

PREPARATION AND ANTIMICROBIAL ACTIVITY OF ZINC OXIDE NANOPARTICLES (*ABIES WEBBIANA*) BASED MOUTHWASH

ABSTRACT ANY RED WORD NEED TO BE CORRECTED IN THE ARTICLE AND IF UNDERLINE IS NEED TO BE CHANGED

Introduction:

Nanotechnology has been taking the scientific community by storm as they have been showing promising results due to their antibacterial, antifungal and cytotoxic activity. Multiple studies have found various plant species to be a rich source of these nanoparticles. *Abieswebbiana* is one of these plants, however, it has not been studied enough. The aim of this study is to synthesize zinc oxide nanoparticles from *a. Webbiana* plant extract and to test its antimicrobial activity against certain microorganisms.

Materials and methods:

1 g of *a. Webbiana* was mixed with 100 ml of distilled water and boiled for 15 minutes to 60°C in a heating mantle. The solution was filtered using Whatman no. 1 filter paper. 20 millimolar (0.574 g) of zinc oxide was dissolved in 60 ml of distilled water. 60 ml of this solution was mixed with 40 ml of *a. Webbiana* filtrate in a beaker, which was then kept on a stirrer. A mouthwash was prepared using the extracted nanoparticles and was used for antimicrobial testing.

Results:

The plasmon resonance band of zinc oxide nanoparticles showed absorbance peak at 450 nm. Maximum zone of inhibition was obtained at 100 µl against *s. Aureus*, 25 µl against *e.faecalis*, 50 µl against *c.albicans* and 100 µl against *s. Mutans*.

Conclusion:

The mouthwash shows effective activity against *c.albicans* and *s.mutans*. Zinc oxide nanoparticles extracted from *a. Webbiana* have a potent antimicrobial activity and can be used for the treatment of orofacial infections.

Keywords: antifungal, green synthesis, innovative technology, nanoparticles, zinc oxide

1. INTRODUCTION

The reason for the “nano-hype” that recently has conquered all disciplines of natural sciences is the observation that properties of materials depend not only on composition but also on morphology (size and shape). For materials with at least one dimension below 50 nm, the contribution of surface energy to total energy becomes more and more significant. The smaller the structure gets, just as chemical and physical properties of surfaces differ from those of the bulk, so do those of nanoscale materials. Zinc oxide (ZnO) is a wide band gap semiconductor with an energy gap of 3.37 eV at room temperature. It has been used considerably for its catalytic, electrical, optoelectronic, and photochemical properties [1]. ZnO nanostructures have a great advantage to apply to a catalytic reaction process due to their large surface area and high catalytic activity. Since zinc oxide shows different physical and chemical properties depending upon the morphology of nanostructures, not only various synthesis methods but also the physical and chemical properties of synthesized zinc oxide are to be investigated in terms of its morphology [2]. ZnO NPs have a very strong antibacterial effect at a very low concentration of gram negative and gram positive bacteria as confirmed by the studies, they have shown strong antibacterial effect than the ZnO NPs synthesized chemically [3-5].

Plant parts like roots, leaves, stems, seeds, fruits have also been utilized for the NPs synthesis as their extract is rich in phytochemicals which act as both reducing and stabilizing agent [6-12]. *A. webbiana* is a medicinal plant which is an extract, evergreen perennial shrub. It is used to prepare ayurvedic formulated drugs because of aphrodisiac, rejuvenating, anti inflammatory, anti tumor properties. The genus *Abies* belongs to the division *Tracheophyta*, class *pinopsida*, order *pinales* and family *Pinaceae*. The studies by Mishra et al [13] and Naidu et al [14] reveal that the leaves of *A. webbiana* (Talisapatra) are anti-oxidant, immune modulatory and haematopoietic in nature. The chemical compounds like phytochemicals are biologically active and can provide health benefits for humans than those attributed to macronutrients and micronutrients.

The aim of this study is to synthesize zinc oxide nanoparticles from *A. webbiana* plant extract and to test its antimicrobial activity against certain microorganisms.

2. MATERIAL AND METHODS

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1g of *A. webbiana* plant extract was mixed with 100mL of distilled water and boiled for 15 minutes to 60°C in a heating mantle.

The solution was filtered using Whatman no 1 filter paper. 20 millimolar(0.574g) of zinc oxide was dissolved in 60 mL of distilled water. 60mL of this solution was mixed with 40mL of *A. webbiana* filtrate in a beaker, which was then kept on a stirrer.

