https://doi.org/10.33472/AFJBS.6.9.2024.3139-3165

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Eco-friendly green synthesis of zinc oxide nanoparticles (ZnO NPs) using *Ocimum sanctum* leaf extract: potential to antibacterial and antibiofilm properties: *in-vitro* analysis

Dr. Atluri Manoj[1], Dr. Manish Ranjan[2], Dr. Chinnasamy Ragavendran[3], Dr. KrishnaKanth Jaju[4] , Dr. Manavalan Arulmani[5]

Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India Email id- 152206004.sdc@saveetha.com

Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India Email Id: manish@saveetha.com

Assistant Professor, Department of Cariology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600 077, India ragavan889@gmail.com

department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India krishnajaju8055@gmail.com

Department of Cariology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600 077, India arulmanim@gmail.com

***Corresponding author: Dr. Chinnasamy Ragavendran**

E. mail: ragavan889@gmail.com.

Volume 6, Issue 9, May 2024

Received: 19 March 2024

Accepted: 12April 2024

Published: 22 May 2024

doi:10.33472/AFJBS.6.9.2024.3139-3165

Abstract

The present study investigated the Zinc oxide nanoparticles (ZnO NPs) synthesized from *Ocimum sanctum* leaf against *Enterococcus faecalis* and *Streptococcus mutans* for antibacterial and antibiofilm properties. *O. sanctum* leaf extracts synthesized zinc nanoparticles sustainably. Synthesized zinc nanoparticles were characterized by UV-visible, transmission electron microscopy, FTIR, and X-ray diffraction. Then, zinc oxide nanoparticles (ZnO NPs) were tested for antibacterial and antibiofilm activity against dental caries-causing organisms such *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 ZnO 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 zinc ions in aqueous solution can create clean, safe, and sustainable zinc nanoparticles for biological use.

Keywords: Sustainable synthesis; ZnO NPs; 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 zinc, 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 ZnO 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 ZnO 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, *Ocimum sanctum* (Tulsi) leaf extract from plants was employed to reduce or cap ZnO nanoparticles greenly. This study additionally examined biosynthesized *O. sanctum* ZnO NPs antibacterial and antibiofilm characteristics needed for inhibiting dental pathogen growth.

2. Materials and methods

2.1 Collection of plant materials

The leaves of *Ocimum sanctum* 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 ºC for ultimately use as a reducing and stabilizing agent.

2.2 Green synthesis of ZnO NPs

Biogenic ZnO NP 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 zinc nitrate hexahydrate ($Zn(NO3)_{2.6}H_2O$) 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 ZnO NPs

The ZnO 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 ZnO and evaluate the crystallite shape and size, powdered material was examined using a CuKα1-X Ray diffractometer operating at 40 kV, 30 mA, and 2θ ranging from 20° to 80°. For further analysis, the ZnO 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 ZnO NPs 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. Zinc 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 ZnO NPs 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. ZnO NPs 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 ºC 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 ZnO 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 ZnO NPs. Following incubation in a microtiter plate, treated with selected concentration of synthesized ZnO NPs 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 µL 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× 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.

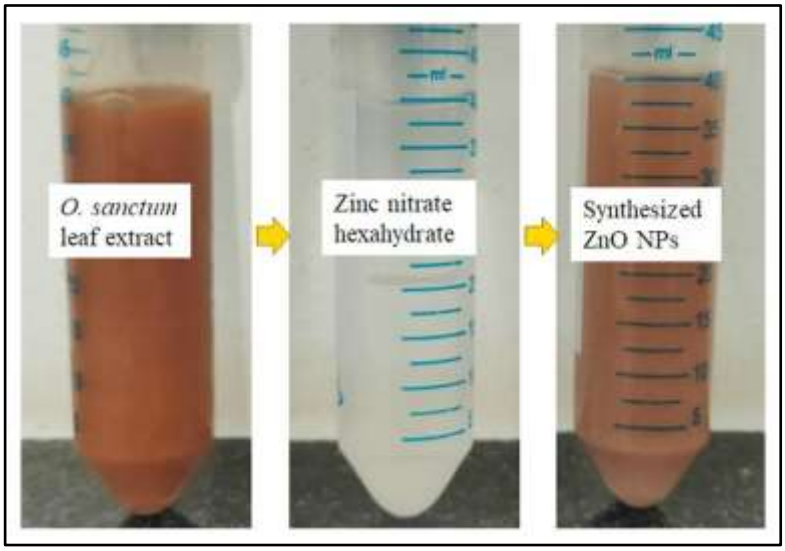

Figure 1 The synthesis of ZnO NPs has been determined using *O. sanctum* leaf extract, zinc nitrate solution, and ZnO NPs.

The figure 1 shows the synthesis of ZnO NPs has been determined using *O. sanctum* leaf extract, zinc nitrate solution, and ZnO NPs.

