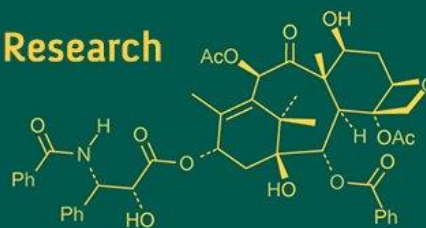


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Poonam

Ph. D. Scholar, Department of
 Zoology, Chaudhary Charan
 Singh Haryana Agricultural
 University, Hisar, Haryana,
 India

Prateek

Master's Degree Holder,
 Department of Zoology,
 Maharshi Dayanand
 University, Rohtak, Haryana,
 India

Nikki

Ph. D. Scholar, Department of
 Zoology, Chaudhary Charan
 Singh Haryana Agricultural
 University, Hisar, Haryana,
 India

Corresponding Author:**Prateek**

Master's Degree Holder,
 Department of Zoology,
 Maharshi Dayanand
 University, Rohtak, Haryana,
 India

Comparison of antibacterial properties of zinc-oxide nanoparticles synthesized from aqueous root extracts of *Rubia Cordifolia*

Poonam, Prateek and Nikki

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Abstract

In recent decades, nanoparticles (NPs) have gained significant research interest due to their unique physicochemical properties and broad applications across various industries, including food, cosmetics and biomedicine. Among these, zinc oxide (ZnO) nanoparticles are particularly notable for their large band gap, high binding energy, low toxicity and tunable size, shape and surface area. The present study focuses on the green synthesis of ZnO nanoparticles using root extract of *Rubia cordifolia*, which serves as a natural reducing and stabilizing agent. This eco-friendly approach not only minimizes the use of hazardous chemicals but also enhances the biocompatibility and functionality of the nanoparticles. The biosynthesized ZnO nanoparticles, ranging in size from 12.3 to 28 nm, were characterized using UV-Visible spectroscopy, zeta potential analysis, FTIR and TEM. Their antibacterial efficacy was evaluated against both Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) using the agar well diffusion method. Five concentrations (25-200 mg/ml in 2 ml DMSO) were tested. The maximum zone of inhibition observed was 8 mm for *K. pneumoniae* and 6 mm for *P. aeruginosa* at the highest concentration (200 mg/ml), indicating strong antibacterial activity. No inhibition was observed in control groups. Additionally, PVA-ZnO nanocomposites demonstrated antibacterial activity at lower concentrations (5-20 mg/ml), while *Rubia cordifolia*-assisted ZnO NPs required higher concentrations (50-200 mg/ml) for similar effects. This study also explores the structural and morphological characteristics of the synthesized nanoparticles and proposes potential mechanisms of bacterial inhibition, highlighting the interaction of ZnO NPs with bacterial cell walls that may lead to membrane disruption and cell death. Overall, green synthesis offers a sustainable, cost-effective and efficient alternative to conventional nanoparticle fabrication methods, with promising antibacterial applications.

Keywords: Green synthesis, Zinc oxide nanoparticles, *Rubia cordifolia*, Antibacterial activity, Nanotechnology

Introduction

Nanotechnology is an evolving field of science and technology with diverse applications in various field like biomedical, industrial, drug delivery and anti-microbial treatment. The potential uses of nanoparticles (NPs) in a variety of fields are based on their unique characteristics, which include their size, shape, charge, high surface area to mass ratio and high reactivity (Kim *et al.*, 2007) [15]. Because of the distinctive properties they possess oxide nanoparticles such as gold, silver, platinum and others have found broad use in the field of nano-medicine (Jeyaraj *et al.*, 2013) [12]. According to Ingle *et al.*, (2013), nanoparticles high surface area to volume ratio makes them remarkable antibacterial agents and a special kind of delivery system. Among these zinc oxide (ZnO) nanoparticles are thought to be non-toxic, bio-safe and biocompatible in a variety of applications, including semiconductors, materials with magnetic properties, electroluminescent materials, piezoelectric sensors and actuators, nano-structure varistors, field emission displaying materials, thermoelectric materials, gas sensors and cosmetic components. Nanoparticles can be synthesized by physical, chemical and biological methods. One such effective method for synthesizing nanoparticles is green synthesis.

