RESEARCH PAPER

Treatment of dental biofilm formed by Pseudomonas aeruginosa using curcumin loaded Zinc oxide nanoparticles

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Abstract

The present study aimed to synthesize and evaluate the antibacterial and antibiofilm efficacy of curcumin-loaded zinc oxide nanoparticles (Cur-ZnONPs) against *Pseudomonas aeruginosa*. The successful formation of Cur-ZnONPs was confirmed by the appearance of a characteristic yellow color and transmission electron microscopy, which revealed nanoscale particle size. Surface modification with curcumin improved nanoparticle stability by reducing aggregation. Elemental and functional group analyses using EDX and FTIR further verified the successful loading of curcumin onto the ZnO nanoparticle surface, indicating its role as a stabilizing and functionalizing agent. Antibacterial studies demonstrated that Cur-ZnONPs exhibited significantly enhanced activity compared to curcumin or ZnO alone, as evidenced by a larger zone of inhibition, a minimum inhibitory concentration (MIC) of 75 µg/mL, and strong bactericidal effects at higher concentrations. The improved efficacy is attributed to the synergistic action of ZnO-induced reactive oxygen species generation and curcumin-mediated enhancement of bacterial membrane disruption. Confocal laser scanning microscopy (CLSM) analysis further supported these findings, showing predominant red fluorescence (dead cells) and disrupted biofilm architecture in treated samples, compared to green fluorescence (live cells) in untreated controls. These results highlight the potential of Cur-ZnONPs as an effective nanotherapeutic strategy for combating biofilm-associated bacterial infections.

***Keywords*:** Curcumin; ZnO nanoparticles; *Pseudomonas aeruginosa*; Antibiofilm activity; Dental biofilm; Nanoformulation.

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Introduction

Dental biofilm is a structured microbial community that develops on tooth surfaces and oral tissues, where microorganisms are embedded within a self-produced extracellular polymeric matrix composed of polysaccharides, proteins, lipids, and extracellular DNA (Bowen et al., 2018). These biofilms play a central role in the pathogenesis of many oral diseases, including dental caries, periodontal infections, and peri-implantitis (Govindarajan et al., 2025). The oral cavity provides an ideal environment for microbial colonization due to the presence of saliva, nutrients, and suitable surfaces such as enamel and dental restorations (Rath et al., 2021). Within this complex ecosystem, several opportunistic pathogens can integrate into polymicrobial biofilms and contribute to disease progression. One such organism is *Pseudomonas aeruginosa*, a Gram-negative opportunistic pathogen that has increasingly been detected in dental plaque, periodontal pockets, and saliva (Sá et al., 2023). Studies have demonstrated that this bacterium can be present in up to 40% of oral biofilm samples from individuals with periodontal infections, suggesting that the oral cavity may serve as an important reservoir for this pathogen (Cruz et al., 2022). Moreover, the presence of *P. aeruginosa* in dental biofilms has been associated with treatment failure in refractory periodontal infections due to its strong biofilm-forming capacity and high level of antibiotic resistance (Wu et al., 2020).

The persistence of *P. aeruginosa* in oral environments is largely attributed to its sophisticated biofilm formation mechanisms and virulence factors (Li et al., 2023). Biofilm development in this bacterium is regulated by signaling pathways such as quorum sensing systems (las, rhl, and PQS) that control the production of extracellular polymeric substances and various virulence factors (Lin & Cheng, 2019). During biofilm formation, bacterial cells transition from planktonic to sessile communities and produce an adhesive matrix that anchors them to surfaces and protects them from environmental stress (Olaimat et

al., 2024). This matrix significantly reduces the penetration of antimicrobial agents and host immune defenses, thereby contributing to chronic and persistent infections. Furthermore, *P. aeruginosa* possesses multiple intrinsic and acquired resistance mechanisms, including β-lactamase production, efflux pumps, and adaptive genetic responses, which further complicate treatment (Alharbi et al., 2025). These characteristics make infections associated with *P. aeruginosa* biofilms extremely difficult to eradicate using conventional antimicrobial therapies. As a result, there is a growing interest in developing alternative therapeutic strategies capable of disrupting biofilm architecture and inhibiting bacterial virulence (Singh & Arsi, 2026).

