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Green synthesis and characterization of zinc oxide nanoparticles and its antimicrobial activity

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Abstract:
The current study presents a method for synthesizing crystalline zinc oxide nanoparticles using green chemistry principles. The study demonstrated a method for synthesizing zinc oxide nanoparticles in a manner that efficiently minimizes the use of dangerous substances and the production of harmful byproducts. The text provided describes the mechanism by which something works and the common methods used to analyze its physical and chemical properties. In this study, we rapidly synthesized zinc oxide nanoparticles using a zinc nitrate solution and investigated the process of nanoparticle formation using the chemical method. Utilize techniques such as infrared spectroscopy (IR), field-emission scanning electron microscopy (FeSEM), transmission electron microscopy (TEM), and X-ray diffractometry in order to investigate and explain the characteristics of zinc oxide nanoparticles. Further investigation has been conducted to see whether or not it is capable of eliminating bacteria like Escherichia coli and Bacillus subtilis.
Keywords: Zinc oxide, Nanoparticles, Antimicrobial, Escherichia coli. Bacillus subtilis.

Introduction

Nanotechnology is a field that is growing very quickly and is all about making new things very small (Attia, *et al.,* 2023). In other words, the goal of nanotechnology is to create, study, and work with things that are between 1 and 100 nm in size (Amarachinta, *et al.,* 2021). Now in the 21st century, nanotechnology has grown into a field that includes many different fields. The process of metal nanoparticle formation is illustrative (Akhilesh, *et al.,* 2012). Food and feed, chemicals, biomedical science, health care, gene transport, pharmaceuticals and cosmetics, electronics, energy science, the space industry, and mechanics are just some of the fields that biosynthetic nanotechnology could help a lot (Aboud, *et al.,* 2016). This smallening of the size of the material causes a lot of different physicochemical properties and a lot of different possible uses in areas like material science and biology (Sabareesh, *et al.,* 2020). Zinc oxide nanoparticles have gotten a lot of interest in research because they can be used for many things, including biosensors, targeted drug delivery, smart UV sensors, antioxidant activity, cleaning up the environment, making plants more resistant to drought, and improving their nutrition (Suryawanshi, *et al.,* 2021). Biosynthesis of nanoparticles has a unique feature called selectivity (Sharma, *et al.,* 2018). This means that the biological source used determines the shape of the nanoparticles that are made. Also, these nanoparticles are more stable than usual. In this study, the goal was to speed up the process of making zinc oxide nanoparticles using a common lab method (Ahire, *et al.,* 2020). Zinc oxide is not made of living things. Most of the time, it looks like a white powder that dissolves easily in water (Sharma, *et al.,* 2016). The vast majority of ZnO used in industrial settings is made in a lab. The chemistry method was used to guide the freezing and lyophilization processes (Sahoo, *et al.,* 2014). Nanomaterials are used in many different ways because they have interesting chemical, physical, and catalytic qualities. Because zinc oxide nanoparticles naturally kill bacteria, scientists have looked into how they might affect medicines (Ramkanth, *et al.,* 2018). Nanoparticles of zinc oxide (ZnO) were made using a chemical process at room temperature (Behera, *et al.,* 2010). This technology has built-in benefits, like a high output and low operating costs. For this study, scanning electron microscopy, TEM analysis, infrared spectroscopy, and powder X-ray diffraction were used to look at how temperature changes the structure of materials (Surana and Mahajan, 2022). There are some chemicals

that can directly stop microorganisms from multiplying at levels that the host creature can handle. Zinc oxide nanoparticles are well known for their ability to kill microbes (Reddy and Gandla, 2022). To stop the growth of Bacillus subtitles Escherichia coli, we use the disc diffusion method to take advantage of this feature. Small particles of zinc oxide (ZnO) can be used for many things, like killing germs, fighting cancer, and getting drugs to the right places they need to go. Because these two types of bacteria are very good at infecting others, it is worth looking into whether zinc oxide nanoparticles could help fight bacteria (Yeola, *et al.,* 2023).

