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Formulation and evaluation of zinc oxide nanoparticles containing ornidazole

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ABSTRACT
The leaves of Delonix regia were extracted using deionized water as the solvent using cold maceration method. The zinc oxide nanoparticles were prepared using the plant extract and zinc nitrate in alkaline condition. The synthesized ZNPs were characterized using for particles size and the lowest size particles were loaded with ornidazole. The drug loaded ZNP was characterized by UV-Visible spectrophotometer, FT-IR spectroscopy, XRD, particle size and SEM.The lowest particles size was obtained with 30% extract concentration in relation to the zinc nitrate solution. The particle size of the synthesized ZNPs ranged from 49.7 nm to 32.4 nm. The increase in concentration of ZNP was found to increase drug loading with maximum loading of 69.4 % using 1% solution of ZNP4. The particle size of the ornidazole loaded ZNP was found to be 49.3 nm. The ZNPs were found to be spherical structures with smooth surface.The ZNP loaded with ornidazole were found to exhibit antibacterial activity against gram positive and gram negative bacteria.

Keywords: Nanoparticles, zinc oxide, ornidazole, Delonix regia, aqueous extract

I. INTRODUCTION

Drug delivery has always been an evolving component of biomedical research, ever since the advent of synthetic drugs and surgical medicine. Nanotechnology is a rapidly growing and diverse field that impacts many areas of science and engineering, and has the potential to revolutionise a vast array of technologies, from power generation and electronics to disease detection and treatment [1]. Due to their size features and advantages over available chemical imaging drug agents and drugs, inorganic particles have been examined as potential tools for medical imaging as well as for treating diseases. Inorganic nonmaterial have been widely used for cellular delivery due to their versatile features like wide availability, rich functionality, good compatibility, and capability of targeted drug delivery and controlled release of drugs [2].

Zinc oxide nanoparticles (ZnO-NPs) are one of the metal oxide nanomaterials and a valuable and versatile inorganic compound due to its unique physical and chemical characteristics. They possess high chemical stability, a broadened radiation absorption spectrum, high electrochemical coupling coefficient, and high photostability with the molecular formula ZnO [**3**]. ZnO-NPs have been widely manufactured and utilized in various commercial and additive products, including ceramics, cement, plastics, glass, ointments, lubricants, adhesives, sealants, pigments, batteries, ferrites, fire retardants, cosmetics, and sunscreens, as well as in foods as a source of zinc nutrient [**4,5**]. Nanosized ZnO particles demonstrate significant antibacterial capabilities due to their small size, which can stimulate different bactericidal mechanisms once inside the bacterial cell, including the bacterial surface or bacterial core, generate ROS (reactive oxygen species), release Zn^{2+}, and even be endocytosed by cells [**6,7**].

Green synthesis of nanoparticles is a common eco-friendly method using plants extract is due to numerous advantages, including better products and worth of NPs obtained, easiness of process and control, safety, the richness of resources, and cost-effective.

Ornidazole (ODZ) chemically known as 1-chloro-3-(2-methyl-5-nitroimidazole-1-yl) propan-2-ol, is a third generation 5 nitroimidazole derivatives that is commonly used in the treatments of infections caused by the bacterial and protozoa [8]. The objective of the present investigation is to perform the green synthesis of Zinc Oxide nanoparticles using Delonix regia leaf aqueous extract and load the nanoparticle with ornidazole and assess its antimicrobial action.

II. MATERIAL AND METHODS

Calibration curve of ornidazole in methanol

A stock solution of ornidazole (100 mg/100 ml) was prepared in methanol. Diluted ornidazole solution (10 mg / 100 ml) in methanol was prepared from the stock solution. Then, serial

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dilutions were prepared from that diluted solution in methanol to obtain different concentrations ranging from 10 to 50 µg/ml. The absorbance of these serial dilutions was determined spectrophotometrically at λmax 311 nm, using ethanol as a reference. The measured absorbance was plotted against the corresponding concentrations to obtain the standard calibration curve.

FT-IR study of ornidazole

The FT-IR spectrum was ornidazole was obtained and analyzed for the stretching and bending vibrations that occurred due to various functional groups present in the molecule.