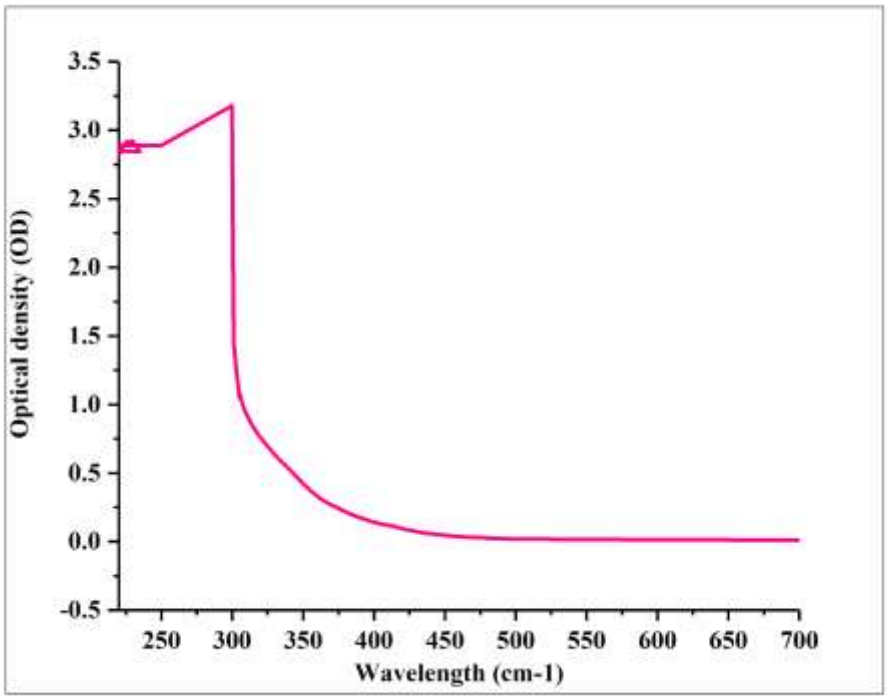

Figure 2 UV-visible spectrum of synthesized ZnO NPs

The Figure 2 shows the UV-visible spectrum of synthesized ZnO NPs

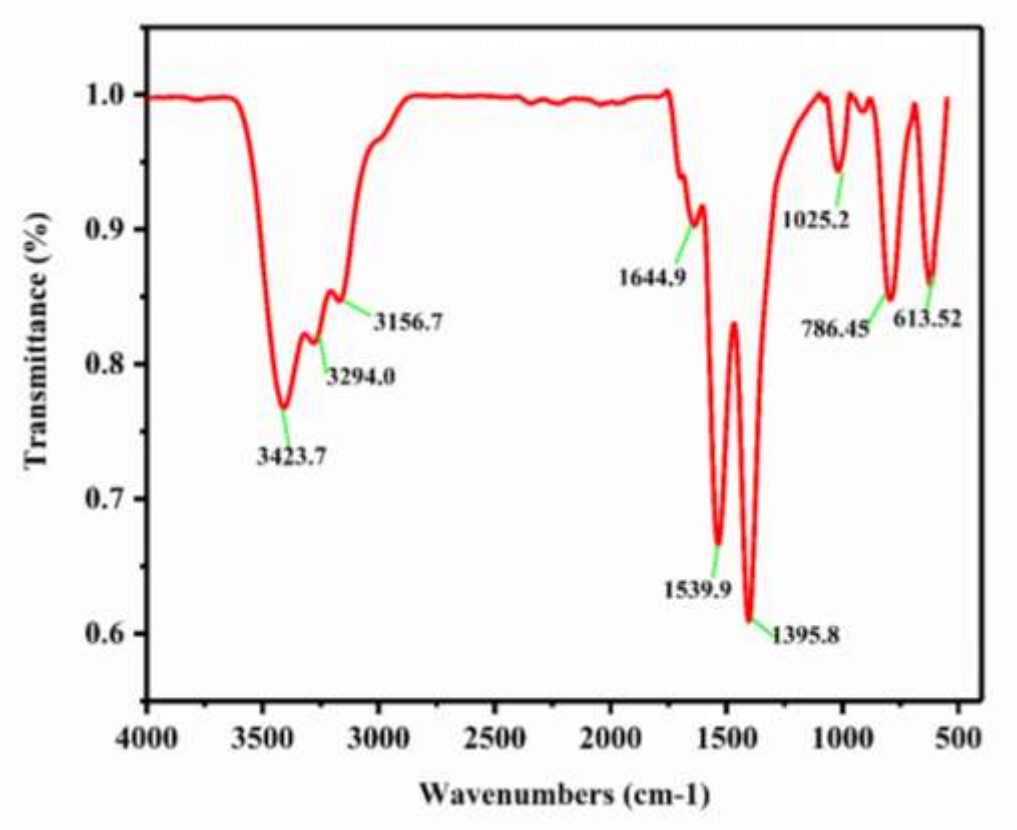

Figure 3 FTIR spectrum of ZnO NPs

The Figure 3 shows the FTIR spectrum of ZnO NPs

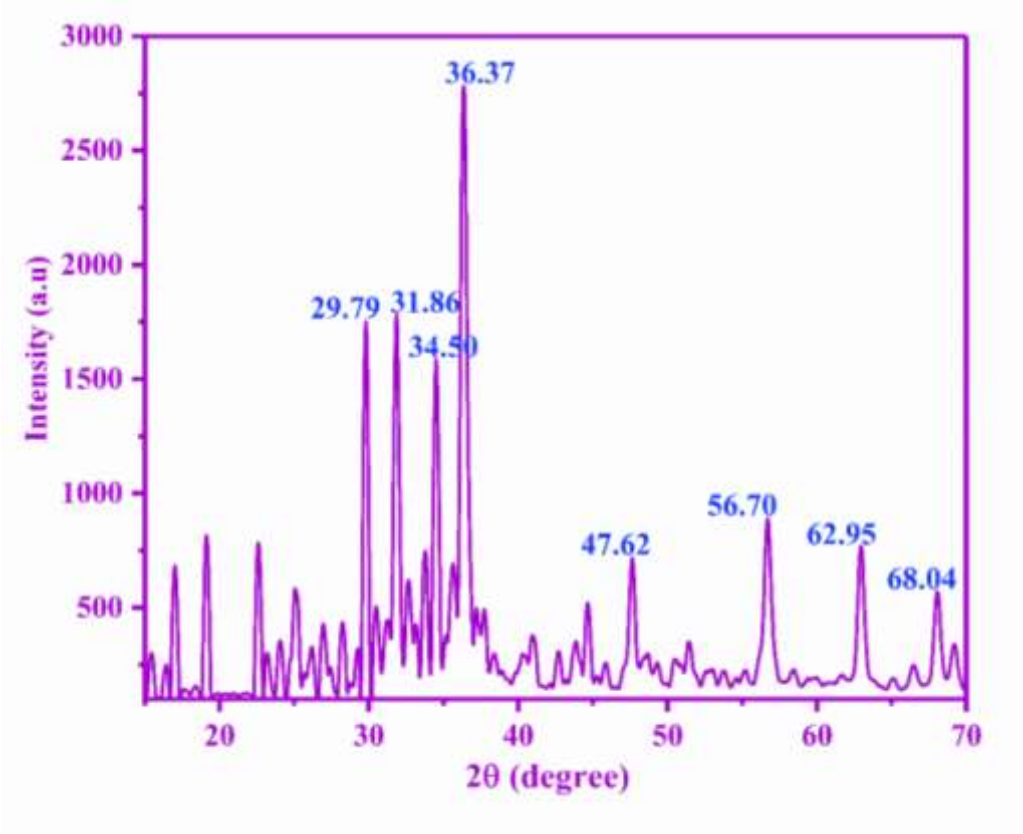

Figure 4 XRD pattern of biosynthesized ZnO NPs from *O. sanctum*

The Figure 4 shows the XRD pattern of biosynthesized ZnO NPs from *O. sanctum*

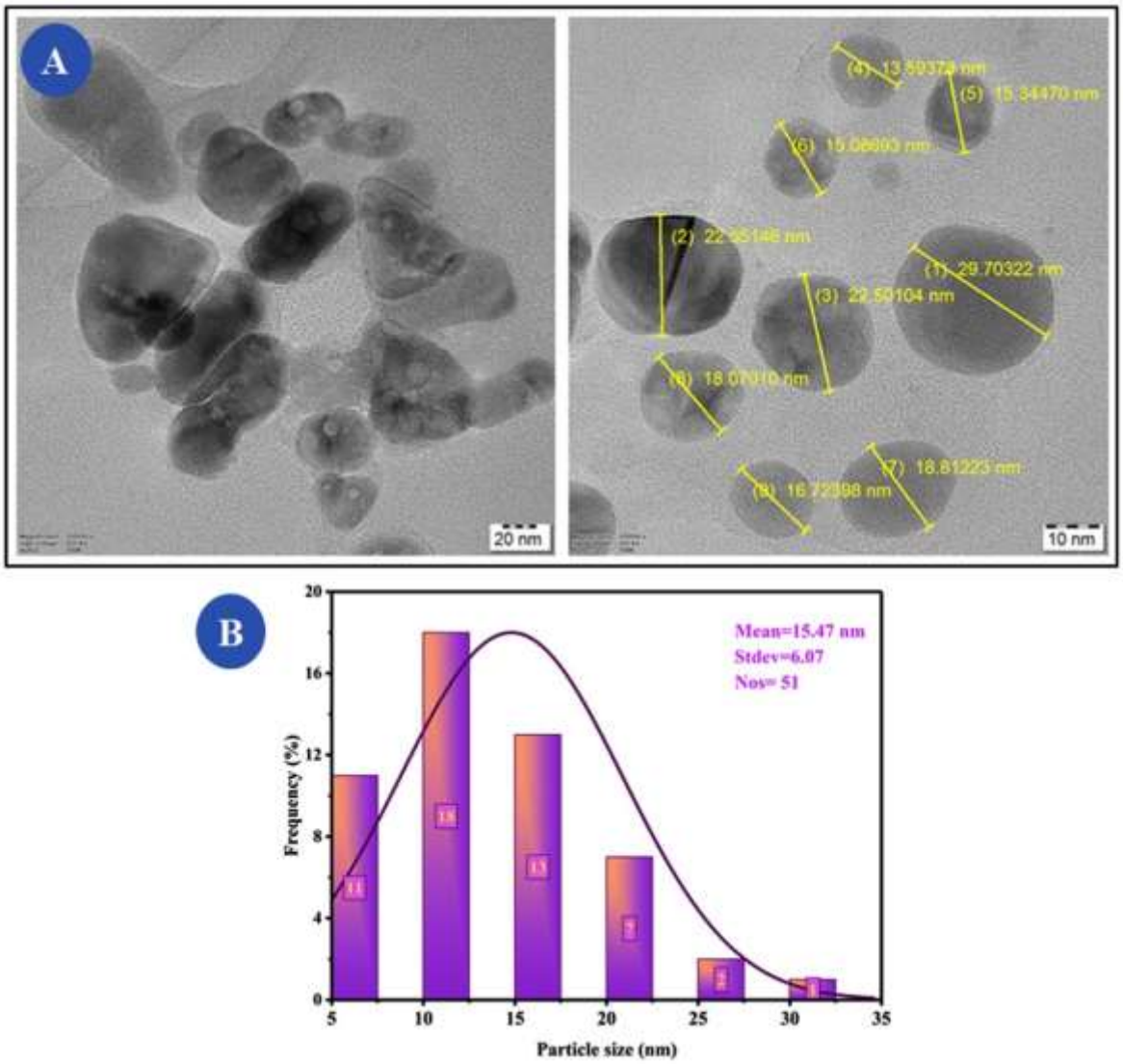

Figure 5 A). TEM images of Zno NPs **B).** Particle size distribution of ZnO NPs

