Open Access

SCHOLARENA

Journal of Nanoscience and Nanotechnology Applications

RESEARCH ARTICLE

ISSN: 2577-7920

Synthesis, Characterization, and biological studies of ZnO Nanoparticles prepared by using Leaf Extract of Ocimum Tenuiflorum

Shikha Bandhu[1,*], Rajalakshmanan Eswaramoorthy[1,2], Thiyaneswaran Nesappan[1],Palanivel Sathishkumar[2], Sahana Selvaganesh[1, 2]

[1]Department of Implantology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, India

[2]Department of Biomaterials, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, India

[*]**Corresponding author:** Dr. Rajalakshmanan Eswaramoorthy Professor, Department of biomaterials Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India, E-mail: rajalakshmanane.sdc@saveetha.com

Dr. Sahana Selvaganesh, Assistant professor, Department of Implantology Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India, E-mail: sahanaselva4@gmail.com.

Citation: Shikha Bandhu, Rajalakshmanan Eswaramoorthy, Thiyaneswaran Nesappan,Palanivel Sathishkumar, Sahana Selvaganesh (2023) Synthesis, Characterization, and biological studies of ZnO Nanoparticles prepared by using Leaf Extract of Ocimum Tenuiflorum. J Nanosci Nanotechnol Appl 7: 103

Abstract

Nanotechnology is a developing interdisciplinary field of research interspersing material science, bionanoscience, and technology. Nanoparticles are studied extensively for their specific catalytic, magnetic, electronic, optical, antimicrobial, wound healing and anti-inflammatory properties. The main aim of the present study was to synthesize Zn nanoparticles using the aqueous extract of tulsi (Ocimum tenuiflorum) leaves and to evaluate their antimicrobial efficacy against some selected microbes, antioxidant assay and biocompatibility. The synthesis Zn nanoparticles were characterized by FT-IR (Fourier Transform Infrared spectroscopy), DLS (Dynamic light scattering) particle size analyzer, Energy-dispersive X-ray spectroscopy and Scanning Electron Microscopy. The synthesized Zn nanoparticles showed significant antimicrobial activity against Gram positive bacteria. The maximum zone of inhibition had been found against Staphylococcus aureus. Thus from this study it can be concluded that tulsi leaf extracts can be effectively used for synthesizing Zn nanoparticles. This study also suggests that green synthesized Zn nanoparticles can be used as an alternative to antimicrobial agents.This study also suggests that green synthesized Zn nanoparticles were found to be excellent antioxidant and biocompatible nanomaterials.

Keywords: Green synthesis; zinc nanoparticles; Ocimum tenuiflorum; scanning electron microscopy

Introduction

Nanotechnology is a developing interdisciplinary field of research interspersing material science,bionanoscience and technology. Nanoparticles are studied extensively for their specific catalytic, magnetic, electronic, optical, antimicrobial, wound healing and anti-inflammatory properties. Zinc Oxide has many impressive properties like large binding ,wide band gap,high piezoelectric property etc.It is used in a large number of applications like to eliminate sulfur and arsenic from water because nano particles have greater surface area than bulk material.Zinc oxide nanoparticles are known to be one of the multifunctional inorganic nanoparticles with effective antibacterial activity.

In literature, ZnO nanoparticles (Ahmed 2013)are synthesized from conventional methods like chemical reduction, laser ablation, solvothermal, inert gas condensation and the sol-gel method. These methods require some toxic chemicals, high pressure, laser radiation, inert gasses like helium ac compared to green synthesis method. Some of these conventional methods are expensive, not easy to operate, require too much attention during the process, and also require special types of vessel like polypropylene vessel for nanoparticle synthesis.

