VOLUME 10 ISSUE 1 2024

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 10, No. 1, 647-655 (2024)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

A Comprehensive Study on Zinc Oxide Nanoparticles: Synthesis, Characterization, Evaluation and Microbiological Insights

Sonawane Vijayraj Narendra[1]*, Pathan Amjadkhan[2] and Sonawane Deepak Devidas[3]

[1]Department of Pharmaceutical Sciences, Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan 333010, India
[2]Department of Pharmacy, Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan 333010, India
[3]Department of Pharmaceutical Sciences, Shree Shakti Shaikshanik Sanstha's, Divinecollege of Pharmacy, Satana, Nashik, Maharashtra 423301, India

**Corresponding Author*

Received: 2nd February, 2024; **Accepted:** 22nd April, 2024; **Published online:** 1st May, 2024

https://doi.org/10.33745/ijzi.2024.v10i01.069

Abstract: Over the past ten years, nanoscale science and technology have become revolutionary areas of study. Due to their ability to withstand challenging processing circumstances, inorganic materials like metal oxides and metals have received an attention in the past 10 years. Metal oxides like zinc oxide, calcium oxide, titanium dioxide, and magnesium oxide are important inorganic materials that are generally considered safe for use by humans and animals. Due to their antibacterial qualities, zinc oxide and silver nanoparticles have been used as an effective approach for preventing infectious infections. Shape, crystallinity, composition, and morphology are the primary determinants of the intrinsic properties of metal nanoparticles. In this research, sodium hydroxide, zinc nitrate and soluble starch were used as precursors and stabilising agents in the chemical creation of zinc oxide nanoparticles. Then zinc oxide nanoparticles were characterized for evaluation of their properties. The ZnO nanoparticle assay, TEM, UV-visible spectroscopy, SEM, EDX, antimicrobial test, and Malvern size distribution report were used to characterize the zinc oxide nanoparticles. The average particle size of zinc oxide nanoparticles was found to be 10-40 nm. The antibacterial activity of the zinc oxide nanoparticles was demonstrated against *Candida albicans* (CA), *Escherichia coli* (EC), *Staphylococcus aureus* (SA), and *Pseudomonas aeruginosa* (PA). During comparison of zone of inhibition it was observed that the zinc oxide nanoparticles show strong antimicrobial activity than zinc oxide powder.

Keywords: Metal Nanoparticles, Characterization, Skin diseases, Antibacterial activity, Nanosize, *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*

Citation: Sonawane Vijayraj Narendra, Pathan Amjadkhan and Sonawane Deepak Devidas: A comprehensive study on zinc oxide nanoparticles: synthesis, characterization, evaluation and microbiological insights. Intern. J. Zool. Invest. 10(1): 647-655, 2024.

https://doi.org/10.33745/ijzi.2024.v10i01.069

Introduction

The antibacterial and antifungal infection of the skin is common problem in whole world. These infections should be treated by using systemic or topical antibiotics. The systemic treatment is more critical as it creates many severe adverse effects. Thus, topical antibiotic treatment is one of the better options to treat the topical infections. However, the consistent use of antibiotics produces the resistance within the bacteria. In addition, this multi-drug resistant bacteria and fungus is the main problem in treating infectious diseases. The amount of multi drug resistant bacteria and fungus is increasing rapidly. Therefore, it is urgently necessary to create new, powerful antimicrobial drugs that can stop these bacteria and fungi that are resistant to a variety of medications (Smith *et al.*, 2020). In the current study, ZnO nanoparticles were synthesized using chemical method and it is evaluated for its antimicrobial, antifungal activity followed by its analytical evaluation. The zinc oxide, due to its nanosize have unique and improved antimicrobial activity. Due to the nanosize of zinc oxide, the penetration in the skin will increase and that will be beneficial in treating topical microbial infections (Kaur *et al.*, 2022).

Nanotechnology:

It would not be an exaggeration to refer to the twenty-first century as the "nanocentury," given how the nano-revolution has affected nearly every aspect of human life, including optical technology, electronics, industries, drug delivery, diagnostics, imaging, biosensing, and consumer goods like sunscreen, clothing, and cosmetics (Alhagiesa *et al.*, 2020).

