



Synthesis Characterization and Antimicrobial Evaluation of Zinc Oxide and Iron Nanoparticles Synthesized Using Stembark of *Tecomellaundulata*

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Abstract

*Synthesis of green nanoparticles is an alternative and safe approach used widely nowadays. In the present study, by using extract of stembark of *Tecomellaundulata*, zinc oxide nanoparticles and Fe nanoparticles were synthesized. Those were characterized by using UV, FTIR, and FESEM. These synthesized nanoparticles were evaluated against bacteria (*E. coli* and *S. aureus*) and fungi (*A. niger* and *P. chrysogenum*) along with standard antibiotic drugs. Results of the study showed that these synthesized nanoparticles had good antibacterial and antifungal potential. The maximum activity by ZnONPs was found to be against *A. niger* while FeNPs showed maximum activity against *E. coli*. From the results of the present investigation, it can be concluded that green nanoparticles using this plant can be used as antimicrobial agents.*

Keywords

Tecomellaundulata, green nanoparticles, antibacterial, antifungal.



1. Introduction

Biology supplies nanotechnology models and bio-assembled components for inspiration, and nanotechnology uses biology to provide the tools and technological platform for the study and modification of biological processes. According to Gericke and Pinches [1], nanobiotechnology is a discipline that employs biological concepts and materials to construct novel devices and systems integrated from the nanoscale. It applies nanoscale principles and techniques to comprehend and modify biosystems (living and non-living). With potential applications in electronics and health, nanotechnology has grown rapidly as a modern study topic [2-4]. To create nanoparticles with specialized functionalities, nanobiotechnology blends physical and chemical processes with biological principles. An affordable substitute for the physical and chemical processes used to create nanoparticles is nanobiotechnology. These synthesis techniques may be separated into external and intracellular categories [5].

Nanoparticle production involves physical, chemical, and biological techniques. Hazardous chemicals were used in the chemical synthesis of nanoparticles, while high temperatures and energy consumption were engaged in the physical procedures, which had an impact on the environment [6]. Green nanotechnology has focused on creating non-toxic, eco-friendly, and economically viable nanoparticles in recent years. The limitations of chemical and physical approaches may be solved by the green synthesis of nanoparticles from a variety of natural sources, including plants and microorganisms (bacteria, fungus, algae, actinomycetes, and viruses) [7].

Tecomella undulata, often referred to as Rohida, is a member of the Bignoniaceae family. It is used to treat syphilis, gonorrhoea, hepatitis, tumors, conjunctivitis, hepatosplenomegaly, and liver and spleen diseases in traditional medicine. It is also used to cleanse blood and hasten the healing of wounds. Compounds that produce TU have been reported to include triterpenoids, fatty alcohol, phytosterol, iridoid glucoside, naphthaquinone derivative, and flavonols.

In the present study, using stem bark extract of *T. undulata*, ZnO and Fe nanoparticles were synthesized and characterized. These nanoparticles were evaluated against some bacteria and some fungi.

2. Materials and methods

Collection of plant materials

The experimental plant material (stem bark) of *Tecomella undulata* was collected from Campus, University of Rajasthan, Jaipur, Rajasthan.

Preparation of plant extracts

After being carefully cleansed with tap water and then distilled water, the recently harvested stem bark of *T. undulata* was examined. The stem bark was then broken up into tiny fragments. One beaker containing 10 g of stem bark and 100 ml of distilled water was added. The mixture was held for 20 minutes at 60°C. It was then allowed to cool at ambient temperature. Whatman filter paper 42 was used to filter it. For 20 minutes, the filtrate was centrifuged at 81 G-force. To be used later on to create Au nanoparticles, the extract was refrigerated.

Preparation of Zinc nanoparticles

A boiling solution of zinc nitrate hexahydrate was mixed with thirty milliliters of stem bark extract. 5 g of zinc nitrate hexahydrate were added to 50 ml of distilled water to create the zinc nitrate solution. A magnetic stirrer heater was used to continuously swirl the mixture at a temperature of 80°C. Stirrer heating was allowed to continue continuously until a paste with a pale-yellow color was achieved. For three hours, this paste was calcined in a furnace set at 400 degrees Celsius. The result was a powder with a yellow hue that was later used.