Plant mediated synthesis of nanoparticles are preferred over chemical synthesis due to its simplicity, eco-friendliness and extensive antimicrobial activity, non-toxic by-products and large-scale synthesis.In recent years, green synthesis of ZnO nanoparticles (Anvekar, Rajendra, and Kadam 2017)was achieved by using leaves extracted from the Ocimum Tenuiflorum plant. Ocimum Tenuiflorum is also called holy basil, tulsi. It belongs to the Lamiacece family. Ocimum Tenuiflorum is mostly present in tropical regions. The chemical constituents of Ocimum Tenuiflorum are linalool, alkaloids, ursolic acid, glycosides, carvacrol, tannins, rosmarinic acid, aromatic compounds etc. Leaves extract of ocimum tenuiflorum plant has been utilized in the synthesis of copper nanoparticles,gold nanoparticles and silver nanoparticles.The aim of the present study is synthesis and characterization of ZnO nanoparticles using aqueous leaves extract of Ocimum Tenuiflorum plant and to evaluate its antimicrobial efficacy.

Material and Methods

Green synthesis of ZnO nanoparticles using tulsi leaf extract

For preparation,(Mulpuri and Guttula 2014) tulsi leaves are washed with distilled water and completely dried in sunlight.Then crush the leaves in its powder form and dilute 25gm of powder in 500ml of distilled water.The composition is mixed well and left overnight for sedimentation.The solution was filtered using filter paper. 100ml of that aqueous extract(Bährle-Rapp 2007) was taken in a beaker and was placed on a magnetic stirrer.2gm of zinc nitrate was diluted in 20ml of water and added to the solution drop by drop.Observe for 2 hours for any color change. A reddish-brown change in color was observed (Vazhacharickal et al. 2019), indicative of the formation of zinc nanoparticles. After the solution was left to cool down,it was transferred in graduated centrifuge tubes of 15ml and placed in the centrifugation chamber at 10,000 rpm for 10 minutes (figure: 1). The extra liquid was drained leaving the residue.Then the tubes were left open and kept in a hot air oven overnight.This led to the complete drying of the residue making it easier to grind it into finer particles.

Figure: 1 represents the preparation of the nanoparticles with tulsi leaf extract.

Characterization of ZnO nanoparticles

Particle size analysis

Particle size analysis determines the size range or mean size of a particle in a powder or liquid form. This testing is done to assess the presence of nanoparticles in the mixture(Ahmed 2013).

DLS Analysis: The particle size distribution of the samples was obtained through Particle Size Analyzer. The liquid samples of ZnO NPs were diluted ten times using Milli-Q water, centrifuged and then transferred to cuvette for analysis. The zeta potential of ZnO NPS was determined in water as a dispersant.

SEM (Scanning Electron Microscope)

The SEM analysis was used to determine the structure of reaction products that were formed. The samples of ZnO NPs were dispersed in methanol (evaporating solvent) at a concentration of 1 mg/20 mL. A single drop of aqueous solution of ZnO NPs was placed on the carbon coated grid to prepare a thin film. Extra solution was removed with the help of blotting paper and the grid was allowed to dry under mercury lamps for around five minutes. The morphological measurements of the ZnO NPs samples were recorded with field emission scanning electron microscope (JEOL, Model: JSM-IT800) in the range of 0.1 nm to 10,000 nm.

FTIR (Fourier Transform Infrared Spectroscopy)

FTIR Spectroscopy,fourier-transform infrared spectroscopy,is concerned with the vibration of molecules(Pradeep and Chandrasekaran 2006).Each functional group has its own discrete vibrational energy which can be used to identify a molecule through the combination of all of the functional groups.This makes FTIR microscopy ideal for sample ID,multilayer film characterization,and particle analysis.

FTIR Analysis: The surface chemistry of NPs was analyzed by FTIR spectroscopy using the FTIR spectrometer(Bruker Alpha II). The functional groups attached to the surface of NPs were detected in the range of 4000–400 nm. The samples were prepared by dispersing the ZnO NPs uniformly in a matrix of dry KBr which was then compressed to form a transparent disc. KBr pellets were used as a standard.

EDX (Energy-dispersive X-ray spectroscopy)

It is an analytical technique used for the elemental analysis or chemical characterization of a sample.Its characterization capabilities are due in large part to the fundamental principle that each element has a unique set of peaks on its electromagnetic emission spectrum.

