Nanotechnology Perceptions
ISSN 1660-6795
www.nano-ntp.com

Assessment of Time and Dose-Dependent Killing Assay of Azadirachta Indica Bark Extract and Solanum Xanthocarpum Seed Extract Developed ZNO NPS against Dental Pathogens

Dr. Atluri Manoj , Dr. Manish Ranjan, Dr. Krishnakanth Jaju, Dr. C. Raghavendran

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

Introduction: The discipline of nanomaterials is now under intensive investigation. Because of their nanoscale size, nanoparticles (NPs) find application in a wide range of industries, including medicine and engineering. Since zinc (Zn) is used in many different medicine delivery methods and other sectors, it seems to be the most attractive metal nanoparticle (NP). The nonpolluting and reasonably priced NPs employed in this work were made using a sustainable synthesis. Both the bark extract of Azadirachta indica and the seed extract of Solanum xanthocarpum have strong secondary metabolite contents and antibacterial effects. In this work, zinc oxide nanoparticles (ZnO NPs) were synthesized from the aqueous extracts of A. indica and S. xanthocarpum seed extract, and their antibacterial activity towards oral bacteria was evaluated. Methodology: After an extracted powder made from the dehydrated extracts of S. xanthocarpum seeds and A. indica bark, an aqueous extract was made. The aqueous extract was then combined with 150 mL of Zinc nitrate solution and mixed in an orbital shaker to produce ZnO NPs. For confirmation of their formation, they were examined both visually and via ultraviolet (UV) spectrophotometry. Streptococcus mutans and Enterococcus faecalis, both different oral bacterial strains, were employed in the well diffusion approach to determine the antimicrobial activity of these ZnO NPs. Results: It was observed that zinc oxide (ZnO) nanoparticles showed a deepening of coloring during the fabrication phase. Furthermore, at a wavelength of 296 nm, the UV spectrum analysis showed a noteworthy absorption value of 1.2. The dimension of the zone of inhibition was used to determine the antibacterial effect against S. mutans and E. faecalis. The inhibition zones for S. mutans and E. faecalis have been shown to be 14.5 ± 0.2 and 17.5 ± 1.2 mm, respectively, at a dose of 100 µg/mL of ZnO NPs. Conclusion: The current study uses seed extracts from A. indica and S. xanthocarpum to synthesize ZnO NPs in an ecologically friendly and sustainable way. The antibacterial properties of ZnO NPs against oral infections suggest that dental materials may contain nanomaterials. These NPs'

antibacterial effects and green production process make them interesting candidates for research in nanomedicine and oral health.

Keywords: Green synthesis, ZnO NPs, Antibacterial activity, Dose response assay.

1. Introduction

The capability of bacteria to overcome the inhibitory properties of antimicrobial agents is referred to as antimicrobial resistance. As these microorganisms become resistant, the efficacy of treatments that work against them decreases (Thomas 2020). These microorganisms may tolerate the effects of numerous drugs, a condition known as multidrug resistance (MDR). Microbes have been observed to demonstrate a variety of resistance mechanisms, including genetic alterations, innate resistance, and conjugation-induced adoption of resistance profiles from other organisms (Abushaheen et al. 2020). The misuse or negligent use of antimicrobial drugs is contributing to the global increase of antibiotic resistance (Medical Association 2022). Managing resistant microorganisms presents considerable difficulties, requiring the use of stronger prescription dosages or different antimicrobial options. However, limitations or an inadequate supply of effective drugs have an adverse impact on the majority of countries (Muloi et al. 2023).

There are many potential uses for nanotechnology in the detection and treatment of many ailments, making it a potentially exciting subject. Nanomaterials may consist of material particles with a size of nanometers. They come in several forms, including nanoparticles (NPs), nanorods, nanosensors, and nanorobots. Because of their enhanced surface-volume proportion, hardness, reactivity, bioavailability, nanoscale size, durability, and optical qualities, metal oxide nanoparticles (NPs) have drawn a lot of interest. Nanomedicine is a more efficient and secure therapeutic approach than traditional medicine because it allows for focused administration of drugs, less cytotoxicity, enhanced solubility, permeability, and absorption with delayed release of pharmaceuticals. Possible anti-inflammatory, antibacterial, antioxidant, and anti-carcinogenic properties of these NPs have been investigated. NPs based on gold, silver, copper, and zinc have all been extensively studied. It should be noted that minimal study has been done on the potential of zinc oxide nanoparticles (ZnO NPs). These metal oxide nanoparticles are very stable, non-toxic, and biocompatible; that is, they may have therapeutic uses.

