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## **Formulation and characterization of *Wrightia Tinctoria* loaded zinc oxide nanoparticles by green synthesis**

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**Abstract**---In the recent years, nanotechnology has become an important research field of modern material sciences. Green synthesis has garnered wide interest due to its inherent features like rapidity, eco-friendly and cost effectiveness. Zinc oxide nanoparticles were synthesized using *Wrightia Tinctoria* leaf extract using sol-gel method. The formation of Zinc oxide nanoparticles is confirmed by FTIR. The characterization of the formulated nanoparticles was done by X-ray diffraction (XRD) studies showed the crystalline nature and revealed the purity of Zinc oxide nanoparticles. The morphological studies Transmission electron microscopic (TEM) the shape of the nanoparticles can be identified. The zinc oxide nanoparticles were found to be rod-shaped on Scanning Electron Microscopy (SEM). The in-vitro antibacterial activity showed that the *Wrightia tinctoria* loaded zinc oxide nanoparticles were found to act against both gram-positive and gram-negative bacteria.

**Keywords**---formulation, characterization, *Wrightia Tinctoria*, zinc oxide nanoparticles, green synthesis.

## Introduction

Nanotechnology has emerged as one of the most significant innovations of our time. It can be defined as a discipline of current research that focuses on designing, synthesizing, and manipulating particles with sizes ranging from 1 to 100 nanometres. The extraordinary development of this technology has paved the way for the emergence of novel fundamental and applied frontiers, such as nanoscale structure synthesis and explorative probing for their application based on their physicochemical. Nanotechnology's rise as a breakthrough tool has shown its importance in various fields, including health care, beauty, food, biomedicine, target medication delivery, energy research, and photo electrochemically applications (Korbekandi et al., 2013). Their unique physicochemical properties, which transform the medical therapy to higher potency and lower toxicity perspective, have received much interest in treatment and diagnosis. Nanoparticles are frequently used as carriers to improve drug specificity, delivery, and efficacy (Yin L, 2020). The emergence of technologies that transformed how nanoscale objects might be imaged and manipulated enabled nanotechnologies to be established. The Scanning Tunnel Microscope (STM) and the Atomic Force Microscope (AFM) were the two instruments that brought in this dramatic change (Schaming & Remita, 2015). Surface modifications are engineered into the particles, which are then modified to meet the requirements of their respective applications. The tremendous diversity of nanoparticles can be due to the numerous possible surface alterations and their various chemical constitution, form, morphologies, and state of dispersion (Franci et al., 2015).

Nano particulate systems offer a lot of promise because they can turn poorly soluble, poorly absorbed, and labile biologically active substances into viable drug delivery methods. The core of this system may encompass a wide range of drugs, enzymes, and genes and has a prolonged circulation time due to the hydrophilic shell, which hinders recognition by the reticular-endothelial system (Bennis et al., 1994). Nanoparticles can increase medication encapsulation, pharmacokinetics, bioavailability, and therapeutic efficacy compared to microparticles. In the field of drug delivery, nanotechnology has created a new age. Polymeric carriers for Nano particulate drug delivery should be simple to manufacture and describe and biocompatible, biodegradable, non-immunogenic, non-toxic, and water-soluble (Kayser et al., 2005; Pandey & Khuller, 2004).

Bacterial infections are recognized as a severe public health problem. New bacterial mutations, antibiotic resistance, pathogenic strain outbreaks, and so on are all on the rise, necessitating the creation of more effective antibacterial drugs. Zinc oxide has long been known for its antimicrobial qualities (Frederickson et al., 2005). The antibacterial mechanism of ZnO N.P.s involves direct interaction between the nanoparticles and the cell surface, affecting the permeability of the cell membrane; these nanoparticles then enter bacterial cells and induce oxidative stress, inhibiting cell growth and eventually cell death; the demonstrated antibacterial activity of ZnO N.P.s suggests its potential application in the food preservation field. It can be used as a solid sanitizing agent to disinfect and sterilize food industry equipment and containers, protecting them from attack and contamination by food-borne pathogenic bacteria. ZnO nanoparticles demonstrated toxicity and antibacterial efficacy against pathogenic

microbes.(Sáez et al., 2006). Zinc oxide is a form of zinc, it is possible to create nanoparticles chemically, physically, or biologically. When high pressure or temperature conditions are required, chemical procedures like precipitation, micro emulsion, chemical reduction, sol-gel, and hydrothermal processes can use much energy.(Naveed Ul Haq et al., 2017).

