Antimicrobial activity of zinc oxide nanoparticles synthesized using leaves extract of *Abies webbiana*

ABSTRACT:

Introduction: Green synthesis of nanoparticles using plant extract, bacteria, fungi and enzymes are eco-friendly and cost effective which do need high pressure, energy, temperature and toxic chemicals for its synthesis protocol. Zinc oxide nanoparticles have various physical, chemical and biological properties which can be applied in the treatment of oral diseases.

Aim: The aim of the present study is to determine the antimicrobial activity of zinc oxide nanoparticles synthesized using leaves extract of *Abies webbiana.*

Materials and Methods: In this report, we used the extract of *A. webbiana* to synthesize zinc oxide nanoparticles and were characterized using U-V visible spectrophotometer and were tested for their antimicrobial activity against oral pathogens.

Results: These biosynthesized zinc oxide nanoparticles exhibited potent antimicrobial activity against oral pathogens, *Streptococcus mutans, Staphylococcus aureus and Enterococcus faecalis, Candida albicans* and can be developed as a novel medicine against pathogenic oral diseases.

Conclusion: With increasing demand in advancements in diagnosis and treatment modalities, green synthesis of zinc oxide nanoparticles using *A. webbiana which was found to have* potent antimicrobial action has wider applications in dentistry.

Keywords: Abies webbiana, antimicrobial activity, innovative technology, green synthesis, zinc oxide nanoparticles

1. INTRODUCTION

Nanoparticles (NPs) are small estimated particles with improved synergist reactivity, warm conductivity, non-straight optical execution, and compound relentlessness due to their enormous surface territory to volume proportion.(1)(2) NPs have begun being considered as nano antibiotics in light of their antimicrobial activities (3)(4). Nanoparticles have been included into a variety of modern health, nutrition, feed, space, chemical, and cosmetic care goods, necessitating a green and environmentally friendly approach to their synthesis. (5)(6) The properties of metallic nanoparticles, for example, gold, silver, iron and zinc nanoparticles have developed interests in biomedical applications.(7)

Zinc oxide is a unique material with semiconducting, piezoelectric, and pyroelectric capabilities that has a wide range of applications in transparent electronics, UV light matters, piezoelectric devices, compound sensors, spin electronics, individual consideration items, coatings, and paints.(8). Biosynthesis of Zinc oxide nanoparticles (ZnO NPS) from plants like *Aloe vera, Borassus flabellifer* fruit*, Sargassum muticum, Eichhornia crassipes,* and furthermore from some bacterial and contagious species like *Bacillus subtilis and Escherichia coli, Ureolytic microorganisms, Lactobacillus plantarum* have been reported.(9) The plants are sometimes described as biosynthetic laboratories where different secondary metabolites are produced and stored. Secondary metabolites are organic compounds such as alkaloids, amino acids, carbohydrates, fixed oils, flavonoids, glycosides, gums, resins, saponins, sugars, tannins, volatile oil etc., which are responsible for their medicinal properties. There are a large number of traditionally used plants on earth having a wide range of medicinal efficacies.(10)(11)

Talispatra in Bengali and Hindi, Talispatram in Sanskrit, and Indian Silver Fir in English are all names for *Abies webbiana Lindl. (Pinaceae),* a huge tall evergreen tree indigenous to the Himalayan region.(12,13) The leaves of this plant have been traditionally used for their carminative, stomachic, expectorant, decongestant, antiseptic, astringent, antihyperglycemic, female antifertility, febrifuge and antispasmodic properties. Characteristics include antibacterial, astringent, antihyperglycemic, female fertility, febrifuge, and antispasmodic. Cough, phthisis, asthma, chronic bronchitis, and other pulmonary infections can be treated with decoctions of the leaves taken orally. Furthermore, leaves of the plant have been used traditionally for its chemotherapeutic efficacies in several ailments like hoarseness, chronic bronchitis, rheumatism, and other pulmonary affections (14,15).Previously it has been reported the extracts from the leaves of the plants have antibacterial, mast cell stabilizing, anxiolytic, anti-tumour, anti-inflammatory, antitussive and CNS depressant actions. Our team has extensive knowledge and research experience that has translated into high quality publications (16–19)(20–23)(24–27)(28–31)(32–35). The present study aims to synthesize, characterize and check antimicrobial activity of zinc oxide nanoparticles synthesized using leaves extract of *A. webbiana.*