Antibacterial activity studies

Zone of inhibition (ZOI) measurement

Antibacterial activity of the ZnONPs synthesized by Ocimum tenuiflorum extract were assessed against Gram-positive bacterial strains such as Staphylococcus aureus, Streptococcus mutans and Enterococcus faecalis using Mueller–Hinton agar (MHA; Himedia, Mumbai, India) plate by agar well-diffusion technique. The MHA was prepared in double distilled water (pH 7.0) and sterilized in autoclave at 121 ˚C for 15 min. Then, the sterilized MHA was poured into the perti plate and allowed to solidify at room temperature in laminar flow. Inoculum containing 106 cfu/mL of the freshly prepared bacterial culture was spread onto the MHA plates with a sterile cotton swab moistened with the suspension of the respective microbial culture. Then, three wells (9 mm in diameter) were punched into the MHA medium and filled with different concentrations (50 µg, 100 µg, and 250 µg) of ZnONPs with the help of micropipette, and kept at room temperature for 4 h to diffuse the ZnONPs into the medium. Then, the culture plates were incubated at 37 °C for 24 h. After incubation, the diameter (mm) of the zone of inhibition was recorded in each plate. The results were expressed as mean value with standard deviation (SD) of triplicate experiment.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) of the ZnONPs synthesized by Ocimum tenuiflorum extract was determined against Staphylococcus aureus and Enterococcus faecalis by the broth dilution method. Briefly, each bacterial culture (0.5 McFarland: 1.5 × 108 CFU/mL) was added to the nutrient broth, which containing different dilution (10-1 to 10-7) of ZnONPs (1 mg/mL). The MIC concentration of the ZnONPs is defined as the lowest concentration inhibiting the visible growth of Staphylococcus aureus and Enterococcus faecalis. After 18 h incubation, the growth of Staphylococcus aureus and Enterococcus faecalis in the tubes was observed as turbidity at 600 nm using UV-visible spectrophotometer. The least concentration which showed more than 75% of bacterial growth inhibition was determined and noted as the MIC value.

Antioxidant Assay

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang et al. (2001). The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm. Ascorbic acid (10mg/ml DMSO) was used as reference. Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. Using the following formula, the % of DPPH inhibition was computed,

% scavenging activity = ((Abs. of control–abs. of test)/ Abs. of control) × 100

Biocompatibility Study

Biogenic ZnO-NPs' biocompatibility was demonstrated using fresh human red blood cells (hRBCs).Blood samples (1 mL) were taken from healthy individuals in ethylenediaminetetraacetic acid (EDTA) tubes after permission of the individual. After collection of blood samples, the samples were subjected to centrifugation for the isolation of RBCs. After centrifugation, supernatant and pellet were obtained; the supernatant was discarded, and the pellet was collected after washing three times with

PBS. For the preparation of PBS–erythrocyte suspension, we mix 200 µL of RBCs with 9.8 mL of PBS (pH 7.2). Then, the suspensions of erythrocytes and green synthesized ZnO-NPs were mixed in Eppendorf tubes. The Eppendorf tubes containing the mixture of erythrocyte suspension and biogenic NPs were then subjected to incubation for 1 h at 35 °C. Reaction mixtures were centrifuged at 1000 rpm for 10 min followed by transfer of 200 µL of supernatant to a 96-well plate; and hemoglobin release absorption spectra were recorded at 540 nm. As a control, Triton X-100 (0.5%) was used, while DMSO was considered as a negative control. %hemolysis was calculated using the following formula

$$\%hymolysis = \left\{ \frac{sampleAb - negativecontrolAb}{PositivecontrolAb - negativecontrolAb} \right\} \times 100$$

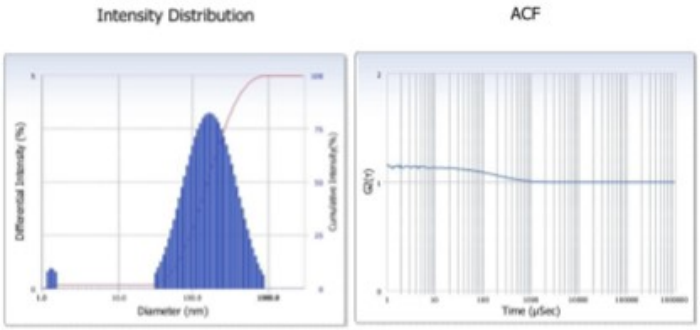
where Ab stands for the absorbance of the samples as recorded.

