



Green synthesis of Chitosan nanoparticles (CHNPs) using *Azardiracta indica* leaf extract: potential to antibacterial and antibiofilm properties: *in-vitro* analysis

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Abstract

The present study investigated the Chitosan nanoparticles (CHNPs) synthesized from *Azardiracta indica* leaf against *Enterococcus faecalis* and *Streptococcus mutans* for antibacterial and antibiofilm properties. *O. sanctum* leaf extracts synthesized chitosan nanoparticles sustainably. Synthesized chitosan nanoparticles were characterized by UV-visible, transmission electron microscopy, FTIR, and X-ray diffraction. Then, chitosan nanoparticles (CHNPs) were tested for antibacterial and antibiofilm activity against dental caries-causing organisms such as *S. mutans* and *E. faecalis* (Deepika et al. 2022). The characterization of the nanoparticles (NPs) indicated that the biosynthesized NPs were spherical in shape. FTIR spectroscopy analysis detected the presence of phenolic and aromatic compounds in the NPs. These biosynthesized NPs demonstrated strong antibacterial activity, leading to a significant reduction in bacterial populations when tested against various pathogens (Janani et al. 2020). Confocal laser scanning microscopy (CLSM) demonstrates 100µg/mL CHNPs NPs minimize colonization and biofilm development. These studies demonstrated the potential of green nanoparticles for root canal disinfection. Thus, this finding could help the green way for developing medicines to treat oral disease-causing microbes. Using *O. sanctum* extract to reduce metallic chitosan ions in aqueous solution can create clean, safe, and sustainable chitosan nanoparticles for biological use.

Keywords: Sustainable synthesis; CHNPs; TEM; Endodontic pathogens; Antibiofilm; CLSM



1.Introduction

Dental caries, endodontic infections, and periodontitis are major oral health disorders (Kurita et al., 2023) (Deschner et al., 2023). The pathogenic bacteria *S. mutans* causes dental caries by producing extracellular glucan-homopolymers from sucrose, generating large organic acids via carbohydrate metabolism, and thriving in low pH settings. Homopolymers are crucial to biofilm adherence, colonization, and deposition on tooth surfaces (Treccani, 2023). Gram-positive *S. mutans* colonizes the supragingival plaque in humans and helps build biofilms (Dubern et al., 2023) Dental caries is currently characterized as a polymicrobial dysbiosis caused by frequent carbohydrate ingestion (Habib et al., 2023) Since quick sugar metabolism acidifies the environment, sugar consumption increases tooth cavities. Biofilms' low pH promotes acidogenic and aciduric microorganisms like *Streptococcus mutans*, which thrives in acid (Park et al., 2023) When dental plaque pH decreases below 5.5, enamel remineralization and demineralization are imbalanced, causing mineral loss and cavities (Nasiri et al., 2023)

The production of metal nanoparticles using green technologies has gained prominence due to its ease of production, non-toxicity, and sustainability (Nasim et al. 2022). Nanotechnology produces 1–100 nm-sized materials with different forms, sizes, and chemical constituents. Nanoparticles are used in medicine, optoelectronics, and catalysis. Due to their size-dependent characteristics noble metal nanoparticles like chitosan, silver and gold are significant in biology, medicine, and electronics (Guglielmelli et al., 2023) Nanoparticles have an excellent surface-to-volume ratio that increases with size. Nanostructures' large surface area boosts beneficial and associated characteristics. Due to surface energy, nanoparticle ecological efficacy improves with surface area (Altammar, 2023). Metal nanoparticles have been established using physical and chemical processes, including lithography, laser ablation, and photochemical reactions reduction (Kaushal et al., 2023) (Agrawal et al., 2024) These procedures are expensive and need toxic substances. (Yadav et al., 2023) This pollution of nanoparticle surfaces has negative effects on their medicinal uses.