Collection and preparation of plant material

The leaves of Delonix regia were collected from the local surroundings of Bhopal. The leaves were washed several times with water to remove the dust particles and allowed to dry at room temperature in dark, crushed to coarse powder and stored in airtight container till use.

Extraction of plant material

The powdered leaf was weighed (42 g) and filled in the extractor of a soxhlet extraction apparatus. Petroleum ether (95 mL) was flown down the extractor and the solvent was heated at 80°C for 2.5 h. The solvent was separated from the marc, the marc was dried. The dried marc was macerated with 500 mL of cold water for 24 h by intermittent shaking for first 6 hours followed by standing for 18 hours. The menstrum was filtered using muslin cloth and was stored in refrigerator till further use [9].

Preparation of Zinc oxide nanoparticles

The zinc oxide nanoparticles were prepared using the plant extract and zinc nitrate in alkaline condition. Various ratio of extract and zinc nitrate were used for preparing the nanoparticles (Table 1).

Table 5.1 Formulation variables for ZnO nanoparticles

Formulation	Delonix regia extract (%)	Zinc nitrate (g)
ZNP1	10	4
ZNP2	20	4
ZNP3	30	4
ZNP4	40	4
ZNP5	50	4
ZNP6	30	5

Accurately weighed quantity of zinc nitrate was added into 50 mL of deionized water to get final concentration of zinc nitrate (8% & 10% w/v). The required milliliters of Delonix regia leaf extract were slowly added to zinc nitrate solution in the beaker. 1 M of NaOH was added drop-by-drop to the solution to control the pH of the solution at 12 [10,11]. The mixture was stirred at room temperature for eight to ten hours until the greenish liquid slowly started to fade with the appearance of yellow-colored suspension followed by the formation of a pale-yellow precipitate. The precipitate was collected with filter paper and cleaned with tap water and ethanol to eliminate insoluble zinc nitrate and other impurities. The precipitate was dried in an oven for about 12 hour at 80 °C, and lastly calcining for 2 hour at 350 °C in a muffle furnace.

Characterization of ZNPs

UV-Visible spectroscopic study

The ZNPs synthesized were dissolved in 0.1N HCl solution and the UV-visible absorption spectra of the solution was obtained between 700-200 nm. The spectra was studied for the observed band gap [12].

Particle Size

The particle size of the ZNPs was determined by a light scattering particle size analyzer. The morphology of the ZNPs was studied with the help of scanning electron microscopy. The particles were coated with gold sputter on a metal stub and was scanned with an electron beam to obtain magnified image of the surface. The surface characteristics were observed from the image.

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Loading of ornidazole on ZNPs

To load the drug into ZNPs, the ZNP was dissolved in solutions of 100 mL of distilled water at three concentrations (0.1, 0.5, and 1%). Then, 40 mg of ornidazole was added to the three solutions before stirring by a magnetic stirrer at 600 rpm for a seven hours at room temperature. The solutions were left overnight, and then centrifuged at 5000 rpm for 10 minutes to get yellow precipitate [13].

Characterization of drug loaded ZNPs

Morphology and size

The particle size of the ZNPs was determined by a light scattering particle size analyzer. The morphology of the ZNPs was studied with the help of scanning electron microscopy. The particles were coated with gold sputter on a metal stub and was scanned with an electron beam to obtain magnified image of the surface. The surface characteristics were observed from the image.

Drug loading

The drug loading was studied by dissolving the ornidazole loaded ZNP in methanol and analyzing the solution at 311 nm by UV spectroscopy and amount of ornidazole was calculated from the calibration curve.

X-ray diffraction study

X-ray diffraction pattern of the prepared ZNPs was studied for obtaining the information about the crystal structure of the particles.

FT-IR spectral study

The stretching vibrations characteristic of the zinc oxide nanoparticles was studied by obtaining the FT-IR spectra of ZNPs.

Antibacterial activity of ZNP loaded with ornidazole

The microorganisms used for the antimicrobial study were procured from Institute of Microbial Technology, Chandigarh (MTCC). Escherichia coli (MTCC 40), and Staphylococcusaureus (MTCC 3160) were used for the present investigation.

Revival of cultures and preparation of plates

The lyophilized cultures were revived by adding 0.3 mL of nutrient broth to the culture ampoules to obtain a suspension of the bacteria. Revival of the fungal culture was done using 0.3 mL of water.

