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Green Synthesis of Zinc Oxide Nanoparticles Using *Pterocarpus santalinus* Leaf Extract: Antioxidant Potential and Antibacterial Efficacy Against *Pseudomonas cichorii* in Chrysanthemum

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Abstract

The green manufacturing of nanoparticles utilising biological systems, particularly plant extracts, is a new field in nanotechnology. Zinc oxide nanoparticles were created in this work using an aqueous extract of Pterocarpus Santalinus leaves and zinc salt (zinc nitrate) as precursors. The green synthesised zinc oxide nanoparticles were assessed using a UV-visible spectrophotometer. Utilising SEM with EDAX, the shape of the zinc oxide nanoparticles was described. Research employing X-ray diffraction (XRD) equipment revealed that zinc oxide nanoparticles are crystalline and pure. Utilising FTIR spectroscopy, the specific functional groups in charge of the reduction, stabilisation, and capping agents seen in the nanoparticles were identified. Using the disc diffusion technique, the antibacterial activity of synthesised ZnO nanoparticles against a multihost bacterium (*Pseudomonas cichorii*) was evaluated. ZnO nanoparticles had superior antibacterial action. The findings of the antioxidant testing were positive. This work demonstrates that zinc oxide nanoparticles made through green synthesis have inherent anti-microbial and antioxidant qualities that might be used to make agricultural insecticides.

Keywords: *Pterocarpus Santalinus*, Zinc Oxide nanoparticles, *Pseudomonas cichorii*, Antibacterial activity, Antioxidant activity, Green Synthesis.

Introduction

A developing topic of study that has the potential to revolutionize other scientific disciplines is nanotechnology. Because of their small size and distinctive form, Nanomaterials have a wide range of uses and are a hot topic in both basic and applied sciences (1). These nanoparticles have become more important in recent years because of their remarkable chemical and thermal endurance (2). There are several ways to produce zinc nanoparticles (NPs), including the sol-gel, hydrothermal, spray pyrolysis, microwave-assisted, ultrasonic condition, chemical vapour deposition, and precipitation processes (3). These preparations are energy-intensive and include toxic and hazardous compounds that may be biologically dangerous.

However, biological methods are becoming more and more popular since they are often easy, clean, safe, and affordable (4,5). The sizes and shapes of nanoparticles produced via biosynthetic pathways are more accurately specified than those produced by other physical and chemical methods (5). Natural substances present in biological systems are vital and flexible in the creation of nanoparticles, as well as capping agents that stabilise them. Compared to other biological systems, using plants offers a number of advantages, as the research study shows. Compared to alternative techniques, the plants provide more stable nanoparticles and are safe to handle (6).

Here, we describe the synthesis of ZNPs employing zinc salt (Nitrate) and an aqueous extract of Pterocarpus Santalinus leaves as precursors. The structural and morphological properties of the synthesised Zinc Oxide NPs have been confirmed using

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the methods of UV-Visible Spectroscopy, FT-IR, XRD, SEM with EDAX. Using the disc diffusion technique, the antibacterial activity of green synthesised nanoparticles were evaluated against the bacterium *Pseudomonas cichorii*, which causes Bacterial Leaf Spot in Chrysanthemum.

A gram-negative soil bacterium called *Pseudomonas cichorii* is harmful to plants. It can affect a variety of hosts and have a significant financial impact on the crops of lettuce, celery, and chrysanthemums (7). *P. cichorii* got its name from the endives (Cichorium endivia) where it was initially discovered. *P. cichorii* has been assigned to the *P. syringae* group by 16S rRNA analysis (8).

The fact that *Pseudomonas cichorii* infects multiple hosts indicates that it is non-host specific. Its host range covers a variety of host plants, such as basil, lettuce, pepper, celery, coffee, and wheat (9) The host and location of the diseased plant affect the causative agent's symptoms differently. Leaf blighting and spots are typically seen to be caused by *Pseudomonas cichorii.* A water-soaked lesion that appears at the leaf's border, midvein, or sporadically throughout the leaf is the initial sign of the condition. These lesions may be encircled by yellow haloes and gradually turn black or brown. These lesions frequently "coalesce and progress to severe leaf blight under favorable conditions for the bacterium"(10). The spots frequently appear brittle and dry when the afflicted leaves are at their driest.