Biomolecules offer an environmentally friendly and dependable approach to producing metal nanoparticles. Numerous bacteria, fungi, and yeasts have been recognized for their ability to



synthesize noble metal nanoparticles (Singhal et al., 2023) (Ahuja et al., 2024) However, the practicality of microbial-mediated nanoparticle production for commercial purposes is limited by the strict hygiene requirements and the high cost of maintaining suitable growth media. (Santomartino et al., 2023) (Passos et al., 2023) In contrast, plant-based components have been utilized for several decades in nanoparticle manufacturing. Plant extracts contain biomolecules capable of converting metal ions into nanoparticles and providing stabilization (Saleh and Fadillah, 2023) Recently, a variety of ethno-botanically and economically valuable plant species have been employed to produce nanomaterials.

Many researchers have investigated about the green synthesis of CHNPs nanoparticles from different plant parts such as *Piper longum*, (Harini et al., 2022) *Bambusa arundinacea*, (Jayarambabu et al., 2021) *Solanum lycopersicum*, (Raliya et al., 2015) and *Deverra tortuosa*. (Selim et al., 2020) This phyto mediated approach utilizing various plant components is considered an innovative, cost-effective, and modes method for bio-synthesizing CHNPs nanoparticles. This plant can be found in tropical areas like India and has great therapeutic properties (Ambedkar et al., 2023) Researchers showed that plant material strongly impacts nanoparticle dimensions and surface appearance in green synthesis. In the present investigation, *Azardiracta indica* (Neem) leaf extract from plants was employed to reduce or cap CHNPs nanoparticles greenly. This study additionally examined biosynthesized *O. sanctum* CHNPs antibacterial and antibiofilm characteristics needed for inhibiting dental pathogen growth.

2. Materials and methods

2.1 Collection of plant materials

The leaves of *Azardiracta indica* L. were freshly obtained and cleaned with running tap water and double purified water to eliminate dirt and other impurities. The plants were properly dehydrated in the shade at room temperature. After drying, 25 g of these leaves were powdered. This powder was boiled for 30 min in 250 mL of clean water. To separate extracts from solid particles, the mixture was sieved using Whatman filter paper No. 1 after warming to room temperature. The filtered extract was refrigerated at 4 °C for ultimately use as a reducing and stabilizing agent.

2.2 Green synthesis of CHNPs



Biogenic CHNPs production followed Iqbal et al. (Iqbal et al., 2021) modified alterations. The leaf extract (25 mL) was magnetically stirred at 60–80 °C. After the extract reached 60 °C, 2.5 g of Chitosan nitrate hexahydrate $\text{CH}(\text{NO}_3)_{2.6}\text{H}_2\text{O}$ was placed in and allowed for 1h until a white precipitate formed. This mixture was kept overnight at 60 °C in a hot air oven to make a paste that is creamy. The paste was extracted and cleaned many times with a 3:1 solution of distilled water and ethanol. The resulting paste was subsequently placed in a ceramic crucible cup and heated at 400 °C for 2 h. Analysing the white powder in a tightly closed container.

2.3 Morphological analysis of CHNPs

The CHNPs nanoparticles were subjected to analysis using a Rigol ultra-3660 UV-visible spectroscopy instrument over a wavelength range of 200 to 800 nm. Ten different FTIR spectra were utilized to identify functional groups and phytochemical substances responsible for reducing and stabilizing the produced nanoparticles. An ATR mode-equipped Jasco FTIR 4100 spectrophotometer from Japan was employed for FTIR analysis, and the resulting data was noted in the range of 4000 to 400 cm^{-1} . To check the content of CHNPs and evaluate the crystallite shape and size, powdered material was examined using a $\text{CuK}\alpha$ -X Ray diffractometer operating at 40 kV, 30 mA, and 2θ ranging from 20° to 80°. For further analysis, the CHNPs nano powder was immersed in ethanol, subjected to sonication, placed onto a copper grid, dried, and subsequently analysed using a JEOL-2100 HR-TEM instrument (Cao et al., 2021)