Nanoparticles demonstrate new or improved qualities depending on particular characteristics like size distribution and form. The discipline of nanotechnology has made great strides in recent years, with a variety of methods developed to synthesize nanoparticles of various shapes and sizes in accordance with specific needs. It deals with substances whose nanoscale architectures display innovative and enhanced physical, chemical, and biological phenomena, characteristics, and functionality. Nanoparticles have more surface area than materials with a larger size due to their tiny size (Khan *et al.*, 2019).

Over the past ten years, nanoscale science and technology have become revolutionary areas of study. Due to their ability to withstand challenging processing circumstances, inorganic materials like metal oxides and metal have received a lot of consideration in the past 10 years. Metal oxides like zinc oxide, calcium oxide, titanium dioxide and magnesium oxide are important inorganic materials that are generally considered safe for use by humans and animals. Due to their antibacterial qualities, zinc oxide and silver nanoparticles have been used as an effective approach for preventing infectious infections. Shape, crystallinity, composition, and morphology are the main factors that determine a metal nanoparticle's intrinsic characteristics (Jubran *et al.*, 2020).

Zinc Oxide Nanoparticles:

ZnO has been the attention of thousands of researched during the last 100 years, some as early as 1935. ZnO was introduced to industry because of its potential for ultraviolet absorbance, piezoelectricity, diverse chemistry, and luminescence at high temperatures, and it is now a crucial component of contemporary science. It is used in the production of rubber, paints, cosmetics, plastics, medications and electronics. It has recently attracted a lot of interest due to its semiconducting qualities (Padmavathy *et al.*, 2008; Bawarski *et al.*, 2008).

Antimicrobial substances can be roughly divided into organic and inorganic types. In comparison to inorganic antibacterial agents, organic antibacterial compounds are shown to be less stable at high temperatures and pressures. As a result, zinc oxide has demonstrated efficacy as an antibacterial agent in the production of therapeutic micro- and nanoscale systems. ZnO nanoparticles outperformed bulk and micro particles in terms of antibacterial activity (Parveen *et al.*, 2017; Tania *et al.*, 2021).

ZnO nanoparticles may be employed as a medicine carrier, a potent antimicrobial, and a treatment for leukemia and carcinoma cancer cells. Due to their strong UV absorption qualities without dispersing visible light, they are widely utilized in cosmetics and sunscreens. Due to their antibacterial qualities, they are also utilized in the food sector as additives and in packaging. They may also be employed as fungicides in agriculture (Sharma *et al.*, 2009).

Topical Treatment of Skin Diseases:

As more infectious diseases develop resistance to synthetic medications, finding new antibiotics is a global problem facing the pharmaceutical and research sectors. The improper use of orthodox medicines has led to problems with resistance, pollution, and environmental damage. As a result, there is a growing interest in nature as a source of safer and more effective alternatives for treating human ailments (Rai *et al.*, 2019; Baraiya *et al.*, 2022). The drug's capacity to enter the skin sufficiently to produce the desired effects is what determines whether topical delivery will be successful. The majority of drug candidates cannot penetrate the stratum corneum on their own and must be combined with physical boosters or unique transporters in order to penetrate the skin (Mamillapalli *et al.*, 2020). Delivery of nanoparticles to the skin is being employed more frequently to support local therapies. The three main locations for topical nanoparticle medication delivery are the stratum corneum surface, dermatoglyphs, and the entry of hair follicles (infundibulum) (Sanchez-Lopez *et al.*, 2020; Mashar *et al.*, 2023).

In this research, sodium hydroxide, zinc nitrate and soluble starch were used as precursors and stabilising agents in the chemical creation of zinc oxide nanoparticles. Then zinc oxide nanoparticles were characterized for evaluation of their properties. The ZnO nanoparticle assay, TEM, UV-visible spectroscopy, SEM, EDX, antimicrobial test, and Malvern size distribution report were used to characterize the zinc oxide nanoparticles. The average particle size of zinc oxide nanoparticles was found to be 10-40 nm. The antibacterial activity of the zinc oxide nano-particles was demonstrated against *Candida albicans* (CA), *Escherichia coli* (EC), *Staphylococcus aureus* (SA), and *Pseudomonas aeruginosa* (PA).

