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Original Article

Green synthesis of zinc oxide nanoparticles using *Boswellia serrata* extract for antibacterial and antibiofilm applications against oral pathogens

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Abstract

Increasing outbreak of antibiotic resistant bacteria in the world has made the discovery of safe and sustainable antimicrobial agents even more vigorous. In this study, zinc oxide nanoparticles (ZnO-NPs) were synthesized through a green methodology using *Boswellia serrata* (Gum Olibanum) extract and tested their antibacterial and antibiofilm activity against oral pathogens. The ZnO-NPs were characterized by X-ray diffraction (XRD) analysis, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), energy dispersive X-ray spectroscopy (EDX) which confirmed the crystalline structure, nanoscopic morphology and the elemental composition of the nanoparticles. The ZnO-NPs were highly antibacterial with a diameter of inhibition zone of 47 mm and 38 mm on *Streptococcus spp.* and *Granulicatella adiacens* respectively. The nanoparticles were also found to exhibit a substantial biofilm inhibitory effect, decreasing adhesion of *Streptococcus pneumoniae, Streptococcus gordonii, Streptococcus mitis*, and *Granulicatella adiacens*, suggesting the ability of the nanoparticles in disrupting the formation of oral biofilm. The results indicate that Boswellia serrata-mediated ZnO-NPs could be used as a potent and environmentally friendly antimicrobial agent in potential oral care products. There in vivo biocompatibility, toxicity and applicability in clinical use need further research.

Keywords: Zinc oxide nanoparticles, *Boswellia serrata*, Green synthesis, Antimicrobial activity, Biofilm inhibition, Oral pathogens, Dental nanotechnology

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Introduction

Nanotechnology has revolutionized various fields of science by offering new solutions in medicine, agriculture, environmental science and materials engineering. Zinc oxide nanoparticles (ZnO-NPs) are commonly explored among other nanomaterials because of their optical, chemical, antimicrobial, and photocatalytic characteristics (Gong et al., 2024; Khan et al., 2019). They are good biomedical candidates because of their high surface area to volume ratio, photostability, and biocompatibility (Yan et al., 2024; Yang et al., 2022).

The traditional ways of producing ZnO-NPs such as sol gel and chemical precipitation techniques usually used toxic substances, high temperatures, and energy demanding protocols, which restrict their clinical safety and environmental sustainability (Jiang et al., 2022; Xu et al., 2021). Green synthesis is an environmentally sustainable where biological materials, particularly plant extracts are used as natural reducing and stabilizing agents. Plant mediated synthesis is preferable because it is simple and economical and has a good phytochemical composition (El-Nour et al., 2023; Gong et al., 2024)

Moringa oleifera, Aloe vera, Trianthema portulacastrum, and Cassia siamea are some examples of plants that have utilized to make ZnO-NPs with remarkable antimicrobial and antibiofilm properties (Asif et al., 2022; Prakash et al., 2024; Ahamad Khan et al., 2023). Nevertheless, the selection of plant species has a substantial impact on the size, morphology, stability, and bioactivity of nanoparticles, and therefore, it is crucial to find new botanical origins.

Boswellia serrata (Indian frankincense) is a botanical candidate of nanomaterial synthesis that is not widely studied. Its gum resin (olibanum) comprises boswellic acids, terpenoids, flavonoids and other bioactive phytochemicals with anti-inflammatory, antioxidant and antimicrobial effects (Sharma and Jana, 2020). Although it is important in terms of pharmacology, there is no literature that has tested its potential in ZnO-NP production and its use as a reducing and capping agent has not been reported.

Gram-positive bacterial species such as *Streptococcus mitis, Streptococcus gordonii*, and *Granulicatella adiacens* are commonly associated with oral infections like periodontitis, negative and positive oral caries and biofilm related diseases. Both the clinical challenge and the growing resistance of these species to antibiotics represent a major clinical problem with the growing complexity of biofilms in which they can form.(Wypij et al., 2021; Husain et al., 2022). New antimicrobial strategies that have the potential to attack both planktonic and biofilm cells are thus in great demand.

ZnO-NPs based on the plant-derived products have been reported to carry out a multimodal mechanism of antibacterial action, including membrane damage, protein release, generation of reactive oxygen species (ROS), and disruption of quorum sensing signaling in the formation of biofilms.(Yang et al., 2022; Yan et al., 2024). All these mechanisms make them more active against planktonic and biofilm forming bacteria. (Husain et al., 2022) .

Despite the previous research on plant mediated ZnO-NPs, oral pathogens and any form of ZnO-NP application to *Boswellia serrata* resin have not been reported before. In addition, it has not been tested against important oral biofilm forming bridging bacteria, so the study is a new contribution to green nanotechnology and oral infectious disease control. The purpose of the study was:

To prepare and characterize the ZnO-NPs on an environmentally friendly green synthesis method through the use of *Boswellia serrata* extract.