Numerous aspects of phytochemical molecules have been studied for a range of biological applications. The green synthesis technique offered a different strategy for producing NPs in a cost-effective, ecologically responsible, and sustainable manner while lowering the use of dangerous chemical substances. Furthermore, the plant extract phytochemical components may function as NP synthesis capping and minimizing agents. To the best of our knowledge, no previous research has examined the biological characteristics of nanoparticles of zinc oxide that are greenly generated (ZnO NPs). The aim of this work was to synthesize ZnO NPs and examine their antibacterial, time- and dose-dependent killing properties(1). The findings from this study will assist with the development of a more sustainable and efficient strategy for dealing with the known risks caused by populations of bacteria.

2. Materials and methods

Collection of plants

A. indica and S. xanthocarpum plant seeds were extracted, and the plants came from Gutoor Village in Krishnagiri District, Tamil Nadu, India. The collected seed and bark were thoroughly cleaned with distilled water, then dried, crushed into a powder, and kept at room temperature until required again.

Green synthesis of ZnO NPs

A 5 mM aqueous solution of zinc nitrate was made using double-distilled water. A 200 mL Znn mixture was then added to a conical flask, and about 200 mL of the previous aqueous extract was added dropwise while being constantly stirred in an orbiting shaker(2). The synthesized material was visually viewed and then subjected to further analysis using an ultraviolet (UV) spectrophotometer operating 8within the 200–800 nm wavelength region(3). Following biosynthesized samples, a 10,000 rpm centrifuge was used. After being divided, the pellets were heated to 65 °C for 24 hours in a hot air oven.

Characterization of ZnO NPs

ZnO-NPs were produced and their properties were assessed using UV spectroscopy (Labman Double Beam UV-Vis spectrophotometer LMSPUV1900S, India, 190-1100 nm) at T0 and T24 between 190 and 800 nm. The interaction between the functional group and the metal ions was investigated at ambient temperature using FTIR (Bruker Alpha II, Germany) in the range of 500–3500 cm-1. FE-SEM (JEOL~800S) was utilized to examine the molecules' morphological characteristics using the chemical makeup of the NPs was investigated using both FE-SEM and EDX (OXFORDX-Plor-30/C-Swift) spectroscopy.

Antibacterial activity of synthesized ZnO NPs

The antibacterial properties of the produced ZnO-NPs were thoroughly evaluated using the bacterial strains S. mutans and E. faecalis. The inhibitory zone width was used to evaluate the antibacterial properties. The well diffusion method is employed to investigate the susceptibility of bacterial strains to antibiotics in a clean zone surrounding the well(4). The inoculum containing the strain of bacteria that will be evaluated was placed on nutrient agar plates using a sterile brush dampened with the suspension of bacteria. Agar media served to create holes with a diameter of around 6 mm. ZnO-NPs were added in different quantities (50 and 100 µg/ml), and streptomycin (10 µg/ml) was employed as a positive control.

Time killing kinetic assay

Following overnight growth in MHB broth, the bacteria were grown again in fresh medium for four hours and then placed in deionized water. This approach was adopted considering PBS's ions of chloride, which interact with metal, are absent from deionized water. Antibiotics (Positive control) and ZnO NPs were applied to the cells for four hours at 37 °C. 100 µL of portions from each sample were taken after the appropriate times to obtain bacteria in order to estimate the CFUs; these samples included a negative control (MHB and inoculum without ZnO NPs) and a positive control (ZnO NPs and MHB media without inoculum). Every sample was made three times(5).

Statistical analysis

IBM SPSS Statistics for Windows, Version 23.0 (which was released in 2015; IBM Corp., Armonk, NY, USA) was used to collect, summarize, and evaluate the results. The antibacterial activity of ZnO NPs was evaluated using a one-way ANOVA compared to the antibiotic standard, with a p value of < 0.05 considered to be significant.

3. Results

UV-visible spectroscopy

The produced ZnO-NPs were found to have particular features and were described for their fundamental investigations. The ZnO-NPs UV-Vis spectrophotometer was examined, and the absorbance was measured. The development of ZnO-NPs was confirmed by the peak that developed after 24 at 296 nm.