The interest in biologically generating ZnONPs has grown significantly during the last decade. The discovery of this unique approach and the accompanying rise in interest is primarily due to the absence of toxic chemicals or a high energy input during biological synthesis, making the process more cost-effective and ecologically friendly than traditional approaches. (Khalid et al., 2017). According to the literature, the biological synthesis of metallic and metal oxide nanoparticles is more environmentally friendly than the current chemical or physical processes (Kharissova et al., 2013). Nanoparticles produced using biological or green technologies have a wide range of qualities, are more stable, and have the right dimensions due to the one-step synthesis process used in their production. Manufacturing nanoparticles can be accomplished using various methods, including chemical, physical, biological, and hybrid processes. (Mohanpuria et al., 2008; Tiwari et al., 2008).

There has been a growing interest in plants as significant sources of novel medications since the 1990s. Many pharmaceutical companies are interested in plant-derived pharmaceuticals, owing to the prevalent perception that "Green Medicine" is safer and more reliable than expensive synthetic drugs with harmful side effects. According to WHO statistics, 80 percent of the world's population currently uses herbal medicine for various parts of primary health care (Al-Daihan & Bhat, 2012).

The present study utilizes *Wrightia tinctoria* from Apocynaceae family, known as "Dogbane," plant-derived products and novel chemicals have garnered a worldwide reputation for their ability to save lives. Members of this family have extensively researched antibacterial activity against several nosocomial pathogens. This family's members have also produced plant-derived components that effectively inhibit microbial proliferation.(Anand et al., 2020). Apocynaceae family members and their phytochemicals are effective antimicrobials and resistance modifying agents (RMAs) against MDR and highly drug-resistant pathogens (EDR) Microorganisms that are Gram-positive and Gram-negative (Aiyambo, 2010).

## Materials and Methods

### Collection and preparation of plant extract



Figure 1: Leaves and flowers of *Wrightia tinctoria*

*Wrightia tinctoria*, often known as Pala indigo or Dyer's oleander, is a flowering plant species in the *Wrightia* genus that can be found in Southeast Asia, India, and Australia. It can be found in both wet and dry areas. The therapeutic benefits of many components of this plant have been discovered (Khyade & Vaikos, 2009). *Wrightia tinctoria*, was obtained from the SRM college of pharmacy in kattankulathur. Dr. P Jayaraman, the Plant Anatomy Research Centre director in Chennai, subsequently confirmed its authenticity. The dried plant sample was allowed to contact with boiling water to obtain the aqueous extract.

The collected plant leaves were shade-dried for two weeks at room temperature. When the leaves are dried to the desired condition, the leaves are ground using a steel blender. The prepared sample is then weighed and stored in an airtight container; it is used for further experiments. The ground leaf powder is subjected to a decoction extraction process.

### Synthesis of Zinc oxide nanoparticles

To synthesize zinc oxide nanoparticles, 10g of finely powdered *Wrightia tinctoria* leaves are accurately weighed and mixed with 100 ml distilled water. The mixture is then heated for 15 minutes on a hot plate at 70 degrees Celsius. The mixture is allowed to cool and then filter. 2.195g of zinc acetate is accurately weighed in an electronic balance and added to 35ml of distilled water that is in constant stirring in a magnetic stirrer at 350 RPM. Add the plant extract in a drop-wise manner to the zinc acetate solution with constant stirring. Maintain 500-600 RPM stirring for 6 hours. The mixture is basified using 2M NaOH till it reaches P.H. 14. The resulting solution is stirred overnight, which accelerates the formation of nanoparticles. The solution is placed in a centrifuge for 15 minutes at 10000 – 12000 RPM. Discard the supernatant solution. The remaining sediment is alcohol washed once using Ethanol and washed with water 5 times. Zinc oxide nanoparticles are dried and stored in an airtight container.