Materials and Methods

Zinc Oxide Nanoparticle Synthesis:

Utilizing soluble starch as a stabilizing agent and sodium hydroxide and zinc nitrate as pioneers, wet chemical synthesis is utilized to produce the ZnO nanoparticles(Mirzaei *et al.*, 2017; Ghorbani *et al.*, 2015).

Evaluation of Zinc Oxide Nanoparticles by Characterization:

UV-Visible Spectral Analysis:

To understand the kinetic behavior of ZnO nanoparticles, the UV-visible spectrophotometer was used to characterize the ZnO nanoparticles. The sample has a 200–400 nm scanning range. Using a blank reference, the spectrophotometer's baseline correction is performed. The sample's UV-Vis absorption spectra was captured (Gautam *et al.*, 2023; Yeola *et al.*, 2023).

Transmission Electron Microscopy (*TEM*):

With an W emitter electron source and LaB, a voltage acceleration of 200 kv, an objective lens of S-TWIN, resolution point of 0.27 nanometre or better, a line resolution of 2.0 nanometre or better, and a magnification of 750000x to 25x or higher and a single CCD camera tilt holder transmission electron microscopy was carried out (Petrushevska *et al.*, 2019).

Scanning electron microscopy (*SEM*):

The resolution of the scanning electron microscopy was 1.4 nanometer at 1 kV, 1.0 nm at 15 kV and 3.5 nm at 100V. The sample navigation was done using a 5 axes motorised, xyz tilt rotate stage with eucentric tilt, tilting from $+75^0$ to -15^0.

Acceleration voltage was 20 V to 30 kV Current of beam was up to 200 nA, X25 to X1000000 magnification, and ultra-high brightness Schottky emitter field emitter is employed (Asano *et al.*, 2021).

Energy dispersive X-ray analysis (EDX):

Energy dispersive X-ay study was carried out on TEAM EDS Analysis system with octane SDDs having Maximum energy resolution of 129eV and quantifiable analysis at up to 200,000 cps mapping rates (Scimeca *et al.*, 2018).

Size distribution report by intensity by Malvern:

With the Malvern analyzer, the size distribution was computed. The system parameters utilized in the analysis were: temperature of 25°C, duration used is 30, count rate (KCPS) of 177.0, and measurement position is of 4.65 mm. Disposable sizing cuvette, attenuator of 8, water as the dispersant, RI of 1.330 for the dispersant, 1.17 for the material, and 0.100 for the material absorption are the characteristics of the cell (Caputo *et al.*, 2021).

Assay of ZnO nanoparticles:

0.15 g of ZnO nanoparticle powder was dissolved in 2 M acetic acid (10 ml), and the mixture was then diluted to 50 ml with water. The resultant solution was given a sufficient amount of hexamine and about 50 mg of xylenol orange triturate to give violet-pink colour. The mixture was titrated with 0.1 M of disodium edetate until it becomes yellow, following the addition of 2 g of hexamine (Ghorbani *et al.*, 2015).

Antimicrobial Activity of ZnO Nanoparticles, ZnO Powder:

ZnO bulk powder and nanoparticles was tested for their antibacterial and antifungal properties against various pathogens using the traditional disc diffusion method (Xie *et al.*, 2011; Sirelkhatim *et al.*, 2015).

Results and Discussion

Evaluation of Zinc Oxide Nanoparticles by Characterization:

UV-Visible Spectral Analysis:

The UV-Vis spectra of ZnO nanoparticles prepared with 0.5% concentration of starch which is soluble is shown in Figure 1.

In UV-visible spectra the absorption peak of the created ZnO nanoparticles was found at 359 nanometre.

Transmission Electron Microscopy (TEM):

The images of nanoparticles captured using Transmission Electron Microscopy was as shown in Figure 2.

From the TEM analysis, the normal (average) size of nanoparticles of zinc oxide was found 10-40 nanometer. Thus, the formation of nanoparticles was confirmed.

Scanning electron microscopy (SEM):

The images of nanoparticles captured with Scanning Electron Microscopy is shown in Figure 3.