Ready to use nutrient agar powder was used for preparing the nutrient agar medium (Microgen). Agar plates were prepared by pouring the sterilized medium into sterilized petridishes suitably marked and labeled. The plates were allowed to solidify in the laminar flow bench and stored packed for culturing with microbes and antimicrobial screening.

Screening Procedure

About 3 mm thick pre-poured nutrient agar plates were inoculated with a few drops of the bacterial suspension by swabbing on the surface of agar. The antimicrobial action was screened using disc diffusion method [14].Wells were bored into the agar plate at equal distances using cork borer (10mm) and 200µL of the ZNPs (50, 75, 100 & 150 µg/mL) were placed in each hole. The plates were incubated for 24h at 37 ± 0.1°C to allow for microbial growth. The zone of inhibition in each plate was measured in millimeters.

III. RESULTS AND DISCUSSION

Standard calibration curve of ornidazole in methanol

The standard calibration curve of ornidazole was constructed in ethanol to obtain different concentrations ranging from 10 to 50 µg/ml, for which the absorbance readings were determined spectrophotometrically at λmax 311 nm (Figure 1). The standard calibration curve was linear over the concentration range studied and obeys Beer-Lambert's law with a correlation coefficient (r2) 0.998. The corresponding regression equation was found to be Y = 0.0177X-0.0058.

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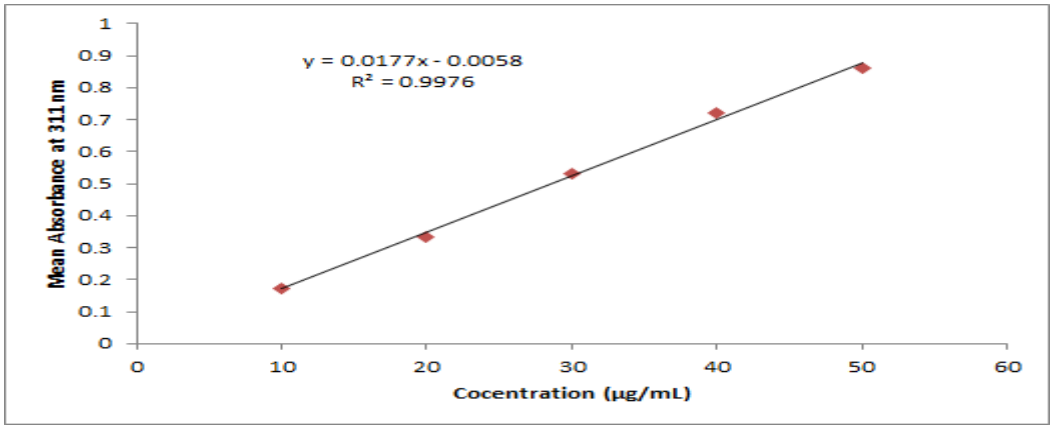

Figure 1Standard calibration curve of ornidazole in methanol

Synthesis of ZNPs

The schematic illustration for the synthesis of ZNPs is presented in Figure 6.4. The precursor used in the synthesis was zinc nitrate in alkaline medium (1 M NaOH). The mixtures containing the precursor and plant extract were stirred and was observed for color change from dark green to light brown through the course of stirring. The change in color of the solution indicates the conversion of zinc nitrate to zinc oxide. The presence of sodium hydroxide causes formation of zinc hydroxide through reduction as the initial intermediate which on thermal decomposition leads to the formation of zinc oxide [15]. Calcination at 400°C was done in muffle furnace to obtain particles with lower particle size. Previous studies have suggested that a temperature higher than 350°C for thermal decomposition of the zinc hydroxide leads to particles with higher particles size [16,17].

Effect of extract ratio on particles size of ZNP

The effect of extract concentration on the particle size of ZNP was observed by varying the concentration of the extract and using fixed amount of zinc nitrate (8 %w/v). The reduction and thermal decomposition were carried out and it was found that the particle size of the ZNP decreased with an increase in concentration of the extract (Figure 2).The lowest particles size was obtained with 40% extract concentration in relation to the zinc nitrate solution.