Results and Discussion

Optical analysis of ZnO NPs formation

For the preparation of green synthesized zinc nanoparticles, zinc Nitrate in leaf extracts of Ocimum Tenuiflorum was added which lead to physio-chemical changes in the aqueous solution. The most obvious of which is a change in the color of the reaction mixture that can be observed within a few minutes. This was considered as an initial sign for the formation of NPs. In present study, change of color from yellow to light brown and red to off-white indicated the formation of ZnO NPs in leaf extracts of Ocimum Tenuiflorum. Flavonoids and phenolic compounds are thought to be responsible for Zn ions to ZnO NPs. In a period of a few hours, the color of the solution stopped changing further suggesting the complete bioreduction of ZnO salt into NPs. A clear illustration of change in color of the reaction mixtures due to formation of ZnO NPs. These results were consistent with the previous reports of color changes in plant based synthesis of ZnO NPs. Temperature is considered an important contributing factor in synthesis of good sized nanoparticles. It is also well established that higher the temperature of the reaction process of NPs synthesis, the smaller the size of the NPs. Therefore, we use a relatively higher temperature of 70 °C for incubating the reactants that leads to the production of very small sized ZnO NPs.

Particle Size analysis



Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	1.3	0.1
2	216.5	158.1
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	212.8	159.2

Figure 2: represents the particle size of the ZnO Nps, in the given solutions

SEM (Scanning electron microscope)

Scanning Electron Microscopy (SEM) images of ZnO NPs synthesized from leaf extracts of Ocimum Tenuiflorum. The images were recorded at magnification of 5 µm, 1 µm. Topographical view shows that nanoparticles are more or less spherical in nature, clustered together and the surface of the aggregates seems to be rough. SEM images also revealed that NPs derived from plants are entirely pure and it can be concluded that the plant has tremendous capability to synthesize ZnO NPs (figure 3). Shape of NPs plays a very crucial role in the effectiveness against pathogens(Choukade, Jaiswal, and Kango 2020). Because spherical NPs tend to be very potent during antibacterial activity owing to their ability to easily penetrate into the cell wall of pathogens. Therefore, ZnO NPs synthesized from the plant species can be of great importance in treating clinical pathogens.

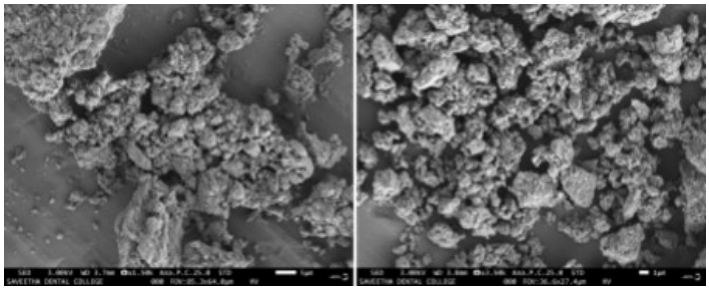
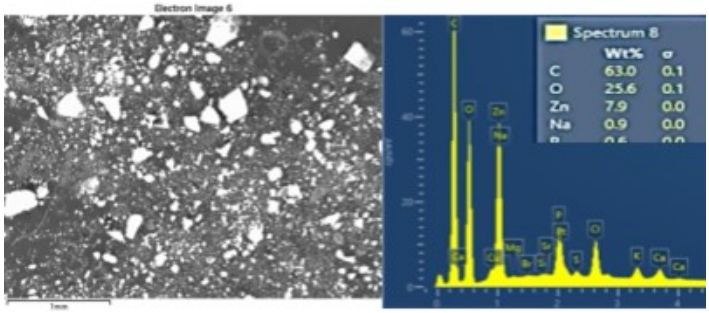


Figure 3: Represents the SEM pictures of the nanoparticle.

Energy Dispersive Analysis of X-Ray (EDAX)



Element	Wt%	At%
Zn	7.9	0
O	25.6	0.1

Table 1: spectral peak of metal nanoparticles formed.

