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ORIGINAL ARTICLE

Green Synthesis of Zinc Oxide Nanoparticles Using *Coleus zeylanicus* (Benth.) L.H.Cramer Leaf Extract: Characterization, Antimicrobial Activity, and Antioxidant Potential

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ABSTRACT
This study presents a green synthesis approach for zinc oxide nanoparticles (ZnO NPs) using Coleus zeylanicus leaf extract, offering a cost-effective and environmentally friendly alternative to conventional methods The size, shape, morphology of nanoparticles synthesised is done using (UV-spec), X-ray diffraction, FTR(Fourier transform infrared), IR (Infra red), and TEM (Transmission Electron Microscopy). The results confirmed the successful synthesis of crystalline ZnO NPs with a hexagonal structure and an average size of 58.49 nm. The ZnO NPs exhibited significant antimicrobial activity against Serratia marcescens, E.coli, Proteus vulgaris, Enterobacter faecalis standard bacterial and Candida albicans fungal strains obtained from MTCC Chandigarh. The primary screening was carried out by disc diffusion assay and MIC was determined by Microbroth dilution technique. The MIC 10 µg, 1 µg, and 10 µg were the MIC values of gram-negative strains Proteus vulgaris, Serratia marcescens, and Escherichia coli, respectively. The fungal strain Candida albicans has the MIC value of 10 µg. The study explores the antimicrobial and antioxidant potential of the nanoparticles, revealing significant activity against bacterial and fungal strains and strong free radical scavenging abilities. By utilizing plant extracts as reducing agents, this research contributes to the growing field of green nanotechnology and emphasizes the significance of sustainable synthesis methods. The results pave the way for further investigations into the biomedical applications of ZnO NPs, such as their potential as anticancer and antidiabetic agents. This study underscores the importance of eco-friendly synthesis methods for functional nanoparticles in healthcare and medicine.
Keywords: *FTR, IR, TEM, MIC, DPPH assay, antifungal, antibacterial, antioxidant*

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INTRODUCTION

Nanotechnology, which involves the design and manipulation of particles at the nanometer scale (10-9 m), has emerged as a rapidly developing field of research in material science [1]. The interest in nanotechnology has expanded over the past decade, with particular emphasis on bio nanotechnology and the synthesis of various nanoparticles [2]. Metal oxide nanoparticles, such as zinc oxide nanoparticles (ZnO NPs), have gained significant attention due to their small size and large surface area, which make them suitable for diverse applications in biotechnology, chemistry, and medicine [3]. Nanoparticles exhibit unique physicochemical properties compared to bulk materials, thanks to their high surface-to-volume ratio and small size. The surface atoms of nanoparticles are highly reactive, making them attractive for various applications in medicine, drug delivery, water purification, agriculture, cosmetics, and electronics [4]. The synthesis of nanoparticles offers advantages such as cost-effectiveness, simplicity, and the ability to tailor their morphology and size for specific applications [1]. However, traditional methods of nanoparticle synthesis, such as physical and chemical approaches, are often expensive, labor-intensive, and environmentally hazardous [5]. To address these challenges, green synthesis approaches have gained attention due to their eco-friendliness, cost-effectiveness, and use of low-toxicity [6]. Plant-mediated biosynthesis is a popular green synthesis method, where plant extracts containing bioactive

compounds act as reducing and stabilizing agents [7]. Among the various metal nanoparticles, zinc oxide nanoparticles have garnered significant interest due to their unique properties and potential applications. Zinc (Zn) is an essential element for animals, humans, and microorganisms, playing a crucial role in cellular processes [8]. ZnO nanoparticles exhibit antimicrobial, antibacterial, and photocatalytic properties, making them suitable for various biomedical and environmental applications [9], [10], [11]. Additionally, ZnO nanoparticles possess antioxidant properties, making them attractive for controlling reactive oxygen species (ROS)-mediated pathogenesis [12]. While several studies have reported the biosynthesis and characterization of ZnO nanoparticles using various plant extracts [13] [14] [3], there is a lack of research on the synthesis of ZnO nanoparticles using *Coleus zeylanicus* leaf extract. In this study, we aim to fill this research gap by presenting a green synthesis method for ZnO nanoparticles using *C. zeylanicus* leaf extract. The synthesized nanoparticles will be characterized using UV-Visible spectrophotometry, Fourier transform infrared spectroscopy, X-ray diffractometry, and transmission electron microscopy. Furthermore, we will evaluate the antioxidant and antimicrobial properties of the nanoparticles. The plant material Coleus zeylanicus is so far not explored for nanoparticle synthesis. Green synthesis from medicinal plants is very significant in aspect of economy and eco-friendly method. This is rapid and single step process without much effort. It can be safely used in human therapeutic studies as already these are proved medicinal plants.