The Figure 5 A) shows the TEM images of Zno NPs B). Particle size distribution of ZnO NPs

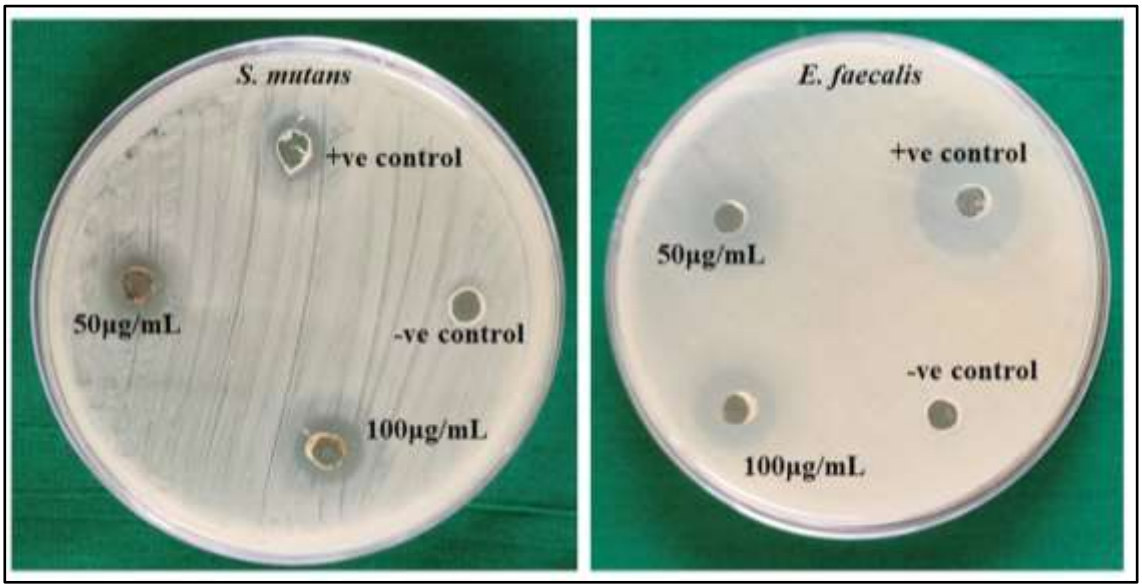

Figure 6 Antibacterial activity of biosynthesized ZnO NPs against dental pathogens.

The Figure 6 shows the Antibacterial activity of biosynthesized ZnO NPs against dental pathogens.

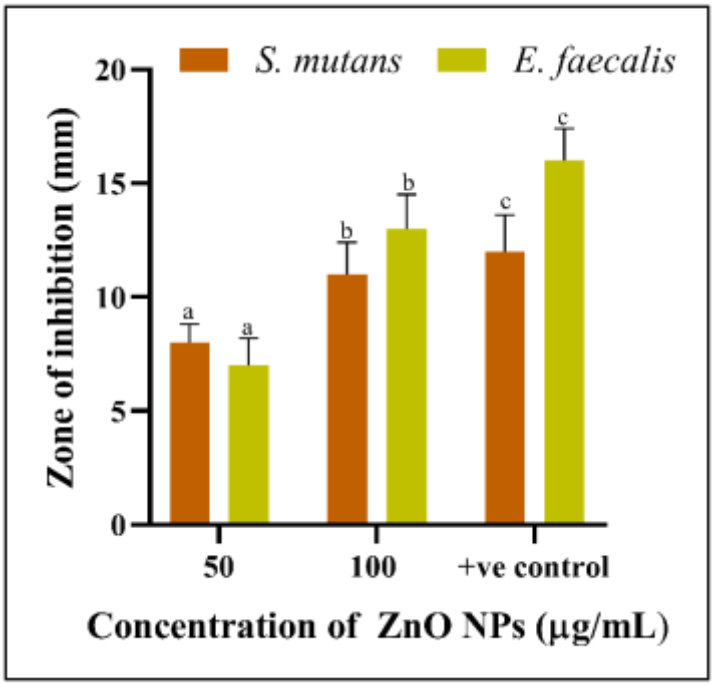

Figure 7 Measurements of zone of inhibition produced by *O. sanctum* synthesized ZnO NPs against *S. mutans* and *E. faecalis*. Mean values within the column followed by the same letter in superscript are not significantly different at $P< 0.05$ level.