Material and Method:

A green synthesis of nanoparticles composed of zinc oxide:

It is recommended that fifty milliliters of deionized water be combined with one mole of zinc nitrate ($Zn(NO_3)_2$). In a glass beaker with a capacity of 250 milliliters, combine potassium hydroxide (KOH) with fifty milliliters of deionized water. While the answers are being churned, they should be stirred continuously until they become clear. Next, small amounts of KOH solution were added to the solution that had already been described (Pawar, *et al.,* 2023). This was done while the mixture was constantly stirred with a magnetic mixer at 150°C for an hour. Upon completion of the procedure, a milky white precipitate emerged, indicating the formation of metal oxide nanoparticles. The resulting solid was treated with many cycles of sonication and centrifugation, and then separated and washed with ethanol. Finally, the solid that formed was subjected to a drying process for duration of 12 hours at a temperature of 120 degrees Celsius in a vacuum-air oven (Fig. 1,2) (Deepika, *et al.,* 2020 and Ahmad, *et al.,* 2017).

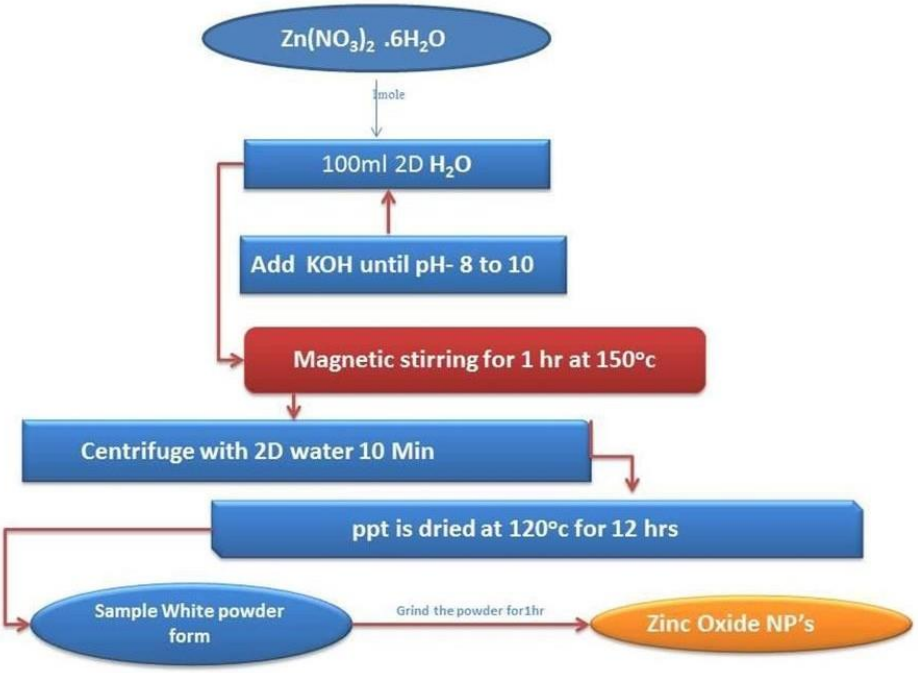

Figure 1: A green synthesis of nanoparticles composed of zinc oxide

Figure 2: Prepared zinc oxide nanoparticles

Characterization:

For the purpose of conducting X-ray diffraction research, analytical XPert PRO X-ray diffractometers equipped with Cu X-ray tubes and operating at 40 kV and 40 mA are utilized. After sputtering a tiny layer of gold onto the items, carbon tape is used to connect them to SEM mounts (Gandra, 2020 and Kakar, *et al.,* 2010). This process is repeated several times. It is used for infrared emission spectroscopy (IES) with the Nicolet Nexus 870 FTIR instrument. When measuring the emission spectra, the temperatures are raised by 50 ºC every time from 100 ºC to 850 ºC. More information has been made public (Sonawane, *et al.,* 2023 and Govindarajan, *et al.,* 2022). The picture shows ZnO nanoparticles that were seen with a transmission electron microscope (TEM) (Aher, *et al.,* 2023). The ZnO particles are made up of spherical grains that are randomly arranged and have an average width of 33 nm (Surana, *et al.,* 2022). The Agar-well diffusion method is used to conduct antibiotic susceptibility tests against B. subtilis, E. coli, and A. niger, following the Performance Standards for antimicrobial susceptibility testing (Keservani, *et al.,* 2010).