The creation of nanoparticles using green chemistry depends on the bioactive substances originating from natural resources including plants, algae and a variety of other sources. More and more studies are being conducted these days on the synthesis of nanoscale metals

using physical, chemical and green synthesis techniques (Horwat *et al.*, 2011) [7]. According to Alsammarraie *et al.* (2018) [3], physical and chemical methods are gradually being replaced by green synthesis techniques. This is because of things like excessive energy consumption (Horwat *et al.*, 2011) [7], toxic and hazardous chemical releases. Comparing green synthesis to chemical and physical approaches provides multiple advantages: it is more affordable (Kataria and Garg, 2018) [13], non-poisonous (Devi *et al.*, 2019) [6], non-polluting (Alsammarraie *et al.*, 2018) [3], more ecologically friendly and more sustainable (Nasrollahzadeh and Mohammad Sajadi, 2016) [22]. Plant components such as roots, leaves, stems, seeds and fruits have also been used for the production of NPs because their extract contains an abundant number of phytochemicals that function as stabilizing and reducing agents (Agarwal *et al.*, 2017) [2].

The *Rubia cordifolia* (Rubiaceae family) is a perennial climbing vine known as a medicinal plant. *R. cordifolia* is commonly referred to as India Madder or Madder in India. India's locals use it as "Manjistha." (Adwankar *et al.* 1980) [1]. In Ayurveda, *R. cordifolia* is externally applied to painful regions, ulcers and fractures. The root of this plant has been used to treat several kinds of prolonged swellings. It has additionally been widely used as a coloring agent for therapeutic oils. (Patil *et al.*, 2009) [20]. Multiple pharmacological actions of *R. cordifolia* including immunosuppression, anti-inflammatory, anti-tumor, neuroprotective, antibacterial and antioxidant effects (Balachandran *et al.*, 2021) [4]. ZnO's antibacterial activity has been extensively studied using a variety of pathogenic and non-pathogenic microorganisms including *klebsiella*, *pseudomonas* and *S. saprophyticus*. The current study is focused on green synthesis of zinc oxide nanoparticles from root extract of *Rubia cordifolia* and examined for its antibacterial properties against gram positive and negative bacteria.

Experimental details

Materials and Methods

All chemical reagents utilized for this research work were of high purity standards (AR grade). Antibiotics discs, LB broth and LB agar medium were procured from Hi Media Laboratories Pvt. Ltd. zinc acetate dihydrate, sodium hydroxide pellets, ethanol and all other chemicals and solvents of reagent grade were procured from Sisco Research Laboratories Pvt. Ltd. (SRL).

Preparation of root extract of *Rubia cordifolia*

Rubia cordifolia roots were obtained from the general shop of the Rohtak market and were well cleaned under tap water. The roots of *Rubia cordifolia* were dried in shade and homogenized to fine powder using a mortar and pestle or electric grinder and then sieve the powder through muslin cloth. 20 grams of powder was weighed and mixed in 200ml of distilled water, then we take this mixture in water bath at 60°C temperature for 24 hours. Next day the mixture filtered into plastic bottle with the help of Whatman filter paper and stored in freeze at -4°C till further use (Chandrashekar *et al.*, 2018) [10].

Biosynthesis of *R. cordifolia*-aqueous zinc oxide nanoparticles (RCAE-ZnONPs)

Biosynthesis of *R. cordifolia* aqueous Zinc oxide nanoparticles done according to the protocol modified from

Naiel *et al.* 2022. Twenty gram of plant extract was mixed into 200 ml of double distilled water. Now 1.31 grams of zinc acetate was added to 600 ml of double distilled water to make the zinc acetate dihydrate solution. 190ml of zinc acetate dihydrate solution was mixed with 10ml of aqueous root extract and kept on heated magnetic stirrer for two hours at 300rpm. The pH of the solution was maintained at 12 by adding 2M NaOH dropwise to achieve complete reduction and the appearance of a white precipitate. Sonicate and then centrifuge the mixture at 10,000 rpm for 10 minutes. The final product was then collected by decantation, cleaned with deionized water to get rid of any leftovers, and oven-dried overnight at 60 °C to produce powdered ZnO nanoparticles. The dried material was kept at 4°C temperatures in an airtight container for further use and analysis.