### Characterization of Zinc oxide nanoparticles

The X-ray diffraction (XRD) of Zinc oxide nanoparticles and leaf extracts were recorded by powder X-ray diffraction using K $\alpha$  radiation with about  $2\theta$ -80

(Scintag x-ray diffractometer). FT-IR (Fourier Transform Infra-Red Spectroscopy) was performed to analyze the functional groups of ZnO nanoparticles (SHIMADZU, IRTRACER 100). The microstructure of the sample was analyzed by Scanning Electron Microscope (SEM, Thermoscientific Apreo S) with and Transmission Electron Microscope (TEM, JEOL Japan, JEM-2100 Plus) was exploited to prove the occurrence of pure elemental Zinc Oxide nanoparticles. Zeta potential, Particle size, and polydispersity index was also recorded.

### **Scanning electron microscopic (SEM)**

Electron microscopy utilizes electrostatic or electromagnetic lenses to concentrate beams of accelerated electrons to produce images. A scanning electron microscope (SEM) uses a high-energy electron beam to produce signals that describe the sample's topography and atomic composition. The sample preparation process can alter the nanomaterial's properties, yet scanning electron microscopy can determine their size, shape, and distribution. The shape and morphology of zinc oxide nanoparticles were studied. The SEM images were captured at an acceleration voltage of 15 kV electron beam.

### **Transmission electron microscope (TEM)**

The morphology of *Wrightia tinctoria*-loaded zinc oxide nanoparticles was examined using an 80 VK JEOL Japan, JEM-2100 Plus transmission electron microscope. Zinc oxide nanoparticles containing *Wrightia tinctoria* are diluted in distilled water, and a drop of the suspension is deposited on copper-coated 400 mesh copper grids. For TEM investigation, the grid was dried at room temperature.

### **Fourier Transform Infra-Red Spectroscopy (FTIR)**

Infrared spectroscopy is a versatile analytical method that permits qualitative investigation. The spectral bands observed by vibrational spectroscopy techniques are unique to molecules. The successful interpretation of spectra depends on the existence of specific bands and the absence of other significant bands. Using SHIMADZU, IRTRACER 100, and Fourier Transform Infra-Red Spectroscopy, the compatibility of *Wrightia tinctoria*-loaded zinc oxide nanoparticles was investigated. Under high pressure, the pellets are produced by gently combining the samples with potassium bromide. The employed scanning range is between 400 and 4000  $\text{cm}^{-1}$ . The spectra of the drug and polymer in formulations were compared to the pure drug and polymer spectra.

### **Zeta potential, Particle size, and polydispersity index**

Polydispersity index, mean particle size, and zeta potential of *Wrightia tinctoria* loaded zinc oxide nanoparticles were determined using a Horiba Scientific SZ-100 nanoparticle Zetasizer to determine the zeta potential of the *Wrightia tinctoria* loaded zinc oxide nanoparticles. A dilution of *Wrightia tinctoria* loaded zinc oxide nanoparticles in Milli Q water was performed, and the results were evaluated at room temperature.

### **In-vitro Antibacterial activity**

The well diffusion test is used to investigate the antibacterial activity of zinc oxide nanoparticles loaded with *Wrightia tinctoria*. As a growth medium, Müller-Hinton agar was utilized. 100 L of the test organism was transferred aseptically and distributed using a sterile L-shaped glass rod onto nutrient agar plates. The organism was given 5 minutes to settle on the medium. On the surface of the bacteria-inoculated plates, wells were punched, and 50 L of the nanoparticle preparation was injected into each well. The plates were incubated at 37°C for 24 hours. The inhibitory zones were evaluated using the standard HiMedia scale. Wells injected with concentrated *Wrightia tinctoria* leaf extract serves as the positive control, while zinc oxide nanoparticles loaded with *Wrightia tinctoria* serve as the negative control.

### **X-ray diffraction method**

This experiment used Cu K radiation and nickel screen at 40kV and 30mA in Scintag x-ray diffractometer, with a voltage of 40kV and an ampere current of 30mA. Nanoparticles containing *Wrightia tinctoria* and zinc oxide were characterized by diffraction patterns. (Sharma et al., 2021).