From the Scanning Electron Microscopy study, the normal (average) size of ZnO nanoparticles was found 10-40 nm. Morphology of Nanoparticles was found to be Spherical with milk white colour.

Energy dispersive X-ray analysis (EDX):

The graph obtained from energy-dispersive X-ray analysis is shown below in Figure 4.

From the EDX study of nanoparticle powder the composition of elements of powder was identified and confirmed. The Oxygen was found to be 39.61 % and Zinc was found to be 60.39%, this confirms the exact composition of Zinc oxide nanoparticles.

Size distribution report by intensity by Malvern:

The graph obtained during size distribution by intensity by Malvern is shown in Figure 5.

Size distribution results showed that zinc oxide nanoparticles were within the Nano metric size (less than 60 nm)

Assay of ZnO nanoparticles:

Assay of Zinc oxide nanoparticles is performed using titration method (Table 1).

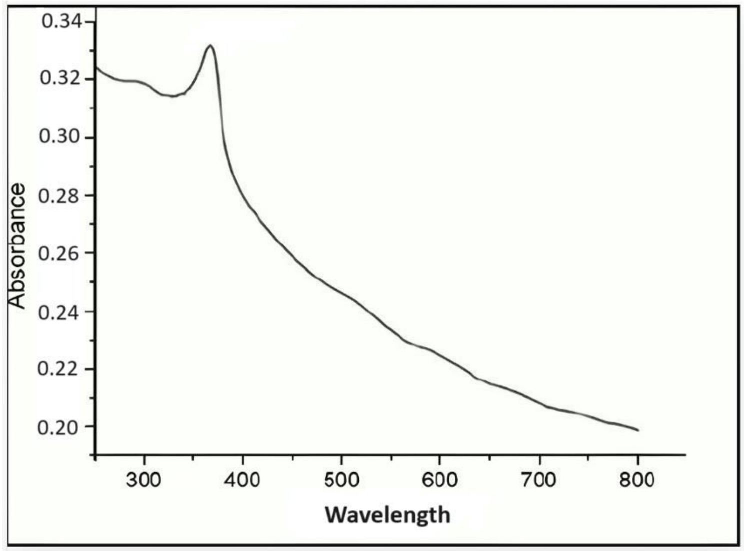


Fig. 1: UV-visible spectra of ZnO nanoparticles.

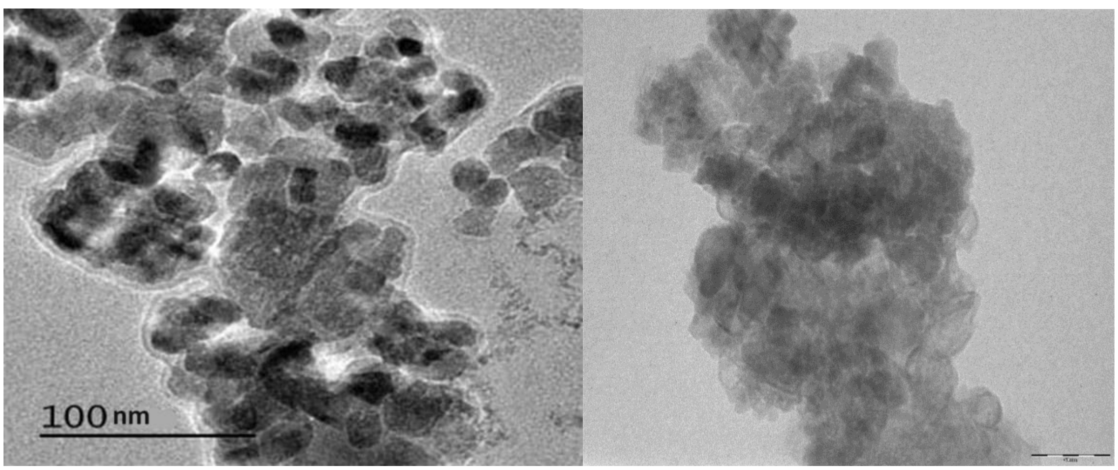


Fig. 2: Images of ZnO nanoparticles by TEM.