MATERIAL AND METHODS

Plant collection and identification

The plant material Coleus is collected from Palakkad district of Kerala. It is a genus belonging to Lamiaceae family. Fresh plants were collected from the areas in sterile polypropylene covers. Using Flora of Madras presidency, the material was properly identified in Kerala Forest Research Institute, Peechi, Thrissur, Kerala. The voucher specimen is labelled and kept in research lab .

Preparation of plant extract

The fresh leaves of *Coleus zeylanicus* (Benth.) L.H. Cramer were collected and processed to obtain the plant extract. The leaves were thoroughly washed with tap water and distilled water to remove any impurities. After air-drying at room temperature, the leaves were sliced into small pieces. A total of 100 grams of these plant parts were boiled with 500 mL of distilled water at 60°C for one hour. The resulting mixture was then cooled, centrifuged at 5000 rpm for 10 minutes, and the supernatant was collected and stored in dark bottles. [15]

Synthesis of Zinc oxide nanoparticles

A 10 millimolar solution of Zinc nitrate hexahydrate was prepared by dissolving 2.975 grams of the chemical in 1000 mL of distilled water. In a 1:9 ratio, 10 mL of the prepared plant extract was added dropwise to 90 mL of the 10 millimolar zinc nitrate hexahydrate solution. The initial pH of the mixture was noted (pH 5) and the solution was subjected to a water bath at 70°C for 30 minutes. The pH was then adjusted to 11 by adding NaOH and further heated in a water bath for another 30 minutes. This resulted in the formation of a powdery ash precipitate. The solution was allowed to cool, centrifuged at 1000 rpm for 10 minutes, and the pellets were collected. The pellets were subjected to repeated centrifugation, suspended in distilled water, and collected. The collected pellets were dried in a hot air oven at 80°C for one day, and the powder was obtained. The powder was then transferred to a crucible, calcinated at 300°C for 3 hours, and preserved for further studies.

Characterization of nanoparticles

Visual observation

For the primary detection of nanoparticle synthesis visual observation was carried out. The colour change of the solution after synthesis indicated the formation of nanoparticles, as the plant extract acted as a reducing agent to convert the metal ions in the precursor solution into nanoparticles.

UV-Vis spectrophotometry

The optical properties of the synthesized zinc oxide nanoparticles were characterized using UV-Visible spectroscopy (T70/T80 series UV/Vis Spectrophotometer, PG Instruments Ltd, U.K.). The absorption spectra were recorded in the wavelength range of 200-800 nanometers.

X-ray diffraction (XRD)

X-ray diffraction analysis was performed using a Bruker D8 Advance X-ray diffractometer to determine the particle size, crystal structure, and purity of the synthesized zinc oxide nanoparticles. The analysis was carried out at Sophisticated Test and Instrumentation Center, Cochin University of Science and Technology, Kerala, India.

Fourier-transform infrared (FTIR) spectroscopy

Surface chemistry was detected using FTIR. Fourier-transform infrared spectroscopy was conducted using a Thermo Nicolet iS50 spectrometer to identify the functional groups involved in the synthesis of zinc oxide nanoparticles. The analysis covered the wave number range of 4000 cm^{-1} to 100 cm^{-1} and was performed at Sophisticated Test and Instrumentation Center, Cochin University of Science and Technology, Kerala, India.

Transmission electron microscopy (TEM)

Transmission electron microscopy was employed to investigate the particle size distribution, size, and surface morphology of the synthesized zinc oxide nanoparticles. TEM imaging was carried out using a Jeol/JEM 2100 instrument at Sophisticated Test and Instrumentation Center, Cochin University of Science and Technology, Kerala, India.

In-vitro antioxidant assay

The free radical scavenging potential of the zinc oxide nanoparticles was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Solutions of different concentrations of zinc oxide nanoparticles were prepared and incubated with the DPPH solution. The absorbance was measured at 517 nm, and the scavenging activity was calculated using the formula provided in the previous section.

Antimicrobial activity

Microorganisms

The antibacterial and antifungal activity of the synthesized zinc oxide nanoparticles were evaluated against selected microbial strains obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. The strains used in the study are listed in Table 1.