### **Antibacterial studies**

The *in-vitro* antibacterial activity of *Wrightia tinctoria* loaded Zinc oxide nanoparticles was investigated using well diffusion method. The culture is prepared using Muller-Hinton agar as growth media. Wells were made on the surface of the bacteria inoculated plates, and 50 µL of the nanoparticle preparation was injected into the wells. The bacteria used for the *in-vitro* antibacterial studies are *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* and *Proteus*. The formation of zone of inhibition can be seen clearly around the wells. The Aqueous extract of *Wrightia Tinctoria* leaves was found to show lesser activity than the *Wrightia tinctoria* loaded Zinc oxide nanoparticles.

### **Results and Discussions**

The present study was conducted to prepare *Wrightia Tinctoria* loaded Zinc oxide nanoparticles using sol-gel method.

#### **Structural analysis**

##### **X-ray diffraction (XRD)**

This study investigated the X-ray diffraction (XRD) patterns of green Zinc Oxide nanoparticles that were created. The pattern was found to be in the range of  $20^{\circ} < 2\theta < 80^{\circ}$ . The XRD spectrum reveals sharp and narrow diffraction peaks, indicating that the nanoparticles produced are pure and crystalline in their natural state. To determine the crystalline size of the formed nanoparticles, the Debye Scherrer formula can be utilized. As an example, the Scherrer formula can be written as follows:

$$D = \frac{0.9\lambda}{\beta \cos\theta}$$

Where D is the crystallite size,  $\lambda$  is the X-ray wavelength (1.5406),  $\beta$  is the full width at half maximum (FWHM), and  $\theta$  is Bragg's angle.

Figure 2 shows the XRD diffraction peak of *Wrightia tinctoria* loaded zinc oxide nanoparticles. The diffraction peak at  $2\theta$  of 27.61, 31.78, 34.42, 36.27, 40.81, 47.54, 56.58, 62.34, 66.34, 67.90, 72.53 and 76.89 degrees which were assigned to the 110, 002, 101, 102, 110, 103, 200, 112, 201, 004, and 202 planes respectively. The presence of narrow and strong diffraction peaks indicates that zinc oxide is a well-crystalline compound. The presence of even smaller particles than those found in the SEM test was confirmed by an XRD analysis. We have an aggregation of smaller nanoparticles as a result of the fact that we are working with biological material as well as the presence of moisture in the surrounding environment (Bhuyan et al., 2015).

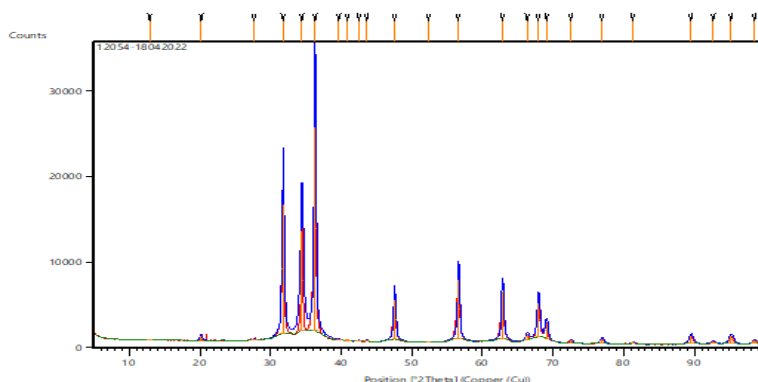


Figure 2 X-ray diffraction (XRD) of *Wrightia tinctoria* loaded zinc oxide nanoparticles

## Optical characterization

### Fourier Transform Infra-Red Spectroscopy (FTIR)

The FTIR spectra of zinc oxide nanoparticles loaded with *Wrightia tinctoria* were recorded. Figure 6.1 depicts the FTIR spectra of *Wrightia tinctoria* leaf extract and green ZnO N.P.s production. The FTIR spectra of zinc oxide nanoparticles loaded with *Wrightia tinctoria* were observed between 400 and 4,000  $\text{cm}^{-1}$ . The FTIR spectra reveal the presence of multiple chemical moieties derived from phytochemicals in the zinc oxide nanoparticles loaded with *Wrightia tinctoria*. FTIR spectrum of green Zinc Oxide nanoparticles. Upon production of nanoparticles, the majority of the significant stretching frequencies of bands, as depicted in the figure, are either moved or have their intensities drastically reduced. In the FTIR spectrum of Zinc Oxide N.P.s, the elimination of the stretching vibrations of the O-H group, C=O, C-O, and C=C bonds has been observed to be substantial. Simultaneously, shifted bands with very low intensity are identified, such as O-H group stretching at 3553.00  $\text{cm}^{-1}$  and 3020.63  $\text{cm}^{-1}$ ,