FTIR

To identify the functional groups associated with the ZnO NPs formation, FTIR spectrometry was performed. Spectral peaks at 683–500 cm−1 and 698–505 cm−1proposed the formation of ZnO nanoparticles in Ocimum Tenuiflorum extract (figure 4). Absence of peaks in the region of 3500 and 2500 cm−1 indicated no characteristic OH and N-H stretching of aldehydes. The bands at 1600–1510 cm−1correspond to amide I and amide II regions arising due to carbonyl stretching in proteins and that of 1400 to 1000 cm−1 correspond to C-N stretching vibrations of amine. Peaks from 1460–1410 cm−1 suggested C-C stretching vibration of alcohol, carboxylic acid, ether and ester. Although many changes were not observed at these frequencies, all peaks showed a shift to lower frequency and a decrease in intensity on binding with the nanoparticles. This trend of free carbonyl and NH2 groups from proteins and amino acid residues indicates that they have the ability to bind to a metal and that the proteins could possibly form a layer around the metal for preventing agglomeration and thereby stabilizing the nanoparticles. It is revealed

from the FTIR spectra that in fact, the protein molecules present in the leaf extract possibly cause the reduction of metal ions which is in agreement with the previous reports. These findings suggest that not only the OH group of flavonoids but also the protein molecules and their functional groups play an important role in bioreduction of salts and capping of NPs.

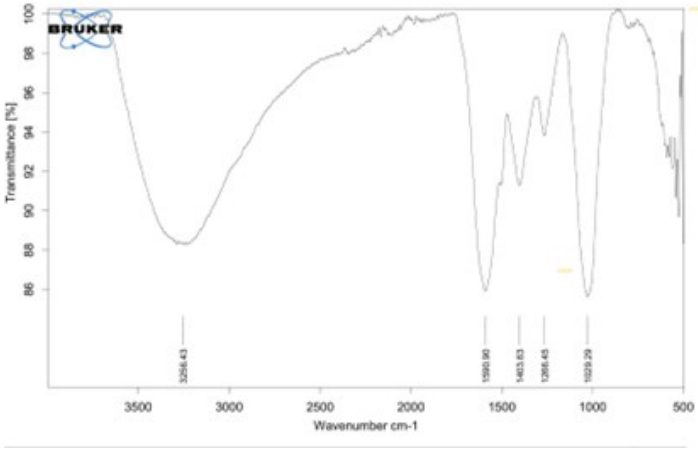


Figure 4: Represents the spectral peaks of the green synthesized zinc nanoparticles.

Zone of inhibition (ZOI) and minimum inhibitory concentration (MIC)

The antibacterial activity of the ZnONPs synthesized by Ocimum tenuiflorum extract was assessed against Gram-positive organisms Staphylococcus aureus, Streptococcus mutans and Enterococcus faecalis at three different concentrations (50 µg, 100 µg, and 250 µg). As shown in Fig. 5, the result evidently indicates that the ZOI for ZnONPs was observed as follows: 11.5±1.5 mm (50 µg), 12.0±1.0 mm (100 µg), and 15.5±1.5 mm (250 µg) in diameter against

Staphylococcus aureus; whereas, 10±0.5 mm (50 µg), 11±0.5 mm (100 µg), and 13.5±1.0 mm (250 µg) in diameter against Enterococcus faecalis. It confirms that the ZnONPs synthesized by Ocimum tenuiflorum extract exhibits excellent antibacterial activity against Gram-positive organisms such as Staphylococcus aureus and Enterococcus faecalis. However, there was no antibacterial activity was found against another tested Gram-positive organisms Streptococcus mutans. Based on the ZOI results, further study was carried out with Staphylococcus aureus and Enterococcus faecalis to find out the minimal inhibitory concentration (MIC) of the biosynthesised ZnONPs. The MIC result denotes that the growth of both bacterial strains was inhibited (above 75%) by the biosynthesised ZnONPs at a concentration of 60 µg (range of 50-100 µg) and 200 µg (range of 100-500 µg), respectively, as illustrated in Fig. 6. Finally, this antibacterial study proves that the biosynthesised ZnONPs by Ocimum tenuiflorum extract might be a good nanodrug to treat the disease caused by Staphylococcus aureus and Enterococcus faecalis.