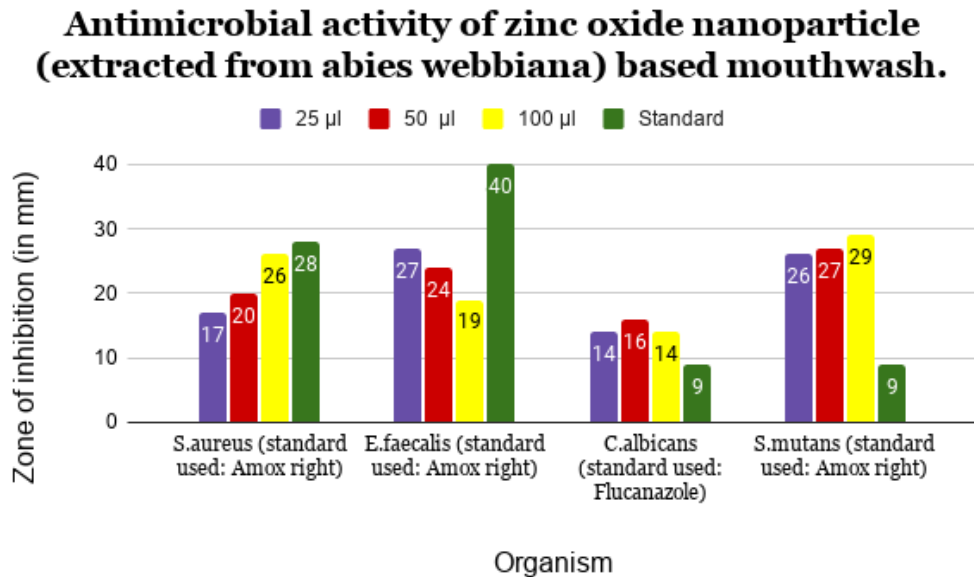
The solution was checked periodically for colour change. The synthesised nanoparticles were measured using a double beam UV spectrophotometer.

The mouthwash was prepared by dissolving 2g of sodium benzoate into 10mL of distilled water using a glass rod in a beaker and adding 3 drops of clove oil and leaving the solution idle for 15 minutes. Then, 20µl of zinc oxide nanoparticles was added into the mouthwash and stirred. An antimicrobial test was done via agar well diffusion method against *S.aureus*, *E.faecalis*, *C.albicans* and *S.mutans*. The zones of inhibition which were obtained were measured.

3. RESULTS

Zinc oxide nanoparticles were initially identified by their characteristic dark brown colour with the surface plasma resonance at its peak position at 450nm. The antimicrobial activity of the mouthwash was assayed by a well diffusion method against oral pathogens and the respective zone of inhibitory concentration was found to be as shown in the graph. Maximum zone of inhibition was obtained at 100µL against *S.aureus*, 25µL against *E.faecalis*, 50µL against *C.albicans* and 100µL against *S.mutans*. The inhibition zone achieved against *S.aureus* was the lowest at 25µL, against *E.faecalis* was at 100µL, against *C.albicans* and *S.mutans* was when the standard solution was used.

Fig 1:



4. DISCUSSION

Our team has extensive knowledge and research experience that has translate into high quality publications.[15-27], [28-32], [33], [34]

There are similar studies done on copper nanoparticles [35-38], iron nanoparticles [39-40], silver nanoparticles [41] and selenium nanoparticles [42] with varying degrees of success. The current study shows Zinc oxide nanoparticles (extracted from *A.webbiana*) based mouthwash has a potent antimicrobial property where it is more effective against bacteria when compared to *C.albicans*. It is also shown to have a similar action as the standard on *S.aureus*. The action of the mouthwash on *E.Faecalis* is less effective than the standard solution. In case of *S.mutans*, it shows a higher percentage of zone of inhibition than the standard solution, making it a potent agent to be used in a mouthwash

In a study done by Lakshmi et al, similar findings were seen where the zone of inhibition was higher for the solutions containing the nanoparticles than the standard [43]. In another study done by Elumalai et al on *Azadirachtaindica*, zinc oxide nanoparticles showed interesting antimicrobial activity against both gram positive and gram negative bacteria [44]. A study done by Ismail et al found that the optimum calcination temperature for the preparation of zinc oxide nanoparticles for a superior antimicrobial action against gram positive and gram negative bacteria is 300 °C [45].

In the present study, the zone of inhibition against fungal organisms was considerably larger than that of the standard solution (which was fluconazole), which suggests a potent antifungal activity. This is similar to results of studies done by He et al [46] and Karimian et al[47] where, especially in the later, zinc oxide nanoparticles are shown to be extremely effective against *C.albicans*.