-C-O acid stretching at 1383.01  $\text{cm}^{-1}$ , and aldehyde stretching at 1768.78  $\text{cm}^{-1}$  (1768.78  $\text{cm}^{-1}$ ). The bands near 500.8  $\text{cm}^{-1}$ , 459.07  $\text{cm}^{-1}$ , and 418.57  $\text{cm}^{-1}$ , which are caused by the typical stretching vibration of the Zn-O link, reveal the production and stabilization of the Zinc Oxide nanomaterials, which validates the presence of zinc oxide nanoparticles even further.

A reduction or an oxidation mechanism is likely responsible for forming zinc oxide nanoparticles from *Wrightia tinctoria* leaf extract. Phytocompounds and enzymes found in the biological materials assisted in the metal compound's transformation into specific nanoparticles. The leaf extract includes several metabolites, including Tannin, organic acids, Terpenoids, Aromatic dicarboxylic acid, amides, etc., that are responsible for the antioxidant or reducing property that allows the fast reduction of Zinc ions into nanostructured Zinc oxide. Zinc oxide nanoparticles are stable because of these factors. (Yuvakkumar et al., 2015).

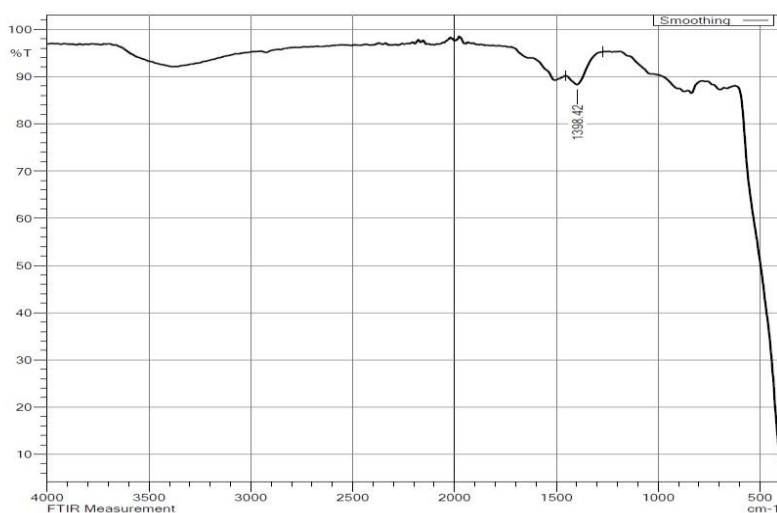


Figure 3 FTIR of *Wrightia tinctoria* loaded Zinc oxide nanoparticles

Peak at 1398.42  $\text{cm}^{-1}$  indicates the presence of oxide, the peak at 469  $\text{cm}^{-1}$  indicates the presence of zinc oxide.

### Microscopic analysis

#### Transmission electron microscopic (TEM)

An examination of the *Wrightia tinctoria* loaded zinc oxide nanoparticles using transmission electron microscopy (TEM) reveals that the nanoparticles are rod-shaped and well isolated from one another. This demonstrates that zinc oxide nanoparticles have been formed. The structures are depicted in figure 4, which follows.