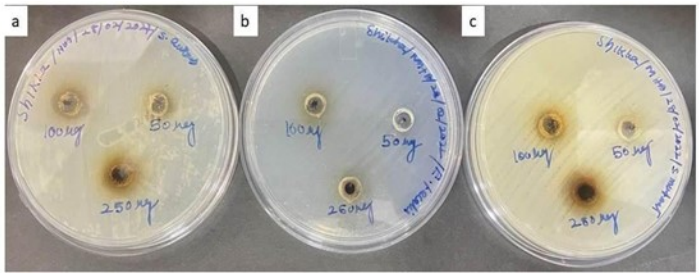


Figure 5. Zone of inhibition (ZOI) test on MHA plate for the ZnO for Gram-positive organisms

(a) Staphylococcus aureus, (b) Enterococcus faecalis, and (c) Streptococcus mutans.

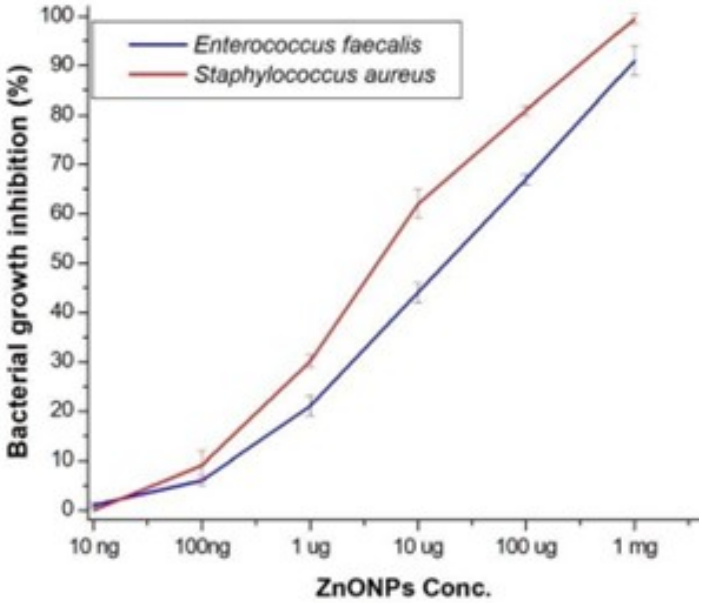


Figure 6: Minimum inhibitory concentration (MIC) test for the biosynthesized AgNPs against Gram positive Staphylococcus aureus and Enterococcus faecalis.

Antioxidant Assay

The change in plant metabolic pathways is attributed to environmental stress that results in reactive oxygen species (ROS) destroying membrane lipids, plant cells, DNA, and proteins. Many metabolically important compounds like flavonoids, terpenoids, and oxidative stress-responsive agents play a promising role in the capping and stabilization of the nanoparticles. In accordance with it, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay (FRSA), was conducted to assess the in vitro antioxidant potential of plant-synthesized NPs. DPPH is a stable free radical that is reduced by accepting hydrogen or electrons from a donor based on formation of a yellowish diphenyl picryl hydrazine molecule. These spectrophotometric methods are based on quenching of stable-colored radicals of DPPH, indicating the scavenging ability of the antioxidant sample. In the study, excellent free radical scavenging activity of all test concentrations was revealed.

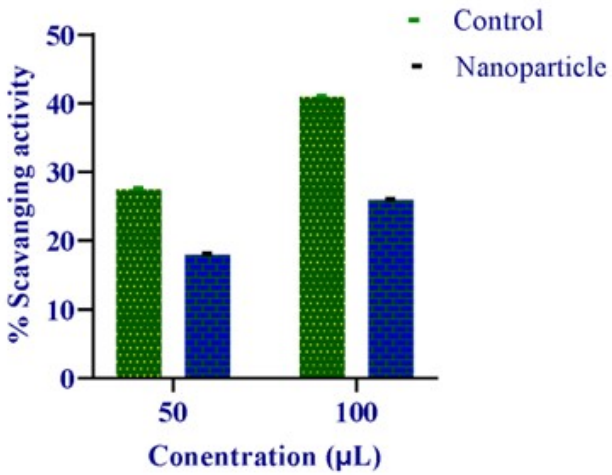


Figure 7: Antioxidant activity of ZnO/Ocimum Tenuiflorum nanoparticles compared with Ascorbic acid.