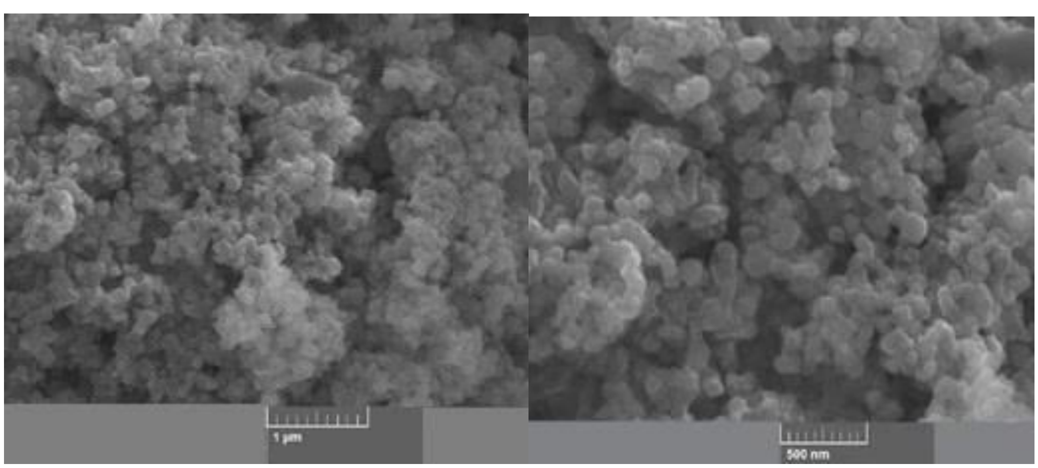
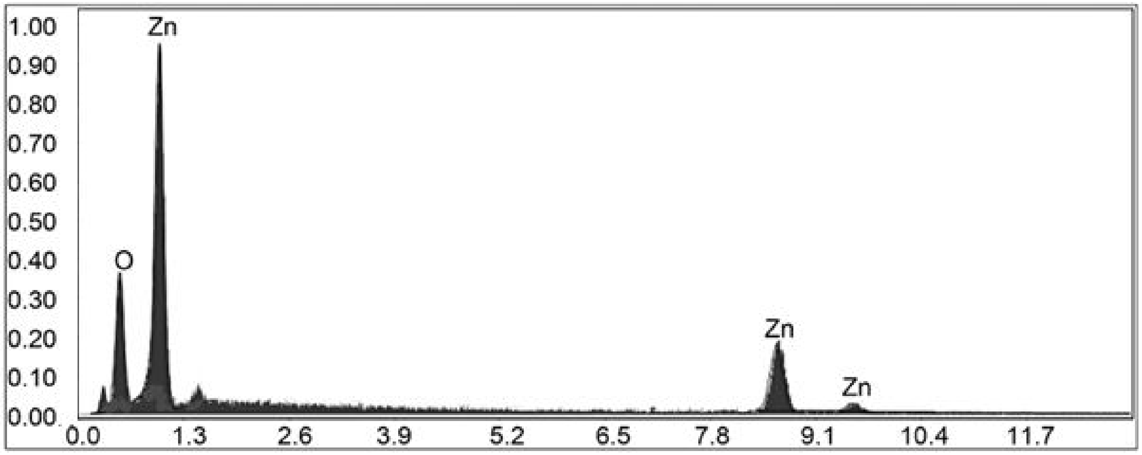


Fig. 3: Images of ZnO nanoparticles by SEM.



Element	Weight %	Atomic %
O	39.61	72.83
Zn	60.39	27.17

Fig. 4: Energy dispersive X-ray analysis graphs.

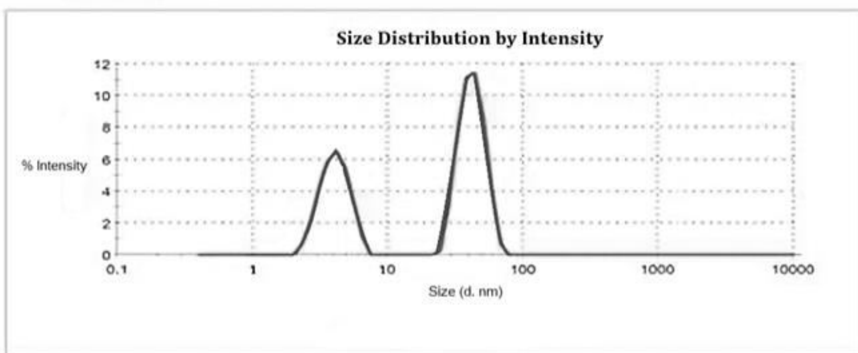


Fig. 5: Graph of size distribution by intensity.