Preparation of Fe Nanoparticles

FeNPs were synthesized by mixing plant extract and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in the ratio of 1:10. The mixture was kept on shaker for overnight at 37°C . The change in color from yellow to black indicated that phytoconstituents present in stem bark extract caused the reduction of Fe into FeNPs. These FeNPs were collected by centrifugation process and used further.

Characterisation of ZnO and Fe nanoparticles

Using a UV-vis spectrophotometer, the decrease of the produced nanoparticles was seen between 200 and 2000 nm in wavelength. The powdered NPs were recorded using a KBr pellet technique with an FTIR spectrophotometer throughout a frequency range of $4000\text{--}400\text{ cm}^{-1}$ at a resolution of 4 cm^{-1} for FTIR analysis. FESEM analysis was performed to ascertain the size, shape, and other morphological properties of the nanoparticles.

Antibacterial activity

For the in-vitro antibacterial test, the standard microbiological approach known as the Agar Well Diffusion method [8] was employed. Plant extracts were made at three different concentrations (50 mg/L, 100 mg/L, and 200 mg/L) and the various samples were diluted using 10% dimethyl sulphoxide (DMSO). Test microorganisms (*S. B. subtilis* and *E. coli*) were inoculated into nutritional agar (NA) medium-containing sterile petri plates. Using a spreader, the inoculum was applied to the whole dish and let to stand for thirty minutes. In the seeded agar plates, wells of 6 mm in diameter were created. A control well was also constructed at the same distance. Prearranged wells of seeded plates were filled with varying amounts of manufactured nanoparticles, plant extracts, and standard medication (30 μl). The plates were incubated for twenty-four hours at 37°C . The inhibition zone (IZ) surrounding each prepared well was used to assess the test sample's antibacterial spectrum. A comparison was made between the test samples and the commercial control antibiotics' (1 mg/ml) inhibitory zone diameters.

Determination of antifungal assay

For the in-vitro antifungal assay, a standard microbiological technique called the Agar Well Diffusion method [9] was employed. Three distinct concentrations (50 mg/ml, 100 mg/ml, and 200 mg/L) of manufactured nanoparticles and plant extract were generated, and the various samples were diluted using 10% dimethyl sulphoxide (DMSO). Test microorganisms (*A. niger* and *P. chrysogenum*) were inoculated onto sterile petri dishes containing the potato dextrose agar (PDA) medium. The inoculum was applied evenly throughout the dish using a spreader and allowed to stand for 30 minutes. In the seeded agar plates, wells of 6 mm in diameter were created. A control well was also constructed at the same distance. Prearranged wells of seeded plates were filled with a conventional medication (30 μl) and various quantities of plant extract and nanoparticles. The plates were incubated for seventy-two hours at 37°C . The inhibition zone (IZ) surrounding each prepared well was used to assess the test sample's antifungal spectrum. The test sample and the commercial control antibiotic ketoconazole (1 mg/ml) were compared for the sizes of the inhibitory zones they created.

3. Results

Characterization of ZnO and FeNPs

UV-visible spectroscopy

Figures 1 and 2, respectively, display the UV-visible absorption spectra of the produced ZnONPs and FeNPs. As time passed, the peaks' strength in relation to their height steadily grew. Iron nanoparticles' dimensions and morphology show the ab-

sorption peak. Because of the existence of singly ionized oxygen vacancies, ZnO nanoparticles exhibited two peaks in the UV-visible absorption spectra, with an absorption band at 204 nm. In contrast, a single peak was found at 205 nm for the absorption band of FeNPs. The form and size of the nanoparticles, together with other factors that are similar to the features of the UV-visible spectrum of metallic Zn and Fe, greatly influence the peak's location.