2.4 Antibacterial properties

Biosynthesized CHNPs were evaluated for their antibacterial properties against *Streptococcus mutans* and *Enterococcus faecalis* using the well diffusion assay on Mueller-Hinton agar (MHA) plates. The MHA agar medium was sterilized by autoclaving at 121°C for either 15 pounds or 20 min. Afterward autoclaving, 20 mL of the medium were aseptically shifted to sterile petri-dish and permitted to solidify in a laminar hood. Inoculation involved adding 200 μL of overnight-cultured



bacterial cells onto MHA plates and spreading them evenly using a sterile glass spreader. Bores with a width of 6 mm were carefully placed on the MHA plates containing the bacterial cells. chitosan nanoparticle stock solution (1 mg/mL) was prepared, and dosages of 50 and 100 µg/mL were loaded into distinct wells, with streptomycin serving as a positive control. The sample petri dish was then shifted to a bacteriological incubator and kept at 37°C for a period of 24 h. After the incubation period, antibacterial properties were assessed by measuring the zone of inhibition of growth surrounding each well. The zone of inhibition was quantified in millimeters (mm).

2.5 Antibiofilm activity

The assessment of the antibiofilm potential of biosynthesized CHNPs was conducted following the protocol described in reference (Adebayo-Tayo et al., 2019) with slight modifications. Initially, a suspension was prepared and mixed with 200 µL of Brain Heart Infusion (BHI) in a 96-well microtiter plate, followed by kept at 37 °C for 48 h. CHNPs and antibiotic (positive control- cefotaxime) have been added to the solution at different amounts ranging from 25 to 100 µg/mL. The wells were then kept for 24 h at 37 °C in bacteriological incubator. After incubation, the entire mixture was carefully removed, and the microtiter plate was subsequently washed three times with sterile water to eliminate non-adherent bacteria. The adherent cells were then fixed using 95% ethanol for 5 mins and rinsed with sterilized water. Next, 200 µL of crystal violet was added to the static cells and left for 15 mins, after which they were cleaned again with sterile water and allowed to air-dry. To evaluate the inhibitory effect on biofilm formation with help of microtiter wells. The quantification of biofilm inhibition was performed utilizing an ELISA reader (BIOTEX model: ELx800, Biotex Instruments, USA) at a wavelength of 560 nm (OD600). The % of biofilm inhibition was then determined as follows:

$$\% \text{ biofilm inhibition} = \text{OD control} - \text{OD treatment} / \text{OD control} \times 100$$

2.6 Confocal laser scanning microscopy (CLSM) analysis

A biofilm was produced by growing *S. mutans* and *E. faecalis* (1×10^8 CFU/mL) on a 24-well microtiter plate with 100µg/mL concentrations of biosynthesized CHNPs NPs at 37°C for 24 h. After three sterile phosphate buffer washes, the biofilm was cleaned of unattached cells. In the experiment, both controlled and treated wells were exposed to predetermined concentrations of



biosynthesized CHNPs. Following incubation in a microtiter plate, treated with selected concentration of synthesized CHNPs were stained with a 5 mmol/L solution of Acridine orange (AO) dye (Thermo Fisher Scientific, Waltham, MA, USA) for a duration of 10 minutes. AO binds to nucleic acids in both living and dead cells as well as extracellular DNA in biofilms. For biofilm cells with compromised cell membrane integrity, 200 μ L of propidium iodide dye (0.02 mg/mL, Sigma-Aldrich, USA) was introduced and incubated for 10 min in the absence of light to visualize nucleic acids. Using a confocal laser scanning microscope (CLSM, Leica DMI8, Germany), the dyed biofilms were captured and their thickness assessed. Five random regions were viewed at 40 \times resolution for each sample. (Kodeš et al., 2021)

2.7 Statistical analysis

All tests performed triplicate. The data was analysed using ANOVA and unpaired Student's t-test. The statistically significant level was calculated by *P*-values < 0.05.