Materials and Methods

Preparation of Plant Extract

Pterocarpus Santalinus leaves were collected fresh at the Yogi Vemana University Botanical Garden in Kadapa, Andhra Pradesh, India. After being cleaned, the leaves were crushed, stored for later use, and dried in the shade to eliminate moisture. Separately, 100 ml of deionised water and 10g of powdered material were added to a beaker, which was then heated to a boil at 80 °C for 25 to 30 minutes while being stirred. The liquid was brought to a boil and then allowed to cool to room temperature. The solution was centrifuged for ten minutes at 3000 rpm after being filtered using filter paper (Whattman No. 1) to exclude heavy biomaterials. Plant extracts were prepared into clear solutions and stored at 4°C for future research(21).

Synthesis and purification of zinc oxide nanoparticles

In order to synthesis zinc oxide nanoparticles, 80 millilitres of a 0.02 M zinc nitrate (Zn $(NO_3)_2.6H_2O$)solution were mixed with 20 millilitres of the plant extract. The liquid was constantly stirred for half an hour at room temperature using a magnet stirrer. To get a brown to white colour change, the mixture was agitated for two more hours. A series of re-dispersions in deionised water and an ethanol wash were used to purify the precipitate. After being dried overnight at 60°C, the final result was a white powder (11).

Characterization of zinc oxide nanoparticles

An X-ray diffraction device (XRD) with Cuk radiation (= 1.5412) was used to study zinc oxide nanoparticles in the scanning range of 100 to 800. To verify the absorbance of ZnO NPs, UV-visible (UV-vis) spectra were obtained in the wavelength range of 200–600 nm using UV Spectrophotometer. Using Fourier transform infrared (FTIR) spectra of zinc oxide nanoparticles were obtained in the 400–4000 cm^{-1} range to identify the unique functional groups on the ZnO surface. All synthesised ZnO NPs surface morphology was examined using SEM, and elemental analyses were performed using energy dispersive x-ray analysis.

Method used for Anti-Bacterial Activity

Materials used for Anti-Bacterial activity of Zinc Oxide Nanoparticles, commercially available Nutrient Broth (HiMedia), Nutrient Agar Media (HiMedia), Petri plates, Sterile Disks, Cotton Swabs, Zinc Oxide Nanoparticles sample, Bacteria *(Pseudomonas cichorii)* was collected from Chrysanthemum; Disk Diffusion Method was used for antibacterial activity of Zinc Oxide Nanoparticles.

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Preparation of Inoculam

Diseased Chrysanthemum leaves were collected from the nearby fields of Yogi Vemana University, Kadapa. The samples were first washed and surface sterilized. Infected portion of the leaves were excised with a sterile scalpel and placed on onto a sterile mortar and pestle and then the leaves were crushed into liquid by adding two drops of sterile distilled water. 2mL of the crushed liquid portion was added to the 100 mL of Nutrient Broth media. Place the Broth media in an incubator at 37^0C for overnight.

Disk Diffusion Method

Nutrient agar medium (100 mL) was prepared and sterilised. In a laminar air flow chamber, the medium was poured into the sterile Petri dishes while maintaining sterility. Following the solidification, a cotton swab was dipped into the broth culture of the organism. Use the swab to make a lawn of growth on a nutrient agar plate and gently press it against the tubes within to remove any excess fluid. After putting three different quantities of zinc oxide nanoparticles (10, 20, and 30 µg/mL) in eppendorf tubes, sterile discs were obtained and dipped in the tubes holding the nanoparticles. Discs were applied to the agar surface using flame-sterilized forceps (discs of nanoparticles in three concentrations are present on all plates). After that, the plates were incubated at 37°C for the whole night (11). The antibacterial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Finally, we were measured the (mm) diameters of the inhibitory zones. In this case, the blank is sterile discs immersed in distilled water.

Anti Oxidant Assay

In order to conduct antioxidant testing, 15 days old, healthy chrysanthemum plant lings were collected from the fields nearby Yogi Vemana University in Kadapa. We maintained four batches of gathered chrysanthemum plants to conduct the experiment. The first batch from them were given simply distilled water and served as the experiment's control. Nanoparticles and distilled water were given to the second batch. *Pseudomonas cichorii* (pathogen) and distilled water were given to the third batch and, *Pseudomonas cichorii* (pathogen) along with nanoparticles and distilled water were given to the fourth batch.