Table 1: Average reading for assay of ZnO nanoparticles powder

S. No.	0.1 M disodium edetate (ml)	Average reading (ml)
1	18.3	18.3
2	18.2	
3	18.4	

ZnO equivalent to 1 ml of 0.1 M disodium edetate is 0.008138 g.

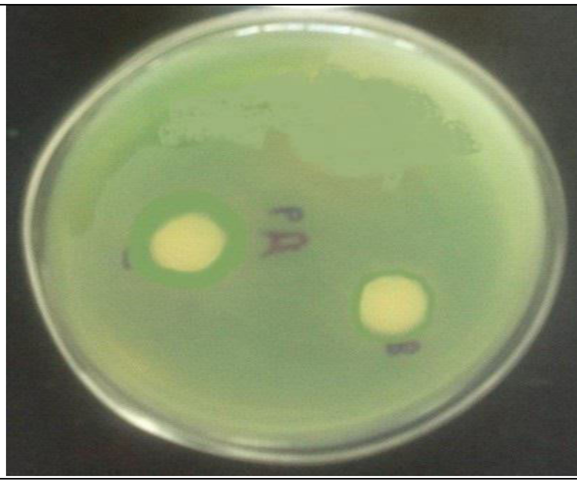
Therefore, ZnO equals X g in 18.3 ml of 0.1 M disodium edetate.

X = 18.3 × 0.008138 / 1 = 0.1489254 g of ZnO

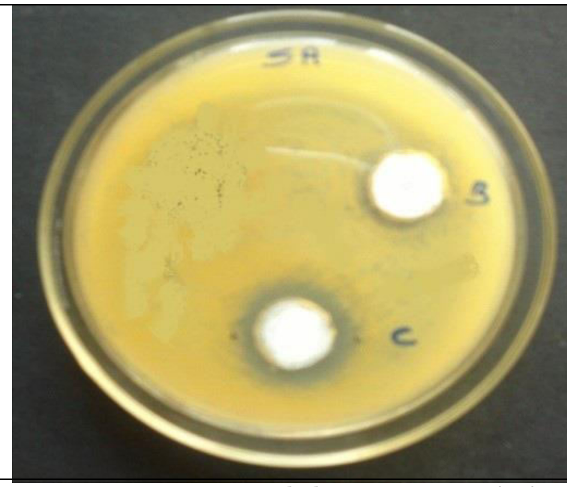
Now, 0.15 g of ZnO is equivalent to 100%

Therefore, 0.1489254gm is equivalent to Y%

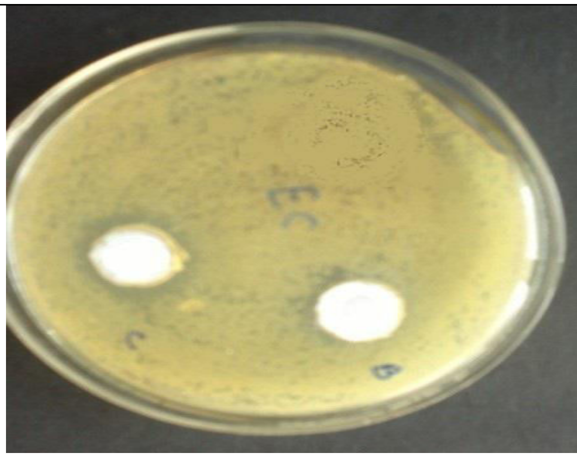
Y = 0.1489254 × 100 / 0.15 = 99.28%



Activity against *Pseudomonas aeruginosa* (PA).



Activity against *Staphylococcus aureus* (SA).



Activity against *Escherichia coli* (EC)

Activity against *Candida albicans* (CA)

Fig. 6: Antimicrobial activity of *Rubia cordifolia* extract, ZnO bulk powder and ZnO nanoparticles.