Characterization of ZnO NPs

Using a UV-Visible spectroscopy, the optical transmission/absorption spectra of ZnO dispersed in water was examined. The absorbance spectrum was obtained at the range of 200-800nm. The absorbance was used to measure the concentration of a solution using Beer-Lambert law. The functional groups of ZnONPs were identified using the FTIR analysis. No special FTIR sample preparation was done. The spectra were acquired in the 400-4000cm⁻¹ wave number range in an attenuated total reflection (ATR) mode using a BRUKER FTIR Spectrometer. The synthesized samples FTIR confirmed presence of several reducing agents functional group present in the *Rubia cordifolia* root extract as well as production of zinc nanoparticles. Zeta Potential measurements were performed using (Zeta sizer Nano-ZS, ZEN3600, Malvern) analyzer to investigate particles' surface charge of prepared green-synthesized RCAE-ZnONPs. The electrostatic potential of the particle was determined in room temperature deionized water ultrasonic dispersion at 0.01 g per 100 ml. TEM analysis was done to determine the morphology, size and foam of Zn NPs.

Anti-bacterial activity

To check the antibacterial activity of zinc oxide nanoparticles, a zone of inhibition was observed around the wells. The larger the diameter of the zone of inhibition, higher the antibacterial activity of ZnO nanoparticles. RCAE-ZnO-NPs in DMSO at different concentrations (50mg/ml, 100mg/ml, 150mg/ml, 200mg/ml) showed antibacterial activity against *K. pneumonia*, *S. saprophyticus* and *P. aeruginosa*. The higher concentration of ZnO caused higher bacterial inhibition (Sirelkhatim *et al.* 2015) [27]. Our results are in accordance with the earlier studies. In control groups, no antibacterial activity was observed but significant antibacterial activity was observed in aqueous root extract and RCAE-ZnO nanoparticles in different concentrations with a maximum zone of inhibition of 8mm and 6 mm at 200 mg/ml with Agar well diffusion assay, for *K. pneumoniae* and *P. aeruginosa* respectively. After that, a decrease/saturation in the inhibition zone was observed for other bacteria. The ZnO-NPs made in other solvents like methanol or ethanol and at some other concentrations may show more antibacterial activity against *K. pneumoniae*, *S. saprophyticus* and *P. aeruginosa*. Many researchers have noted that ZnO-NPs exhibit a broad range of antibacterial activity against different microbes (Sirelkhatim *et al.* 2015) [27].

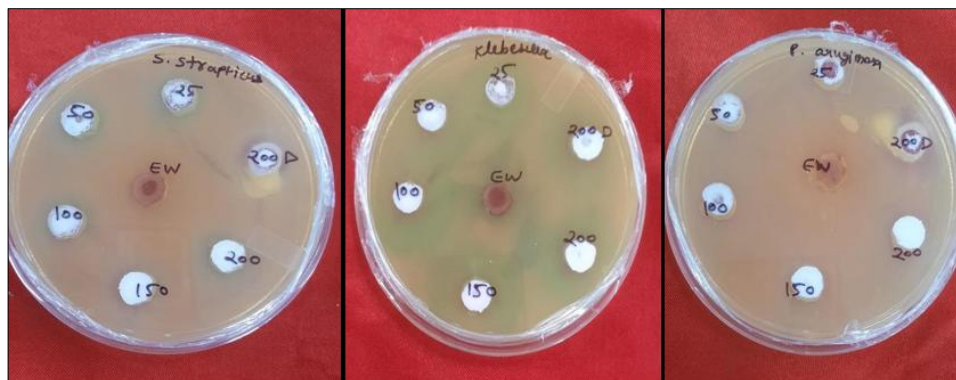


Fig 1: Antibacterial activity of RCAE-ZnONPs' against (A) *S. saprophyticus* (B) *P. aeruginosa* (C) *K. pneumoniae*

Table 1: Antibacterial Activity of Aqueous Extract and Zinc Oxide Nanoparticles Against Selected Bacterial Strains