The Figure 7 shows the Measurements of zone of inhibition produced by *O. sanctum* synthesized ZnO NPs against *S. mutans* and *E. faecalis*. Mean values within the column followed by the same letter in superscript are not significantly different at $P< 0.05$ level.

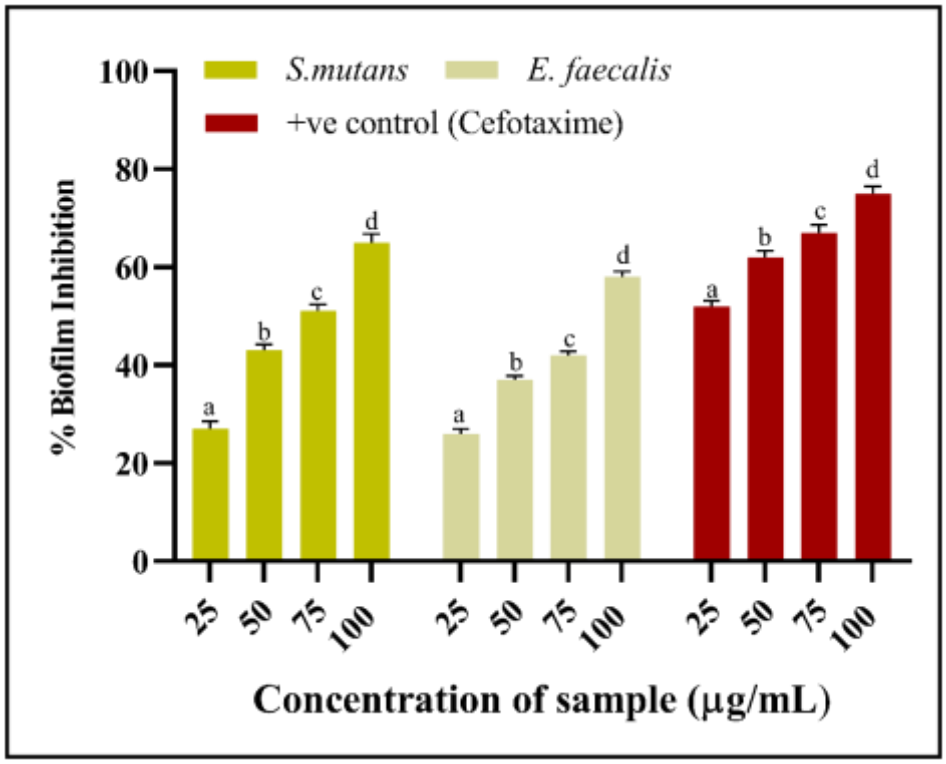

Figure 8 Percent biofilm inhibition of dental pathogens by ZnO nanoparticles at different concentrations. Mean values within the column followed by the same letter in superscript are not significantly different at $P< 0.05$ level.

The Figure 8 shows the Percent biofilm inhibition of dental pathogens by ZnO nanoparticles at different concentrations. Mean values within the column followed by the same letter in superscript are not significantly different at $P< 0.05$ level

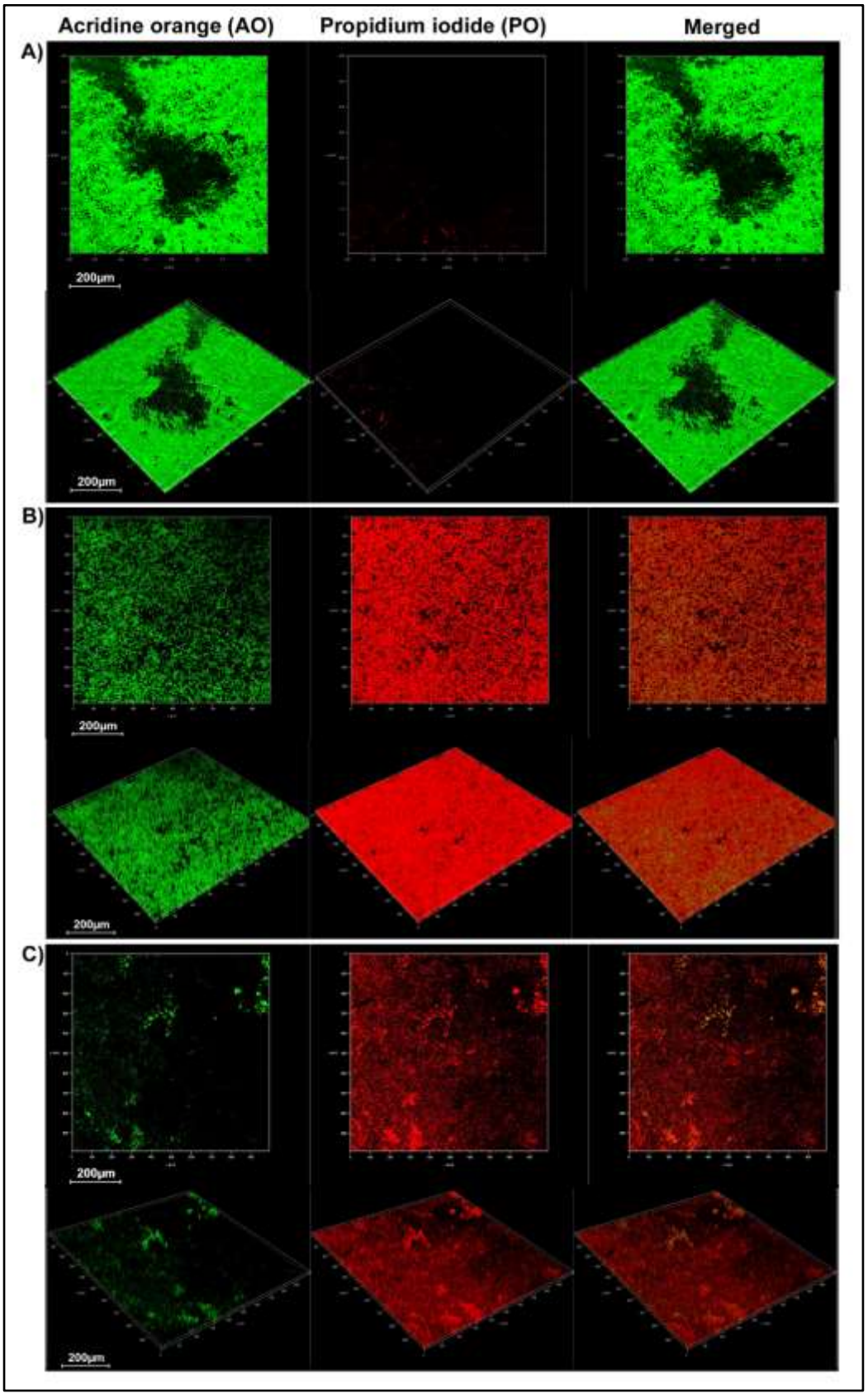
Acridine orange (AO)
Propidium iodide (PO)
Merged
A)
B)
C)
200µm

Figure 9 Live and Dead cell fluorescent staining of **A**). Control cells (without any treatment) **B**). *S. mutans* and **C**). *E. faecalis* biofilm treated with biosynthesized ZnO NPs at 100µg/mL.