In Vitro Biocompatibility Studies

Current hemocompatibility assay shows a maximum of 0.13% of lysis that exhibits the bioactive materials have good agreement with blood cells. Hence, development of bioactive materials with improved hemocompatibility certainly enhances the tolerability and reduces side effects. ZnO nanoparticles showed lower eryth- rocyte rupture rate. We evaluated the blood compatibility with 5 mg, and 10 mg/mL of bioactive materials. 10mg/mL of ZnO nanoparticles showed superior compatibility and exhibited 0.13% of lysis (10 mg/mL). Red blood cells lysis percentage exhibits a decrease in survival erythrocytes with the proportion of increasing concentration of bioactive materials. Bioactive compositions have tremendous biocom- patible as well as biodegradable properties. Hence, it was methodically analyzed for toxicology as well as biocompatible properties of bioactive material formulations in-vitro . After several investigations, it was summarized that bioactive material induces cell growth along with cell division, which exhibits improved biocompatibility with host tissue . In accordance with aforementioned reports, our results indicate that bioactive material formulations are always exhibiting optimal biocompatibility in the physiological environment. Further, the assessed erythrocyte compatibility by varying the sodium precursors shows that tulsi with zinc oxide nanoparticles registered improved erythrocyte compatibility (Fig.7).Synthesized biogenic ZnO-NPs are hem-compatible, and even at a high concentration of 10mg/mL, we observed no hemolytic activity. The biocompatibility results of our study thus show that plant-based synthesized nanoparticles are biosafe and we can use ZnO-NPs for therapeutic purposes.

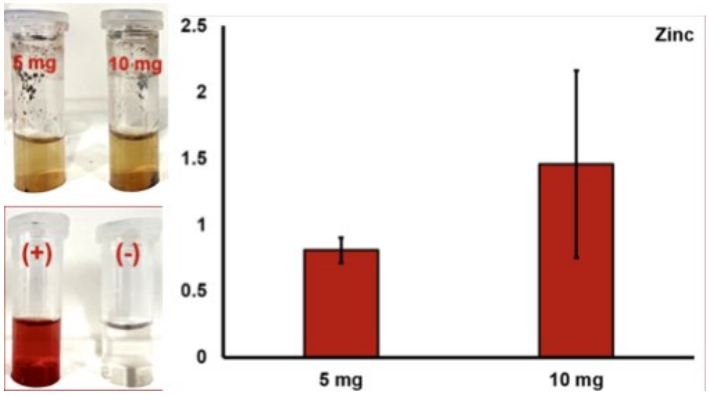


Figure 8: Erythrocyte rupture rate while treating with bioactive materials (a) photographical images represent the blood compatibility of various concentrations and b) percentage of hemolysis.

Conclusion

Leaf extracts of Ocimum Tenuiflorum showed excellent potential as reducing agents in the formation of NPs. Structural and optical studies conducted using FTIR, DLS, EDAX and SEM analysis confirmed the formation of efficient ZnO NPs. Antibacterial analysis revealed that ZnO NPs synthesized from leaf extracts exhibited significant capability of inhibition against the clinical pathogens when compared to traditional drugs. Moreover, some plant extracts are more effective than that of others in synthesizing NPs and biological activities due to their diverse biochemical compositions. In conclusion, synthesis of NPs using extracts of medicinal plants can have useful medicinal applications in treatment of numerous human infectious pathogens. However, further studies will be required to validate the efficacy of these NPs in medical applications and their capacity to overcome the risks associated with conventional drugs.

References

1. Ahmed Sumair (2013) Synthesis & Characterization of Nano Zinc Oxide by Solution Combustion: NanoCrystalline Zinc Oxide, Synthesis, Characterization, Analyzing. LAP Lambert Academic Publishing.

2. Anvekar, Tushar, Vrajeshwari Rajendra, Hari Kadam (2017) "Green Synthesis of ZnO Nanoparticles, Its Characterization and Application." Material Science Research India.