To compare their anti-bacterial and anti-biofilm effects on selected oral pathogens.

Material and Methods

Material

All the chemicals used were of analytical grade. Zinc chloride ($ZnCl_2$), ethanol and hydrochloric acid (HCl) were supplied by Merck Research Laboratories (Germany). *Boswellia serrata* gum resin (Arabic gum/olibanum) was purchased locally. The experiment was done using distilled water. Four oral bacterial isolates (*Streptococcus pneumoniae, Streptococcus gordonii, Streptococcus mitis and Granulicatella adiacens)* were utilized in the antibacterial and antibiofilm assays.

Preparation of *Boswellia serrata* extract

In order to prepare the plant extract, 5g of Boswellia serrata gum resin was dissolved in 100mL of distilled water in a 200mL Erlenmeyer flask. The mixture heated at 80°C on a magnetic hot plate and mixed for 1 hour. The extract was cooled down and then filtered using Whatman No. 1 filter paper and stored at 4ºC until use.(Jamdagni et al., 2018).

Green synthesis of ZnO nanoparticles using *Boswellia serrata*

ZnO-NPs were prepared by a green synthesis process. $ZnCl_2$ (0.2M) solution was prepared by dissolving 6.05g of $ZnCl_2$ in 100mL of distilled water and 100uL of HCl was added to enhance solubility. A stabilizing agent in the form of 50mL of Arabic gum solution was added, followed by the addition of 100mL of Boswellia serrata extract.

The pH of the reaction mixture was brought to 11 with the help of 1M NaOH because ZnO nanoparticles are sensitive to alkaline conditions and require it to successfully be nucleated and grown. Then the mixture was stirred continuously and heated in a water bath at 80ºC for 2hours. A light yellow precipitate was obtained and left to settle 18hours. Precipitate was then centrifuged at 10,000rpm over 25minutes and rinsed three times with distilled water and dried in hot air oven at 90ºC overnight. Lastly, the dried powder was heated in 400ºC for 2hours to increase crystallinity and eliminate organic matter. The synthesis was conducted thrice to make it reproducible. (Jamdagni et al., 2018).

Characterization of ZnO nanoparticles

Scanning electron microscopy (SEM)

A Scanning Electron Microscope (Inspect 550, FEI, Netherlands) was used to investigate the surface morphology, the shape, and the distribution of the ZnO-NPs that had been synthesized. Samples were placed on carbon tape, sputtered to gold and observed under high vacuum at the right accelerating voltages (El-Belely et al., 2021).

X-ray powder diffraction (XRD)

The crystallographic and identification of phase of ZnO-NPs was done by employing Powder X-ray Diffraction (XRD-6000, Shimadzu, Japan) using Cu Kα (λ = 1.5406A). The patterns of diffraction were taken in a range of 2θ in 20°–80° and at a scanning rate that was appropriate to measure a nanoparticle. The resulting peaks were subsequently compared to standard JCPDS data of ZnO.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy (Perkin Elmer, USA) was used to help identify functional groups that were associated with ZnO-NPs. Mixing of the sample was done with ZnO-NPs and potassium bromide (KBr) before the samples were pelletized. The spectra were measured at 400-4000 cm^{-1}. This method was applied to identify phytochemical groups (e.g., phenolics, terpenoids, and boswellic acid related moieties) that can have their origin in *Boswellia serrata* which can have a reducing and capping effect. (El-Belely et al., 2021).

Energy dispersive X-ray spectroscopy (EDX)

The elemental composition of the ZnO-NPs was determined using energy dispersive X-ray spectroscopy (EDX), combined with the SEM instrument. The typical peaks of zinc and oxygen were recorded to ascertain the formation of ZnO nanoparticles (El-Belely et al., 2021).

Bacterial isolates

Plaque samples on patients diagnosed as periodontitis and with pocket depths of 3.5 to 7mm were collected in the Faculty of Dentistry, the University of Kufa. The samples were grown on Brain Heart Infusion (BHI) agar and isolates were identified using VITEK 2 Compact system (BioMerieux, France) according to the conventional microbiological procedures. The isolates were clinically relevant Gram-positive pathogens of the mouth in connection with biofilm formation and periodontal disease(Jamdagni et al., 2018).

Preparation of bacterial suspensions

The bacterial cultures were incubated in the brain heart infusion broth at 37º C 18-24 hours. The bacterial suspensions were brought to a scale of 0.5 McFarland using the Kirby-Bauer standard with the help of a spectrophotometer at 625nm (Jamdagni et al., 2018).