The present study shows that zinc oxide nanoparticles extracted from *A. webbianashow* a potent antimicrobial activity, especially an antifungal activity. Further, large scale research should be done to expand on the application of these findings.

5. CONCLUSION

The zinc oxide nanoparticles were found at the peak position of 450nm. The mouthwash showed potent action against *C.albicans* and *S.mutans* when compared to the standard solution, while showing similar action against *S.aureus* and *E.faecalis*. The zinc oxide nanoparticles have showed potent antimicrobial property which promises similar therapeutic effectiveness against various infectious conditions (the conclusion of *F.Faecalis* is not correspondind to the result)

REFERENCES CORRECT THE REFERENCES THAT ARE MARKED BY RED COLOR

1. Ischenko V, Polarz S, Grote D, Stavarache V, Fink K, Driess M. Zinc oxide nanoparticles with defects. *AdvFunct Mater.* 2005 Dec;15(12):1945–54.
2. Kumar SS, Venkateswarlu P, Rao VR, Rao GN. Synthesis, characterization and optical properties of zinc oxide nanoparticles. *International Nano Letters.* 2013 May 7;3(1):30.
3. Vimala K, Sundarraj S, Paulpandi M, Vengatesan S, Kannan S. Green synthesized doxorubicin loaded zinc oxide nanoparticles regulates the Bax and Bcl-2 expression in breast and colon carcinoma. *Process Biochem.* 2014 Jan 1;49(1):160–72.
4. Venkatachalam P, Jayaraj M, Manikandan R, Geetha N, Rene ER, Sharma NC, et al. Zinc oxide nanoparticles (ZnONPs) alleviate heavy metal-induced toxicity in *Leucaena leucocephala* seedlings: A physiochemical analysis. *Plant PhysiolBiochem.* 2017 Jan;110:59–69.
5. Hazra C, Kundu D, Chaudhari A, Jana T. Biogenic synthesis, characterization, toxicity and photocatalysis of zinc sulfide nanoparticles using rhamnolipids from *Pseudomonas aeruginosa* BS01 as capping and stabilizing agent. *J Chem Technol Biotechnol.* 2013 Jun;88(6):1039–48.
6. Zong Y, Li Z, Wang X, Ma J, Men Y. Synthesis and high photocatalytic activity of Eu-doped ZnO nanoparticles. *Ceram Int.* 2014 Aug 1;40(7, Part B):10375–82.
7. Nachiyar V, Sunkar S, Prakash P, Others. Biological synthesis of gold nanoparticles using endophytic fungi. *Der Pharma Chem.* 2015;7(11):31–8.
8. Ramesh M, Anbuvaran M, Viruthagiri G. Green synthesis of ZnO nanoparticles using *Solanum nigrum* leaf extract and their antibacterial activity. *Spectrochim Acta A Mol Biomol Spectrosc.* 2015 Feb 5;136Pt B:864–70.
9. Xiao L, Liu C, Chen X, Yang Z. Zinc oxide nanoparticles induce renal toxicity through reactive oxygen species. *Food Chem Toxicol.* 2016 Apr;90:76–83.
10. Rajeshkumar S. Anticancer activity of eco-friendly gold nanoparticles against lung and liver cancer cells. *J Genet Eng Biotechnol.* 2016 Jun;14(1):195–202.
11. Nagajyothi PC, Minh An TN, Sreekanth TVM, Lee J-I, Joo Lee D, Lee KD. Green route biosynthesis: Characterization and catalytic activity of ZnO nanoparticles. *Mater Lett.* 2013 Oct 1;108:160–3.
12. Gnanajobitha G, Paulkumar K, Vanaja M, Rajeshkumar S, Malarkodi C, Annadurai G, et al. Fruit-mediated synthesis of silver nanoparticles using *Vitis vinifera* and evaluation of their antimicrobial efficacy. *Journal of Nanostructure in Chemistry.* 2013 Aug 2;3(1):67.
13. Mishra L-C, Singh BB, Dagenais S. Scientific basis for the therapeutic use of *Withania somnifera* (ashwagandha): a review. *Altern Med Rev.* 2000;5(4):334–46.
14. Naidu PS, Singh A, Kulkarni SK. Effect of *Withania somnifera* root extract on haloperidol-induced orofacial dyskinesia: possible mechanisms of action. *J Med Food.* 2003 Summer;6(2):107–14.
15. Ramesh A, Varghese S, Jayakumar ND, Malaiappan S. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. *J Periodontol.* 2018 Oct;89(10):1241–8.
16. Paramasivam A, Priyadharsini JV, Raghunandhakumar S, Elumalai P. A novel COVID-19 and its effects on cardiovascular disease. *Hypertens Res.* 2020 Jul;43(7):729–30.
17. S G, T G, K V, Faleh A A, Sukumaran A, P N S. Development of 3D scaffolds using nanochitosan/silk-fibroin/hyaluronic acid biomaterials for tissue engineering applications. *Int J Biol Macromol.* 2018 Dec;120(Pt A):876–85.
18. Del Fabbro M, Karanxha L, Panda S, Bucchi C, Nadathur Doraiswamy J, Sankari M, et al. Autologous platelet concentrates for treating periodontal infrabony defects. *Cochrane Database Syst Rev.* 2018 Nov 26;11:CD011423.
19. Paramasivam A, Vijayashree Priyadharsini J. MitomiRs: new emerging microRNAs in mitochondrial