Sr. No.	Name of bacteria	Control	Aqueous Extract alone	Diameter of zone of inhibition(mm)				
				Aqueous +Zn NPs (50mg/ml)	Aqueous +ZnNPs (100mg/ml)	Aqueous +ZnNPs (150mg/ml)	Aqueous +ZnNPs (200mg/ml)	Aqueous +DMSO (200mg/ml)
1	<i>S. saprophyticus</i>	No zone of inhibition	No zone of inhibition	-	-	3	4	5
2	<i>K. pneumonia</i>	No zone of inhibition	No zone of inhibition	2	3	4	5	6
3	<i>P. aeruginosa</i>	No zone of inhibition	No zone of inhibition	-	-	3	5	8

Further, the RCAE-ZnO-NPs were coated with polyvinyl alcohol (PVA) and nanocomposites were made and their antibacterial activity was also studied by Agar well diffusion assay. PVA nanocomposites also showed antibacterial

activity with bacterial strains at lower concentration i. e. at 5mg/ml to 20mg/ml (table 4) compared to RCAE-ZnO nanoparticles which showed antibacterial activity at higher concentration i. e. 50 to 200 mg/ml

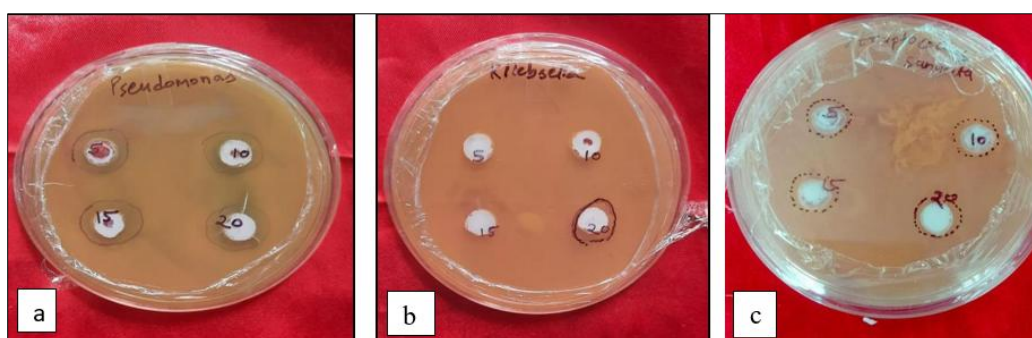


Fig 2: Antibacterial activity of RCAE-ZnONPs' PVA nanocomposites against (a) *S. saprophyticus* (b) *P. aeruginosa* (c) *K. pneumoniae*

Table 2: Antibacterial Activity of PVA-Coated Nanoparticles at Different Concentrations Against Selected Bacterial Strains

Sr. No.	Name of bacteria	Control	Diameter of zone of inhibition (mm)			
			PVA coated nanoparticle concentration			
			5mg/ml	10mg/ml	15mg/ml	20mg/ml
1	<i>S. saprophyticus</i>	No zone of inhibition	2	4	6	7
2	<i>K. pneumonia</i>	No zone of inhibition	4	5	6	7
3	<i>P. aeruginosa</i>	No zone of inhibition	6	7	8	9

Antibacterial activity of PVA nanocomposites against *Pseudomonas*, *klebsiella* and *saprophyticus*

Result and Discussion

Visible Observations

Before the addition of Zinc acetate, the colour of *Rubia cordifolia* solution was dark brown which changes to light brown after the addition of zinc acetate solution. The colour change indicates the formation of *Rubia cordifolia* zinc oxide nanoparticle (RC-ZnO NPs). The colour was changed due to a size dependent property of nanoparticles.

UV-Visible spectroscopy analysis

Nanoparticles possess exceptional optical qualities that are sensitive to the shape, agglomeration state, size, refractive index near the nanoparticle surface and concentration which

makes UV-Vis an invaluable technique for the identification and characterization of nanoparticles. In the present study, UV-visible spectroscopy was used to determine the size and form of the zinc oxide nanoparticles in the aqueous extract of *Rubia cordifolia*. To confirm the generation of RCAE-ZnONPs', the UV-vis spectroscopy method was used. The absorbance peak of RCAE -ZnONPs was at 263 nm. The UV absorption peak of Zinc nanoparticles generally lies in the 280-380 nm range (Kebede Urge *et al.* 2023) ^[14]. This confirms that the proper shape and sized RCAE-ZnO NPs were prepared.