Antibacterial assay

Agar well diffusion method was used to determine the antibacterial activity of the ZnO-NPs synthesized. Muller Hinton Agar (MHA) plates were prepared with the aid of sterile cotton swab and 0.1mL of 0.5 McFarland bacterial suspension was placed and left to dry within 10-15minutes. A sterile cork borer was used to make the wells of 6mm diameter and 100µL of ZnO-NP suspension at a concentration of 1mg/mL (or µg/mL) was added to each well. The plates were kept at 37°C and allowed to incubate after 24hours and the areas of inhibition were realized in millimeters with a digital caliper. All tests were conducted three times (n = 3) (Romadhan et al., 2016).

Biofilm inhibition assay

A flat bottom 96 well microtiter plate was used to determine the antibiofilm activity of ZnO-NPs. BHI broth (200µL), ZnO-NPs (100µL at 1mg/mL) and bacterial suspension (20µL) were added to each well. Only BHI broth and bacterial inoculum but without nanoparticles were used in control wells. The plates were incubated at 37°C and 24hours.

Following incubation, the wells were washed three times using phosphate buffered saline (PBS) to remove non adherent cells and dried. Biofilms were incubated with 100µL of 1% crystal violet during 15minutes, washed in order to eliminate the excess Crystal Violet and destained with 200µL of absolute methanol. A microplate (ELISA) reader was used to record the absorbance of the released dye at 630nm. (Jamdagni et al., 2018). Biofilm inhibition was calculated using:

$$\textbf{Percentage inhibition}\ (\%) = \left[\frac{\textbf{Acontrol} - \textbf{Atreated}}{\textbf{Acontrol}}\right] \times \textbf{100}$$

Statistical analysis

The experiments were replicated three times and the results presented as mean ± standard deviation (SD). The analysis of data was performed by means of SAS software version 9.1 (SAS Institute Inc., USA). Statistical significance was determined by using one-way ANOVA with post-hoc test by Tukey. The results of statistical significance were accepted when $p < 0.05$ (Sedgwick, 2014).

Results

FTIR analysis

The FTIR spectrum of the synthesized ZnO-NPs is depicted in figure 1. The broad peak at 3481.51 cm^{-1} is attributed to the presence of the OH stretching vibrations, as it is actually the presence of phenolic and hydroxyl groups that are rich in the resin of Boswellia serrata. C=O stretching and bending vibrations of carboxylic or carbonyl groups (represented by 1662.64 and 1635.64cm^{-1}) are also the common features of boswellic acids and terpenoids found in the extract.

The C-H bending and symmetric COO^- bands at 1440.83 and 1379.15cm^{-1} indicate the presence of organic stabilizing molecules in the resin. C-O and C-H out of plane bending vibrations of aromatic or terpenoid compounds are linked to peaks of 817.82 and 754.17cm^{-1}.

Notably, the presence of a distinct ZnO stretching band in the typical area of 600-800 cm^{-1} proves the formation of ZnO nanoparticles.

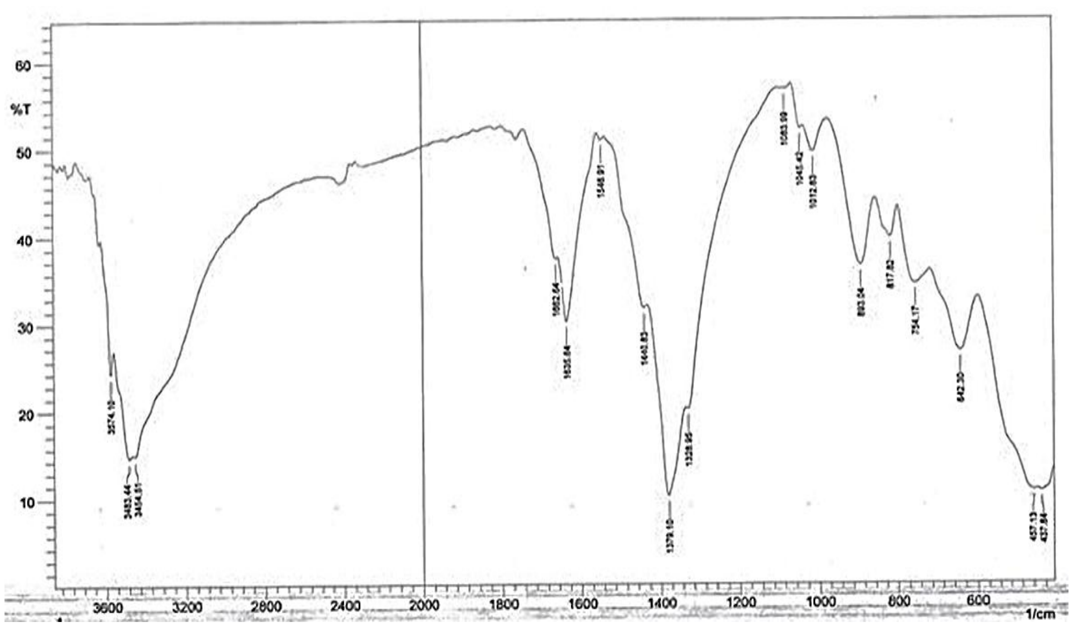

Figure-1. FT-IR spectra of synthesized ZnO nanoparticles using Boswellia serrata.