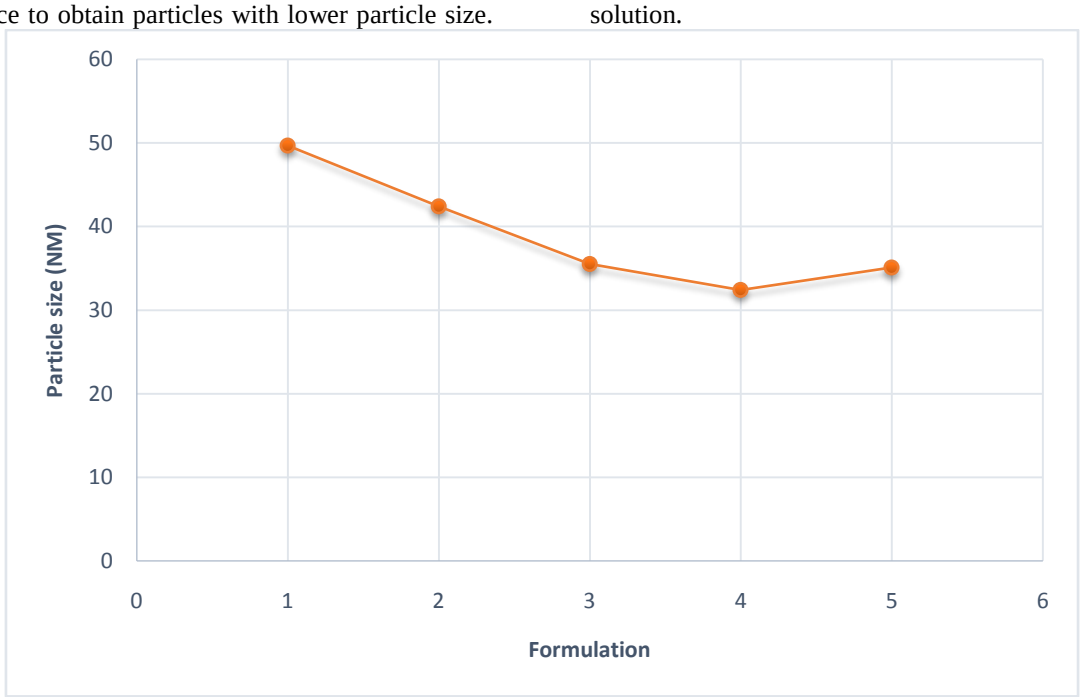

Figure 2 Effect of extract concentration on ZNP particles size

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Effect of zinc nitrate on particle size of ZNP

In order to assess the effect of zinc nitrate concentration on the size of the particles synthesized, nanoparticles were synthesized using 40% extract and 10%w/v zinc nitrate. The particles size was found to remain unaffected by the amount of zinc nitrate in the solution. The particles size of the formulation was obtained to be 35.1 nm, almost equal to the particle size obtained with 40% extract and 8% w/v zinc nitrate.

Characterization of ZNPs

The synthesized ZNPs were characterized with respect to their size, surface morphology, crystallinity, FT-IR spectral study and UV absorption spectra.

Particle size

The particle size of the ZNPs was measured using dynamic light scattering principle with the aid of particle size analyzer. The particle size of the synthesized ZNPs ranged from 49.7 nm to 32.4 nm (Table 2). The particles size was found to be affected by the concentration of the extract whereas the concentration of zinc nitrate did not affect the particle size.

Table 2 Particle size and drug loading of synthesized ZNPs

Formulation	Particle size (nm)
ZNP1	49.7
ZNP2	42.4
ZNP3	35.5
ZNP4	32.4
ZNP5	35.1

Amount of Drug loading

The lowest particle size was obtained in ZNP4 and hence three different concentration of ZNP4 were used to load ornidazole on the surface of the nanoprtiles. The loading of ornidazole was calculate using UV spectrophotometry. The amount of drug loaded in the samples was found to be increased with increasing the concentration of ZNP (Table 3).

Table 3 Drug loading

S. No.	Concentration of ZNP4	Drug loading
1	0.1 %	47.3 %
2	0.5 %	56.1 %
3	1.0 %	69.4 %

Particle size and morphology of drug loaded ZNP

The particle size of ZNP4 was found to increase on loading ornidazole on the ZNP. The particle size of 1.0 % ZNP4 loaded with ornidazole was found to be 49.3 nm.