Zeta potential

RC-ZnO NPs were found to have a Zeta potential of -17.5 mV in distilled water (DW), which indicates that they are strongly anionic. Particle stability of NPs is determined by the magnitude of their Zeta potential, which in turn determines the variety of applications they can be used for. Particles having a high ZP have a high level of stability (Iqbal *et al.*, 2021) [9].

TEM (Transmission electron microscope) analysis

Transmission electron microscopy (TEM) was performed to know morphology

(size and shape) of biosynthesized RC-ZnO NPs. TEM

results had shown that the diameter of prepared nanoparticles in the solution was about 12.3 nm to 28 nm. The RC-ZnO NPs were of varied shapes like spheres, dumbbell shaped, polyhedrons, flowers, boxes, hexagonal prismatic rods and plates etc. Our results are similar to other investigations (Wahab *et al.* 2010) [28] who used the similar methods for preparing ZnO nanoparticles and obtained structure of spherical surface that showed high antibacterial activity against the tested pathogens. Similarly, spherical shaped ZnO-NPs in another investigation (Wahab *et al.* 2014) [28] were obtained and it was used for the treatment of bacteria (*S. saprophyticus*, *P. aeruginosa* and *B. subtilis*,) and cancer cells (MCF-7 and HepG2 cell lines).

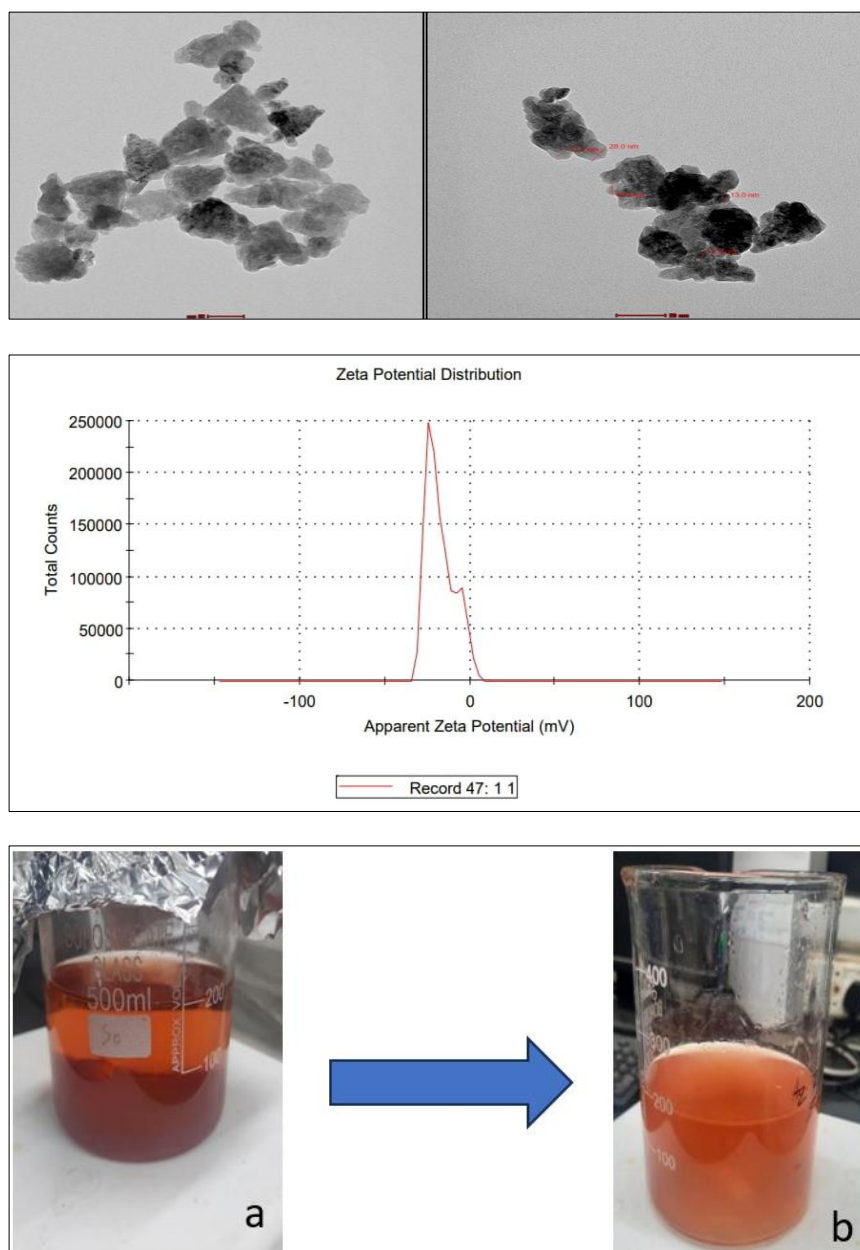


Fig 3: Aqueous plant extract solution(a) before the addition of Zinc acetate (b) after the addition of zinc acetate