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X-rays diffraction (XRD)

The XRD analysis has verified the crystalline structure of ZnO-NPs synthesized. The sharp diffraction patterns at 2θ of 31.88, 34.54, 36.43, 47.58, 56.68, 62.98, 68.03 angles were represented as lattice plane of (100), (002), (101), (102), (110), (103), and (112), respectively (in Figure 2). These diffraction patterns are consistent with the reference JCPDS card of ZnO, which shows the presence of hexagonal wurtzite-phase ZnO (Haiouani et al., 2024). The sharp and intense peaks indicate that the nanoparticles are pure with high crystallinity.

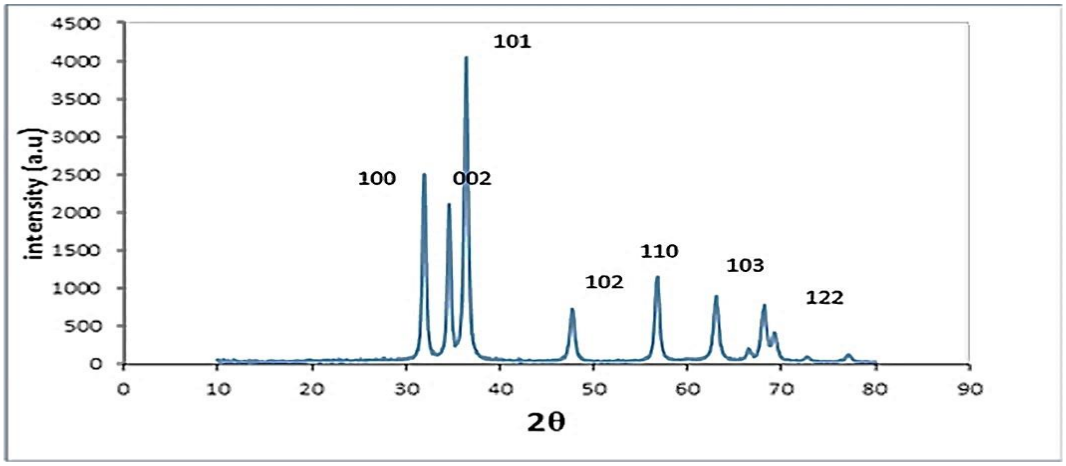

Figure-2. XRD Patterns for Boswellia serrate-ZnO-NPs.

Scanning electron microscope (SEM)

As depicted in Figure 3, the nanoparticles exhibited mostly spherical and slightly irregular shapes, and an approximate size range of 25-35nm. Light aggregation was noted.