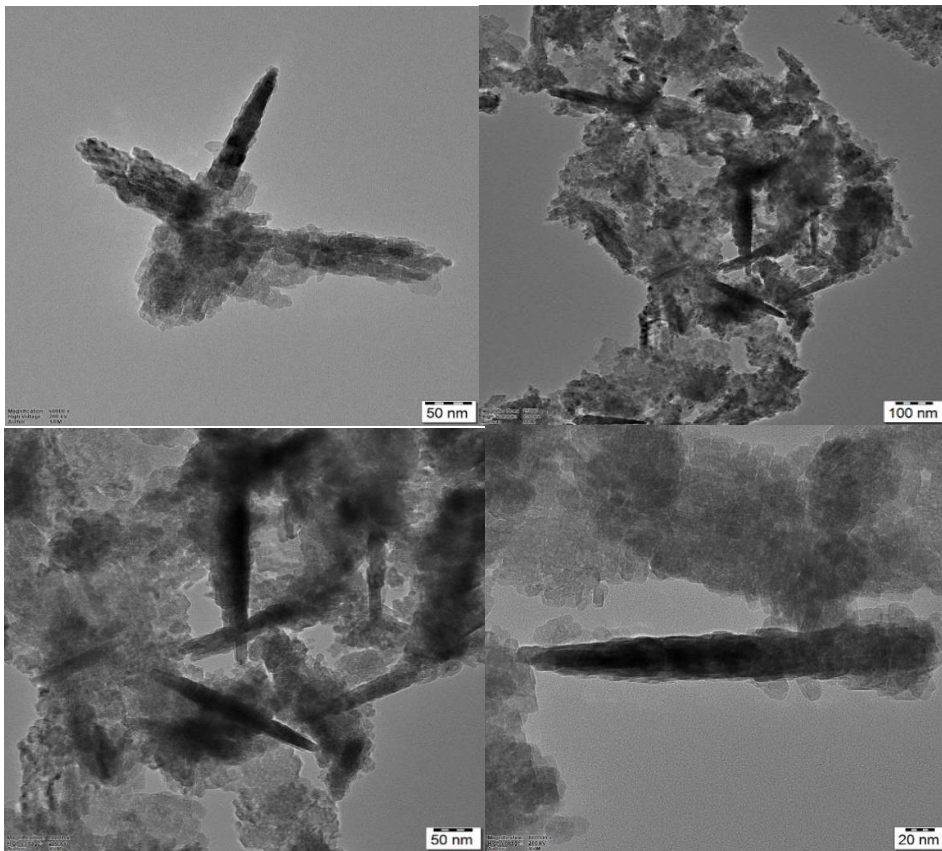


Figure 4 TEM images of *Wrightia tinctoria* loaded Zinc Oxide nanoparticles

### Scanning Electron Microscope (SEM)

Various types of Zinc Oxide nanostructures, including nanorods, nanotubes, nanobelts, nano springs, and many others, have been identified. In addition, due to their one-dimensional nanostructures, rod-like nanostructures are superior to others. As a result of zinc acetate being utilized as a precursor, morphology research has determined that produced Zinc Oxide has a rod-like structure. According to the claim, the shape of Zinc Oxide nano-powder made with zinc acetate is smoother than that made with zinc nitrate. Moreover, precursor concentration has a significant effect on nanorod morphology.

The SEM examination of *Wrightia tinctoria* loaded zinc oxide nanoparticles revealed rod-shaped nanoparticles with rough and porous surfaces. The *Wrightia tinctoria* loaded zinc oxide nanoparticles SEM analysis pictures are given in figure 5 below (Santos et al., 2018)