Natural phytochemicals have recently attracted significant attention as potential antibiofilm agents due to their biocompatibility and diverse biological activities (Zhou et al., 2025). Among these compounds, Curcumin a polyphenolic compound derived from the rhizome of Curcuma longa has been extensively studied for its antimicrobial, anti-inflammatory, antioxidant, and antibiofilm properties (El-Saadony et al., 2025). Curcumin has been reported to interfere with bacterial cell membrane integrity, inhibit quorum sensing signaling pathways, and reduce the expression of virulence factors responsible for biofilm formation (Wang et al., 2025). However, the clinical application of curcumin is limited due to its poor aqueous solubility, rapid degradation, and low bioavailability (Pan-On et al., 2022). To overcome these limitations, nanotechnology-based delivery systems have been explored to enhance the stability and therapeutic efficacy of curcumin (Sathishkumar & Khan, 2024). In particular, Zinc oxide nanoparticles (ZnO NPs) have emerged as promising antimicrobial nanomaterials due to their strong bactericidal activity (Lebaka et al., 2025), ability to generate reactive oxygen species, and capacity to disrupt bacterial membranes (Thamayandhi et al., 2024). When curcumin is incorporated into ZnO nanoparticles, the resulting nanocomposite can exhibit synergistic antibacterial and antibiofilm effects, enabling improved penetration into the extracellular polymeric matrix and more efficient eradication of biofilm-forming pathogens such as *P. aeruginosa* (G et al., 2025). In this study, curcumin-loaded ZnO nanoparticles represent a promising nanotherapeutic strategy for the effective treatment of dental biofilms and the prevention of biofilm-associated oral infections.

2. Materials and methods

2.1. Chemicals

Mueller-Hinton agar (MHA), Mueller-Hinton broth (MHB), crystal violet (CV), MTT reagent, Nutrient broth, and standard antibiotics disc were procured from HiMedia (Mumbai, India). Natural components such as quercetin, morin, hesperetin, naringin, and diosmetin were acquired from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Phosphate buffered saline (PBS) and dimethyl sulphoxide (DMSO) were obtained from Sisco Research Laboratories (Maharashtra, India). The remaining chemicals and reagents used in this investigation are of analytical grade and were purchased from standard companies.

2.2. Isolation and identification of *P. aeruginosa*

The bacterial strain *P. aeruginosa* isolated from the oral infection of hospitalized patients were used in this study. The isolates were identified using the VITEK®2 Compact System at Saveetha Medical College and Hospital, SIMATS, India. The isolated strains were preserved as glycerol stocks and stored at −80 °C for further analysis.

2.3. Synthesis of zinc oxide nanoparticles

Zinc oxide nanoparticles (ZnONPs) were synthesized using a chemical precipitation method with zinc acetate as the precursor. Briefly, 0.1 M zinc acetate dihydrate was dissolved in 100 mL of deionized water under continuous magnetic stirring at room temperature. The solution was stirred for 30 min to ensure complete dissolution of the precursor. Subsequently, 0.2 M sodium hydroxide solution was added dropwise to the zinc acetate solution under constant stirring until the pH reached approximately 10–11. The reaction mixture was maintained under stirring for 2 h, resulting in the formation of a white precipitate of zinc hydroxide. The precipitate was collected by centrifugation and washed several times with deionized water and ethanol to remove impurities. The obtained product was then dried in a hot air oven at 80 °C for 12 h. The dried powder was kept at 400 °C for 2 h in a muffle furnace to obtain crystalline zinc oxide nanoparticles (Vijayakumar et al., 2018).