The surface morphology of the synthesized ZNP was studied using scanning electron microscopy (SEM). The gold sputter coated particles were scanned under beam of electron and the image obtained was used to study the surface characteristics of the ZNPs. The ZNPs were found to be spherical structures with smooth surface. Moreover the clusters of particles were also observed in the images (Figure 3).

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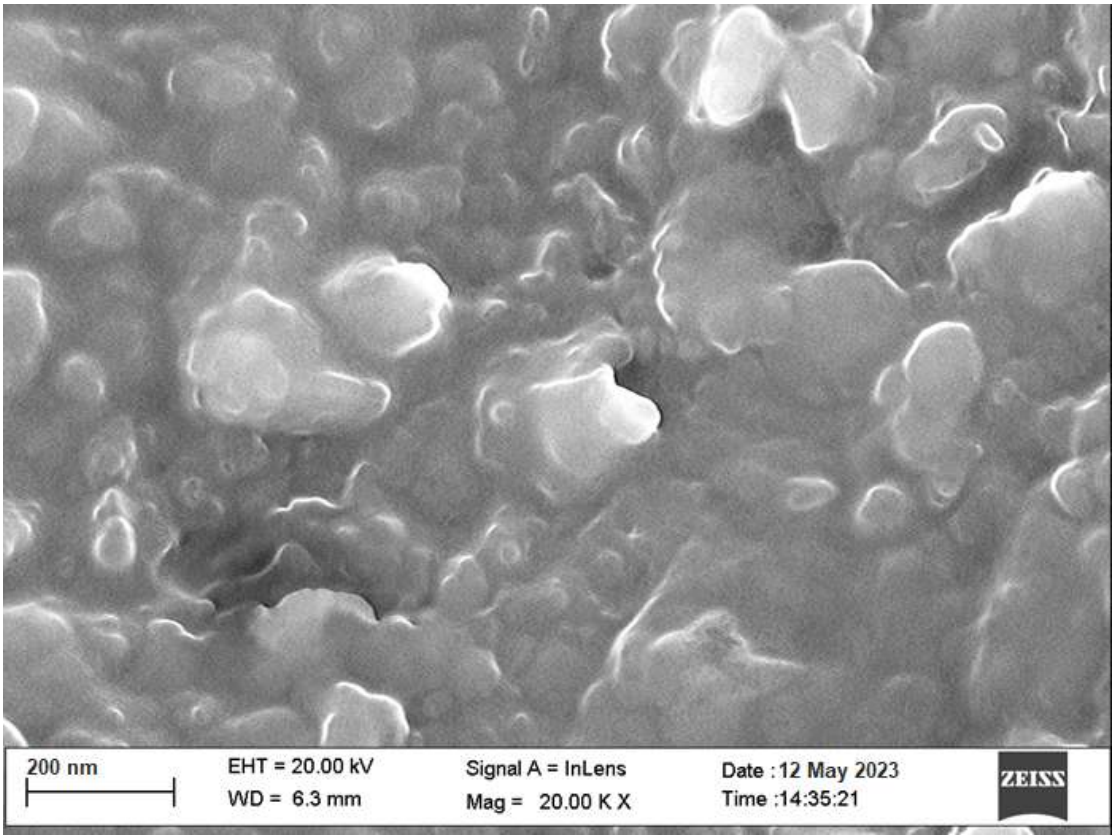

Figure 3 SEM image of ZNP4

X-ray diffraction study

The crystallinity of the synthesized ZNP was studied using XRD. Diffraction peaks was observed at 36°, 41°, 43°, 47.5° and 50° indicating the pattern of pure Zinc oxide with a hexagonal wurtzite polycrystalline structure with lattice planes.