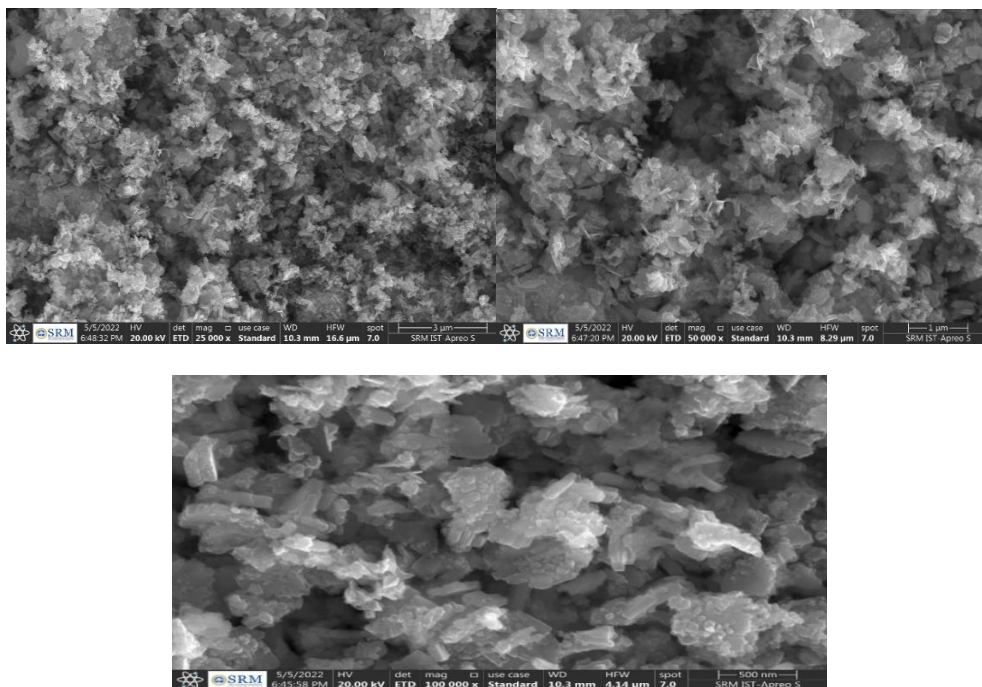


Figure 5 SEM images of *Wrightia tinctoria* loaded zinc oxide nanoparticles

### Particle size and polydispersity index

Zeta sizer is used to analyze the particle size and polydispersity index of zinc oxide nanoparticles, and the results are given below. The mean particle size was 110.3 nm with moderate levels of polydispersity index. Therefore, with the known values, the PDI was 0.452 (Liu et al., 2015).

Table 1 Particle size, PDI, and Zeta potential of nanoparticles

Mean Particle size (nm)	PDI	Zeta potential (mV)
110.3	0.452	-17.7 Mv

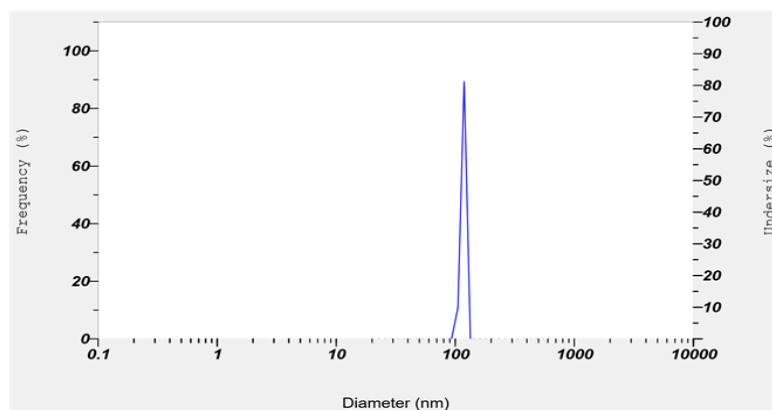


Figure 6 Mean particle size of *Wrightia tinctoria* loaded zinc oxide nanoparticles

## Zeta potential

Zeta potential analyzer is used to analyze the zeta potential of *Wrightia tinctoria* loaded zinc oxide nanoparticles. In the study, the results show that the zeta potential is -17.7 mV. The zeta potential results show that the nanoparticles possess excellent stability since it is in the ideal range. The zeta potential result is shown in table number 7 (Liu et al., 2015).