2.4. Fabrication of curcumin-loaded ZnO nanoparticles (Cur-ZnONPs)

Curcumin loading onto ZnO nanoparticles was carried out by a physical adsorption method. Curcumin was dissolved in ethanol or dimethyl sulfoxide to prepare a stock solution (1 mg/mL). A known amount of synthesized ZnONPs (100 mg) was dispersed in 50 mL of the curcumin solution using ultrasonication for 30 min to obtain a homogeneous suspension. The mixture was then magnetically stirred for 12–24 h at room temperature in dark conditions to facilitate adsorption

of curcumin onto the surface of ZnONPs. After incubation, the suspension was centrifuged at 10,000 rpm for 15 min to collect the curcumin-loaded nanoparticles. The resulting Cur-ZnONPs were washed with ethanol to remove unbound curcumin and dried under vacuum (Khan et al., 2022).

2.5. Characterization of Cur-ZnONPs

The synthesized nanoparticles were characterized to evaluate their structural and physicochemical properties. The morphology and particle size of the curcumin-loaded zinc oxide nanoparticles (Cur-ZnONPs) were analyzed using transmission electron microscopy (TEM). The elemental composition and purity of the nanoparticles were determined by energy-dispersive X-ray spectroscopy (EDX) coupled with the TEM instrument. Furthermore, the presence of functional groups and the successful loading of curcumin onto the ZnO nanoparticles were confirmed using Fourier transform infrared spectroscopy (FTIR) by analyzing the characteristic vibrational peaks corresponding to Zn–O bonds and curcumin functional groups (Suresh et al., 2026).

2.6. Antibacterial activity against *P. aeruginosa*

The antibacterial activity of curcumin, zinc oxide nanoparticles, curcumin mediated zinc oxide nanoparticles, and positive control (ciprofloxacin) against *P. aeruginosa* was evaluated using the Kirby-Bauer disk diffusion method (Jan Hudzicki, 2009). An overnight culture was then swabbed onto MHA plates using sterile cotton swabs. Four wells were created in the agar using a well cutter, and each was filled with 100 µL solution. The plates were incubated at 37 °C for 24 h. The zones of inhibition were measured using the HiAntibiotic ZoneScale™ (Himedia, India) (J. R. S. M. Reddy et al., 2023).

2.7. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of Cur-ZnONPs against *P. aeruginosa* was determined using the broth microdilution method in a sterile 96-well microtiter plate. Cur-ZnONPs were dispersed in sterile distilled water and sonicated to obtain a homogeneous suspension. The nanoparticle suspension was then serially two-fold diluted in Mueller–Hinton broth (MHB) across the wells of the microtiter plate. Each well was inoculated with a standardized bacterial suspension of *P. aeruginosa* adjusted to 0.5 McFarland turbidity. Positive control wells contained bacterial inoculum without Cur-ZnONPs, while negative control wells contained only sterile broth to ensure media sterility. The microtiter plates were incubated at 37 °C for 24 h, and bacterial growth was assessed by measuring the optical density at 600 nm using a microplate reader. The MIC was defined as the lowest concentration of Cur-ZnONPs that inhibited visible bacterial growth (C. S. S. Reddy et al., 2026).

2.8. Minimal bactericidal concentration (MBC) determination

MBC of Cur-ZnONPs against *P. aeruginosa* was determined following the MIC assay. Aliquots from the MIC wells showing no visible bacterial growth were sub-cultured onto fresh nutrient agar plates and incubated at 37 °C for 24 h. After incubation, the plates were examined for bacterial colony formation. The MBC was defined as the lowest concentration of Cur-ZnONPs that resulted in complete inhibition of bacterial growth, indicated by the absence of visible colonies on the agar plates (Mihaylova et al., 2025).

2.9. Antibiofilm assay

The antibiofilm activity of Cur-ZnONPs against *P. aeruginosa* was evaluated using a 96-well microtiter plate assay. Cur-ZnONPs were tested at sub-MIC concentrations in wells containing nutrient broth inoculated with bacterial culture and incubated for biofilm formation. After incubation, the wells were gently washed with phosphate-buffered saline (PBS) to remove planktonic cells. The adhered biofilms were stained with crystal violet, excess stain was removed by washing, and the bound dye was dissolved in ethanol. Biofilm biomass was quantified by measuring the absorbance at 570 nm using a microplate reader. In addition, the structural changes in the biofilm after treatment with Cur-ZnONPs were visualized by confocal laser scanning microscopy (CLSM) after appropriate fluorescent staining to assess biofilm architecture and viability (Omole et al., 2025).