- dysfunction and cardiovascular disease. *Hypertens Res.* 2020 Aug;43(8):851–3.
20. Jayaseelan VP, Arumugam P. Dissecting the theranostic potential of exosomes in autoimmune disorders. *Cell Mol Immunol.* 2019 Dec;16(12):935–6.
 21. Vellappally S, Al Kheraif AA, Divakar DD, Basavarajappa S, Anil S, Fouad H. Tooth implant prosthesis using ultra low power and low cost crystalline carbon bio-tooth sensor with hybridized data acquisition algorithm. *ComputCommun.* 2019 Dec 15;148:176–84.
 22. Vellappally S, Al Kheraif AA, Anil S, Assery MK, Kumar KA, Divakar DD. Analyzing Relationship between Patient and Doctor in Public Dental Health using Particle Memetic Multivariable Logistic Regression Analysis Approach (MLRA2). *J Med Syst.* 2018 Aug 29;42(10):183.
 23. Varghese SS, Ramesh A, Veeraiyan DN. Blended Module-Based Teaching in Biostatistics and Research Methodology: A Retrospective Study with Postgraduate Dental Students. *J Dent Educ.* 2019 Apr;83(4):445–50.
 24. Venkatesan J, Singh SK, Anil S, Kim S-K, Shim MS. Preparation, Characterization and Biological Applications of Biosynthesized Silver Nanoparticles with Chitosan-Fucoidan Coating. *Molecules* [Internet]. 2018 Jun 12;23(6). Available from: <http://dx.doi.org/10.3390/molecules23061429>
 25. Alsubait SA, Al Ajlan R, Mitwalli H, Aburaisi N, Mahmood A, Muthurangan M, et al. Cytotoxicity of Different Concentrations of Three Root Canal Sealers on Human Mesenchymal Stem Cells. *Biomolecules* [Internet]. 2018 Aug 1;8(3). Available from: <http://dx.doi.org/10.3390/biom8030068>
 26. Venkatesan J, Rekha PD, Anil S, Bhatnagar I, Sudha PN, Dechsakulwatana C, et al. Hydroxyapatite from Cuttlefish Bone: Isolation, Characterizations, and Applications. *Biotechnol Bioprocess Eng.* 2018 Aug 1;23(4):383–93.
 27. Vellappally S, Al Kheraif AA, Anil S, Wahba AA. IoT medical tooth mounted sensor for monitoring teeth and food level using bacterial optimization along with adaptive deep learning neural network. *Measurement.* 2019 Mar 1;135:672–7.
 28. PradeepKumar AR, Shemesh H, Nivedhitha MS, Hashir MMJ, Arockiam S, Uma Maheswari TN, et al. Diagnosis of Vertical Root Fractures by Cone-beam Computed Tomography in Root-filled Teeth with Confirmation by Direct Visualization: A Systematic Review and Meta-Analysis. *J Endod.* 2021 Aug;47(8):1198–214.
 29. R H, Ramani P, Tilakaratne WM, Sukumaran G, Ramasubramanian A, Krishnan RP. Critical appraisal of different triggering pathways for the pathobiology of pemphigus vulgaris-A review. *Oral Dis* [Internet]. 2021 Jun 21; Available from: <http://dx.doi.org/10.1111/odi.13937>
 30. Ezhilarasan D, Lakshmi T, Subha M, Deepak Nallasamy V, Raghunandhakumar S. The ambiguous role of sirtuins in head and neck squamous cell carcinoma. *Oral Dis* [Internet]. 2021 Feb 11; Available from: <http://dx.doi.org/10.1111/odi.13798>
 31. Sarode SC, Gondivkar S, Sarode GS, Gadbaill A, Yuwanati M. Hybrid oral potentially malignant disorder: A neglected fact in oral submucous fibrosis. *Oral Oncol.* 2021 Jun 16;105390.
 32. Kavarthapu A, Gurumoorthy K. Linking chronic periodontitis and oral cancer: A review. *Oral Oncol.* 2021 Jun 14;105375.
 33. Vellappally S, Abdullah Al-Kheraif A, Anil S, Basavarajappa S, Hassanein AS. Maintaining patient oral health by using a xeno-genetic spiking neural network. *J Ambient Intell Humaniz Comput* [Internet]. 2018 Dec 14; Available from: <https://doi.org/10.1007/s12652-018-1166-8>
 34. Aldhuwayhi S, Mallineni SK, Sakhamuri S, Thakare AA, Mallineni S, Sajja R, et al. Covid-19 Knowledge and Perceptions Among Dental Specialists: A Cross-Sectional Online Questionnaire Survey. *Risk ManagHealthc Policy.* 2021 Jul 7;14:2851–61.
 35. Devi RS, Jeevitha M, Preetha S, Rajeshkumar S. Free radical scavenging activity of copper nanoparticles synthesized from dried ginger. *J Pharm Res Int.* 2020 Aug 26;1–7.
 36. Trisha S, Jeevitha M, Preetha S, Rajeshkumar S. GREEN SYNTHESIS OF COPPER NANOPARTICLES USING TURMERIC-TULSI EXTRACT AND ITS CHARACTERIZATION. **PLANT CELL BIOTECHNOLOGY AND MOLECULAR BIOLOGY.** 2020 Nov 17;79–84.
 37. Rieshy V, Jeevitha M, Rajeshkumar S. CYTOTOXICITY OF COPPER NANOPARTICLES SYNTHESIZED USING DRIED GINGER. **PLANT CELL BIOTECHNOLOGY AND MOLECULAR BIOLOGY.** 2020 Nov 17;1–6.
 38. Rajeshkumar S, Tharani M, Jeevitha M, Santhoshkumar J. Anticariogenic activity of fresh aloe Vera gel mediated copper oxide nanoparticles. *Indian j public health res dev.* 2019;10(11):3664.
 39. Begum A, Jeevitha M, Preetha S, Rajeshkumar S. Cytotoxicity of Iron Nanoparticles Synthesized Using Dried Ginger. **Journal of Pharmaceutical Research International.** 2020 Nov 2;112–8.
 40. Free radical scavenging activity of iron nanoparticles synthesized using dried ginger. *Int J Pharm Res* [Internet]. 2020 Nov 2;12(04). Available from: <http://www.ijpronline.com/ViewArticleDetail.aspx?ID=18249>
 41. Jeevitha M, Rajeshkumar S. Antimicrobial activity of silver nanoparticles synthesized using marine brown seaweed *Spatoglossumasperum* against oral pathogens. *Indian j public health res dev.* 2019;10(11):3568.