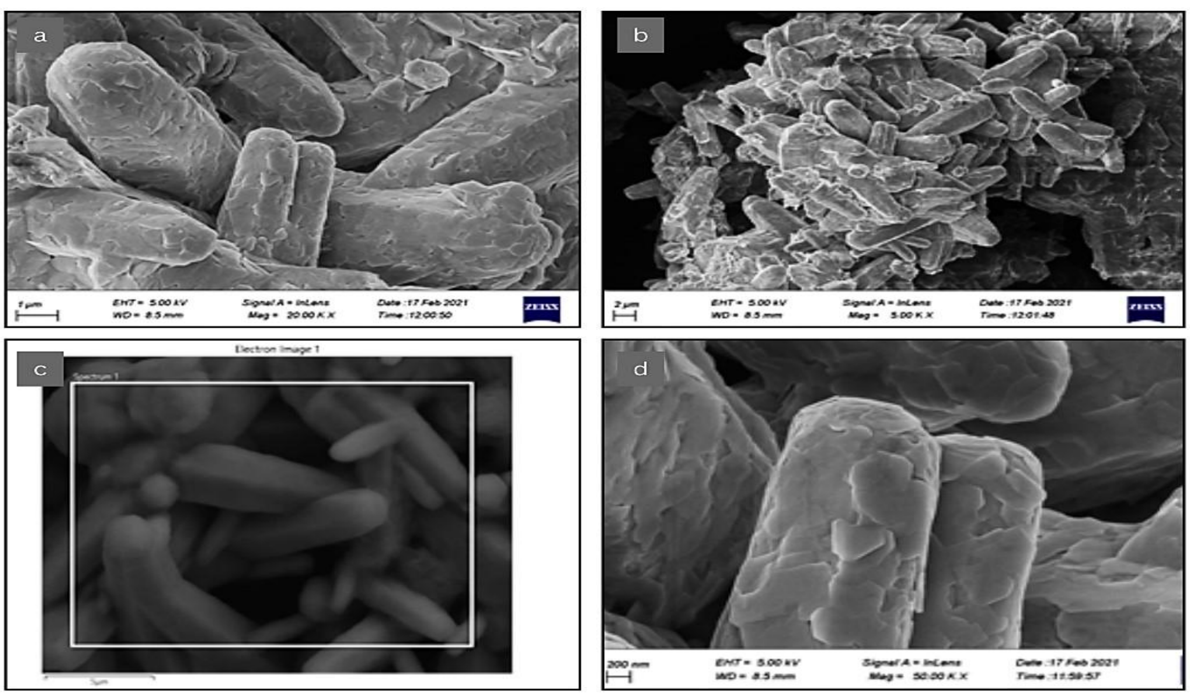

Figure-3. SEM images for the Boswellia serrata-ZnO-NPs at different magnifications.

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EDX spectroscopy

As shown in Figure 4, the spectrum revealed sharp and strong Zn (Zn) peaks at around 1.0, 8.6 and 9.6keV which are the typical Lα and Kα emission lines of Zn. A clear oxygen (O) peak at 0.5keV also confirmed the existence of ZnO. Minor peaks were also observed at the positions of calcium (Ca) (3.638keV) and chlorine (Cl) (2.6keV).

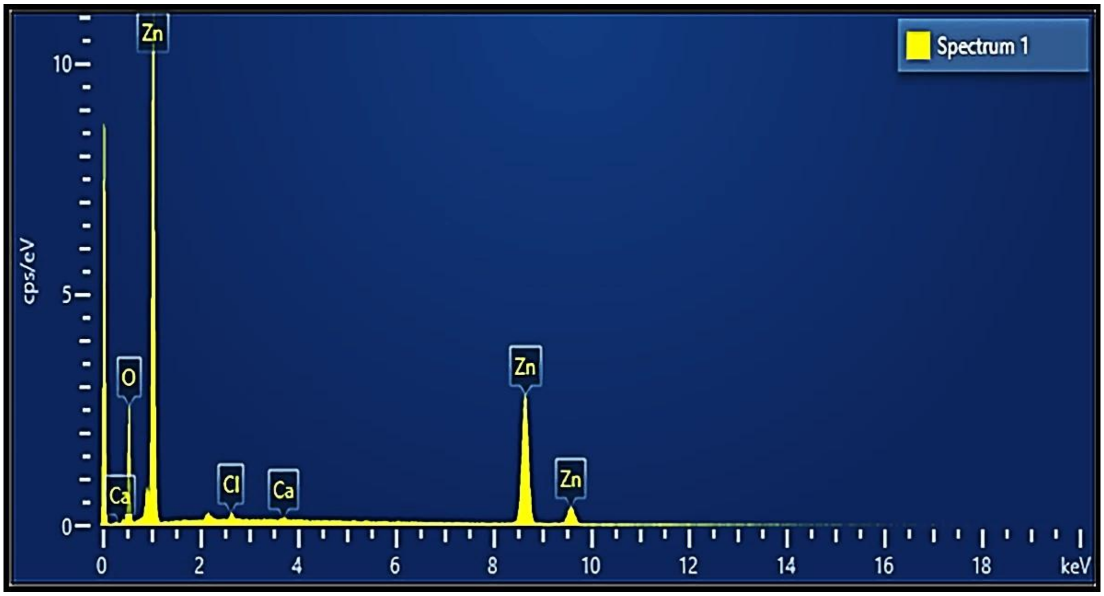

Figure-4. EDX for the Boswellia serrate-ZnO-NPs.

Effect of Boswellia serrata-ZnO-NPs on biofilm formation

The *Boswellia serrata* ZnO-NPs potential as an antibiofilm was determined on oral pathogens. It was found that the amount of biofilm mass decreased significantly in comparison to untreated control (Figure 5). Among the bacteria which were tested, *Streptococcus gordonii* was found to have the largest decrease in absorbance (0.134nm) with *Granulicatella adiacens* having the slightest decrease (0.103nm). Table 1 showed that *Boswellia serrata* ZnO-NPs reduced biofilm growth by 67-84% in all the Gram positive pathogens used in this study. *Streptococcus gordonii* was most inhibited (69%), whereas the minimum inhibition was demonstrated by *Granulicatella adiacens* (72%). The statistical analysis showed that all the reductions were significant ($p < 0.05$).