3. Results

3.1. Synthesis of zinc Cur-ZnONPs

Cur-ZnONPs were successfully synthesized by incorporating curcumin during the nanoparticle formation process. The reaction resulted in the formation of a stable precipitate, indicating the successful interaction between zinc ions and curcumin molecules. After purification and drying, the obtained product appeared as a fine yellowish powder, suggesting the presence of curcumin on the surface of the ZnO nanoparticles (El-Kattan et al., 2022). The successful formation of Cur-ZnONPs indicates that curcumin acted as a stabilizing and functionalizing agent during the synthesis process. **Fig. 1** illustrates the

images of curcumin, synthesized ZnONPs, and Cur-ZnONPs.

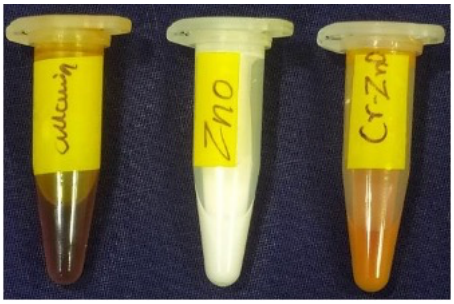

Fig. 1. Fabrication of curcumin loaded Zinc oxide nanoparticles (Cur-ZnONPs)

3.2. TEM-EDX analysis of Cur-ZnONPs

The TEM images provide detailed information on the morphology and structural characteristics of the synthesized nanoparticles. In **Fig. 2**(a), the TEM micrograph of ZnO nanoparticles shows aggregated clusters composed of small quasi-spherical particles with relatively uniform contrast. The individual particles appear to be nanosized and closely packed, forming irregular agglomerates, which is commonly observed for metal oxide nanoparticles due to their high surface energy. The scale bar (50 nm) indicates that the primary particle size is within the nanometer range, confirming the successful synthesis of ZnO nanostructures. In contrast, **Fig. 2**(b) illustrates the TEM image of Cur-ZnO nanoparticles, where ZnO particles are modified or stabilized by curcumin molecules. Compared with the bare ZnO nanoparticles, the Cur-ZnO nanostructures exhibit a slightly more dispersed morphology with particles embedded within a lighter amorphous matrix. This surrounding layer is likely attributed to the organic curcumin coating on the nanoparticle surface. The presence of this coating reduces the degree of particle agglomeration and provides a stabilizing effect, indicating successful functionalization of ZnO nanoparticles with curcumin. The nanoscale dimensions remain consistent with those observed for bare ZnO, suggesting that the modification process does not significantly alter the particle size but rather improves the surface characteristics and dispersion of the nanoparticles.

Fig. 2C demonstrates the energy dispersive X-ray (EDX) spectrum of Cur-ZnONPs. The results confirm the presence of elemental composition in the synthesized Cur-ZnO such as zinc (Zn), oxygen (O), and carbon (C). The strong Zn peaks observed around the characteristic energy levels indicate the presence of zinc as the primary metallic element in the nanoparticles, while the oxygen peak confirms the formation of zinc oxide (ZnO). Quantitative analysis reveals that zinc constitutes approximately 63.7 wt%, while oxygen accounts for about 22.1 wt%, which is consistent with the expected composition of ZnO nanostructures. Additionally, the presence of carbon with a weight percentage of approximately 14.2 wt% further supports the successful incorporation of curcumin onto the surface of the ZnO nanoparticles (Faisal et al., 2021). The carbon signal originates from the organic functional groups present in the curcumin molecules used for modification. The absence of other significant elemental peaks in the spectrum indicates the high purity of the synthesized Cur-ZnO nanoparticles without detectable impurities. Overall, the EDX analysis verifies the elemental composition and confirms the successful formation and surface functionalization of ZnO nanoparticles with curcumin.