2. MATERIALS AND METHODS:

2.1 Study Organisms:

The test organisms used in this study were obtained from the culture collections of the Nanomedicine Laboratory, Saveetha Dental College, Chennai. The organisms used in this study were *Streptococcus mutans, Staphylococcus aureus and Enterococcus faecalis, Candida albicans.*

2.2 Biosynthesis of zinc oxide nanoparticles (ZnO NPs) using *A.webbiana* leaf extract:

1g of *A. webbiana* mixed with 100 mL of distilled water and boiled in 60-70 degree celsius in the heating mantle for 10-15 minutes and filtered using Whattman no 1 filter paper. 20 milli molar (0.574g) of Zinc sulphate was dissolved in 60 mL of distilled water. 40 mL of filtered *A. webbiana* extract is mixed with 60 mL of metal solution to obtain ZnO NPs.

2.3 Characterization of ZnO NPs:

The optical properties of the synthesis of ZnO NPs were determined using UV- vis spectroscopy wavelengths ranging from 250 to 650 nm. They were measured at different time periods.to determine the synthesis of ZnO NPs

2.4 Antimicrobial activity of zinc oxide nanoparticles:

The ZnO nanoparticles synthesized using *A. webbiana* were tested for antimicrobial activity by agar well diffusion method against pathogenic organisms namely *S. aureus, E.faecalis, S. mutans, C. albicans.* The pure culture of the bacterium was subcultured on Mueller Hinton agar. Wells of 9 mm diameter were made on Muller Hinton agar plates using gel puncture and each strain was uniformly swabbed onto the individual plates using sterile cotton swabs. Using a sterile micropipette, a standard antibacterial agent was loaded into one well and three different concentrations of zinc oxide nanoparticles sample solutions (25 µl, 50 µl and 100 µl) were poured into three other wells on all plates. The isolates of *C. albicans* were subcultured onto Rose Bengal agar. A standard antifungal disc (voriconazole) was placed on the agar plate and then nanoparticle sample solutions (25 µl, 50 µl and 100 µl) were poured onto three other wells on the plate. After incubation at 37°C for 24 hours, the different levels of the zone of inhibition of bacteria were observed and measured.

2.4.1 Measurement of Zone of Inhibition:

The test plates were held in front of a desk lamp, and the zones were measured with a ruler held against the back of the petri plate. The diameters of the zones of inhibited growth were measured to the nearest whole millimeter.

3. RESULTS AND DISCUSSION:

3.1 Visual identification:

The present study showed the synthesis of ZnO NPs by using *A. webbiana*. The colour changes in the reaction mixture was observed continuously at various periods of incubation. The zinc oxide was reduced into ZnO nanoparticles that is visually identified by colour changes (Figure 1). The colour of the solution was changed to light brown at 1 h incubation time. The color was changed to dark brown after 24 h of incubation. There was no color change observed after 24 h which indicated that the ZnO NPs synthesis process was completed.