The Figure 9 shows the Live and Dead cell fluorescent staining of **A**). Control cells (without any treatment) **B**). *S. mutans* and **C**). *E. faecalis* biofilm treated with biosynthesized ZnO NPs at 100µg/mL.

3. Results

Synthesis of ZnO NPs 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 Zinc nitrate solution, pH-12, and a 2h reaction at room temperature. ZnO NPs were formed by monitoring UV–Visible wavelengths from 200 to 700 nm. The UV-visible wavelength band of bio-produced NPs exhibited a ZnO NPs peak at 350 nm (Fig.2).

The reducing potential and stabilizing ability of biomolecules in *O. sanctum* extract was estimated using FTIR (Fig.3). FTIR analysis of *O. sanctum* synthesized ZnO NPs 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 ZnO NPs 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 zinc ions in aqueous extracts. Tulsi leaf extracts include plant-based constituents such as eugenol, flavonoids, and phenols, encapsulated on manufactured ZnO NPs. Proteins can attach to zinc salts via free amine groups or cysteine residues, stabilizing produced zinc nanoparticles (Singh and Chaudhuri, 2018).

The XRD pattern of produced ZnO NPs from *O. sanctum* leaf extracts is demonstrated in Fig.4 The XRD evaluations revealed Zinc colloids in the reaction mixture. The results demonstrated clearly prominent and narrow diffraction peaks, indicating that the produced ZnO

NPs were crystalline in nature. Bragg's reflection of 2θ values for ZnO NPs synthesized from *O. sanctum* leaf extract exhibited peaks at 31.86º, 36.37º, 47.62º, 56.70º, 62.95º and 68.04º, respectively. Additionally, the lack of impurity peaks in the diffraction pattern indicates efficient Zn precursor transformation into ZnO NPs. The phenols and flavonoids in *O. sanctum* leaf extract reduce and protect zinc acetate's outer surface. ZnO NPs developed because of them. Narrow and strong diffraction peaks indicate a clearly stated crystalline structure in the product's nanoparticles. The ZnO NPs made are very crystalline, indicated by their valuable peak intensity. HR-TEM pictures showed that these *O. sanctum* leaf synthesized ZnO NPs 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 zinc and bioactive substances coated zinc nanocomplex. *O. sanctum* leaf biosynthesized ZnO NPs 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 ± 0.51 mm to 16 ± 1.5 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 ZnO NPs at 50 µg/mL dose for *E. faecalis* was 6.7±1.2 mm, while the highest zone of growth inhibition observed was 7.0±1.2 and 11.7±1.5 mm for *S. mutans*, respectively. Results indicate that botanical extracts may have antibacterial properties due to bioactive components. Antibacterial investigation using zinc 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 ZnO NPs were stained against bacterial biofilm to determine their antibiofilm efficacy. The biofilm layer frequently contains gram (+ve) and gram (-ve) microorganisms. The synthesized ZnO NPs 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 ZnO NPs treatments

(Fig. 8). This finding may provide an alternate anti-fouling chemical source. To inhibit biofilm development, ZnO NPs were tested *in vitro*.

Biofilms, with dense cell communities, are critical for pathogenicity in invasive bacterial infections. To assess the impact of ZnO 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). ZnO NPs dose-dependently inhibit glass cover slip biofilm formation. In biofilms that were not treated with ZnO NPs, bacterial cells are held together by an extracellular matrix Fig.9A. In biofilms formed using ZnO NPs, no extracellular matrix was found, and some cells had abnormal structures and were killed. Biofilm production was decreased by > 90% with 100 μg/mL ZnO NPs. Conversely, in biofilms exposed to ZnO NPs, 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 ZnO NPs was applied, biofilm production was inhibited by more than 90% (Fig.9B). These findings provide strong evidence of the anti-biofilm efficacy of ZnO 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. Zinc oxide nanoparticles (ZnO NPs) interact non-toxically and bio-compatible with plant and animal cells, making them notable (Hano and Abbasi, 2021) ZnO NPs 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 Zn+ ions to Zn^{o} 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 Zn+ to Zn nanoparticles may be attributed to phytochemical substances, such as phenolic groups, in plant extracts. Flavonoids, terpenoids, and polysaccharides in plant extracts may reduce Zn^{+} (Król et al., 2017)

FTIR analysis identified promising biomolecules for zinc ion bio reduction and capping in ZnO NPs 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 Zn+ to Znº. All biological components interact with metal salts via functional groups to reduce them to NPs.(Beltrán-Noboa et al., 2023) XRD spectra of biosynthesized zinc NPs reveal peaks at 31.86º, 36.37º, 47.62º, 56.70º, 62.95º, and 68.04º, 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 ZnO NPs at 2.5–4 keV using *Coffea arabica* leaf extracts. Abomuti et al.(Abomuti et al., 2021) produced triangular and spherical ZnO NPs 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 ZnO NPs 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 ZnO NPs showed strong antibacterial properties against *S. mutans* and *E. faecalis*. Similarly, (Álvarez-Chimal et al.,

2022) reported the green synthesized ZnO 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 ZnO NPs 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 zinc ions block respiratory enzymes that produce ROS, enabling ZnO NPs antimicrobial(Kamath et al. 2022). Zinc oxide (ZnO), a class II-VI transition metal oxide and semiconductor with a broad band gap (3.3 eV), has a prominent antibacterial activity. ZnO 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 (·OH), ($\cdot HO^{2}-$), and ($O^{2\cdot}-$), which have bactericidal properties. ZnO nanoparticles (ZnO NPs) 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 ZnO 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 ZnO 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 ZnO 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 ZnO NPs were employed as a solution for dental infections. The ecologically friendly ZnO NPs made from *O. sanctum* leaf extract were effective against dental infections. These biosynthesized ZnO NPs reduced dental pathogen biofilms, promising a tooth infection treatment. This is the first study to test ZnO NPs antibacterial efficiency with *O. sanctum* leaf extract for dental pulp infections. Further study into biosynthesized ZnO NPs therapeutic uses may help eliminate oral *S. mutans* infections.

Acknowledgments

The authors would like to thank for Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai for providing lab space for this endeavour. The authors acknowledge the White Lab-Saveetha Dental College and Hospital, Chennai for FTIR, XRD, and SEM studies.

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 on 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.