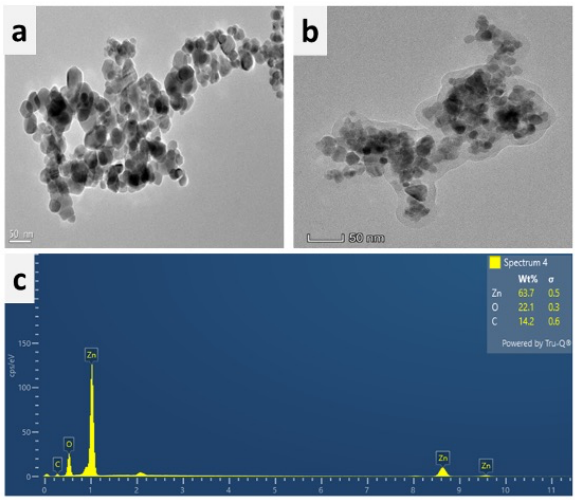

Fig. 2. TEM image of (a) ZnO and (b) Cur-ZnONPs. (c)EDX micrograph of Cur-ZnONPs.

3.4. FTIR spectroscopy of Cur-ZnONPs

As shown in **Fig. 3**, the FTIR spectrum of Cur-ZnONPs confirms the presence of functional groups involved in nanoparticle formation and stabilization. A broad absorption peak observed at 3344 cm^{-1} corresponds to the stretching vibration of hydroxyl (–OH) groups, indicating the presence of phenolic groups from curcumin and surface-bound water molecules. The peaks at 1699 cm^{-1} and 1603 cm^{-1} are attributed to C=O and aromatic C=C stretching vibrations, confirming the presence of curcumin molecules on the nanoparticle surface. Additional bands at 1532 cm^{-1}, 1443 cm^{-1}, and 1310 cm^{-1} correspond to aromatic ring vibrations and C–O stretching, while peaks at 1172 cm^{-1}, 1084 cm^{-1}, and 1013 cm^{-1} are related to C–O–C and C–O functional groups. The characteristic absorption band

at 590 cm^{-1} represents the Zn–O stretching vibration, confirming the successful formation of ZnO nanoparticles. Overall, the FTIR results indicate that curcumin is successfully attached to the ZnO nanoparticle surface and acts as a capping and stabilizing agent during synthesis (Lu et al., 2019).

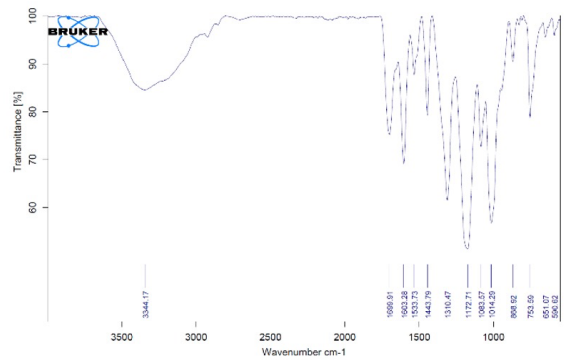

Fig. 3. FTIR spectra of Cur-ZnONPs.

3.5. Antibacterial activity against *P. aeruginosa*

The antibacterial activity of curcumin, zinc oxide nanoparticles, and curcumin-loaded ZnO nanoparticles against *P. aeruginosa* was evaluated using the zone of inhibition (ZOI). Curcumin alone showed a ZOI of 9 mm, while ZnO nanoparticles produced a slightly higher inhibition zone of 10 mm (**Fig. 4**a). Interestingly, the curcumin with ZnO nanoparticles exhibited a larger inhibition zone of 13 mm. The positive control antibiotic, Ciprofloxacin, showed ZOI of 18 mm. As shown in **Fig. 4**b, the MIC results of Cur-ZnONPs indicates that 90% inhibition was observed at 75 µg/mL against *P. aeruginosa*.