FE-SEM and EDAX analysis

The microscopic characteristics of nanomaterials, such as thin films and nanopowders, may be extensively studied using FE-SEM. Furthermore, further information on the composition of the materials and structure may be obtained using the signals from the sample. The ZnO-NPs from the plant extract were subjected to FE-SEM examination, which revealed that their size ranges from 20 to 30 nm. They have an accumulated or spherically cluster-like shape (Fig.1).

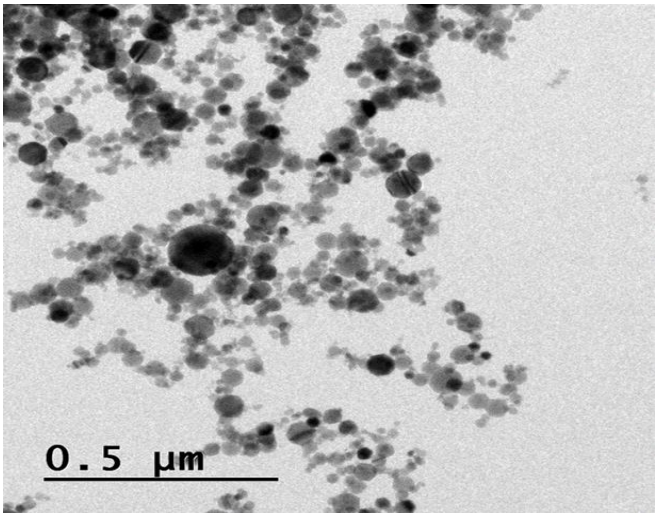

Fig.1. FE-SEM analysis of biosynthesized ZnO NPs.

EDAX analysis

The SEM was applied in combination with the EDX to analyze its elemental composition. When a sample is activated by an energy source, some of the energy it has received goes out as core-shell electrons. After that, the energy difference is released as an X-ray with a certain wavelength that is established by the starting atoms. Whenever a more energetic electron from the outermost layer fills the gap, this occurs. Consequently, it is possible to identify the

contents of a sample volume that has been triggered by an energy source. The high peaks for zinc at 20.2% and oxygen at 45% are shown in Fig. 2. It then determines that ZnO-NPs are present in the sample.

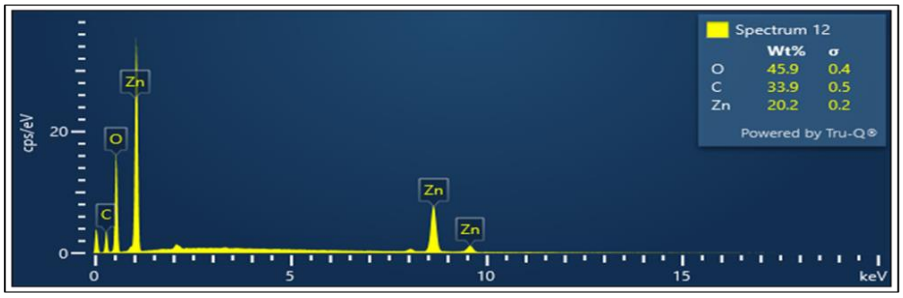

Fig. 2. EDAX analysis of biosynthesized ZnO NPs

Antibacterial activity of ZnO NPs

The zone of inhibition around the wells from the back of the plate was used to measure the antibacterial activity of green-synthesized ZnO NPs against two distinct oral pathogens, S. mutans and E. faecalis (Figure 3 and 4). At two concentrations, the NPs demonstrated remarkable antibacterial activity, with inhibition zones of 14.5 ± 0.2 and 17.5 ± 1.2 mm for S. mutans and E. faecalis at a ZnO NP concentration of 100 µg/mL, respectively. Moreover, the inhibitory zones for the corresponding microorganisms were 12 ± 0.5 and 15 ± 0.5 mm at a concentration of 50 µg/mL.