FT-IR spectral study

The FT-IR of the solid zinc oxide nanoparticles was obtained and observed for the occurrence of stretching of the characteristic groups. The occurrence of transmittance peaks at 510 and 490 cm^{-1} are characteristic of metal-oxygen (ZnO stretching vibrations) (Figure 4). The broad peak at around 3400 cm-1 could be attributed to the O-H stretching of flavonoids and polyphenols of the extract. The FT-IR spectra of ornidazole revealed peaks of C-Cl, C-N, C=N, NO_2, OH stretching and bending (Figure 5). All the peaks of ornidazole were present in the FT-IR spectra of the ZNP loaded with ornidazole suggesting proper loading of drug on the ZNPs (Figure 6). The broad peak of flavonoids was also present in the loaded ZNPs.

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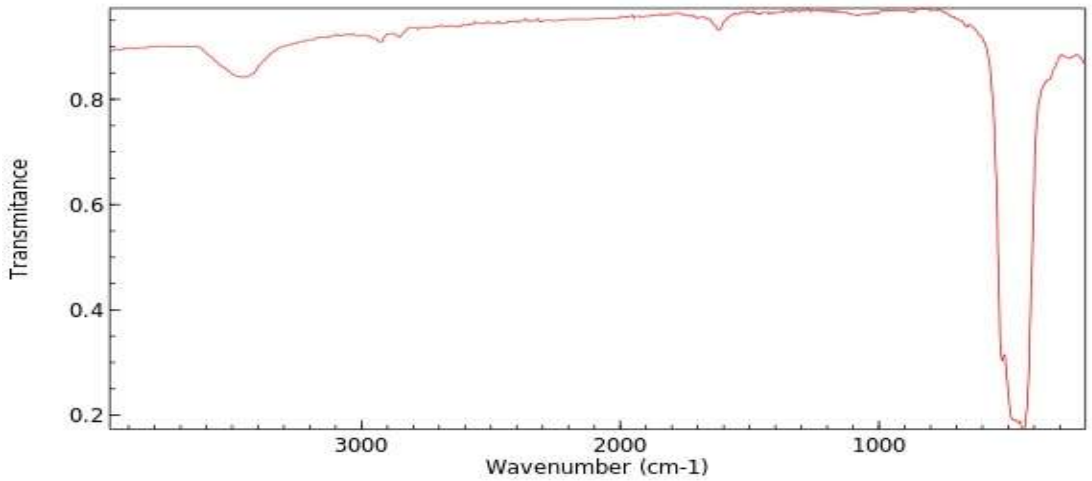