3. Results

Synthesis of CHNPs was confirmed *via* a dark brown to light brown creamy reaction colour (Fig. 1). The experiment used a 1:9 (v/v) ratio of *O. sanctum* leaf aqueous extract to Chitosan solution, pH-12, and a 2h reaction at room temperature. CHNPs were formed by monitoring UV–Visible wavelengths from 200 to 700 nm. The UV-visible wavelength band of bio-produced NPs exhibited a CHNPs peak at 350 nm (Fig.2).

The reducing potential and stabilizing ability of biomolecules in *O. sanctum* extract was estimated using FTIR (Fig.3 & Table 1). FTIR analysis of *O. sanctum* synthesized CHNPs revealed hydrogen-bonded O–H stretching (alcohol groups) at 3423 cm^{-1} . The N–H bend bending of primary amine in protein molecules is 1644 cm^{-1} . The 1539 cm^{-1} pattern indicates C–N dimensions, indicating aliphatic amines. The band at 1395 cm^{-1} represent =C–H bend alkenes groups. The peaks at 1025 cm^{-1} of aliphatic amines from polyamines indicate that molecules that interact with CHNPs contain free and bound amine groups. The peaks at 786 cm^{-1} agree to the C–Cl stretching vibration of alkyl halides, representing the presence of alkyl



alcohols. The bands that appear at 613 cm^{-1} result from alkynes (C–H) bending. The detected bands indicate the biomolecules responsible for reducing and capping chitosan ions in aqueous extracts. Tulsi leaf extracts include plant-based constituents such as eugenol, flavonoids, and phenols, encapsulated on manufactured CHNPs. Proteins can attach to chitosan salts via free amine groups or cysteine residues, stabilizing produced chitosan nanoparticles (Singh and Chaudhuri, 2018).

The XRD pattern of produced CHNPs from *O. sanctum* leaf extracts is demonstrated in Fig.4. The XRD evaluations revealed chitosan colloids in the reaction mixture. The results demonstrated clearly prominent and narrow diffraction peaks, indicating that the produced CHNPs were crystalline in nature. Bragg's reflection of 2θ values for CHNPs synthesized from *O. sanctum* leaf extract exhibited peaks at 31.86° , 36.37° , 47.62° , 56.70° , 62.95° and 68.04° , respectively. Additionally, the lack of impurity peaks in the diffraction pattern indicates efficient CH precursor transformation into CHNPs. The phenols and flavonoids in *O. sanctum* leaf extract reduce and protect chitosan acetate's outer surface. CHNPs developed because of them. Narrow and strong diffraction peaks indicate a clearly stated crystalline structure in the product's nanoparticles. The CHNPs made are very crystalline, indicated by their valuable peak intensity. HR-TEM pictures showed that these *O. sanctum* leaf synthesized CHNPs were widely distributed, 5–35 nm in diameter, and nearly spherical with some truncated NPs (Fig.5 A & B).

Nanomaterials were investigated to determine their bactericidal properties due to an increasing number of antibiotic-resistant bacterial strains. This has prompted modern studies into chitosan and bioactive substances coated chitosan nanocomplex. *O. sanctum* leaf biosynthesized CHNPs at two different concentrations were evaluated against two dental bacteria for antibacterial properties in agar well diffusion assay. Fig.6 shows the nanoparticle sensitive to all tested pathogens. The zone of inhibition ranges from $6 \pm 0.51\text{ mm}$ to $16 \pm 1.5\text{ mm}$, with ciprofloxacin exhibiting the highest zones. The nanoparticles act was dose-dependent, with zone dimensions expanding with concentration (Fig.7). The antibacterial outcomes demonstrated that the minimal zone of inhibition observed with the treatment of CHNPs at $50\text{ }\mu\text{g/mL}$ dose for *E. faecalis* was $6.7 \pm 1.2\text{ mm}$, while the highest zone of growth inhibition observed was 7.0 ± 1.2 and $11.7 \pm 1.5\text{ mm}$ for *S. mutans*, respectively. Results indicate that botanical extracts may have antibacterial properties due to bioactive components. Antibacterial investigation using chitosan nanoparticles