FTIR analysis

Major functional groups that are involved in the reduction, stabilization, and synthesis of NPs can be found using Fourier transform infrared (FT-IR) spectroscopy in a variety of phytoconstituents (Maheswari *et al.*, 2021) [17]. The FTIR spectrum of *Rubia cordifolia* mediated Zinc oxide nanoparticles showed that peaks were observed at 659.21,

1019.20, 1167.03, 1260.04, 1464.73, 1521.94, 1650.72, 1695.47, 1744.10, 2356.61 cm⁻¹. The peak at 659.21 showed the formation of RC-ZnO NPs. The peak at 1019.20 was due to the presence of alkyl and aryl halides and C-F stretching. The peak observed at 1167.03 was related to C-H groups. The peak at around 1260.04 indicates C-C stretching aromatic ring. The peak value at 1464.73

confirmed the presence of C=C stretching of the aromatic compounds. The peaks at 1521.94 was because of the presence of NO₂ in the environment. The peaks at 1650.72 was due to ester group. Peak value at 2356.61 indicated the presence of -SH group. The alkaloids, essential oils, polyphenolic compounds, terpenoids etc and other phytochemicals present in the *R. cordifolia* were involved in the bio-reduction of Zn²⁺ to RC-ZnO NPs (Sirelkhatim *et al.*, 2015) [27]. These results of FTIR spectra analysis of RC - ZnO NPs are in accordance with other previous studies (Iqbal *et al.*, 2021) [9]

Conclusion and Future prospectus

Over the years, the "green" production of metal and metal oxide nanoparticles has become a very popular field of study in the previous ten years. A wide variety of natural extracts (e. g., biological components such as fungi, bacteria, plants, yeast, etc plant extract) are used as effective resources for the creation and/or synthesis of materials. Plant extract has been shown to be highly effective among them as stabilizing and reducing agents in order to create controlled materials, or controlled architectures, sizes, forms, and other distinctive characteristics (Singh *et al.*, 2018) Considering the growing reports of medication resistance in infections, looking for the need for viable antimicrobial therapy options has increased. ZnO-NPs synthesized with RC root extract display antimicrobial efficaciousness against *Staphylococcus*, *klebsiella* and *pseudomonas*. The current study methodology has numerous significant benefits. Because the synthesis technique uses low-cost, non-toxic components on a big scale, it is both affordable and environmentally beneficial amalgamation (Prachi *et al.*, 2017) [21] So nanoparticles of zinc were prepared from plant extract of *Rubia cordifolia* and various techniques were used for the characterization of nanoparticles like UV-Spectroscopy, FTIR(Fourier transform infrared), Zeta potential, TEM (Transmission electron microscopy), XRD(X-Ray diffraction). These techniques gives complete information about the nanoparticles like size, shape, stability of nanoparticles. To check the antibacterial activity of nanoparticles three strains were used *staphylococcus*, *klebsiella*, *pseudomonas*. These three strains are pathogenic in nature causes various diseases. Petri plates were prepared and antibacterial activity was observed. Antibacterial activity was observed when zinc nanoparticles were mixed with DMSO. The diameter of zone of inhibition was increased as concentration of nanoparticles was increased. So, we can conclude that nanoparticles of zinc show antibacterial activity on the strains. Researchers revealed that nanoparticles prepared using different plant extract have variety of properties including antimicrobial, antibacterial, antioxidant. According to Salem *et al.*, 2015 [23] the antibacterial activity was checked on *Vibrio cholerae* and enterotoxin *Escherichia coli* using zinc and silver nanoparticles using plant extract of *C. procera*. size of the nanoparticles was between 90nm-100nm. Antibacterial was more shown by zinc nanoparticles then silver nanoparticles. Their investigation demonstrated that zinc nanoparticles, which lower the amount of secondary messenger, a known regulator of biofilm formation, decreased adenylyl cyclase activity. subsequently, they demonstrated how a single injection of silver nanoparticles can reduce the rate of colonization by

75-100 times in baby mice containing colonized ETEC or *V. cholerae*.