The enzyme extract for Catalase, Glutathione peroxidase, Glutathione reductase, Glutathione S transferase, Lipid peroxidase and Superoxide dismutase was prepared by grinding plant material with 20 ml ofextraction buffer 0.1 M potassium phosphate buffer, pH 7.5 containing 0.5 mM Ethylene Di Amine Tetra Acetic Acid (EDTA). The extract was centrifuged for 20 min at 15,000 rpm and the supernatant was used for enzymatic assay. Superoxide dismutase, Catalase, and Glutathione reductase were determined using the method of Elavarthi, S et al., 2010 with some minor modifications (17). Lipid peroxidase activity was assessed using the technique developed by Jambunathan, N. et al., 2010 (18), Glutathione peroxidase was calculated using the technique developed by Ahmed, A. Y. et al., 2021 (19) with minor modifications, and lastly the Glutathione S-transferase test was carried out using the technique developed by Vontas, J. G. et al., 2000 (20).

Results

Characterization of nanoparticles

Uv-Spectrophotometer

Secondary metabolites found in plants convert zinc ions in the fluid into zinc oxide nanoparticles. The plant extract acts as a reducing and stabilising agent at the same time. This was confirmed by analysing the UV-visible spectra between 190 and 800 nm. ZnO nanoparticles had a noticeable peak in the spectra at 320 nm (Figure 1). It has been reported that the absorbance peak of ZnO nanoparticles occurs between 310 and 360 nm in wavelength(12).

SEM with EDAX

The surface morphology of green synthesised ZnO nanoparticles was investigated using a scanning electron

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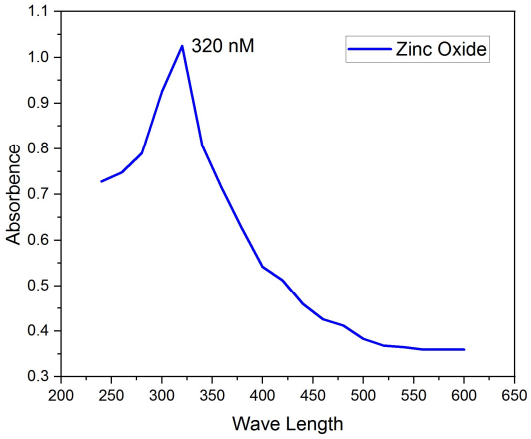

Figure1: UV spectra of Green synthesized Zinc Oxide Nanoparticles (λ max at 320 nM)

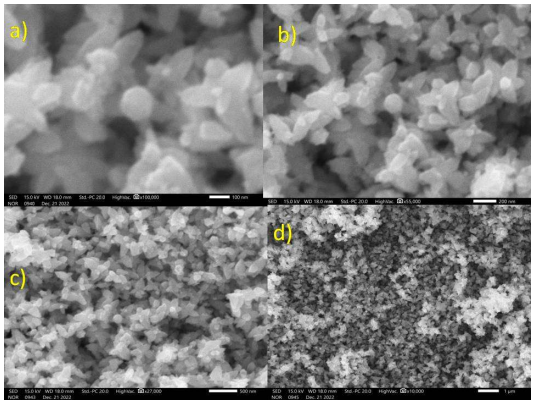

Figure 2: Green synthesised nanoparticles SEM images at various magnifications 100 nm, 200 nm, 500 nm, and 1µm is the range

microscope (SEM). Figure 2 shows SEM pictures of green synthesised ZnO nanoparticles with five-petal flower-shaped structures and a cupola-shaped surface in the middle of each flower. In a recent work (13), iron nanoparticles (FeONPs) synthesised using *Solanum lycopersicum* showed similar flower-like agglomerated patterns in FE-SEM images. These structures were linked to the unique nano-structure of nanoparticles created utilising plant extracts. As a result, we demonstrate that, in addition to iron, zinc can also form structures with a large

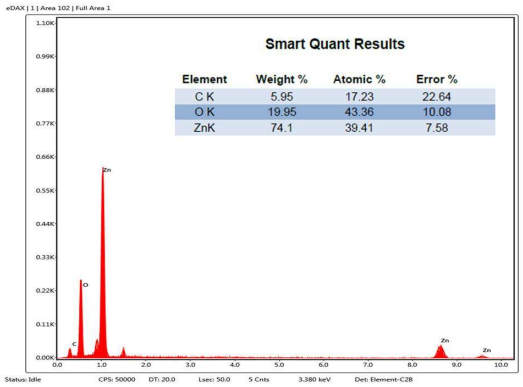

Figure 3: EDAX image displaying zinc and oxygen peaks, as well as their respective percentages