Results and discussion:

Infrared emission spectroscopy (FTIR):

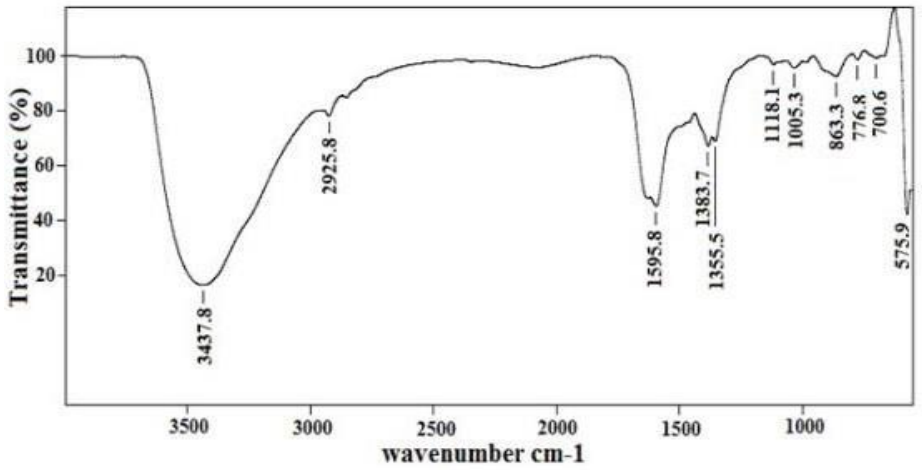

Figure 3: The pattern of zinc oxide nanoparticles using infrared spectroscopy

The solid-state FT-IR spectra of the complex is in complete agreement with its structural data, as evidenced by the peak observed at 3437.8 cm, indicating the presence of an O-H group undergoing stretching vibration. The measured absorption peaks at roughly 2952.8 cm are attributed to the CO_2 mode. The presence of ambient CO_2 in the sample is evident in the FTIR spectra due to the CO_2 mode. During the FTIR characterisation process, it is possible that the sample has absorbed carbon dioxide (CO_2) from the surrounding atmosphere. This absorption could be the cause of the observed mode. The 15.95.8 cm absorption band is mostly due to the C=O bond bending. At a frequency of 1383.7, the hydroxyl group shows a clear absorption bending. The stretching mode is shown by the absorption band at 575.9 cm-1

in ZnO, while the c-o bending wave is shown by the peak at 863.3 cm-1 (Fig. 3).

X-ray diffraction pattern of Zinc oxide nanoparticles:

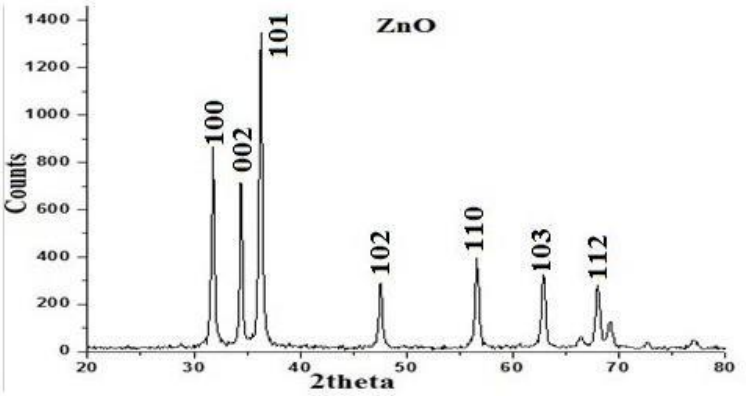

Figure 4: Pattern of X-ray diffraction of Zinc oxide nanoparticles

As can be seen in Figure 4, the XRD pattern of the ZnO nanoparticle is displayed. The peaks can be found at index readings of 23.7 degrees (1188), 30.7 degrees (1342), 33.00 degrees (4680), 49.99 degrees (500), 55.10 degrees (840), and 65.10 degrees.To be more specific, the diffraction peaks of the sample are identical to the structure of zinc oxide nanoparticles, which has lattice values of a=0.315 nm and c=0.529 nm.This is consistent with the trend that is typically observed for ZnO as well as the work that has been already been published. There is a similarity between the X-ray diffraction patterns of C. According to the Scherrer equation, specifically equation 37, the typical size of ZnO nanoparticles is determined to be 76 nanometers when calculated. The diffraction pattern that is identified as being associated with contaminants has been found to be absent. By means of a synthesis procedure, this experiment demonstrates the generation of ZnO nanoparticles that have not been refined in any way (Fig. 4).