This antibacterial activity will be very useful to the medical field in developing medicine for various diseases. There are various applications used regarding antibacterial activity are:

In coating of implanted devices.

- In wound dressing.
- In Dental material and in bone cementing.

So, we can conclude that based on our experiment ZnNPs are highly effective in antibacterial activity and various other metals and strains can be used to check antibacterial activity. Antibiotics can be successfully substituted by nanoparticles, which also hold enormous potential in treating the problem of bacterial multi-drug resistance. The current in-depth analysis of antibacterial processes may aid in the creation of effective antibacterial. Nanoparticles and the avoidance of nanoparticle cytotoxicity (Wang *et al.*, 2017). Due to limited period of time the experiment was done to some extent but this study can be further elaborated.

References

1. Adwankar MK, Chitnis MP, Khandalekar DD, Bhadsavale CG. Anti-cancer activity of the extracts of *Rubia cordifolia* Linn. (NSC b668893). Indian J Exp Biol. 1980.
2. Agarwal H, Kumar SV, Rajeshkumar S. A review on green synthesis of zinc oxide nanoparticles an eco-friendly approach. Resour Effic Technol. 2017;3(4):406-413.
3. Alsammaraie FK, Wang W, Zhou P, Mustapha A, Lin M. Green synthesis of silver nanoparticles using turmeric extracts and investigation of their antibacterial activities. Colloids Surf B Biointerfaces. 2018;171:398-405.
4. Balachandran K, Vijayakumar G, Mageswari S, Preethi A, Senan MV. Synthesis and characterization of Ag-decorated TiO₂ nanoparticles for photocatalytic application. J Environ Nanotechnol. 2021;10(4):13-18.
5. Batool F, Iqbal MS, Khan SUD, Khan J, Ahmed B, Qadir MI. Biologically synthesized iron nanoparticles (FeNPs) from *Phoenix dactylifera* have anti-bacterial activities. Sci Rep. 2021;11(1):22132.
6. Devi HS, Boda MA, Shah MA, Parveen S, Wani AH. Green synthesis of iron oxide nanoparticles using *Platanus orientalis* leaf extract for antifungal activity. Green Process Synth. 2019;8(1):38-45.
7. Horwat D, Zakharov DI, Endrino JA, Soldara F, Anders A, Migot S, *et al.* Chemistry, phase formation, and catalytic activity of thin palladium-containing oxide films synthesized by plasma-assisted physical vapor deposition. Surf Coat Technol. 2011;205:S171-S177.
8. Ingale AG, Chaudhari AN. Biogenic synthesis of nanoparticles and potential applications: an eco-friendly approach. J Nanomed Nanotechnol. 2013;4(165):1-7.
9. Iqbal J, Abbasi BA, Yaseen T, Zahra SA, Shahbaz A, Shah SA, *et al.* Green synthesis of zinc oxide nanoparticles using *Elaeagnus angustifolia* L. leaf extracts and their multiple *in vitro* biological applications. Sci Rep. 2021;11(1):20988.
10. Jagadish K, Shiralgi Y, Chandrashekar BN, Dhananjaya BL, Srikantawamy S. Eco-friendly synthesis of metal/metal oxide nanoparticles and their application in