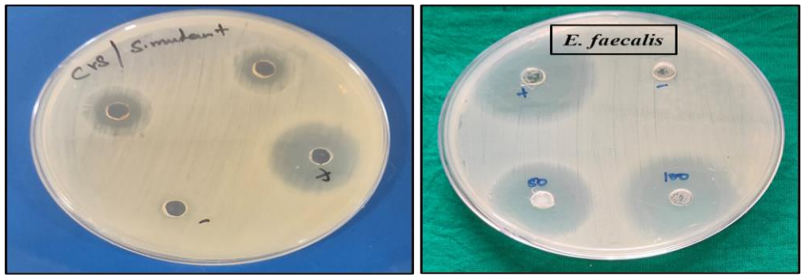

Fig.3. Antibacterial activity of synthesized ZnO NPs against dental caries causing pathogens

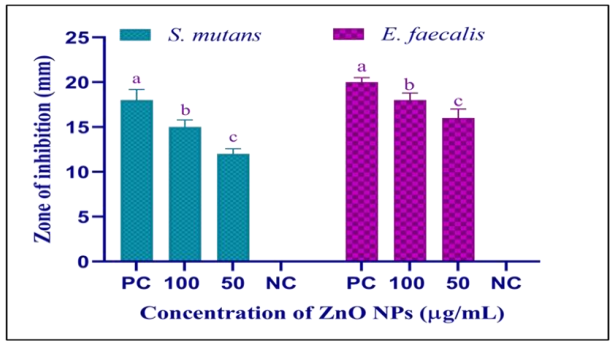

Fig. 4. Zone of inhibition observation on ZnO NPs against S. mutans and E. faecalis. Mean values within the column followed by the same letter in superscript are not significantly

different at p< 0.05 level.

Dose dependent killing kinetic assay

ZnO NPs did not significantly differ in their antibacterial inhibition of the different bacteria; thus, the killing kinetic test was carried out to evaluate the survivability of the bacteria after treatment and to find the minimum duration required to have an inhibitory or bactericidal effect. (Fig.5) shows the time-kill curve of ZnO NPs against strains of S. mutans and E. faecalis(6). The bacterial population was completely eliminated after 8 hours of exposure to ZnO NPs at the appropriate MBC values for both strains (as determined by the McFarland Standard). Bacteriocidal activity increased gradually over this period. ZnO NPs caused an early stationary phase by demonstrating fast and time-dependent bactericidal effects against the pathogens that were examined.

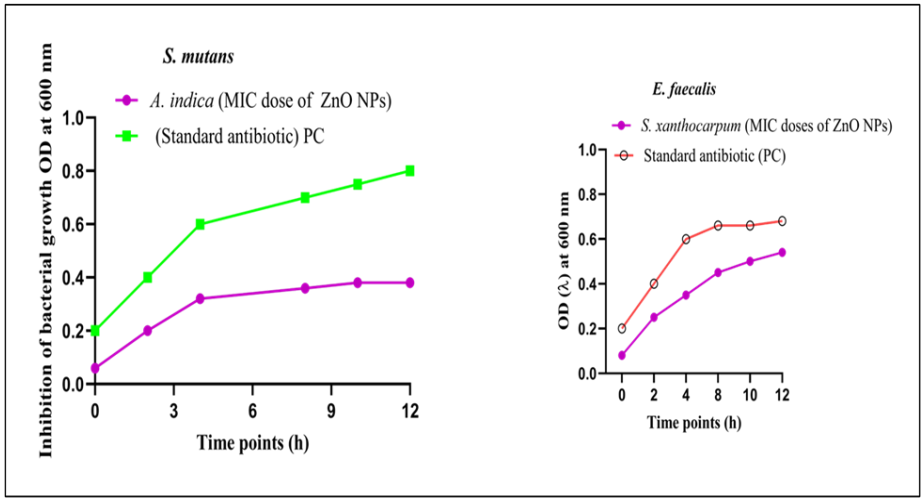

Fig.5. Estimation of time- and concentration-dependent killing kinetic assays of ZnO NPs against dental bacterial strains. S. mutans & E. faecalis. A respective MIC value was applied to observed significant time points. OD was measured in a spectrophotometer at 600 nm wavelength. Nutrient broth was used as a blank. Positive control represents the bacterial culture without ZnO NPs treatment. Here, 0: 0 h exposure, 2: 2 h exposure, 4: 4 h exposure, 6 6 h exposure, 8: 8 h exposure, 10: 10 h exposure, and 12: 12 h exposure.