References

Abdelbaky, A.S., Abd El-Mageed, T.A., Babalghith, A.O., Selim, S., Mohamed, A.M.H.A., 2022. Green Synthesis and Characterization of ZnO Nanoparticles Using Pelargonium odoratissimum (L.) Aqueous Leaf Extract and Their Antioxidant, Antibacterial and Anti-inflammatory Activities. Antioxidants 11, 1444. https://doi.org/10.3390/antiox11081444

Abebe, B., Zereffa, E.A., Tadesse, A., Murthy, H.C.A., 2020. A Review on Enhancing the Antibacterial Activity of ZnO: Mechanisms and Microscopic Investigation. Nanoscale

Res. Lett. 15, 190. https://doi.org/10.1186/s11671-020-03418-6

Abel, S., Tesfaye, J.L., Shanmugam, R., Dwarampudi, L.P., Lamessa, G., Nagaprasad, N., Benti, M., Krishnaraj, R., 2021. Green Synthesis and Characterizations of Zinc Oxide (ZnO) Nanoparticles Using Aqueous Leaf Extracts of Coffee (Coffea arabica) and Its Application in Environmental Toxicity Reduction. J. Nanomater. 2021, 1–6. https://doi.org/10.1155/2021/3413350

AboElmaaty, S.A., Shati, A.A., Alfaifi, M.Y., Elbehairi, S.E.I., Sheraba, N.S., Hassan, M.G., Badawy, M.S.E.M., Ghareeb, A., Hamed, A.A., Gabr, E.Z., 2022. Biofilm Inhibitory Activity of Actinomycete-Synthesized AgNPs with Low Cytotoxic Effect: Experimental and In Silico Study. Microorganisms 11, 102. https://doi.org/10.3390/microorganisms11010102

Abomuti, M.A., Danish, E.Y., Firoz, A., Hasan, N., Malik, M.A., 2021. Green Synthesis of Zinc Oxide Nanoparticles Using Salvia officinalis Leaf Extract and Their Photocatalytic and Antifungal Activities. Biology (Basel). 10, 1075. https://doi.org/10.3390/biology10111075

Adebayo-Tayo, B., Salaam, A., Ajibade, A., 2019. Green synthesis of silver nanoparticle using Oscillatoria sp. extract, its antibacterial, antibiofilm potential and cytotoxicity activity. Heliyon 5, e02502. https://doi.org/10.1016/j.heliyon.2019.e02502

Agrawal, S., Sen, S.K., Bhui, A.K., Singh, R., 2024. Biological synthesis of nanoparticles, in: Nanotechnology and In Silico Tools. Elsevier, pp. 15–29. https://doi.org/10.1016/B978-0-443-15457-7.00017-4

Ahuja, P., Rami, E., Singh, A., Pathak, D., 2024. Green synthesis of silver nanoparticles and their potenial biological applications, in: Nanotechnology and In Silico Tools. Elsevier, pp. 97–115. https://doi.org/10.1016/B978-0-443-15457-7.00022-8

Altammar, K.A., 2023. A review on nanoparticles: characteristics, synthesis, applications, and challenges. Front. Microbiol. 14. https://doi.org/10.3389/fmicb.2023.1155622

Álvarez-Chimal, R., García-Pérez, V.I., Álvarez-Pérez, M.A., Tavera-Hernández, R., Reyes-Carmona, L., Martínez-Hernández, M., Arenas-Alatorre, J.Á., 2022. Influence of the particle size on the antibacterial activity of green synthesized zinc oxide nanoparticles using Dysphania ambrosioides extract, supported by molecular docking analysis. Arab. J. Chem. 15, 103804. https://doi.org/10.1016/j.arabjc.2022.103804

Ambedkar, A.K., Gautam, D., Vikal, S., Singh, M., Kumar, A., Sanger, A., Sharma, K., Singh, B.P., Gautam, Y.K., 2023. Ocimum sanctum Leaf Extract-Assisted Green Synthesis of Pd-Doped CuO Nanoparticles for Highly Sensitive and Selective NO 2 Gas Sensors. ACS Omega 8, 29663–29673. https://doi.org/10.1021/acsomega.3c03765

Baker, J.L., Mark Welch, J.L., Kauffman, K.M., McLean, J.S., He, X., 2023. The oral microbiome: diversity, biogeography and human health. Nat. Rev. Microbiol. https://doi.org/10.1038/s41579-023-00963-6

Banerjee, M., Mallick, S., Paul, A., Chattopadhyay, A., Ghosh, S.S., 2010. Heightened Reactive Oxygen Species Generation in the Antimicrobial Activity of a Three Component Iodinated Chitosan−Silver Nanoparticle Composite. Langmuir 26, 5901–5908. https://doi.org/10.1021/la9038528

Beltrán-Noboa, A., Jordan-Álvarez, A., Guevara-Terán, M., Gallo, B., Berrueta, L.A., Giampieri, F., Battino, M., Álvarez-Suarez, J.M., Tejera, E., 2023. Exploring the Chemistry of Ocimum Species under Specific Extractions and Chromatographic Methods: A Systematic Review. ACS Omega 8, 10747–10756. https://doi.org/10.1021/acsomega.3c00043

Cao, Y., Dhahad, H.A., El-Shorbagy, M.A., Alijani, H.Q., Zakeri, M., Heydari, A., Bahonar, E., Slouf, M., Khatami, M., Naderifar, M., Iravani, S., Khatami, S., Dehkordi, F.F., 2021. Green synthesis of bimetallic ZnO–CuO nanoparticles and their cytotoxicity properties. Sci. Rep. 11, 23479. https://doi.org/10.1038/s41598-021-02937-1

Deschner, J., Schröder, A., Weber, M., Galler, K., Proff, P., Kirschneck, C., Bozec, A., Jantsch, J., 2023. Advancing oral immunology for improving oral health. J. Orofac. Orthop. / Fortschritte der Kieferorthopädie. https://doi.org/10.1007/s00056-023-00473-3

Donga, S., Chanda, S., 2022. Caesalpinia crista Seeds Mediated Green Synthesis of Zinc Oxide Nanoparticles for Antibacterial, Antioxidant, and Anticancer Activities. Bionanoscience 12, 451–462. https://doi.org/10.1007/s12668-022-00952-8

Dubern, J.-F., Hook, A.L., Carabelli, A.M., Chang, C.-Y., Lewis-Lloyd, C.A., Luckett, J.C., Burroughs, L., Dundas, A.A., Humes, D.J., Irvine, D.J., Alexander, M.R., Williams, P., 2023. Discovery of a polymer resistant to bacterial biofilm, swarming, and encrustation. Sci. Adv. 9. https://doi.org/10.1126/sciadv.add7474

Faisal, S., Jan, H., Shah, S.A., Shah, S., Khan, A., Akbar, M.T., Rizwan, M., Jan, F., Wajidullah, Akhtar, N., Khattak, A., Syed, S., 2021. Green Synthesis of Zinc Oxide (ZnO) Nanoparticles Using Aqueous Fruit Extracts of Myristica fragrans : Their Characterizations and Biological and Environmental Applications. ACS Omega 6, 9709–9722. https://doi.org/10.1021/acsomega.1c00310