wrapped with bioactive substances from several plant species demonstrates excellent inhibition of novel bacteria strains, especially multidrug resistance. The size and quantity of nanomaterials influence their antibacterial action, which is stronger against Gram (-ve) bacteria than Gram (+ve) pathogens(Lakshmi 2021).

The examined *O. sanctum* leaf produced CHNPs were stained against bacterial biofilm to determine their antibiofilm efficacy. The biofilm layer frequently contains gram (+ve) and gram (-ve) microorganisms. The synthesized CHNPs were evaluated against *S. mutans* and *E. faecalis*. A dose-dependent impact was observed, with the maximum percentage of anti-biofilm activity at 100 µg/mL being 64.5 and 61.8%, respectively, for CHNPs treatments (Fig. 8). This finding may provide an alternate anti-fouling chemical source. To inhibit biofilm development, CHNPs were tested *in vitro*.

Biofilms, with dense cell communities, are critical for pathogenicity in invasive bacterial infections. To assess the impact of CHNPs nanoparticles (NPs) on biofilms formed by *S. mutans* and *E. faecalis* on glass cover slips, a dual-labelling approach involving propidium iodide (PI) and acridine orange (AO) was employed in combination with confocal laser scanning microscopy (CLSM) (Fig.9). CHNPs dose-dependently inhibit glass cover slip biofilm formation. In biofilms that were not treated with CHNPs, bacterial cells are held together by an extracellular matrix Fig.9A. In biofilms formed using CHNPs, no extracellular matrix was found, and some cells had abnormal structures and were killed. Biofilm production was decreased by > 90% with 100 µg/mL CHNPs. Conversely, in biofilms exposed to CHNPs, the extracellular matrix was absent, and some of the cells displayed abnormal structures and were effectively killed (see Fig. 9A). When a concentration of 100 µg/mL of CHNPs was applied, biofilm production was inhibited by more than 90% (Fig.9B). These findings provide strong evidence of the anti-biofilm efficacy of CHNPs nanoparticles(Lakshmi and Dean -International Affairs, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences 2021).

4. Discussion

Nanotechnology's many applications have transformed medical, genetic, and industrial research. Nanomaterials have different shapes, sizes, surface patterns, and physico-chemical, electrical, and photosensitive characteristics (Verma et al., 2021) Electrochemical, hydrothermal, laser-based



lithography, microwave, and thermal breakdown technologies are used to synthesize nanomaterials. Microorganism and plant-based nanomaterials are important in material science and biology (Oza et al., 2020) Nanomaterials are used in sensors, catalysis, anticancer medications, antioxidants, larvicides, antimicrobials, nanofluids, agriculture, and drug delivery systems. chitosan nanoparticles (CHNPs) interact non-toxically and bio-compatible with plant and animal cells, making them notable (Hano and Abbasi, 2021) CHNPs are used in gene and drug delivery, labelling, nanomedicine, and antibacterial, antifungal, anticancer, antioxidant, anti-angiogenic, and anti-inflammatory drugs (Faisal et al., 2021)^{gr} Present results, during a 2h incubation stage, the reduction of pure Ch^{+} ions to Ch^{0} were tracked by a pictorial colour altered (from light brown to dark brown) and prominent UV-visible absorbance peaks for *O. sanctum* (350nm). The alteration in color in the response of the solution is prompted by surface plasmon vibrations. According to (Faisal et al., 2021) the conversion of Ch^{+} to Ch nanoparticles may be attributed to phytochemical substances, such as phenolic groups, in plant extracts. Flavonoids, terpenoids, and polysaccharides in plant extracts may reduce Ch^{+} (Król et al., 2017)