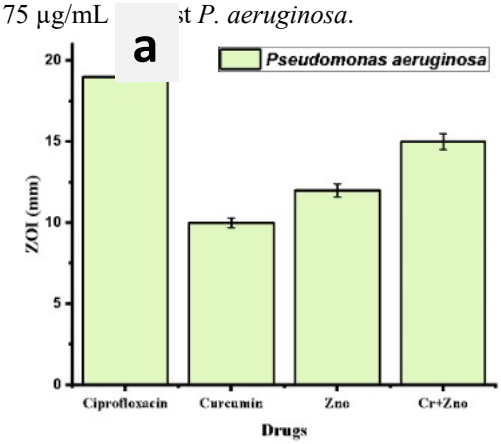

Fig. 4. (a) Zone of inhibition (ZOI) measurement for ZnO, curcumin and Cur-ZnONPs against *Pseudomonas aeruginosa* and (b) MIC

3.6. Minimum bactericidal concentration

The minimum bactericidal concentration analysis demonstrated that Cur-ZnONPs exhibited a clear concentration-dependent antibacterial effect against *P. aeruginosa*. The percentage of bacterial inhibition increased gradually with increasing nanoparticle concentration, showing approximately 20% inhibition at 10 ng/mL, 40% at 100 ng/mL, 60% at 1 µg/mL, 85% at 10 µg/mL, and reaching about 98% inhibition at 100 µg/mL, which was considered the MBC (**Fig. 5**).

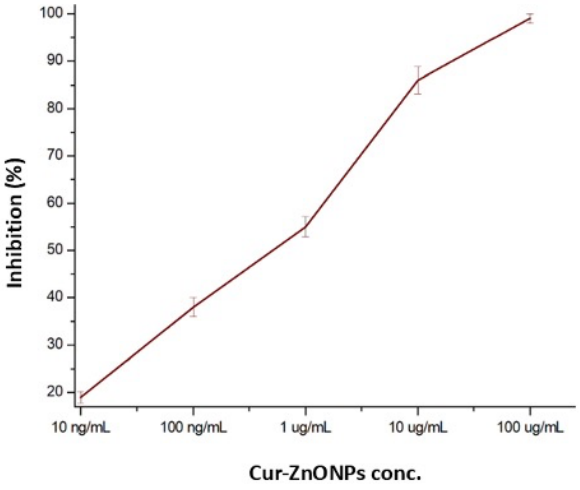

Fig. 5. Minimum Bactericidal Concentration (MBC) of Cur-ZnONPs against *Pseudomonas aeruginosa*

3.7. Antibiofilm studies

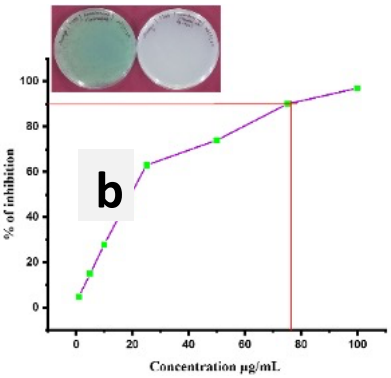

Fig. 6. Biofilm study. (a) Biofilm formation on teeth and (b) Treatment of *Pseudomonas aeruginosa* biofilm formed on teeth.

P. aeruginosa biofilm formation on molar teeth was carried out in nutrient broth medium. The control (*P. aeruginosa* biofilm without treatment) and Cur-ZnONPs treated *P. aeruginosa* biofilm was used to analysis the preventions of biofilm]m formation by CV staining. As shown in **Fig. 7** indicates that more than 95% prevention was achieved at 0.75xMIC (56 µg) and a complete inhibition was achieved at 1.0xMIC (75 µM).

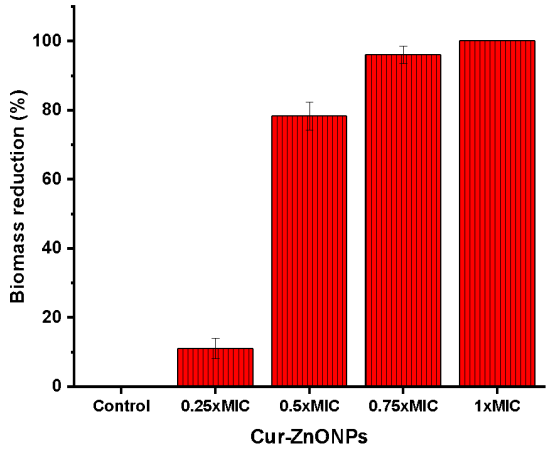

Fig. 7. Prevention of *P. aeruginosa* biofilm formation by Cur-ZnONPs.