Table 2: Zone of inhibition for antimicrobial test ofZnObulk powder and ZnO nanoparticles powder

Bulk drugs	**Concentration**	**Inhibition Zone (millimeter)**			
		***P. aeruginosa* (PA)**	***S. aureus* (SA)**	***E. coli* (EC)**	***Candida albicans* (CA)**
ZnO powder	10 mg/ml	12	15	-	20
ZnO nanoparticle powder	10 mg/ml	19	21	20	27

Thus, from the above observation and calculations, it was found that the zinc oxide nanoparticle powder prepared by chemical reduction method contains 99.28% of ZnO nanoparticles. Thus, the powder produced by chemical reduction methodis in pure form having almost 100% purity.

Antimicrobial Activity of ZnO Nanoparticles, ZnO Powder:

The activity against microbes of ZnO nanoparticles powder, ZnO bulk powder was studied against *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), *Escherichia coli* (EC) and *Candida* *albicans* (CA). The results of zone of inhibition is shown in Table 2 and Figure 6.

It was found that the ZnO nanoparticle powder showed larger zone of inhibition as compared to ZnO bulk powder against *Pseudomonas aeruginosa* (PA), *Escherichia coli* (EC), *Staphylococcus aureus* (SA), and *Candida albicans* (CA). Thus, ZnO nanoparticles powder was found to be a good antibacterial and antifungal agent as compared to bulk ZnO powder.

Conclusion

Zinc oxide nanoparticles were created using a chemical synthesis approach in the current study. Size distribution results showed that zinc oxide nanoparticles were within the nanometric size (less than 60 nm). The UV visible analysis, EDX and assay test indicates the purity of synthesized nanoparticles. TEM, SEM and Size distribution test by intensity results shows the nanoparticles are within the nanometric size and put focus on their morphological characteristics. The zinc oxide nanoparticles were effective towards *Candida albicans* (CA), *Staphylococcus aureus* (SA), *Escherichia coli* (EC), and *Pseudomonas aeruginosa* (PA), according to the antimicrobial test. The zone of inhibition produced during antimicrobial activity shown by zinc oxide nanoparticles was more than the zinc oxide powder indicating the enhancement in activity due to its nanosize.

Acknowledgements

The authors would like to express their sincere gratitude to authorities at Shri JJT University, Vidya Nagari, Jhunjhunu, Rajasthan for technical support and guidance. The authors would also like to thank the ShreeshaktiShaikshanik Sanstha's Divine College of Pharmacy, Satana, Nashik.

References

Alhagiesa AW and Ghareeb MM. (2020) Formulation and evaluation of nimodipine nanoparticles incorporated within orodispersible tablets. Int J Drug Deliv Technol. 10(4): 547-552.

Asano N, Lu J, Asahina S and Takami S. (2021) Direct observation techniques using scanning electron microscope for hydrothermally synthesized nanocrystals and nanoclusters. Nanomaterials 11(4): 908.

Baraiya J, Chauhan B and Shingala Z. (2022) At glance to the plants having anti-inflammatory activity: A review. J Phytopharmacol. 11(3): 200-203.

Bawarski WE, Chidlowsky E, Bharali DJ and Mousa SA. (2008) Emerging nanopharmaceuticals. Nanomed Nanotechnol Biol Med. 4(4): 273-282.

Caputo F, Vogel R, Savage J, Vella G, Law A, Della Camera G, Hannon G, Peacock B, Mehn D, Ponti J and Geiss O. (2021) Measuring particle size distribution and mass concentration of nanoplastics and microplastics: addressing some analytical challenges in the sub-micron size range. J Colloid Interface Sci. 588: 401-417.

Gautam S, Das DK, Kaur J, Kumar A, Ubaidullah M, Hasan M, Yadav KK and Gupta RK. (2023) Transition metal-based nanoparticles as potential antimicrobial agents: recent advancements, mechanistic, challenges, and future prospects. Discover Nano. 18(1): 1-20.

Ghorbani HR, Mehr FP, Pazoki H and Rahmani BM. (2015) Synthesis of ZnO nanoparticles by precipitation method. Orient J Chem. 31(2):1219-1221.