Guglielmelli, A., D'Aquila, P., Palermo, G., Dell'Aglio, M., Passarino, G., Strangi, G., Bellizzi, D., 2023. Role of the Human Serum Albumin Protein Corona in the Antimicrobial and Photothermal Activity of Metallic Nanoparticles against Escherichia coli Bacteria. ACS Omega 8, 31333–31343. https://doi.org/10.1021/acsomega.3c03774

Habib, T., Rahman, A., Nair, A.B., Islam, S.M.S., 2023. Application of Three Compounds Extracted from Cynodon dactylon against Streptococcus mutans Biofilm Formation to Prevent Oral Diseases. Biomolecules 13, 1292. https://doi.org/10.3390/biom13091292

Hano, C., Abbasi, B.H., 2021. Plant-Based Green Synthesis of Nanoparticles: Production, Characterization and Applications. Biomolecules 12, 31. https://doi.org/10.3390/biom12010031

Harini, B., Rajeshkumar, S., Roy, A., 2022. Biomedical Application of Chitosan and Piper Longum-assisted Nano Zinc Oxide–based Dental Varnish. Appl. Biochem. Biotechnol. 194, 1303–1309. https://doi.org/10.1007/s12010-021-03712-8

Iqbal, J., Abbasi, B.A., Yaseen, T., Zahra, S.A., Shahbaz, A., Shah, S.A., Uddin, S., Ma, X., Raouf, B., Kanwal, S., Amin, W., Mahmood, T., El-Serehy, H.A., Ahmad, P., 2021. Green synthesis of zinc oxide nanoparticles using Elaeagnus angustifolia L. leaf extracts and their multiple in vitro biological applications. Sci. Rep. 11, 20988. https://doi.org/10.1038/s41598-021-99839-z

Jayarambabu, N., Venkatappa Rao, T., Rakesh Kumar, R., Akshaykranth, A., Shanker, K.,

Suresh, V., 2021. Anti-hyperglycemic, pathogenic and anticancer activities of Bambusa arundinacea mediated Zinc Oxide nanoparticles. Mater. Today Commun. 26, 101688. https://doi.org/10.1016/j.mtcomm.2020.101688

Kamli, M.R., Malik, M.A., Srivastava, V., Sabir, J.S.M., Mattar, E.H., Ahmad, A., 2021. Biogenic ZnO Nanoparticles Synthesized from Origanum vulgare Abrogates Quorum Sensing and Biofilm Formation in Opportunistic Pathogen Chromobacterium violaceum. Pharmaceutics 13, 1743. https://doi.org/10.3390/pharmaceutics13111743

Kang, M.-G., Khan, F., Jo, D.-M., Oh, D., Tabassum, N., Kim, Y.-M., 2022. Antibiofilm and Antivirulence Activities of Gold and Zinc Oxide Nanoparticles Synthesized from Kimchi-Isolated Leuconostoc sp. Strain C2. Antibiotics 11, 1524. https://doi.org/10.3390/antibiotics11111524

Kaushal, A., Khurana, I., Yadav, P., Allawadhi, P., Banothu, A.K., Neeradi, D., Thalugula, S., Barani, P.J., Naik, R.R., Navik, U., Bharani, K.K., Khurana, A., 2023. Advances in therapeutic applications of silver nanoparticles. Chem. Biol. Interact. 382, 110590. https://doi.org/10.1016/j.cbi.2023.110590

Kodeš, Z., Vrublevskaya, M., Kulišová, M., Jaroš, P., Paldrychová, M., Pádrová, K., Lokočová, K., Palyzová, A., Maťátková, O., Kolouchová, I., 2021. Composition and Biological Activity of Vitis vinifera Winter Cane Extract on Candida Biofilm. Microorganisms 9, 2391. https://doi.org/10.3390/microorganisms9112391

Król, A., Pomastowski, P., Rafińska, K., Railean-Plugaru, V., Buszewski, B., 2017. Zinc oxide nanoparticles: Synthesis, antiseptic activity and toxicity mechanism. Adv. Colloid Interface Sci. 249, 37–52. https://doi.org/10.1016/j.cis.2017.07.033

Kumar, R., Kumar, M., Luthra, G., 2023. Fundamental approaches and applications of nanotechnology: A mini review. Mater. Today Proc. https://doi.org/10.1016/j.matpr.2022.12.172

Kurita, H., Umeda, M., Ueno, T., Uzawa, N., Shibuya, Y., Nakamura, N., Nagatsuka, H., Hayashi, T., Mizoguch, I., Tomihara, K., Ikegami, Y., Noguchi, K., Takiguchi, Y., Yamamoto, N., Sakai, H., 2023. Management of odontogenic foci of infection (dental caries, periodontal disease and odontogenic infections) in the oral functional management of patients receiving cancer treatments: Guidelines based on a systematic review. Oral Sci. Int. https://doi.org/10.1002/osi2.1209

Mendes, C.R., Dilarri, G., Forsan, C.F., Sapata, V. de M.R., Lopes, P.R.M., de Moraes, P.B., Montagnolli, R.N., Ferreira, H., Bidoia, E.D., 2022. Antibacterial action and target mechanisms of zinc oxide nanoparticles against bacterial pathogens. Sci. Rep. 12, 2658. https://doi.org/10.1038/s41598-022-06657-y

Nasiri, K., Masoumi, S.M., Amini, S., Goudarzi, M., Tafreshi, S.M., Bagheri, A., Yasamineh, S., Alwan, M., Arellano, M.T.C., Gholizadeh, O., 2023. Recent advances in metal nanoparticles to treat periodontitis. J. Nanobiotechnology 21, 283. https://doi.org/10.1186/s12951-023-02042-7

Okamoto, K., Kudo, D., Phuong, D.N.D., Iwamoto, Y., Watanabe, K., Yoshioka, Y., Ariyoshi, W., Yamasaki, R., 2023. Magnesium Hydroxide Nanoparticles Inhibit the Biofilm Formation of Cariogenic Microorganisms. Nanomaterials 13, 864. https://doi.org/10.3390/nano13050864

Ong, T.H., Chitra, E., Ramamurthy, S., Siddalingam, R.P., Yuen, K.H., Ambu, S.P., Davamani, F., 2017. Chitosan-propolis nanoparticle formulation demonstrates anti-bacterial activity against Enterococcus faecalis biofilms. PLoS One 12, e0174888. https://doi.org/10.1371/journal.pone.0174888

Oza, G., Reyes-Calderón, A., Mewada, A., Arriaga, L.G., Cabrera, G.B., Luna, D.E., Iqbal, H.M.N., Sharon, M., Sharma, A., 2020. Plant-based metal and metal alloy nanoparticle synthesis: a comprehensive mechanistic approach. J. Mater. Sci. 55, 1309–1330. https://doi.org/10.1007/s10853-019-04121-3

Park, D.-Y., Hwang, J., Kim, Y., Lee, D., Kim, Y.-Y., Kim, H.-S., Hwang, I., 2023. Antimicrobial activity of Limosilactobacillus fermentum strains isolated from the human oral cavity against Streptococcus mutans. Sci. Rep. 13, 7969. https://doi.org/10.1038/s41598-023-35168-7