CLSM was used to evaluate the effect of Cur-ZnONPs on *P. aeruginosa* biofilms. Before treatment, the biofilm appeared dense with predominantly green fluorescence, indicating a high number of live bacterial cells. After treatment with Cur-ZnONPs, there was a significant increase in red fluorescence, representing dead cells, along with a disrupted biofilm structure and reduced bacterial density. This indicates that Cur-ZnONPs effectively penetrate and damage the biofilm, causing bacterial cell death. The antibacterial effect is likely due to the synergistic action of ZnO nanoparticles generating reactive oxygen species and curcumin improving nanoparticle stability and penetration into the biofilm matrix. These findings confirm that Cur-ZnONPs can effectively eradicate biofilm-associated *P. aeruginosa*, highlighting their potential as a therapeutic antibiofilm agent.

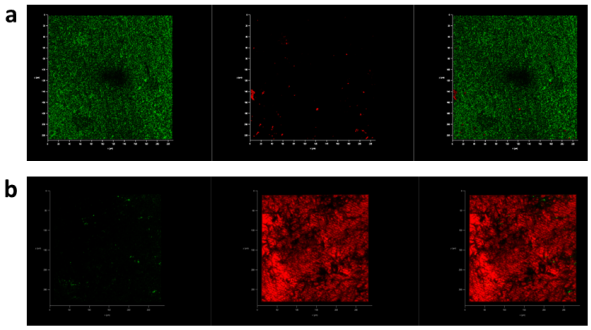

Fig. 8. CLSM bioimaging study (a) before and (b) after treatment of biofilm by Cur-ZnONPs

4. Discussion

The results of this study clearly show that curcumin-loaded zinc oxide nanoparticles (Cur-ZnONPs) were successfully prepared and exhibited improved antibacterial activity against *P. aeruginosa*. The formation of a yellow color and TEM analysis confirmed nanoscale particle formation, while the curcumin coating reduced particle aggregation and enhanced stability. EDX and FTIR results further confirmed the successful attachment of curcumin onto the ZnO nanoparticle surface, indicating its role as a stabilizing and functionalizing agent. In antibacterial studies, Cur-ZnONPs showed better activity than curcumin or ZnO alone, with a larger zone of inhibition, an effective MIC at 75 µg/mL, and strong bactericidal activity at higher concentrations. This enhanced effect can be explained by the combined action of ZnO nanoparticles generating reactive oxygen species and curcumin improving penetration and damage to bacterial cells. CLSM analysis also supported these findings, where untreated biofilms showed more green fluorescence (live cells), while treated samples showed increased red fluorescence (dead cells) along with disrupted biofilm structure (Vadakkan et al., 2025). Similar results have been reported by Shome et al., (2023), where curcumin-loaded ZnO nanocomposites showed significant antibiofilm activity against *Pseudomonas aeruginosa*, achieving around 49% biofilm inhibition at 300 µg/mL

through mechanisms such as oxidative stress, lipid peroxidation, and enhanced bacterial cell death compared to ZnO nanoparticles alone.

5. Conclusion

This study shows that Cur-ZnONPs are effective in controlling and removing dental biofilms formed by *P. aeruginosa*. The combination improves the limitations of curcumin, such as poor solubility, and enhances its antibacterial activity with the help of zinc oxide nanoparticles. The results showed that as the concentration increased, bacterial growth decreased and the biofilm was effectively disrupted. This effect may be due to better penetration of nanoparticles into the biofilm and damage to bacterial cells. Overall, Cur-ZnONPs can be considered a promising and safe approach for treating dental biofilm-related infections, especially those caused by resistant bacteria, and further studies are needed to confirm their use in clinical applications.

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