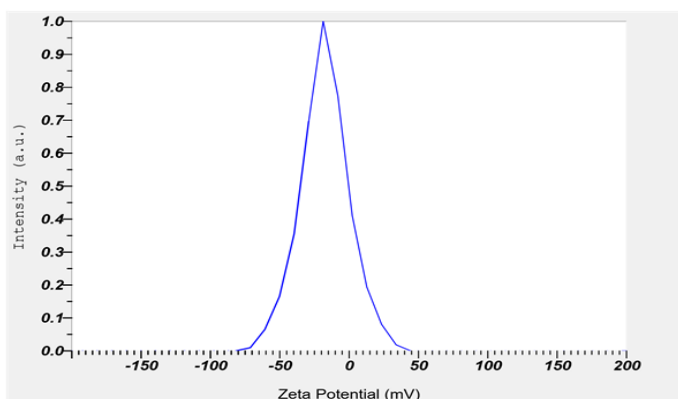


Figure 7 Zeta potential of *Wrightia tinctoria* loaded zinc oxide nanoparticles

## *In-vitro* Antibacterial activity

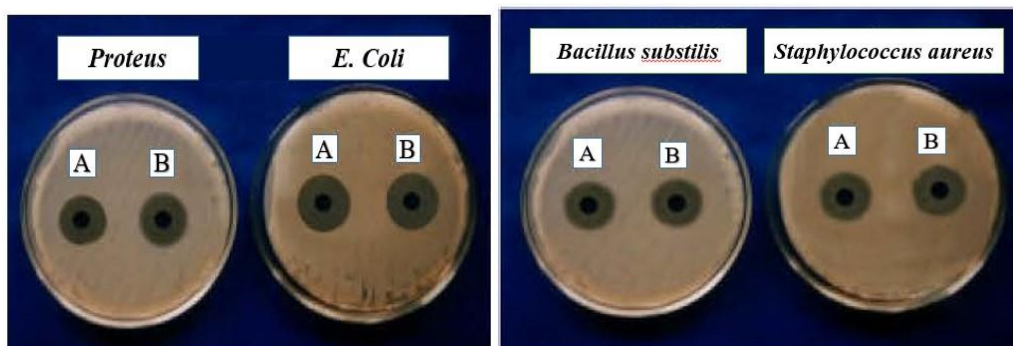
The *in-vitro* antibacterial activity of *Wrightia tinctoria* loaded Zinc oxide nanoparticles was investigated using well diffusion method. The culture is prepared using Muller-Hinton agar as growth media. Wells were made on the surface of the bacteria inoculated plates, and 50  $\mu$ L of the nanoparticle preparation was injected into the wells. Aqueous extract of *Wrightia tinctoria* leaves are used as standard, and the *Wrightia tinctoria* loaded zinc oxide nanoparticles are used as a test. With which two wells were made

The bacteria used for the *in-vitro* antibacterial studies are *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* and *Proteus*. The formation of zone of inhibition can be seen clearly around the wells. The Aqueous extract of *Wrightia Tinctoria* leaves was found to show lesser activity than the *Wrightia tinctoria* loaded Zinc oxide nanoparticles.

*Wrightia tinctoria* loaded zinc oxide nanoparticles were found to show better activity against gram-negative bacteria in comparison to gram-positive bacteria. The *Wrightia tinctoria* loaded zinc oxide nanoparticles was seen to have better activity compared to concentrated crude drug. This is observed because zinc oxide by itself possesses antibacterial activity. Therefore, the presence of zinc oxide nanoparticles aid in the increase of antibacterial activity (Raja et al., 2018).

Table 2 Antibacterial activity of *Wrightia tinctoria* loaded zinc oxide nanoparticles

S. No	Name of the sample	Zone of inhibition (mm)			
		Gram-Positive Bacteria		Gram-Negative Bacteria	
		<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Proteus</i>
1.	Aqueous Extract of <i>Wrightia Tinctoria</i>	16±0.12	15±0.24	17±0.38	19±0.30
3.	<i>Wrightia Tinctoria</i> Loaded Zinc Oxide nanoparticles	22±0.12	20±0.35	25±0.24	24±0.38

Figure 8 Zone of inhibition of the bacterial mediated *Wrightia tinctoria* Zinc oxide nanoparticles against pathogenic bacteria

A) *Wrightia tinctoria* Plant Extract    B) *Wrightia tinctoria* Zinc oxide nanoparticles

### Conclusion

The present study concludes that green, eco-friendly and cost effective approach is best suited for the synthesis of rod shaped Zinc oxide nanoparticles with potent anti-microbial using leaf extract of *Wrightia tinctoria*. These nanoparticles were characterized by XRD, FTIR, SEM, TEM, Zeta potential, particle size and polydispersity index. The biosynthesized Zinc oxide nanoparticles were found to have destructive effects on microorganism. The plant product when combined with Zinc oxide nanoparticles have shown to have appreciable effects on microorganism inhibition. This work has a great social relevance due to its non-toxic and inexpensive material which is suitable for environment and health related application.

### Conflicts of interest

The authors certify that they have no conflicts of interest.

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