Passos, T.F., de Camargo Bertuso, P., Nitschke, M., 2023. Biosurfactants as food additives: New trends and applications, in: Biosurfactants. Elsevier, pp. 85–106. https://doi.org/10.1016/B978-0-323-91697-4.00005-3

Raliya, R., Nair, R., Chavalmane, S., Wang, W.-N., Biswas, P., 2015. Mechanistic evaluation of translocation and physiological impact of titanium dioxide and zinc oxide nanoparticles on the tomato (Solanum lycopersicum L.) plant. Metallomics 7, 1584–1594. https://doi.org/10.1039/C5MT00168D

Saleh, T.A., Fadillah, G., 2023. Green synthesis protocols, toxicity, and recent progress in nanomaterial-based for environmental chemical sensors applications. Trends Environ. Anal. Chem. 39, e00204. https://doi.org/10.1016/j.teac.2023.e00204

Sans-Serramitjana, E., Jorba, M., Pedraz, J.L., Vinuesa, T., Viñas, M., 2017. Determination of the spatiotemporal dependence of Pseudomonas aeruginosa biofilm viability after treatment with NLC-colistin. Int. J. Nanomedicine Volume 12, 4409–4413. https://doi.org/10.2147/IJN.S138763

Santomartino, R., Averesch, N.J.H., Bhuiyan, M., Cockell, C.S., Colangelo, J., Gumulya, Y., Lehner, B., Lopez-Ayala, I., McMahon, S., Mohanty, A., Santa Maria, S.R., Urbaniak, C., Volger, R., Yang, J., Zea, L., 2023. Toward sustainable space exploration: a roadmap for harnessing the power of microorganisms. Nat. Commun. 14, 1391. https://doi.org/10.1038/s41467-023-37070-2

Selim, Y.A., Azb, M.A., Ragab, I., H. M. Abd El-Azim, M., 2020. Green Synthesis of Zinc Oxide Nanoparticles Using Aqueous Extract of Deverra tortuosa and their Cytotoxic Activities. Sci. Rep. 10, 3445. https://doi.org/10.1038/s41598-020-60541-1

Shehabeldine, A.M., Doghish, A.S., El-Dakroury, W.A., Hassanin, M.M.H., Al-Askar, A.A., AbdElgawad, H., Hashem, A.H., 2023. Antimicrobial, Antibiofilm, and Anticancer Activities of Syzygium aromaticum Essential Oil Nanoemulsion. Molecules 28, 5812. https://doi.org/10.3390/molecules28155812

Siddiqi, K.S., ur Rahman, A., Tajuddin, Husen, A., 2018. Properties of Zinc Oxide Nanoparticles and Their Activity Against Microbes. Nanoscale Res. Lett. 13, 141. https://doi.org/10.1186/s11671-018-2532-3

Singh, D., Chaudhuri, P.K., 2018. A review on phytochemical and pharmacological properties of Holy basil (Ocimum sanctum L.). Ind. Crops Prod. 118, 367–382.

https://doi.org/10.1016/j.indcrop.2018.03.048

Singhal, A., Baweja, P., Gupta, S., Chopra, H., Gandhi, P.B., 2023. Biogenesis of nanoparticles using microorganisms: A Review. Plant Sci. Today. https://doi.org/10.14719/pst.2373

Sondi, I., Salopek-Sondi, B., 2004. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J. Colloid Interface Sci. 275, 177–182. https://doi.org/10.1016/j.jcis.2004.02.012

Treccani, L., 2023. Interactions Between Surface Material and Bacteria: From Biofilm Formation to Suppression, in: Surface-Functionalized Ceramics. Wiley, pp. 283–335. https://doi.org/10.1002/9783527698042.ch8

Ureña-Castillo, B., Morones-Ramírez, J.R., Rivera-De la Rosa, J., Alcalá-Rodríguez, M.M., Cerdán Pasarán, A.Q., Díaz-Barriga Castro, E., Escárcega-González, C.E., 2022. Organic Waste as Reducing and Capping Agents for Synthesis of Silver Nanoparticles with Various Applications. ChemistrySelect 7. https://doi.org/10.1002/slct.202201023

Verma, R., Pathak, S., Srivastava, A.K., Prawer, S., Tomljenovic-Hanic, S., 2021. ZnO nanomaterials: Green synthesis, toxicity evaluation and new insights in biomedical applications. J. Alloys Compd. 876, 160175. https://doi.org/10.1016/j.jallcom.2021.160175

Yadav, A., Kumar, H., Sharma, R., Kumari, R., 2023. Synthesis, processing, and applications of 2D (nano)materials: A sustainable approach. Surfaces and Interfaces 39, 102925. https://doi.org/10.1016/j.surfin.2023.102925

Choudhari, Sahil, Jogikalmat Krithikadatta, Ipsitha Vejendla, Swathi, and Mukesh Doble. 2023. “Microbial Interactions in Oral Biofilm: Evaluating Therapeutic Interventions and the Emergence of Resistance: A Narrative Review.” *Cureus*, October. https://doi.org/10.7759/cureus.48021.

Deepika, Burra Anand, Jaiganesh Ramamurthy, Smiline Girija, and Nadathur Duraisamy Jayakumar. 2022. “Evaluation of the Antimicrobial Effect of Ocimum Sanctum L. Oral Gel against Anaerobic Oral Microbes: An in Vitro Study.” *World Journal of Dentistry* 13 (S1): S23–27.

Janani, Krishnamachari, Kavalipurapu Venkata Teja, P. Ajitha, and Raghu Sandhya. 2020. “Evaluation of Tissue Inflammatory Response of Four Intracanal Medicament - An Animal Study.” *Journal of Conservative Dentistry: JCD* 23 (3): 216–20.

Kamath, Ajith K., Iffat Nasim, N. P. Muralidharan, and Ravalika N. Kothuri. 2022. “Anti-Microbial Efficacy of Vanilla Planifolia Leaf Extract against Common Oral Micro-Biomes: A Comparative Study of Two Different Antibiotic Sensitivity Tests.” *Journal of Oral and Maxillofacial Pathology: JOMFP* 26 (3): 330–34.

Lakshmi, and Dean -International Affairs, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences. 2021. “Green Synthesis of Copper Nanoparticles Synthesized Using Black Tea and Its Antibacterial Activity against Oral Pathogens.” *International Journal of Dentistry and Oral Science*, August, 4156–59.

Lakshmi, Nanobiomedicine. n.d. “Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai.” *International Journal of Dentistry and Oral Science*, 2985–87.

Nasim, Iffat, Zohra Jabin, S. Rajesh Kumar, and V. Vishnupriya. 2022. "Green Synthesis of Calcium Hydroxide-Coated Silver Nanoparticles Using Andrographis Paniculata and Ocimum Sanctum Linn. Leaf Extracts: An Antimicrobial and Cytotoxic Activity." *Journal of Conservative Dentistry: JCD* 25 (4): 369–74.