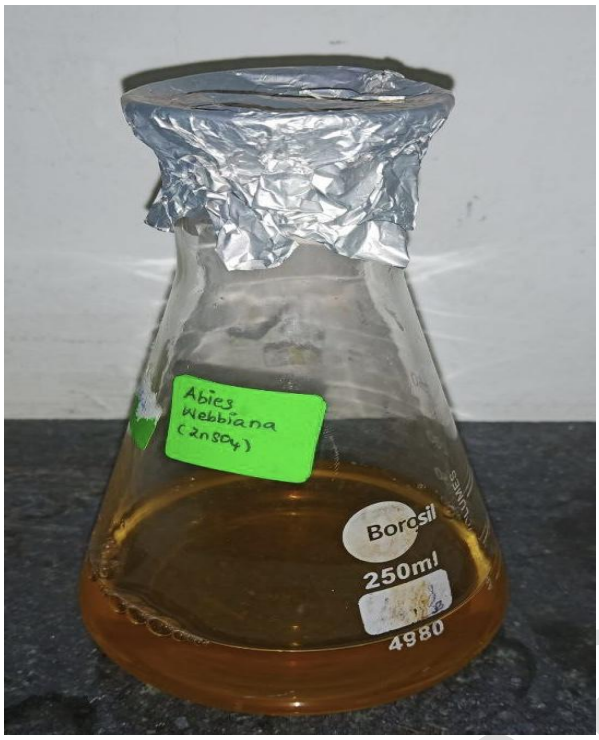

Fig. 1. Reduction of Zinc oxide ions to ZnO nanoparticles visually identified by colour changes at various periods of incubation time.

3.2 UV vis spectrophotometer analysis:

UV - vis spectroscopy analysis depends on the arising of colour in the reaction due to excitation of the surface plasmon resonance band in a reaction mixture. Figure 2 shows the UV absorption spectra of the synthesized ZnO NPs. The spectrum clearly shows that the absorbance steadily decreased with decrease in incubation time. The plasmon resonance band for ZnO NPs sythesized using *A. webbiana* showed high absorption at 370 nm.

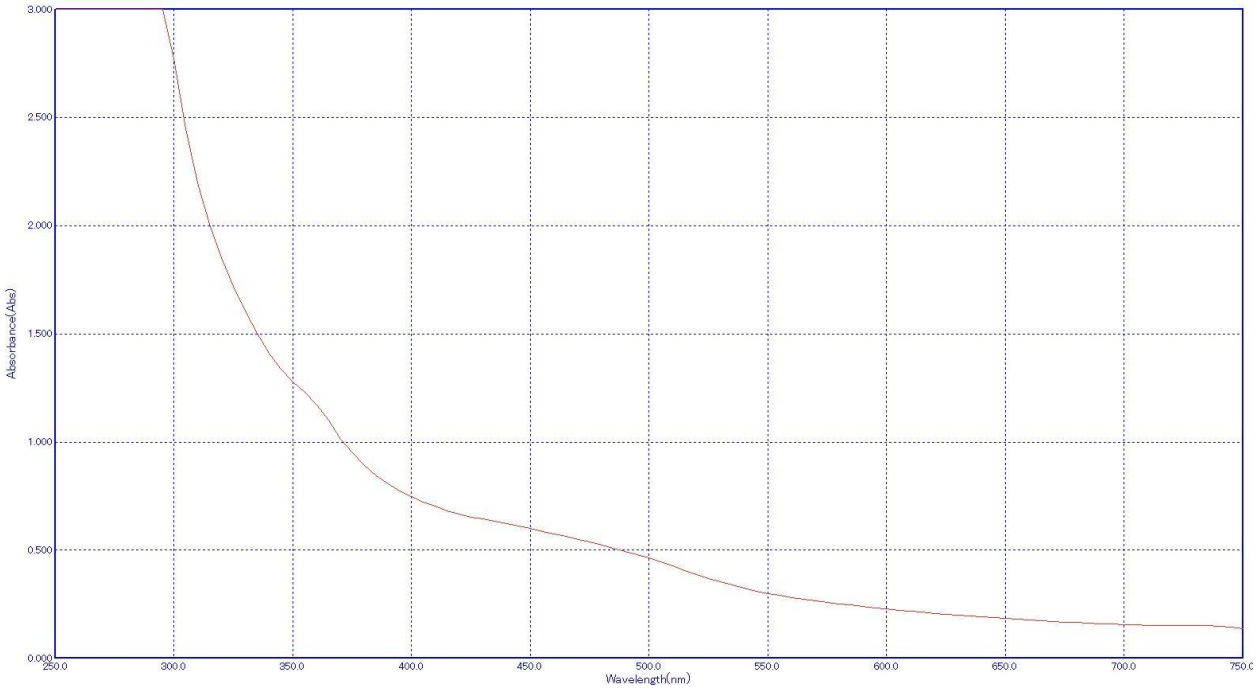

Fig. 2. UV Spectroscopic analyses of Zinc oxide nanoparticles synthesized from the leaf extract of *A. webbiana* recorded as function of time.

