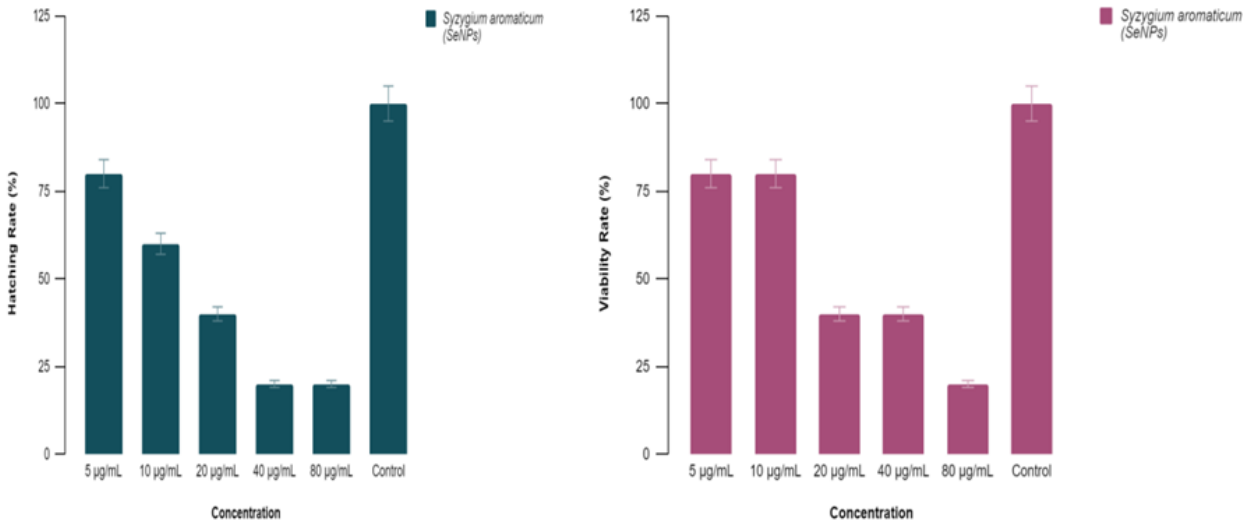

Figure 10. Toxicity studies of Syzygium aromaticum containing selenium nanoparticles on zebrafish embryo at different concentrations

The decrease in hatch success indicates that the SeNPs at high concentrations are advancement in development which may interfere with stress or cell functions. The low toxicity of SeNPs is confirmed with only 5 µg/mL of SeNPs causing insignificant changes in hatching and viability rates. Compared to the control, contents of 10-20 µg/mL result in decreased hatching rates and mild toxic effects. it is possible that oxidative stress or NPs tendency to accumulate begins to influence normal development. Its concentration above 40-60 µg/mL causes considerable toxic effects on the hatching and viability of the embryos. It indicates that lower concentration tagged with AFM could be biocompatible for any biomedical-related applications but for maximum concentration, further modification could be incorporated for future therapeutic or antimicrobial applications.

A conventional approach by Hernández-Díaz *et al.,* synthesizing the NPs using physical, chemical and mechanochemical ways exposes the end products to higher toxicity and poses tremendous threats to the environment [49]. This requires development of green technology, which gives an environmentally friendly solution alongside a sound economic plan for making NPs. In this method, phytochemicals extracted from plants are employed for the stabilization of NPs with no toxic byproducts formed and biocompatibility confirmed.

4. Conclusion

In this study, SeNPs were successfully synthesized using S. aromaticum extract, which served as a natural reducing and stabilizing agent. Characterization confirmed the formation of stable, well-dispersed nanoparticles with favorable size and morphology for biomedical use. SeNPs demonstrated strong antimicrobial activity against oral pathogens, along with low cytotoxicity towards zebrafish. Their dual properties of antimicrobial efficacy and biocompatibility make them especially promising as plant-based nanotherapeutics. However, the present work is limited by its in vitro nature. The interactions of SeNPs with host tissues, microbiota and immune responses under in vivo conditions remain to be clarified. Additionally, the long-term stability, pharmacokinetics and potential cumulative effects of SeNPs in biological systems warrant further investigation. Overall, this work highlights S. aromaticum used for synthesis of SeNPs for the sustainable nanomedicine approaches to address oral infections and antibiotic resistance.

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Acknowledgement

We would like to express our sincere gratitude to the Department of Biochemistry, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, India, for providing support for this research work.

Authors Contribution Statement

Archana Behera: Conceptualization, Data curation, Formal analysis; Methodology, Writing - original draft, and Writing - review & editing. Karthik Krishnasamy Ravichandran: Writing– original draft, Writing– review & editing. Shanmugam Gurusamy: Writing– original draft, Writing– review & editing. Harshini Udumalaipettai Subramanian: Writing– original draft, Writing– review & editing. Iadalin Ryntathiang: Conceptualization, Data curation, Formal analysis, Methodology, Writing - original draft, and Writing - review & editing. Namrutha Dhonthi Shekar: Writing - original draft, and Writing - review & editing. Mukesh Kumar Dharmalingam Jothinathan: Investigation, Visualization. All the authors read and approved the final version of this manuscript.

Competing Interests

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Funding

The authors declare that no funds, grants or any other support were received during the preparation of this manuscript.

Data Availability

The data supporting the findings of this study can be obtained from the corresponding author upon reasonable request.

Has this article screened for similarity?

Yes