FTIR analysis identified promising biomolecules for chitosan ion bio reduction and capping in CHNPs produced from *O. sanctum* leaf. *Ocimum* genera contain flavonoids, anthocyanins, and essential oils such 1,8-cineole, estragole, and eugenol. The functional components found in those polymers and protein-based materials may reduce Ch^{+} to Ch^{*} . All biological components interact with metal salts via functional groups to reduce them to NPs.(Beltrán-Noboa et al., 2023) XRD spectra of biosynthesized chitosan NPs reveal peaks at 31.86° , 36.37° , 47.62° , 56.70° , 62.95° , and 68.04° , corresponding to Miller indices (1 00), (11 0), (111), (2 0 0), (2 0 0), and (311). Bragg peak growing confirms metal nanocrystal production. The significant spectrum signals were probable related to X-ray emission from plant extract functional biomolecules (Donga and Chanda, 2022)(Abdelbaky et al., 2022). The TEM images reveal that synthesis of NPs were found to be shapes such as spherical with smooth edges. Abel et al. (Abel et al., 2021) developed spherical CHNPs at 2.5–4 keV using *Coffea arabica* leaf extracts. Abomuti et al.(Abomuti et al., 2021) produced triangular and spherical CHNPs from *Salvia officinalis* leaf extracts at 2–3.5 keV.

One of the biggest worldwide health concerns is dental plaque, or caries. It happens when oral bacteria develop biofilms.(Okamoto et al., 2023)(Choudhari et al. 2023) The oral cavity contains about 300 bacterial species, however only a small number cause tooth decay or periodontal disease



(Baker et al., 2023) Traditional medicines are not effective to treat multidrug-resistant bacteria-caused dental plaque infections. Due to this increasing worry, dental experts are studying safe and novel techniques to mitigate and eliminate oral infections. (Baker et al., 2023) Nanotechnology plays an important role to science and technology due of its superior features (Kumar et al., 2023) These qualities render nanoparticles important in biological, chemical, environmental, and medical fields. Due to environmental concerns, plant-mediated CHNPs production is gaining fashionable. Various plant components serve as reducing capping and stabilizing agents (Ureña-Castillo et al., 2022). To prevent NP aggregation, carboxylate, carbonyl, and amine functional groups can attach their surfaces. The antibacterial properties of *O. sanctum* biosynthesized CHNPs showed strong antibacterial properties against *S. mutans* and *E. faecalis*. Similarly, (Álvarez-Chimal et al., 2022) reported the green synthesized CHNPs nanoparticles using *Dysphania ambrosioides* showed the better antibacterial activity against dental pathogens like *S. mutans*, *S. sanguinis*, *Porphyromonas gingivalis*, and *Prevotella intermedia*. Antibacterial properties of CHNPs depends on Gram (+ve) or (-ve) bacteria wall structure (Abdelbaky et al., 2022). Nanoparticles' antimicrobial activity has three basic justifications, but their mechanism is unknown. (Sondi and Salopek-Sondi, 2004) found that AgNPs directly disrupt bacterial cell membranes and complex with cell components. (Banerjee et al., 2010) believed their antibacterial activity comes from thiol (–SH) group interaction and ROS generation. Finally, (Mendes et al., 2022) proposed that chitosan ions block respiratory enzymes that produce ROS, enabling CHNPs antimicrobial (Kamath et al. 2022). chitosan oxide (CHNPs), a class II-VI transition metal oxide and semiconductor with a broad band gap (3.3 eV), has a prominent antibacterial activity. CHNPs forms electron-hole pairs when exposed to radiation over its band gap. (Siddiqi et al., 2018) Electrons enter the conduction band, while the hole in the valence band (VB) becomes strongly oxidative, forming oxidizing sites that can oxidize water molecules or hydroxide anions and generate powerful reacting species. This redox chain reaction produces ROS, such as ($\cdot\text{OH}$), ($\cdot\text{HO}^2-$), and ($\text{O}^{2\cdot-}$), which have bactericidal properties. Chitosan nanoparticles (CHNPs) cause oxidative stress in bacteria, affecting protein synthesis and DNA replication. Oxidative stress inhibits bacterial development and destroys pathogens (Mendes et al., 2022) (Abebe et al., 2020)