Table-1. Percentage inhibition of biofilm formation in oral Gram-positive pathogens treated with *Boswellia serrata* mediated ZnO nanoparticles.

Bacteria	Treated OD (±SD)	Control OD (±SD)	% Inhibition	Significance
S. gordonii	0.1343 ± 0.0404	0.4353 ± 0.0251	69.15%	*p < 0.05
S. pneumoniae	0.1300 ± 0.0142	0.4280 ± 0.0229	69.63%	
S. mitis	0.1107 ± 0.0276	0.3430 ± 0.0389	67.73%	
G. adiacens	0.1037 ± 0.0054	0.3623 ± 0.0444	71.40%	

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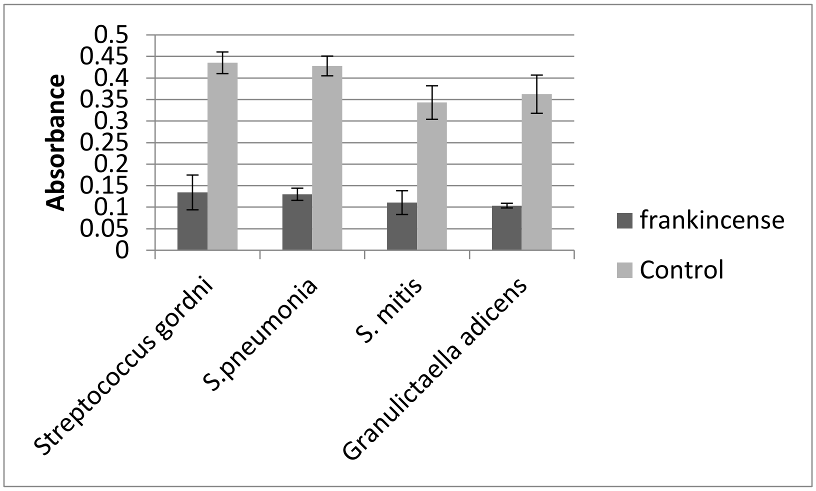

Figure-5. The pathogenic bacterial isolates are shown on the (X) axis, and the readings taken with an ELIZA device at a wavelength of 630 nm are represented on the (Y) axis.

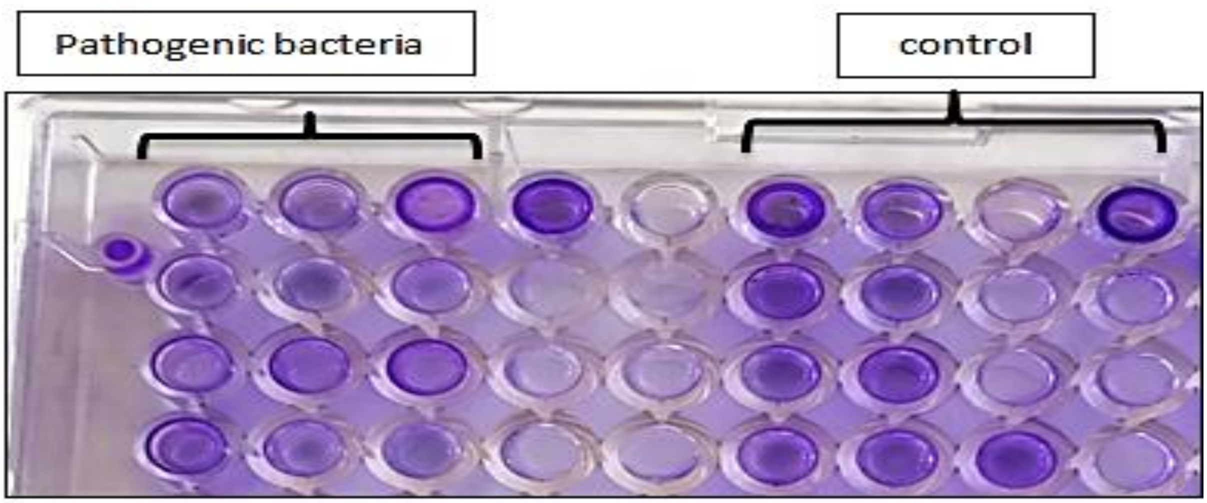

Figure-6. Shows the treatment of the bacterial isolates with the Boswellia serrata-ZnO-NPs.

Figure 6 demonstrated the anatomic visualization of the decreased adherence in that the staining of the control wells was purple (meaning that there was thick biofilm coverage), whereas the treated wells showed lighter staining (indicating less biofilm coverage).