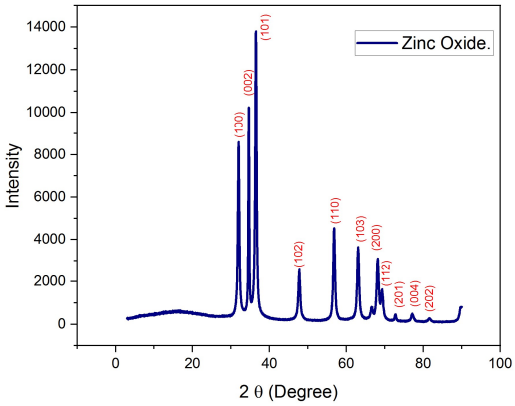

Figure 4: XRD of green synthesized Zinc Oxide nanoparticles

surface area that resemble flowers. The presence of zinc in the oxide form was verified by EDX data (Figure 3), which revealed a significant signal for zinc and oxygen. The EDX yields notable peaks of 19.95% oxygen and 74.1% zinc, whose weight percentage peaks match those previously documented for the fabrication of ZnO nanoparticles, enabling one to ascertain the makeup of each element contained in the analyte. Other than the traces for zinc and oxygen, none were found (14).

XRD

Green synthesised ZnO NPs X-Ray Diffraction (XRD) patterns are seen in Figure 4.

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Lattice constants a=3.249 and c=5.206 promote ZnO NPs crystalline nature and hexagonal form (wurtzite structure). The ZnO planes [100], [002], [101], [102], [110], [103], [200], [112], [201], [004], and [202] may be correlated with the reflections that were captured at 31.80, 34.40, 36.30, 47.50, 56.60, 62.80, 66.40, 67.90, 69.10, 72.50, and 76.90. It is found that the average crystallite size of ZnO nanoparticles is 42.29 nm.
Using the Scherer equation:

$$D = \frac{0.9\,\lambda}{\beta cos\theta} \quad (15)$$

The absence of any diffracted peaks indicating the absence of impurities demonstrates the high purity of the materials synthesised utilising the green synthesis technique(16).

FT-IR

Plant extracts that may have biological properties or functional groups that stabilise zinc oxide nanoparticles (ZnO NPs) were categorised using FT-IR. The green synthesis of nanoparticles is supported by the FTIR spectra in the 400–5000 cm^{-1} range. The results of the analysis revealed that the samples possessed vibrational bands between 400 and 2000 cm^{-1} (Figure 5). Absorbing peaks were seen in the 450–1500 cm^{-1} range of the ZnO NPs data. The following significant peaks were observed: 1433 cm^{-1}, 1096 cm^{-1}, 841 cm^{-1}, 599 cm^{-1}, and 479 cm^{-1}. Zn-O stretching is detected as the peak located at 479 cm^{-1}(11).

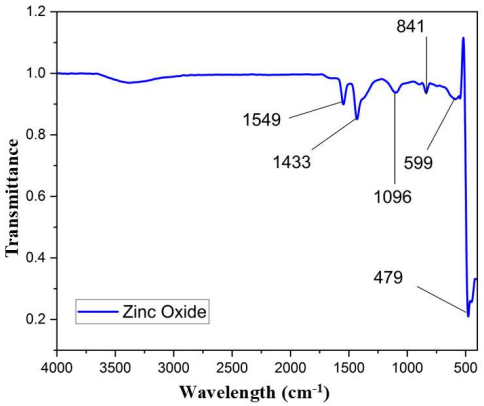

Figure 5: FT-IR analysis of green synthesised ZnO NPs

Anti-Bacterial Activity:

Using the disc diffusion method, the antibacterial efficacy of green synthesised zinc oxide nanoparticles against *Pseudomonas cichorii* was effectively demonstrated, and during a 24-hour incubation period, distinct zones growth inhibition was detected. For the experiment, four plates of Nutrient Agar Medium were produced, one as a blank (control) and the other three as test plates (triplicates). Here, we employed three different concentrations of the nanoparticle sample, and for each concentration, we obtained a zone of inhibition that was 1 cm, 1.7 cm, and 2.3 cm, respectively, for concentrations of 10 µg/ml, 20 µg/ml, and 30 µg/ml. These findings show that zinc oxide nanoparticles have a significant ability to inhibit *Pseudomonas cichorii* (Table 1).