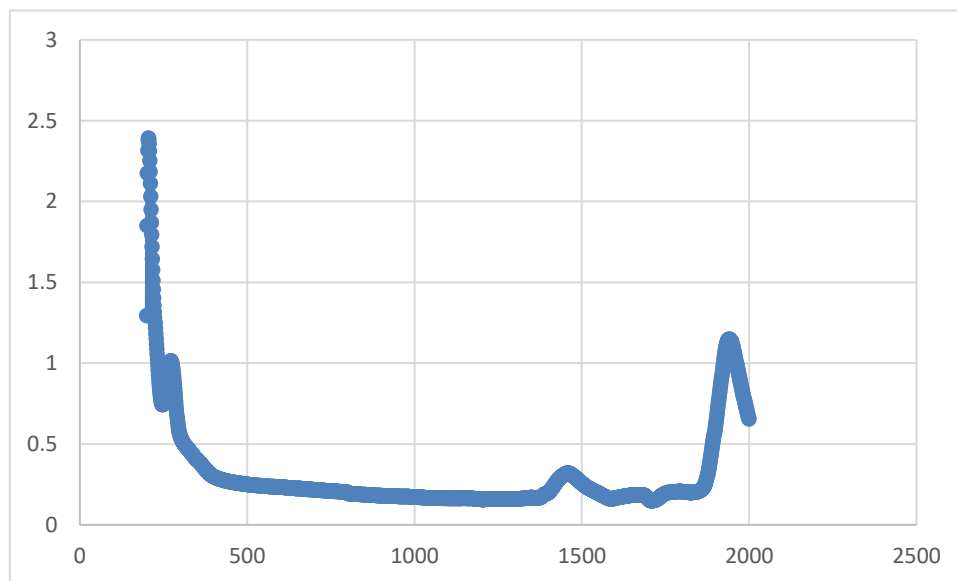


Figure 1. UV-visible spectra of ZnO nanoparticles

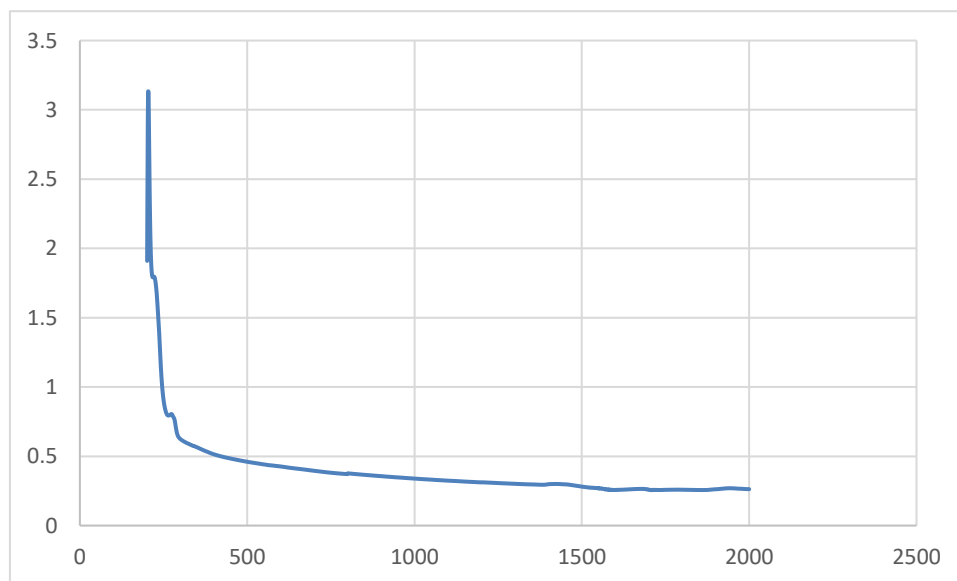


Figure 2. UV-visible spectra of FeNPs.

FTIR

The functional groups that are responsible for the production of ZnONPs and FeNPs from an aqueous extract of *T. undulata* stem bark were identified using FTIR spectroscopy. The absorbance bands of ZnO nanoparticles in the wave area between 400 and 4000 cm^{-1} are shown in Figure 3. There were notable peaks at 1578.16 cm^{-1} and 3422 cm^{-1} . Because of the presence of the -OH functional group, the band at 3422 cm^{-1} reflected O-H stretching, whereas the band at 1578.17 cm^{-1} demonstrated C=C stretching.

The absorbance bands of FeNPs in the wave area spanning 400–4000 cm^{-1} are depicted in Figure 4. There were peaks in the characteristics at 1627.22 cm^{-1} and 3425.01 cm^{-1} . The presence of alcohol or phenol as a functional group caused the band at 3425.01 cm^{-1} to reflect O-H stretching, while the presence of an alkene functional group at 1627.22 cm^{-1} corroborated C=C stretching.