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Antibacterial activity of *Boswellia serrata*-ZnO-NPs

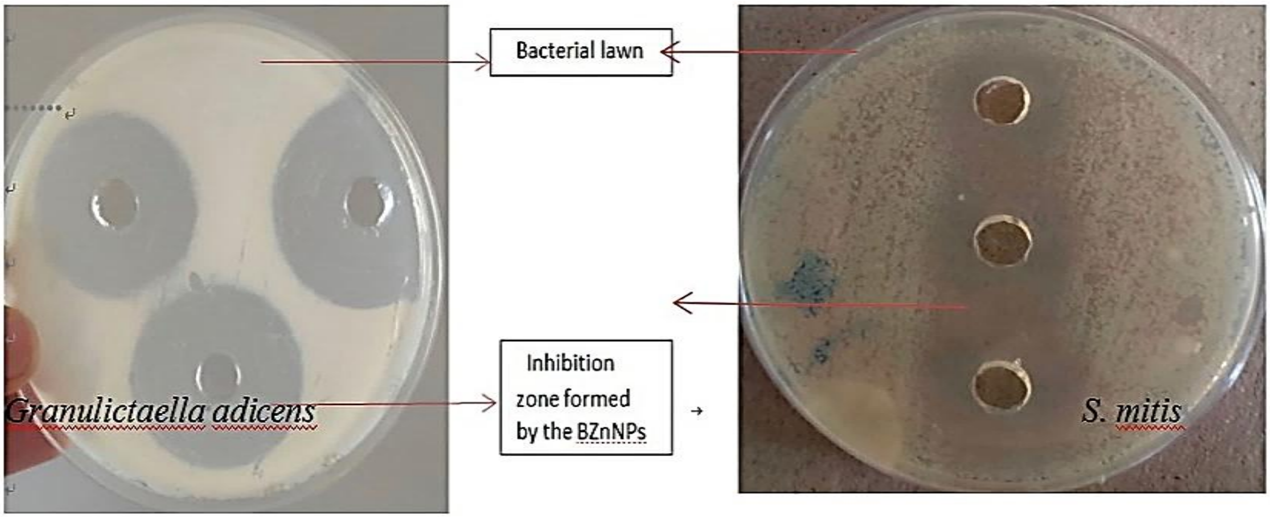

Figure-7. a and b. Zone inhibition of Boswellia serrata ZnO-NPs against pathogenic bacteria.

As highlighted in Figure 7, the nanoparticles showed high inhibitory power against gram positive and the zone of inhibition was observed to be 47mm against *Streptococcus* spp and 38mm against *Granulicatella adiacens*.

Discussion

Green metal oxide nanoparticles synthesized are of high attention because of their biocompatibility, sustainability, and increased biological activity. In the given case, ZnO-NPs produced by means of *Boswellia serrata* demonstrated typical physicochemical characteristics, along with a high level of antibacterial and antibiofilm activity, which characterizes their possible applicability in the sphere of oral healthcare.
FTIR analysis showed that the *Boswellia serrata* extract was majorly functional groups of phytochemical compounds, including phenolics, flavonoids, terpenoids, and boswellic acid derivatives, and this fact demonstrates that the extract was an effective reducing, capping, and stabilizing agent during the synthesis of ZnO nanoparticles (Yuvakkumar et al., 2015). It is well known that such functional groups can contribute an electron, lower metal ions and bind onto the surfaces of nanoparticles and thus stabilize the newly formed nanostructures. This validates that the biomolecules in *Boswellia serrata* were notably involved in the synthesis of stable and uniformly shaped ZnO-NPs using a green synthesis pathway.
SEM micrographs also reinforced this finding as they revealed nanoparticles which were mostly spherical to moderately irregular in nature with a size estimated to be between 25-35nm. Aggregation of light is commonly seen in the case of materials that are green-synthesized, which was probably as a result of the hydrogen bonding and van der Waals forces among phytochemical remains on the surfaces of the nanoparticles. These morphological characteristics are in line with previous reports and support the successful biosynthesis of nanoscale ZnO through plant mediated pathways (Raha and Ahmaruzzaman, 2022). The nanoscale dimension increases the ratio between surface and volume which is closely linked to better antibacterial and antibiofilm activity.
The EDX analysis elemental composition also indicated the presence of ZnO formation because strong peaks of zinc and oxygen were predominant in the spectrum. Calcium and chlorine were found in very low concentrations and were probably of precursor material or plant constituent. The relative intensity of Zn and O peaks is high, which proves the purity of the prepared nanoparticles (Naiel et al., 2022; Kamal et al., 2023). The elemental purity is of particular relevance to the biomedical applications, where impurities may modify the biological activity or cause toxicity and, therefore, the clean EDX profile

increases the confidence of the prepared material in its safety and functionality.