3.3 Antimicrobial activity of zinc oxide nanoparticles against oral pathogens:

The antimicrobial activity of ZnO NPs of *A. webbiana* assayed by a well diffusion method against oral pathogens showed minimum inhibitory concentrations at different concentrations. Table:1 shows the inhibition of bacterial growth in various concentrations of biosynthesized ZnO NPs of *A. webbiana*. The antimicrobial activity of ZnO NPs synthesized using *A. webbiana* was evaluated according to their zone of inhibition and the results were compared with the activity of the standard antimicrobial agent. The antibacterial activity of ZnO NPs against gram positive *S. aureus* showed a zone of inhibition of 15.03 ± 0.23 mm at the concentration of 100 µl. The antimicrobial activity was high against *S. mutans* and *E. faecalis* at concentrations of 100 µl. These results indicate the ZnO NPs of *A. webbiana* were assuring as an antibacterial activity against the pathogens employed. (36)

Some researchers have reported that ZnO NPs disrupt bacterial cell membrane integrity, reduce cell surface hydrophobicity, and downregulate the transcription of oxidative stress-resistance genes in bacteria which leads to its lysis. Rajeshkumar et al., reported that the positively charged AgNPs accumulate on negatively charged bacterial cell membranes to bring about structural alteration in cell membranes. Various mechanisms contribute to its antimicrobial activity such as generation of reactive oxygen species and bacterial protein denaturation.(37)

Anti-bacterial activity of mouthwash incorporated with silica nanoparticles were assessed against oral pathogens. Silica nanoparticles were synthesized using the sol-gel method. In sol-gel reaction, there is hydrolysis and condensation of Tetra-ethoxy silane, where it was first hydrolyzed to silicic acid, followed by a condensation reaction leading to the formation of Si-O-Si bonds. Silica nanoparticles incorporated in mouthwash showed a good potential as an antimicrobial agent that maintains control of biofilm, preventing initial colonization of bacteria.(38)

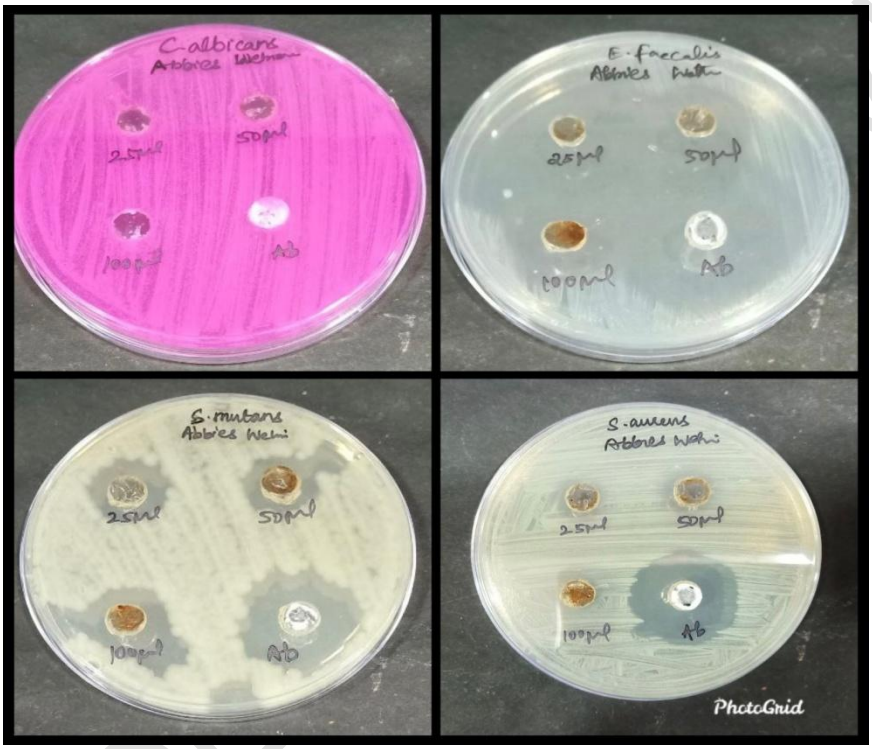

Fig. 3. Antimicrobial activity of *Abies webbiana* mediated zinc oxide nanoparticles against oral pathogens