Growing biofilm-forming bacterium resistance threatens healthcare, agriculture, and environmental preservation, which is concerning (AboElmaaty et al., 2022). Biofilms, complex bacterial colonies in a protective matrix, are more antibiotic and disinfectant-resistant than planktonic microorganisms. This resistance can cause clinical infections, food spoilage, and industrial processes and infrastructural issues. Antibiotic-resistant biofilm-forming microorganisms must be addressed to protect human and environmental health (Kang et al., 2022). Plant-derived nanoparticle-based biofilm eradication is promising research the discipline for biofilm-related illnesses. They are biocompatible and low-toxic, making them excellent biofilm removers (Shehabeldine et al., 2023). Crystal violet was used to measure biofilm suppression. This work enables the development of nano-based medicines with antibacterial and anti-biofilm capacities. Present CLSM results describe synthesized CHNPs NPs inhibited *S. mutans* and *E. faecalis* biofilms at 100µg/mL. Live/Dead stain in CLSM provided qualitative biofilm inhibition evaluation. The untreated biofilm had many well-integrated living adherent cells. Our results match with Kamli et al. (Kamli et al., 2021), who found that *P. aeruginosa* biofilm disintegration occurred when live cells were absent. The study conducted by (Ong et al., 2017) suggested that conventional antimicrobial agents struggle to reach and control biofilm structures. They proposed the use of chitosan-propolis nanoparticles as a potential solution for managing biofilms formed by *E. faecalis*. In our research, we have uncovered that CHNPs nanoparticles effectively combat biofilms produced by *S. mutans*. Additionally, our findings indicate that *E. faecalis* biofilm matrix structures are also disrupted by the penetration of CHNPs nanoparticles. Our data support these previous findings, as we have observed that a concentration of 100µg/mL is effective in eliminating *E. faecalis* when compared to untreated bacteria. (Sans-Serramitjana et al., 2017) demonstrated the effectiveness of utilizing nanostructured lipid carriers combined with colistin to combat biofilms formed by the gram-negative bacterium *Pseudomonas aeruginosa*. Their study employed CLSM analysis and revealed a substantial decrease in the viability of the biofilm following this treatment approach.

5. Conclusion

To treat multidrug-resistant bacteria in the mouth, researchers have investigated alternate treatment. They use nanotechnology for medication delivery, medicinal advances, and detection. In this context, antibacterial CHNPs were employed as a solution for dental infections. The



ecologically friendly CHNPs made from *O. sanctum* leaf extract were effective against dental infections. These biosynthesized CHNPs reduced dental pathogen biofilms, promising a tooth infection treatment. This is the first study to test CHNPs antibacterial efficiency with *O. sanctum* leaf extract for dental pulp infections. Further study into biosynthesized CHNPs therapeutic uses may help eliminate oral *S. mutans* infections.

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Author contributions

Chinnasamy Ragavendran: Project administration and supervision, conception, design, methodology, validation, data analysis and interpretation, paper drafting and critical revision.: Design, data curation, acquisition, manuscript drafting and critical modification, **Manavalan Arulmani & :** Prepared and wrote the paper. All authors carefully evaluated and agreed it.

Data Availability

The datasets used and analyzed in this study are available from the corresponding author on reasonable request

Declarations

Not Applicable

Ethical Approval

Not Applicable

Competing Interests

The authors declare no competing



Consent to Publish

Not Applicable

Consent to Participate

Not applicable.

Conflict of Interests

The authors report no conflicts of interest.

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