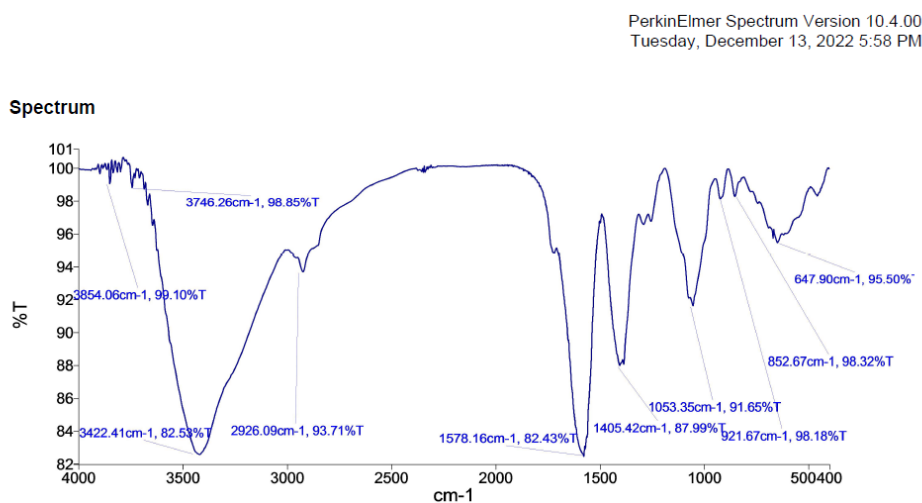


Figure 3. FTIR spectra of ZnONPs

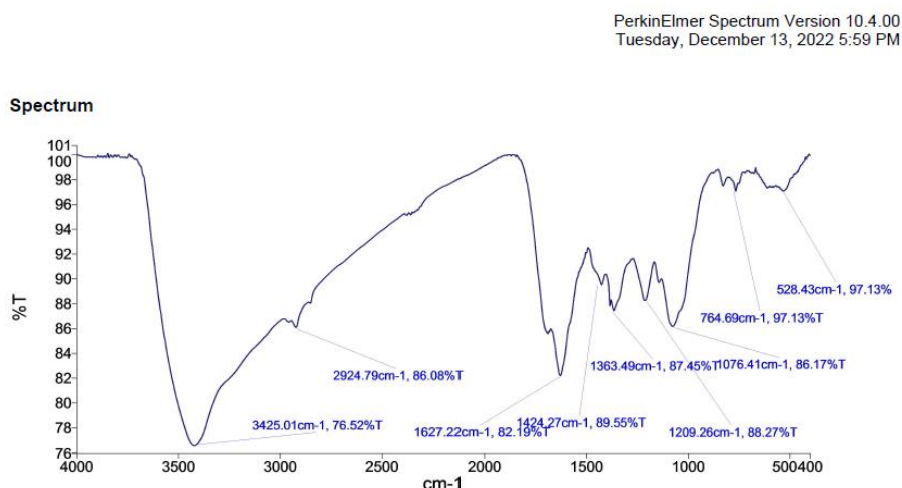


Figure 4. FTIR spectra of FeNPs.

FESEM

The sizes, shapes, and any other relevant morphological characteristics of the nanoparticles were determined using the microscope approach. The FESEM method was used to see the nanoparticles' size and shape. Figures 5 and 6 display FESEM images of ZnONPs and FeNPs, respectively. Iron nanoparticles were spherical with irregular morphologies and a size range of 20-80 nm, whereas the generated zinc oxide nanoparticles were rectangular in shape and had a size range of 40-100 nm.