42. Ali SJ, Preetha S, Jeevitha M, Prathap L, Rajeshkumar S. Antifungal Activity of Selenium Nanoparticles Extracted from Capparis decidua Fruit against Candida albicans. *Journal of Evolution of Medical and Dental Sciences*. 2020 Aug 25;9:2452+.
43. Venkataraju JL, Sharath R, Chandraprabha MN, Neelufar E, Hazra A, Patra M. Synthesis, characterization and evaluation of antimicrobial activity of zinc oxide nanoparticles. *J Biochem Technol*. 2014;3(5):151–4.
44. Elumalai K, Velmurugan S. Green synthesis, characterization and antimicrobial activities of zinc oxide nanoparticles from the leaf extract of Azadirachta indica (L.). *Appl Surf Sci*. 2015 Aug 1;345:329–36.
45. Ismail AM, Menazea AA, Kabary HA, El-Sherbiny AE, Samy A. The influence of calcination temperature on structural and antimicrobial characteristics of zinc oxide nanoparticles synthesized by Sol–Gel method. *J Mol Struct*. 2019 Nov 15;1196:332–7.
46. He L, Liu Y, Mustapha A, Lin M. Antifungal activity of zinc oxide nanoparticles against Botrytis cinerea and Penicillium expansum. *Microbiol Res*. 2011 Mar 20;166(3):207–15.
47. Karimiyan A, Najafzadeh H, Ghorbanpour M, Hekmati-Moghaddam SH. Antifungal effect of magnesium oxide, zinc oxide, silicon oxide and copper oxide nanoparticles against candida albicans. *Zahedan J Res Med Sci [Internet]*. 2015 Oct 25;17(10). Available from: <https://sites.kowsarpub.com/zjrms/articles/2179.html>

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