Anti-Oxidant Activities

The findings displayed in Figure 6 reveal that green synthesised zinc oxide nanoparticles have antioxidant properties. All test results were positive. Adding green synthesised zinc oxide nanoparticles to this boosts the activity of superoxide dismutase, glutathione peroxidase, reductase, glutathione S transferase, and catalase. The Anti-Oxidant activity was suppressed when infected with the pathogen (*Pseudomonas cichorii*). And finally in the presence of nanoparticles they show some increase in activity even though they were in

Table 1: Diameter of zone of inhibitions with Green synthesized Zinc Oxide Nanoparticles

S. No	Concentration of nanoparticles	Zone of inhibition with synthesized ZnO nanoparticles
1.	10 µg/ml	1 cm
2.	20 µg/ml	1.7 cm
3.	30 µg/ml	2.3 cm

Pseudomonas cichorii in Chrysanthemum

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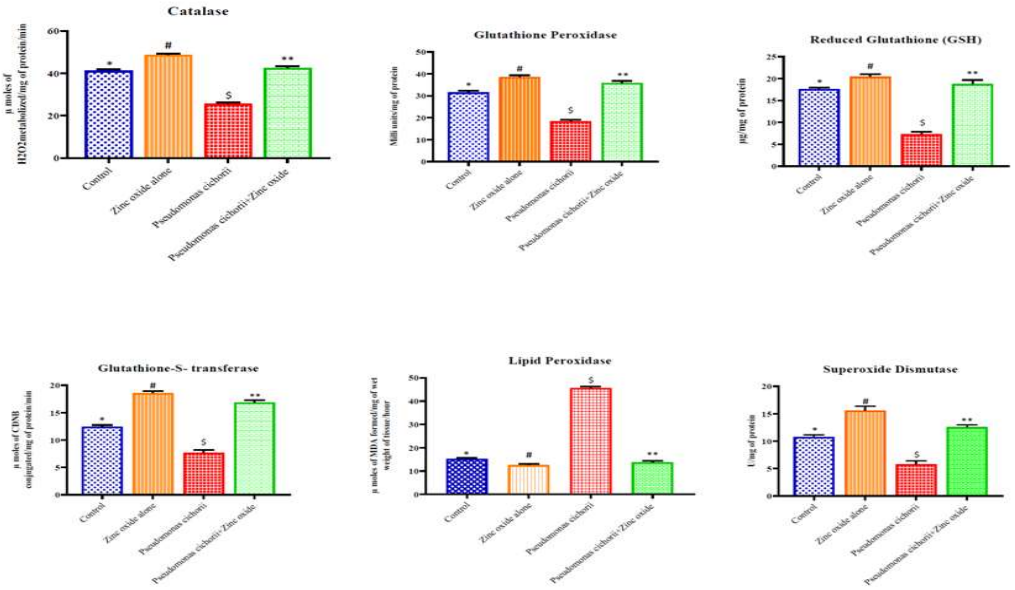

Figure 6: Showing results of Anti Oxidant Activity 1) Catalase, 2) Glutathione Peroxidase, 3) Glutathione Reductase, 4) Glutathione S Transferase, 5) Lipid Peroxidase and 6) Super Oxide Dismutase

infected condition. When it comes to Lipid Peroxidase activity, nanoparticles suppress it. Infected plants had high Lipid Peroxidase activity, but when exposed to nanoparticles, this activity was reduced.

Conclusion

Recent studies have effectively synthesized zinc oxide nanoparticles using an environmentally safe green method from *Pterocarpus Santalinus* leaf extract. The characterization of green synthesized ZnO NPs using several methods, including XRD, UV-Visible, FTIR, SEM, and EDX, was effective. Results show that green-synthesized ZnO NPs perform much better in terms of antioxidant and antibacterial properties. Due to the synergistic inclusion of biologically active adsorbed Phytochemicals, the synthesized NPs displayed improved biological activity. Therefore, this research work has further demonstrated that an affordable and viable alternative to traditional chemical processes would be an environment-friendly and modest green synthesis of ZnO NPs with increased and/or new biological capabilities using the leaf extract of *Pterocarpus Santalinus.* And these properties may serve for the production of pesticides in the field of agriculture.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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