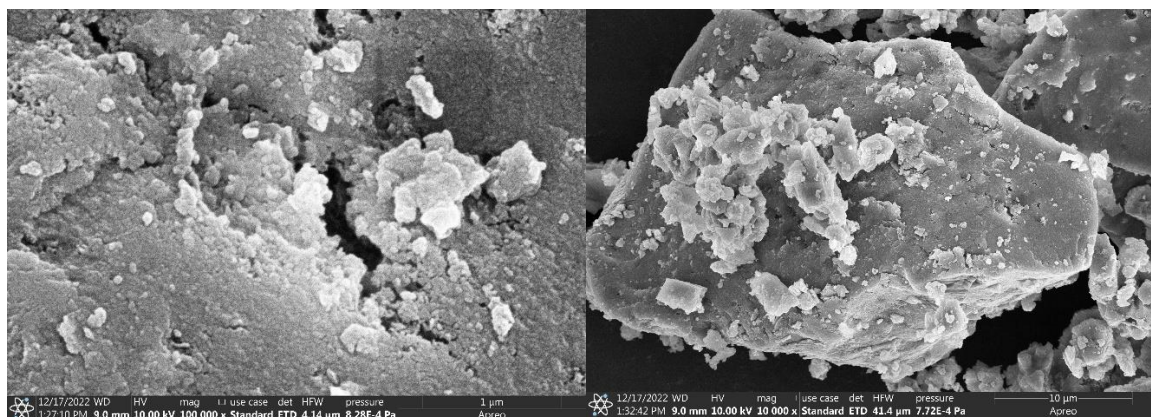


Figure 5. FESEM images of ZnO nanoparticles

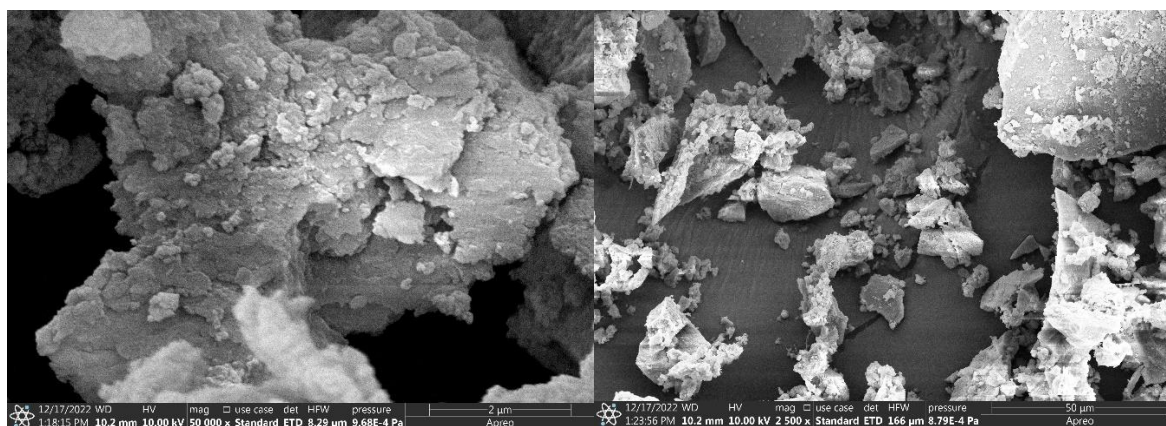


Figure 6. FESEM images of FeNPs

3.1. Antibacterial and antifungal activity

It has been demonstrated that metal oxide nanoparticles, such as those of silver, iron, zinc, and nickel, exhibit a variety of biological properties, including antibacterial, antifungal, and antioxidant properties [10]. *Tecomella undulata* has long been used to treat a wide range of illnesses, including wound healing, liver infections, and cancer. The plant is abundant in health-promoting phytoconstituents, including terpenoids, flavonoids, and phytosterols [11]. Many phytochemicals included in plant extracts function as capping agents in the nanoparticles during their production, which is what gives these greenly generated nanoparticles their increased biological activity [12].

In the current work, stem bark extract from *T. undulata* was used to create ZnO and Fe nanoparticles. These nanoparticles were tested against fungus (*P. chrysogenum* and *A. niger*) and bacteria (*E. coli* and *S. aureus*). Table 1 displayed the inhibitory

zone and activity index of nanoparticles in combination with a standard treatment for bacteria, and Table 2 displayed the same information in combination with a standard drug for fungus. Results revealed that both nanoparticles showed good inhibition of both bacteria and fungi at the concentration ranging from 50-200 mg/L. The maximum activity by ZnONPs was found to be against *A. niger* (0.16-0.45 activity index) while FeNPs showed maximum activity against *E. coli* (0.12-0.28 activity index). ZnONPs have not shown any inhibition at 50 mg/L against *E. coli* and *P. chrysogenum* while FeNPs have not shown any activity at 50 mg/L against *S. aureus* and *A. niger*. Our findings align with those of earlier research. Anti-gram positive and anti-gram-negative bacteria and fungi, ZnO nanoparticles have been demonstrated to have inhibitory effects [13] [14] [15]. Iron nanoparticles have demonstrated strong inhibitory effects against a variety of bacteria and fungus in research [16].