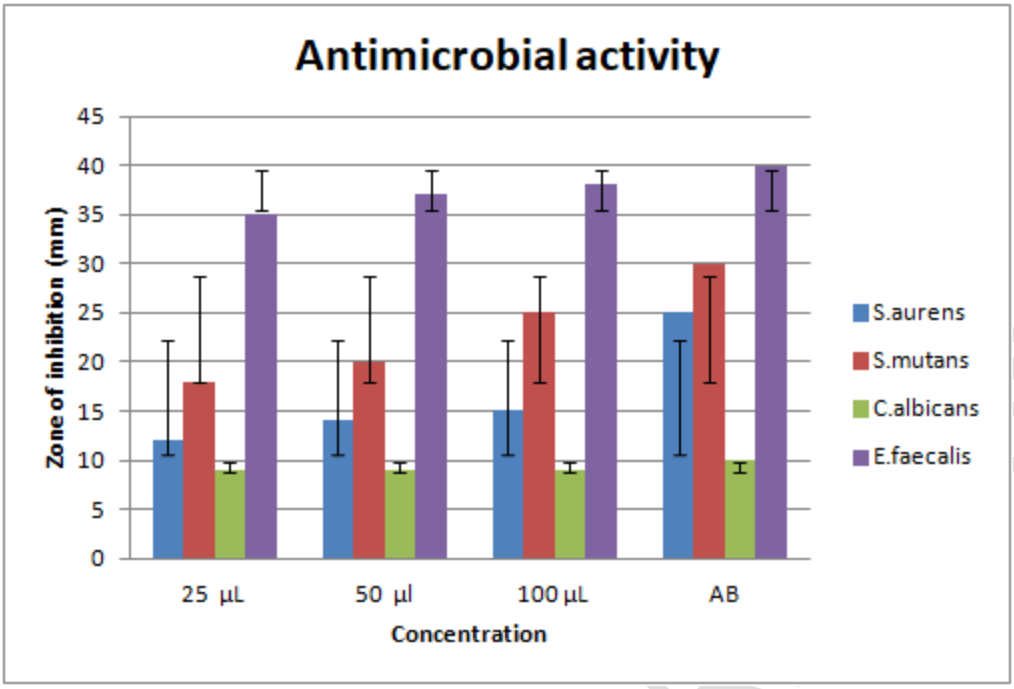

Fig. 4. Graph showing antimicrobial activity of *A. webbiana* assisted Zinc oxide nanoparticles

Table 1: Antimicrobial activity of ZnO NPs against oral pathogens.

ORAL PATHOGENS	Zone of inhibition (mm)			
	25 µL	**50 µL**	**100 µL**	**Ab**
S. aureus	12.24 ± 0.32	14.14 ± 0.31	15.03 ± 0.23	25.14 ± 0.23
S. mutans	18.03 ± 0.22	20.15 ± 0.23	25.15 ± 0.32	25.15 ± 0.32
E. faecalis	35.15 ± 0.24	37.04 ± 0.34	38.02 ± 0.24	38.23 ± 0.24
C. albicans	9.24 ± 0.12	9.12 ± 0.23	9.12 ± 0.32	40.32 ± 0.23

4. CONCLUSION

This study has demonstrated that ZnO NPs synthesis was initially identified by dark brown colour and the surface plasmon resonance was peak positioned at 370 nm. These ZnO NPs showed potent antimicrobial activity against oral pathogens assuring its effective therapeutic application in several infectious oral diseases.

CONSENT

It is not applicable

ETHICAL APPROVAL

It is not applicable

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