Disc diffusion assay

The disc diffusion method was employed to assess the antimicrobial activity of the zinc oxide nanoparticles. The nanoparticles were impregnated on sterile discs and placed on Mueller-Hinton Agar (MHA) plates for bacteria and Sabouraud dextrose agar (SDA) plates for fungi. Streptomycin discs were used as a standard reference. The plates were incubated, and the diameter of the zone of inhibition was measured to determine the antimicrobial activity. [16]

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the zinc oxide nanoparticles was determined using the broth microdilution method. Different concentrations of the nanoparticles were prepared from 0.1 to 100 microgram and added to nutrient broth and Sabouraud dextrose broth for bacteria and fungi, respectively. The microtiter plates were incubated, and the minimum inhibitory concentration was determined as the lowest concentration of nanoparticles that inhibited visible growth of the microorganisms.[17]

RESULT AND DISCUSSION

Plant identification

The collected plant materials is identified as *Coleus zeylanicus* and authenticated from Kerala Forest Research Institute (KFRI) (fig 1) .

Synthesis of nanoparticles

A total of 5 gm powder of nanoparticles were synthesised at 70^{o}C from freshly collected leaf extract. The calcinated powder was stored at room temperature for further studies and analysis.

Visual observation

The visual color change is the preliminary test for nanoparticle synthesis. The formation of an ash- to whitish colored precipitate represents the formation of zinc oxide nanoparticles, and further confirmations were done.

UV–vis spectrophotometry

UV-Visible spectroscopy is an available technique that allows the identification of nanoparticles in a faster and simpler way. Spectra of UV–Vis spectroscopy displayed a peak absorption correlated with the surface plasmon resonance (SPR) and collects conduction band electrons oscillations in reacting with electromagnetic waves, representing metal ion reduction and nanoparticle formation. The UV-Visible spectroscopy analysis was done at a range of 200-800 nanometers at a bandwidth of 1 nanometer. The spectrum showed the absorbance peak at 380 nanometers. The high-intensity peaks for zinc and oxygen justify that the sample contains mainly ZnO. As a rule, the absorption peak maximum for zinc oxide nanoparticles ranges between 300-380 nanometers [6]. Figure 2 represents the UV-VIS graph of ZnO NPs.

X-ray diffraction (XRD) analysis of ZnO NPs

X-Ray diffraction analysis of biosynthesized zinc oxide nanoparticles using *C. zeylanicus* leaf extract as a reducing agent is explained in Figure 3. The purity, phase identification, and structure of bio-assisted zinc

oxide nanoparticles were predicted by XRD analysis. Through the analysis, the crystalline nature and purity of the biosynthesized nanoparticles were confirmed. According to the PDF2 01-070-0870 matched XRD pattern of synthesized zinc oxide nanoparticles confirms their hexagonal structure. The X-Ray diffraction pattern shows 2θ values at 31.85°, 34.51°, 36.27°, 47.65°, 56.69°, 63.04°, 68.05°, and 69.17° corresponding to the (100), (002), (101), (102), (110), (103), (200), (112) planes of the crystal lattice, respectively. The absence of a secondary phase and impurities suggests the purest form of the sample [9].

Fourier transform infrared (FT-IR) spectroscopy of ZnO NPs

FT-IR was performed to reveal the composition of synthesized ZnO NPs. The FT-IR response was performed through the wave number range from 500-4000 cm^{-1}. In short, the IR spectra are divided into three wave number regions: Far-IR spectra (<400 cm^{-1}), Mid IR spectra (400-4000 cm^{-1}), and Near IR spectra (4000-13000 cm^{-1}). The mid-IR spectra are further divided into four regions: the single bond region (2500-4000 cm^{-1}), the triple bond region (2000-2500 cm-1), the double bond region (1500-2000 cm^{-1}), and the fingerprint region (600-1500 cm-1). The broad peak at 3414.11 cm^{-1} was attributed to the H-bonded OH stretch in alcohol, phenol, or water molecules present in the plant extract. The absorption peak at 2341.26 cm^{-1} was attributed to amino-related compounds, i.e., N-H compounds. The band around 1520 cm^{-1} was assigned to the aromatic nitro compounds, the stretching vibrations of C=N. The peak around 1410.43 cm^{-1} is assigned to carbon-related compounds, representing the symmetric stretching of C-N. The peak around 1329.47 cm^{-1} is attributed to the aromatic primary amine. The peak at 1045.96 cm^{-1} is assigned to the aliphatic fluoro compounds, C-F stretch. The peak at 917.38 cm^{-1} indicates the presence of silicate ions. The broad peak at 428.72 cm^{-1} shows the existence of zinc oxide nanoparticles [19] .(fig 4)

TEM imaging of the ZnO NPs

The TEM was used to investigate the ZnO NPs size and surface morphology. The TEM image characterizing the surface morphology of ZnO NPs is presented in Figure 5. A key factor in regulating the physiochemical characteristics of nanoparticles is their surface morphology. TEM analysis of biosynthesized ZnO NPs shows an average size of 58.49 nm. It can be noticed that most ZnO NPs are roughly triangles in morphology [9].