Table 1. Antibacterial activity of synthesized nanoparticles.

| Concentration | | 50 mg/L | | 100 mg/L | | 200 mg/L | |
|------------------|----------|---------|------|----------|------|----------|------|
| | | IZ | AI | IZ | AI | IZ | AI |
| <i>E. coli</i> | ZnONPs | NA | NA | 3 | 0.10 | 7 | 0.21 |
| | FeNPs | 3 | 0.12 | 5 | 0.17 | 9 | 0.28 |
| | Standard | 24 | | 28 | | 32 | |
| <i>S. aureus</i> | ZnONPs | 3 | 0.09 | 8 | 0.22 | 12 | 0.29 |
| | FeNPs | NA | NA | 7 | 0.19 | 11 | 0.26 |
| | Standard | 32 | | 36 | | 41 | |

Note: IZ- Inhibition zone (mm), AI- Activity index

Table 2. Antifungal activity of synthesized nanoparticles.

| Concentration | | 50 mg/L | | 100 mg/L | | 200 mg/L | |
|-----------------------|----------|---------|------|----------|------|----------|------|
| | | IZ | AI | IZ | AI | IZ | AI |
| <i>A. Niger</i> | ZnONPs | 4 | 0.16 | 8 | 0.29 | 14 | 0.45 |
| | FeNPs | NA | NA | 3 | 0.11 | 5 | 0.16 |
| | Standard | 24 | | 27 | | 31 | |
| <i>P. chrysogenum</i> | ZnONPs | NA | NA | 3 | 0.07 | 5 | 0.11 |
| | FeNPs | 2 | 0.05 | 5 | 0.12 | 6 | 0.13 |
| | Standard | 36 | | 40 | | 43 | |

Note: IZ- Inhibition zone (mm), AI- Activity index.



Figure 7. Antibacterial activity of FeNPs, ZnONPs and standard antibiotic against *E. coli*.



Figure 8. Antibacterial activity of FeNPs, ZnONPs and standard antibiotic against *S. aureus*

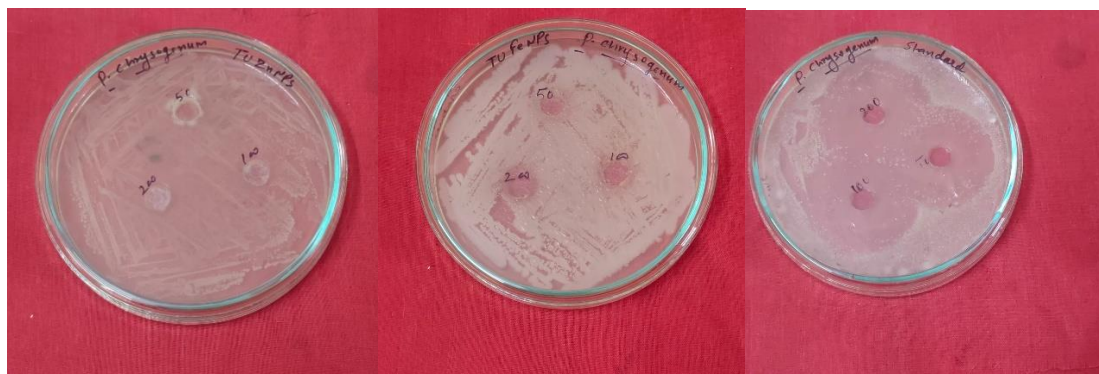


Figure 9. Antibacterial activity of FeNPs, ZnONPs and standard antibiotic against *P. chrysogenum*



Figure 10. Antibacterial activity of FeNPs, ZnONPs and standard antibiotic against *A. niger*

4. Conclusion

From results of the present study, it can be concluded that ZnO and Fe nanoparticles synthesized using stem bark extracts of *T. undulata* can be used as good antimicrobial agents.

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