4. Discussion

Finding efficient therapy techniques that attempt to completely eradicate the pathologic causes of the illnesses is crucial given the increase in the frequency and severity of periodontal disease. Although this is the goal of mechanical therapy, we can more effectively control the established, advanced, and aggressive types of ailments by using additional therapies that have antibacterial and antioxidant qualities(11). In the current work, the antibacterial and dose-dependent kinetic assays of green-produced ZnO NPs utilizing A. indica and S. xanthocarpum were evaluated. The color shift of the nanoparticles—from a light yellow to brown precipitate—confirmed the biosynthesised ZnO-NPs visibly. The phytochemical substances

found in S. xanthocarpum and A. indica are what cause the reduction and serve as a stabilizing element. The existence of ZnO-NPs is confirmed by the evidence of color change. UV-Vis spectroscopy was used to analyze the ZnO-NPs, and after 24 hours, a peak developed at 296 nm. In contrast to a related investigation conducted by Pillai et al. (2020), the primary peak was seen at 284 nm, hence verifying the synthesis of ZnO-NPs. Zinc nanoparticles biosynthesized from Lupinus albus aqueous extract demonstrated the maximum absorbance at 374 nm, similar to a previous investigation (Muhammed, Asere, and Diriba 2024). ZnO NPs with a maximal absorbance peak at 352 nm were produced from Atalantia monophylla leaf extract in a different investigation (Ragavendran et al. 2023).

The synthesized ZnO-NPs derived from plant extract showed a maximal zone of inhibition against S. mutans in their antibacterial effect against E. faecalis. It was demonstrated that ZnO-NPs significantly affected E. faecalis. Proteins and cell structures become non-functional with the release of metal ions (Maret and Wedd 2014). The principal mechanisms of ZnO-NPs antibacterial impact are their electrostatic interaction with the bacterial outer membrane or cell wall and the generation of nano Zn+ ions. ZnO NPs that were biosynthesized using A. marina leaf extract demonstrated an inhibitory zone against S. aureus with a width of 10.87 ± 1.33 mm in a different investigation (Gnanadesigan et al. 2012). The findings showed that at both low and high concentrations, the green-synthesis ZnO NPs exhibited outstanding antibacterial efficacy against both bacterial species. ZnO NPs demonstrated an expanded zone of inhibition and relatively higher antibacterial activity than the antibiotic standard. It has been demonstrated that ZnO NPs create H_2O_2, which causes oxidative damage in microbial species(6). Reactive species are produced as a result, and this results in cell death. ZnO NPs have also been connected to cellular rupture of membranes and fluid leakage after physical contact(7). Their larger surface area facilitates stronger cell contacts, and their comparatively small size helps them penetrate the cells more quickly(Al-Shabib et al. 2020). It has been proven that higher ZnO NP doses harm DNA, proteins, and cells. The findings of this study antibacterial test are consistent with previous research showing ZnO NPs ability to combat both gram-positive and gram-negative bacteria.

According to the growth curves of the bacteria exposed to ZnO NPs, the substance may inhibit the development and reproduction of bacteria. The MIC values of biogenic ZnO NPs were determined using a broth culture of certain bacteria. ZnO NPs showed greater antibacterial activity against E. faecalis and S. mutans (MIC 4.50 µg/mL). For every bacterial strain, the absorbance (λmax 600) decreased as the quantity of nanoparticles increased. When ZnO NPs were used as a therapy, the drug concentration was discovered when there was no longer any detectable proliferation on the agar plate. Similarly, biogenic silver nanoparticles made from Phyllanthus niruri leaf extract showed broad-spectrum antibacterial and antibiofilm activities, as reported by (Kumar et al. 2023). Overall, using A. indica and S. xanthocarpum in a herbal formulation to synthesize zinc oxide nanoparticles shows promise for managing oral infections. Zinc oxide nanoparticles produced via green synthesis and their antibacterial properties. The full potential of zinc oxide nanoparticles as useful tools for managing oral health may be revealed by more research in this domain(22).

5. Conclusion

The current work presents a successful green method for the synthesis of ZnO NPs using the bark and seed extracts of A. indica and S. xanthocarpum as the bio-reductants. Evaluation approaches, including FE-SEM, EDX, and UV-Vis, further validated it. When our ZnO-NPs were evaluated against S. mutans and E. faecalis, they demonstrated significant antibacterial efficacy against both organisms. Furthermore, the results showed that ZnO-NPs that are biosynthesized had potent antibacterial and dose-dependent characteristics. To fully comprehend ZnO-NPs' biological possibilities in vivo and in vitro, more investigation is required. ZnO-NPs addressed above are therefore being explored for use in biological applications, including medicines and therapeutics.

Acknowledgement

The authors express their gratitude for the provision of research laboratory facilities by the Department of Cariology at Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University.

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