In vitro antioxidant assay

The *in vitro* antioxidant assay of ZnO NPs was assessed using the DPPH method, which is dominantly used due to its abundant free radical. DPPH is a stable synthetic free radical that is easily reduced by antioxidants, either by accepting or donating electrons. (Fig 6) The extent of discoloration shows the antioxidant ability to scavenge free radicals, which is observed as a decrease in the absorbance intensity of DPPH. The DPPH assay is simple and a high-potential method for scavenging free radicals during the examination of the antioxidant activity of various nanoparticles. Figures 8 and 9 represent the results of the radical scavenging activity of the test of nanoparticles in comparison with the standard ascorbic acid. There was a significant increase in free radical scavenging activity observed with an increase in the concentration of zinc oxide nanoparticles (Table 2). The same result was repeated in the case of the standard, i.e., ascorbic acid . Higher radical scavenging activity is achieved by ZnO NPs due to their improved ability to donate hydrogen ions to DPPH free radicals [12] [20].

Disc diffusion assay

In the present study, biosynthesized ZnO NPs were analyzed against human pathogenic strains, which include bacterial and fungal strains, on Muller Hinton Agar (MHA). Streptomycin was used as a standard disc against all selected microbial strains. However, the control or standard disc showed a higher zone of inhibition. The diameter of the zone of inhibition varied for the selected microbes. Among the selected microbes, the zinc oxide nanoparticles exhibited higher activity against the fungal strain *Candida albicans (MTCC 183)*. The diameter of the zone of inhibition of *Candida albicans* was 9 mm, and that of the standard disc was 20 mm. Among the bacterial strains, *Staphylococcus aureus (MTCC 96)*, *Enterococcus faecalis (MTCC 439)*, *Proteus vulgaris (MTCC 426)*, and *Serratia marcescens (MTCC 97)* showed higher antibacterial activity. The zone of inhibition was 8 mm for all. Their standard disc showed a zone of inhibition of 15 mm, 15 mm, 25 mm, and 20 mm for *Staphylococcus aureus (MTCC 96)*, *Enterococcus faecalis (MTCC 439)*, *Proteus vulgaris (MTCC 426)*, and *Serratia marcescens (MTCC 97)*, respectively. *Escherichia coli (MTCC 452)* showed the least zone of inhibition. The diameter of the zone of inhibition was 6 mm, and the standard showed a diameter of the zone of inhibition of 20 mm. (Fig 7)The disc diffusion assay was done using the lowest concentration of zinc oxide nanoparticles, which provided a significant result. The antimicrobial property of zinc oxide nanoparticles was concentration-dependent. If more concentrations of the nanoparticles are present, the inhibitory zone will be more effective. Thus, the diameter of the zone of inhibition will increase with an increase in the concentration of the zinc oxide nanoparticles [9], [20], [18].

Determination of minimum inhibitory concentration

ZnO NPs minimum inhibitory concentration was determined by the microdilution method. A series of different concentrations (1 µg/mL, 10 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL, 400 µg/mL, 600 µg/mL, and 800 µg/mL) were performed. All the studied antimicrobial strains were sensitive to ZnO NPs. 1 µg and 10 µg were the MIC values for the gram-positive bacterial strains *Staphylococcus aureus (MTCC 96)* and *Enterococcus faecalis (MTCC 439)*, respectively. 10 µg, 1 µg, and 10 µg were the MIC values of gram-negative strains *Proteus vulgaris (MTCC 426)*, *Serratia marcescens(MTCC 97)*, and *Escherichia coli (MTCC 452),* respectively. The fungal strain *Candida albicans (MTCC 183)* has the MIC value of 10 µg.(Fig 8). The antibacterial and biocompatibility properties of zinc oxide nanoparticles have been well reported. They possess potent bactericidal properties against several gram-positive and gram-negative bacteria, and they are not harmful to human cells. In the present study, both gram-positive and gram-negative microbes were analyzed. Gram-positive microbes have a rigid cell wall network that makes them resistant to rupture, whereas gram-negative bacteria have a network of cell membranes, made up of one molecule thick. However, it is not yet known how these compounds exert their antimicrobial effect. The antibacterial activity is due to the presence of carboxy groups and amines on their cell surface, which offers a higher affinity for zinc ions to bind and results in antimicrobial activity [21]. The accumulation of ROS is the fundamental reason for the antimicrobial activity [22]. Nanoparticles have antibacterial activity due to a chemical interaction between membrane proteins and hydrogen peroxide or other chemical agents generated in the presence of nanoparticles by the bacterial cell's lipid bilayer[9], [20], [18], [23].