Figure 4 FT-IR spectrum of ZNP4

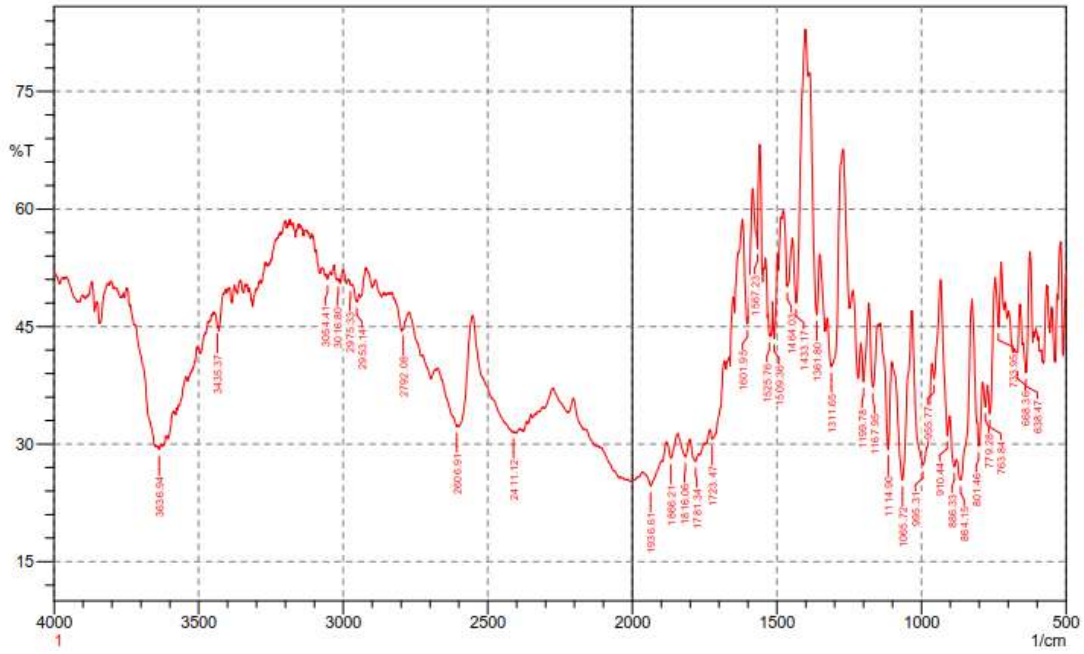

Figure 5 FT-IR spectrum of ornidazole

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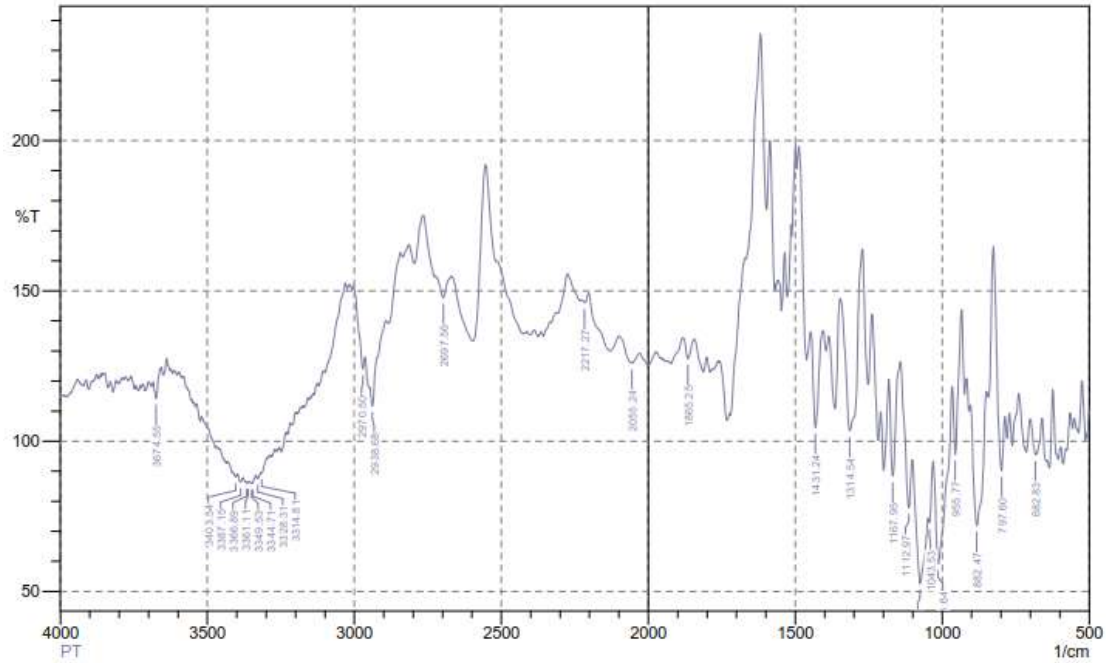

Figure 6 FT-IR spectrum of ornidazole loaded ZNP

UV-Visible spectrophotometric study

The solid white zinc oxide nanoparticles were dissolved in 0.1N HCl and UV absorption was studied. The spectrophotometric absorptive pattern normally depends on the variables like the temperature, size, and shapes of the synthesized nanostructures. The UV–Visible absorption of the ZNPs is correlated with their size. The UV–Vis spectrum of the ZnO-HPNs was measured in deionized water. Broadband can be observed at 367 nm, which was similar to the bandgap of zinc oxide "1s–1s electron transition".

Antibacterial activity

The antibacterial action of ZNP4 (1% solution) loaded with ornidazole was studied using disc diffusion method. The zone of inhibition obtained was taken as a measure of antibacterial activity. The ZNP was found to show activity against both gram positive and gram negative bacteria.

IV. CONCLUSION

The aim of the present study was to synthesize zinc oxide nanoparticles using Delonid regia leaf extract, load the ZNP with ornidazole and study the antibacterial action of the produced nanoparticles. The results suggest that Delonix regia aqueous extract is potential source of reducing agent for synthesize of zinc oxide nanoparticles. It was also found that the drug could be easily loaded on the surface of the nanoparticles. The ZNPs were evaluated for particle size, surface morphology, XRD and UV absorption.

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