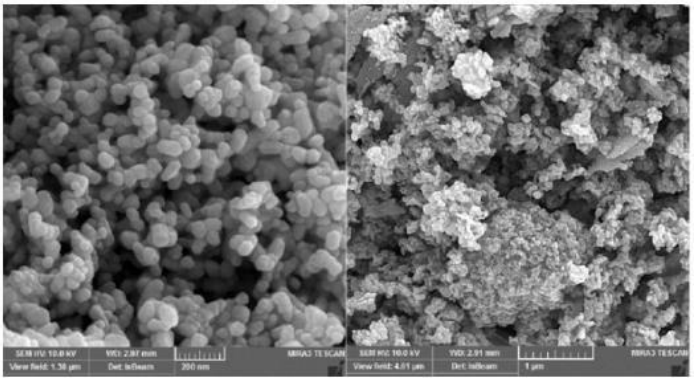

Figure 5: Zinc oxide nanocrystals in a SEM picture

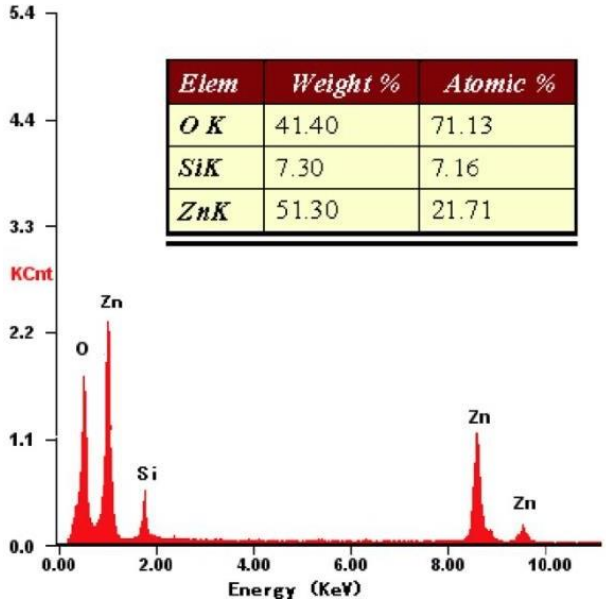

Figure 6: Pattern of EDS of Zinc oxide nanoparticles

The picture of the ZnO nanoparticle taken with a scanning electron microscope (SEM) is shown in Figure 5. At an oven setting of 80°C, the newly made ZnO compound was covered and dried. ZnO nanoparticles with a size range of 20 to 50 nm are made by this process. It is clear to us that ZnO nanoparticles continue to increase in size even at room temperature after the synthesis process is finished.

The scanning electron microscope (SEM) image was obtained at a magnification of X331.68. The figure illustrates that the ZnO particles possess an approximate diameter of 100 nm, have a spherical morphology, and display a sleek surface (Fig. 5).

EDX evaluated the chemical purity of the samples. The EDX spectrum obtained (Figure 6) reveals that the ZnO nanoparticles consist solely of oxygen and zinc components, demonstrating a high level of purity in the product (Fig. 6).

Transmission Electron Microscopy (TEM) Analysis:

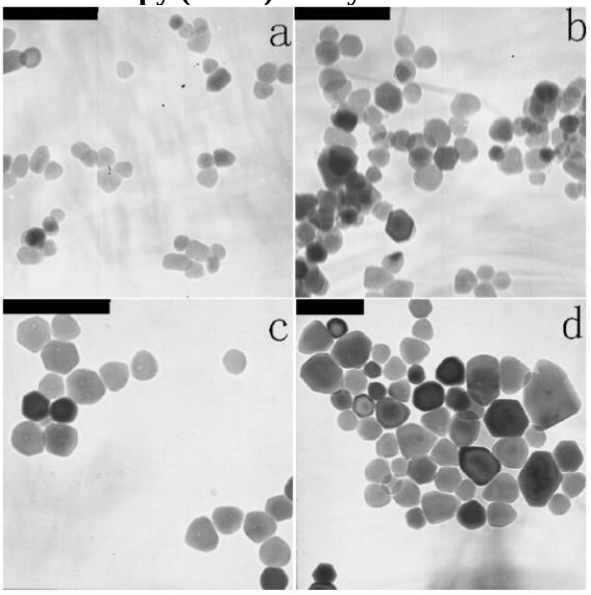

Figure 7: TEM analysis of Zinc oxide nanoparticles.