Jubran AS, Al-Zamely OM and Al-Ammar MH. (2020) A study of iron oxide nanoparticles synthesis by using bacteria. Int J Pharm Qual Assur. 11(1): 1-8.

Kaur J, Anwer MK, Sartaj A, Panda BP, Ali A, Zafar A, Kumar V, Gilani SJ, Kala C and Taleuzzaman M. (2022) ZnO nanoparticles of *Rubia cordifolia* extract formulation developed and optimized with QbD application, considering ex vivo skin permeation, antimicrobial and antioxidant properties. Molecules 27(4): 1450.

Khan I, Saeed K and Khan I. (2019) Nanoparticles: Properties, applications and toxicities. Arabian J Chem. 12(7): 908-931.

Mamillapalli V, Katamaneni M, Tiyyagura VM, Kanajam P, Namagiri AP, Thondepu H, Appikatla B, Devangam B and Khantamneni P. (2020) Formulation, phytochemical, physical, biological evaluation of polyherbal vanishing cream, and facewash. Res J Pharmaceut Dosage Forms Technol. 12(3):139-149.

Mashar HM, Supriyono T, Ismail I, Ysrafil Y and Dali D. (2023) Exploration of endophytic fungi in the kelakai (*Stenochlaena palustris*) and their antibacterial effect. Int J Pharmaceut Quality Assurance 14(2): 250-254.

Mirzaei H and Darroudi M. (2017) Zinc oxide nanoparticles: Biological synthesis and biomedical applications. Ceramics Int. 43(1): 907-914.

Padmavathy N and Vijayaraghavan R. (2008) Enhanced bioactivity of ZnO nanoparticles- an antimicrobial study. Sci Technol Adv Materials 9: 1-7.

Parveen S, Misra R and Sahoo SK. (2017) Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. Nanomed Cancer 8(2):147-166.

Petrushevska M, Pavlovska K, Laskova J, Zdravkovski P and Dodov MG. (2019) Transmission electron microscopy: Novel application of established technique in characterization of nanoparticles as drug delivery systems. Prilozi 40(2): 67-72.

Rai P, Poudyl AP and Das S. (2019) Pharmaceutical creams and their use in wound healing: A review. J Drug Delivery Therapeut. 9(3-s): 907-912.

Sanchez-Lopez E, Gomes D, Esteruelas G, Bonilla L, Lopez-Machado AL, Galindo R, Cano A, Espina M, Ettcheto M, Camins A and Silva AM. (2020) Metal-based nanoparticles as antimicrobial agents: an overview. Nanomaterials 10(2): 292.

Scimeca M, Bischetti S, Lamsira HK, Bonfiglio R and Bonanno E. (2018) Energy dispersive X-ray (EDX) microanalysis: A powerful tool in biomedical research and diagnosis. European J Histochem. 62(1): 1-10.

Sharma V, Shukla RK, Saxena N, Parmar D, Das M and Dhawan A. (2009) DNA damaging potential of zinc oxide nanoparticles in human epidermal cells. Toxicol Letts. 185(3): 211-218.

Sirelkhatim A, Mahmud S, Seeni A, Kaus NH, Ann LC, Bakhori SK, Hasan H and Mohamad D. (2015) Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. Nano-Micro Letts. 7: 219-242.

Smith R, Russo J, Fiegel J and Brogden N. (2020) Antibiotic delivery strategies to treat skin infections when innate antimicrobial defense fails. Antibiotics 9(2): 56.

Tania IS and Ali M. (2021) Effect of the coating of zinc oxide (ZnO) nanoparticles with binder on the functional and mechanical properties of cotton fabric. Materials Today Proc. 38: 2607-2611.

Xie Y, He Y, Irwin PL, Jin T and Shi X. (2011) Antibacterial activity and mechanism of action of zinc oxide nanoparticles against *Campylobacter jejuni.* Appl Environ Microbiol. 77(7): 2325-2331.

Yeola CA, Sonawane VN, Sonawane VN, Surana KR, Patil DM and Sonawane DD. (2023) Development and validation of simple UV-spectrophotometric method for estimation of diclofenac sodium. Asian J Pharmaceut Anal. 13(3): 183-189.