The *Boswellia serrata* ZnO-NPs synthesized also exhibited a high level of antibiofilm activity towards oral pathogens. The notable decrease in biofilm biomass is an indication that the nanoparticles disrupt the biofilm adhesion mechanisms and prevent the formation of extracellular polymeric substance (EPS) that is crucial in the maturation of biofilms. The high percentage inhibition results (67-84%) also affirm the high antibiofilm potential of the ZnO-NPs mediated by *Boswellia serrata*. These results are consistent with the existing literature proving that green-synthesized ZnO-NPs are capable of breaking biofilms formed by the Gram-positive and Gram-negative bacteria (Husain et al., 2022; Ahamad Khan et al., 2023). Oral infections are of special interest in inhibition of biofilms since biofilms shield bacteria, increase resistance to antibiotic effects and induce progression of chronic diseases. The capacity of these nanoparticles to inhibit the early formations of biofilm is an indication of its potential in the preventive agents of oral healthcare formulations.

In addition to antibiofilm activity, the nanoparticles had high antibacterial activity, and their inhibition zones were similar to those observed in the literature about Salman et al. (2021). This high activity can be attributed to several mechanisms: ZnO is capable of generating reactive oxygen species (ROS), which damage lipids, DNA and proteins; Zn^{2+} ions liberated by the nanoparticles can alter the enzymatic pathways; and the electrostatic interaction between the positively charged ZnO-NPs and the negatively charged bacterial membranes can result in membrane rupture and cellular contents leakage. Moreover, the phytochemicals that *Boswellia serrata* possess, such as boswellic acids, flavonoids, and terpenoids, have the potential to work together to increase antimicrobial effects by increasing nanoparticles surface reactivity and providing their own antimicrobial actions (Nayak et al., 2016; El-Nour et al., 2023; Yang et al., 2022). These metal based and phytochemical based mechanisms may be combined to account the robust and consistent antibacterial and antibiofilm results that were achieved in this research.

Due to the presence of oral pathogens like *Streptococcus spp*. and *Granulicatella adiacens* in dental caries, gingivitis, and periodontal diseases, the identified antimicrobial characteristics indicate that ZnO-NPs prepared with the help of *Boswellia serrata* will be very useful when applied in oral health care. It has potential applications in toothpastes, oral rinses, dental restoratives, implant surfaces, and periodontal gels and can provide new antimicrobial options in treating dental caries that are difficult to treat. Moreover, the green synthesis methodology complies with efforts by the whole world to achieve sustainable nanotechnology and provides a more affordable and harmless alternative to chemically produced nanoparticles (Jiang et al., 2022; Pushpalatha et al., 2022). Dual mode of action the single mix of metal oxide nanoparticles and medicinal plant extract offers the multiplication of therapeutic effects of phytochemicals and antibacterial activity of ZnO.

Conclusion

This study has achieved the synthesis of zinc oxide nanoparticles (ZnO-NPs) by *Boswellia serrata* extract by a green synthesis method. Nanoparticles exhibited significant antibacterial and antibiofilm against oral pathogens, which suggests that it can be used in the future in oral health use. Although the results are encouraging, such studies need more investigation to determine biocompatibility, safety, long term stability, and activity against wider microbial populations. Overall, the work presents *Boswellia serrata* as a new botanical source of ZnO-NPs synthesis and preconditions the construction of sustainable antimicrobial strategies in the dental area and other expertise.

Study Limitations

Although this study had some promising results, it has a number of limitations:

Lack of toxicity and cytocompatibility assays on mammalian cells.

Gram-positive bacteria were only considered; Gram-negative pathogens were not taken into account.

No comparison to normal antibiotics was done.

Hydrodynamic size and stability could not be characterized because DLS and zeta potential analyses were unavailable.

To reinforce the usefulness of ZnO-NPs mediated by *Boswellia serrata* in oral applications in the clinic, future research needs to consider such points.

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Data Availability Statement

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

Contribution of Authors

Al Mansoori DHH & Al Bayati FS: Conceptualization.
Jawad SM & Kahdum BJ: Methodology, formal analysis and data curation.
Al Mansoori DHH & Kahdum BJ: Validation.
Al Mansoori DHH, Al Bayati FS & Alchalabi MKO: Investigation and funding acquisition.
Al Mansoori DHH, Kahdum BJ & Al Bayati FS: Resources provision and literature review
Al Mansoori DHH, Jawad SM & Kahdum BJ: Writing– original draft, writing – review & editing and project administration.
Al Mansoori DHH, Jawad SM & Al Bayati FS: Visualization
Al Mansoori DHH: Supervision

All authors read and approved the final draft of the manuscript.

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