Images of zinc oxide nanoparticles (ZnO NPs) that were produced by heating Z1 for two hours at temperatures of 350 degrees Celsius (Z4) and 600 degrees Celsius (Z7) are displayed in Figure 7. These images were captured by a transmission electron microscope (TEM). The pictures taken with transmission electron microscopy (TEM) (Fig. 7) show that the zinc oxide nanoparticles have a circular shape. It should be mentioned that bigger nanoparticle sizes were frequently the consequence of greater annealing temperatures. The average diameter of the sample that was heated to 350°C was 24.54 nm. Notably, the average crystal diameter (28.33 nm) determined using Scherer's formula nearly corresponds to the value obtained from the analysis of transmission electron microscope images. Nevertheless, the ZnO crystals in the sample that was annealed at 600°C generated clusters that differed significantly from the XRD results. These clusters were several hundred nanometers in size. Scherer's formula is only applicable to tiny particles, frequently those that are less than 100 nm in size. The considerable variations seen in this instance suggest that Scherer's equation is inappropriate for large ZnO crystallites.

Evaluation of Antibacterial activity:

The diameter of the zone of inhibition against the test organisms was determined to evaluate the antibacterial efficacy of ZnO nanoparticles. Bacillus subtilis and Escherichia coli are microorganisms used for their antibacterial properties. The measurements of the growth inhibition zones are presented in Table 1. In order to evaluate the effectiveness of metal oxide nanoparticles as antibacterial agents, the disc diffusion method was utilized. The expansion of the zone of inhibition is the result of an increase in the concentration of metal oxide nanoparticles and a decrease in the size of the particles (Table 1 & Fig. 8).

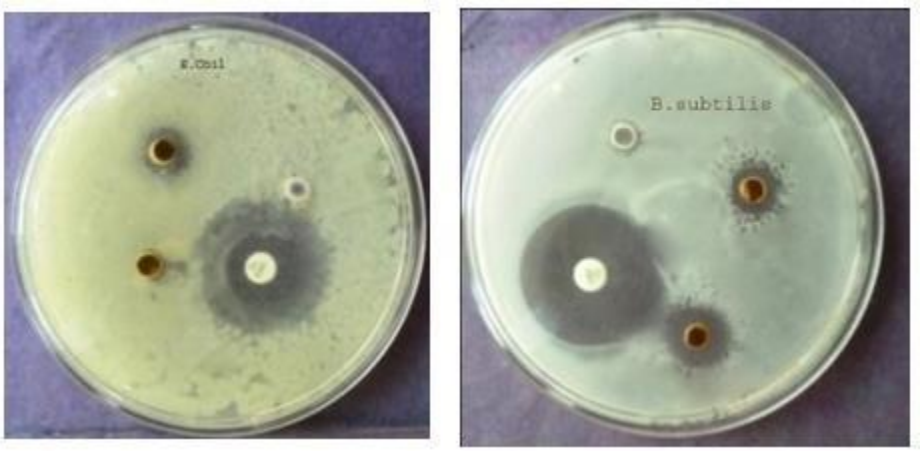

Figure 8: Inhibition zones for complex against *B. subtilis* and *E.coli.*

Table 1: Evaluation of antimicrobial activities of Zinc oxide nanoparticles

Bacteria	**Inhibition zone (mm)**
E.coli	11
B.subtilis	14

Conclusions:

Utilizing a straightforward chemical process, it has been demonstrated that zinc oxide nanoparticles may be efficiently manufactured. In addition to exhibiting improved antibacterial action, the zinc oxide nanoparticles also demonstrated cooperative behavior. The techniques of FT-IR, SEM, FESEM, TEM, and XRD were utilized in order to conduct an analysis on the spherical zinc oxide nanoparticles. After being dried at a temperature of 120 degrees Celsius, the average particle size of zinc oxide nanoparticles was found to be 50 nanometers using Scherrer's equation and 100 nanometers by scanning electron microscopy

(SEM). A high production rate may be achieved by the utilization of this approach, which can be applied for the synthesis of zinc oxide nanoparticles on a large scale.

DECLARATIONS:

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

All the authors approved the manuscript for publication.

Availability of data and material:

All required data is available.

Competing interests:

All authors declare no competing interests.

Funding:

Not applicable.

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