- food packaging and food preservation. In: Impact of Nano-science in the Food Industry. Cambridge: Academic Press; 2018. p. 197-216.
11. Jang J, Hur HG, Sadowsky MJ, Byappanahalli MN, Yan T, Ishii S. Environmental *Escherichia coli*: ecology and public health implications a review. J Appl Microbiol. 2017;123(3):570-581.
 12. Jeyaraj M, Sathishkumar G, Sivanandhan G, MubarakAli D, Rajesh M, Arun R, *et al.* Biogenic silver nanoparticles for cancer treatment: an experimental report. Colloids Surf B Biointerfaces. 2013;106:86-92.
 13. Kataria N, Garg VK. Green synthesis of Fe₃O₄ nanoparticles loaded sawdust carbon for cadmium (II) removal.
 14. Kebede Urge S, Tiruneh Dibaba S, Belay Gemta A. Green synthesis method of ZnO nanoparticles using extracts of *Zingiber officinale* and garlic bulb (*Allium sativum*) and their synergetic effect for antibacterial activities. J Nanomater. 2023;2023(1):7036247.
 15. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, *et al.* Antimicrobial effects of silver nanoparticles. Nanomedicine. 2007;3(1):95-101.
 16. Mahendiran D, Subash G, Arumai Selvan D, Rehana D, Senthil Kumar R, Kalilur Rahiman A. Biosynthesis of zinc oxide nanoparticles using plant extracts of *Aloe vera* and *Hibiscus sabdariffa*: phytochemical, antibacterial, antioxidant and anti-proliferative studies. BioNanoScience. 2017;7(3):530-545.
 17. Maheswari P, Harish S, Ponnusamy S, Muthamizhchelvan C. A novel strategy of nano sized herbal *Plectranthus amboinicus*, *Phyllanthus niruri* and *Euphorbia hirta* treated TiO₂ nanoparticles for antibacterial and anticancer activities. Bioprocess Biosyst Eng. 2021;44(8):1593-1616.
 18. Naiel B, Fawzy M, Halmy MWA, Mahmoud AED. Green synthesis of zinc oxide nanoparticles using Sea Lavender (*Limonium pruinsum* L. Chaz.) extract: characterization, evaluation of anti-skin cancer, antimicrobial and antioxidant potentials. Sci Rep. 2022;12(1):20370.
 19. Njagi EC, Huang H, Stafford L, Genuino H, Galindo HM, Collins JB, *et al.* Biosynthesis of iron and silver nanoparticles at room temperature using aqueous sorghum bran extracts. Langmuir. 2011;27(1):264-271.
 20. Patil R, Mohan M, Kasture V, Kasture S. *Rubia cordifolia*: a review. Adv Tradit Med. 2009;9(1):1-13.
 21. Prachi AM, Patel R, Singh N, Negi DS, Rawat S. Green synthesis of zinc oxide nanoparticles using *Rubia cordifolia* root extract against different bacterial pathogens. Indo Am J Pharm Res. 2017;7(9):759-765.
 22. Sajadi SM, Nasrollahzadeh M, Maham M. Aqueous extract from seeds of *Silybum marianum* L. as a green material for preparation of the Cu/Fe₃O₄ nanoparticles: a magnetically recoverable and reusable catalyst for the reduction of nitroarenes. J Colloid Interface Sci. 2016;469:93-98.
 23. Salem W, Leitner DR, Zingl FG, Schratte G, Prassl R, Goessler W, *et al.* Antibacterial activity of silver and zinc nanoparticles against *Vibrio cholerae* and enterotoxigenic *Escherichia coli*. Int J Med Microbiol. 2015;305(1):85-95.
 24. Senthilkumar N, Nandhakumar E, Priya P, Soni D, Vimalan M, Potheher IV. Synthesis of ZnO nanoparticles using leaf extract of *Tectona grandis* (L.) and their anti-bacterial, anti-arthritis, anti-oxidant and *in vitro* cytotoxicity activities. New J Chem. 2017;41(18):10347-10356.
 25. Singh A, Singh NA, Afzal S, Singh T, Hussain I. Zinc oxide nanoparticles: a review of their biological synthesis, antimicrobial activity, uptake, translocation and bio-transformation in plants. J Mater Sci. 2018;53(1):185-201.
 26. Singh J, Dutta T, Kim KH, Rawat M, Samddar P, Kumar P. Green synthesis of metals and their oxide nanoparticles: applications for environmental remediation. J Nanobiotechnol. 2018;16(1):84.
 27. Sirelkhatim A, Mahmud S, Seeni A, Kaus NHM, Ann LC, Bakhori SKM, *et al.* Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. Nano-Micro Lett. 2015;7(3):219-242.
 28. Wahab R, Mishra A, Yun SI, Kim YS, Shin HS. Antibacterial activity of ZnO nanoparticles prepared via non-hydrolytic solution route. Appl Microbiol Biotechnol. 2010;87(5):1917-1925.
 29. Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: present situation and prospects for the future. Int J Nanomedicine. 2017;12:1227-1249.