3. Bahrle-Rapp, Marina (2007) "Ocimum Tenuiflorum Extract." Springer Lexikon Kosmetik Und Körperpflege.

4. Choukade, Ritumbhara, Amisha Jaiswal, Naveen Kango (2020) "Characterization of Biogenically Synthesized Silver Nanoparticles for Therapeutic Applications and Enzyme Nanocomplex Generation." 3 Biotech 10: 462.

5. Gupta, Monika, Rajesh S Tomar, Shuchi Kaushik, Raghvendra K Mishra, Divakar Sharma (2018) "Effective Antimicrobial Activity of Green ZnO Nanoparticles of." Frontiers in Microbiology.

6. Hymavathi, Atmakur, Sohail Nizamuddin, Himabindu Bantikatla (2022) "Design, Green Synthesis and Characterization of Zinc Oxide Nanoparticles from Ocimum Tenuiflorum Var. Ram Tulsi." Materials Today: Proceedings.

7. Mulpuri, Venkata Ramana, Anuradha Guttula (2014) A Review on Biosynthesis of Metal Nanoparticles. Lulu Press, Inc.

8. Pradeep A, G Chandrasekaran (2006) "FTIR Study of Ni, Cu and Zn Substituted Nano-Particles of MgFe2O4." Materials Letters.

9. Qian Y, Yao J, Russel M, Chen K, Wang X(2015) Characterization of green synthesized nano-formulation (ZnO-A. vera) and their antibacterial activity against pathogens. Environ Toxicol Pharmacol. 39: 736-46.

10. Gupta M, Tomar RS, Kaushik S, Mishra RK, Sharma D(2018) Effective Antimicrobial Activity of Green ZnO Nanoparticles of Catharanthus roseus. Front Microbiol. 9: 2030.

11. Vazhacharickal, Prem Jose, Nissy Mary Joseph, NK Sajeshkumar, Jiby John Mathew, et al. (2019) Green Synthesis of Copper and Zinc Nanoparticles from Plant Extracts and Evaluation of Their Antifungal Activity against Fusarium Oxysporum Cubense: An Overview. Amazon Publishers, USA.

12. Krol A, Pomastowski P, Rafińska K, Railean-Plugaru V, Buszewski B (2017) Zinc oxide nanoparticles: Synthesis, antiseptic activity and toxicity mechanism. Adv Colloid Interface. 249: 37-52.

13. Janaki AC, Sailatha E, Gunasekaran S (2015) Synthesis, characteristics and antimicrobial activity of ZnO nanoparticles. Spectrochim Acta A Mol Biomol Spectrosc. 144: 17-22.

14. Babayevska N, Przysiecka Ł, Iatsunskyi I, Nowaczyk G, Jarek M, et al. (2022) ZnO size and shape effect on antibacterial activity and cytotoxicity profile. 12: 8148.

15. Mohamed AA, Abu-Elghait M, Ahmed NE, Salem SS. (2021) Eco-friendly Mycogenic Synthesis of ZnO and CuO Nanoparticles for In Vitro Antibacterial, Antibiofilm, and Antifungal Applications. Biol Trace Elem Res. 199: 2788-99.

16. Faisal S, Jan H, Shah SA, Shah S, Khan A, et al. (2021) Green Synthesis of Zinc Oxide (ZnO) Nanoparticles Using Aqueous Fruit Extracts of Myristica fragrans: Their Characterizations and Biological and Environmental Applications. ACS Omega. 6:

9709-22.

17. Vinotha V, Iswarya A, Thaya R, Govindarajan M, Alharbi NS, et al. (2019) Synthesis of ZnO nanoparticles using insulin-rich leaf extract: Anti-diabetic, antibiofilm and anti-oxidant properties. J Photochem Photobiol B. 197: 111541.

18. Barani M, Masoudi M, Mashreghi M, Makhdoumi A, Eshghi H (2021) Cell-free extract assisted synthesis of ZnO nanoparticles using aquatic bacterial strains: Biological activities and toxicological evaluation. Int J Pharm. 606: 120878.