Table 1. The test organisms used for Antimicrobial activity studies

Concentration (Ascorbic acid)	Absorbance of control	Absorbance of sample	Radical scavenging percentage (%)
1 mg/mL	1.882	0.052	97%
2 mg/mL	1.882	0.040	97%
2.5 mg/mL	1.882	0.030	98%

Table 2.The antioxidant activity

S/N	Microorganism	Culture name	Gram stain
1.	*Serratia marcescens*	MTCC 97	Gram -ve
2.	*Proteus vulgaris*	MTCC 426	Gram -ve
3.	*Escherichia coli*	MTCC 452	Gram -ve
4.	*Staphylococcus aureus*	MTCC 96	Gram +ve
5.	*Enterococcus faecalis*	MTCC 439	Gram +ve
6.	*Candida albicans*	MTCC 183	Gram +ve

Fig 1. Habit of *Coleus zeylanicus (Benth.) L.H. Cramer*

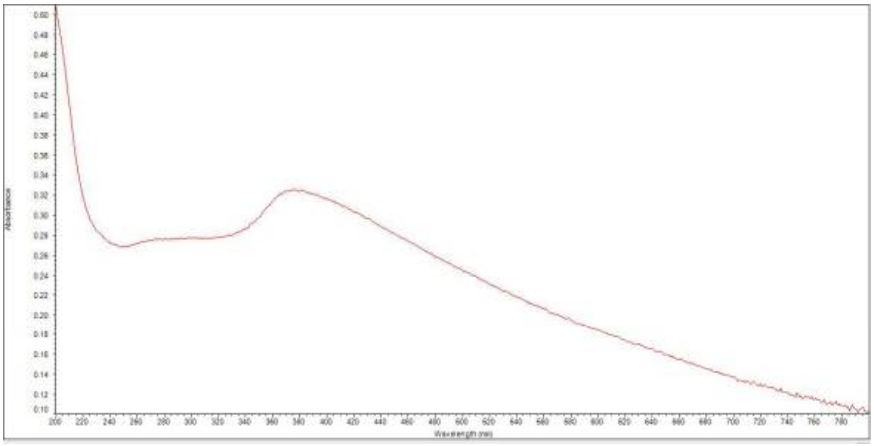


Fig 2. UV-Vis graph of ZnO NPs showing a peak at 380nm

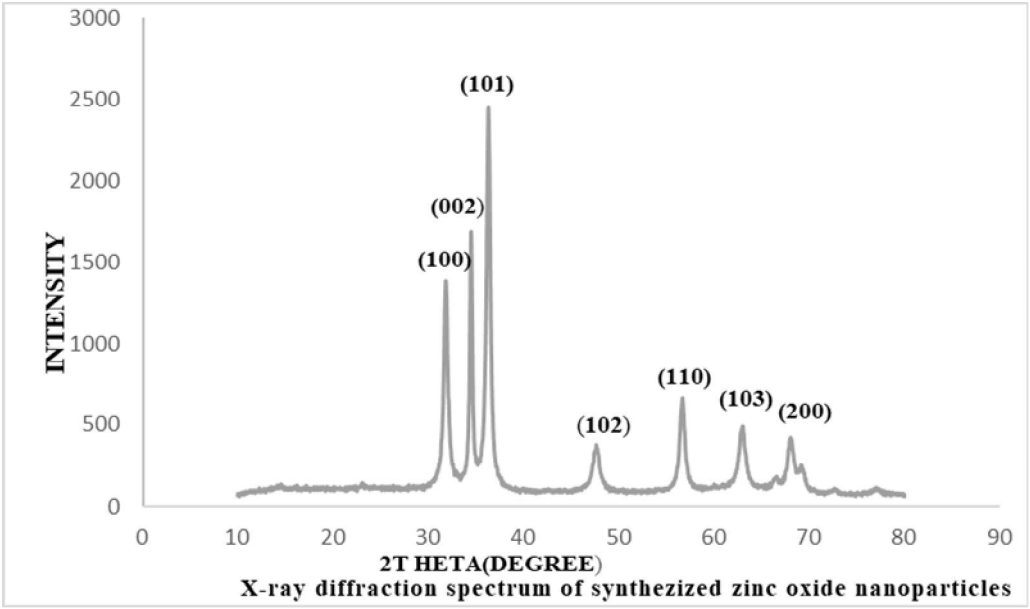


Fig3. Shows the XRD spectrum of ZnO NPs

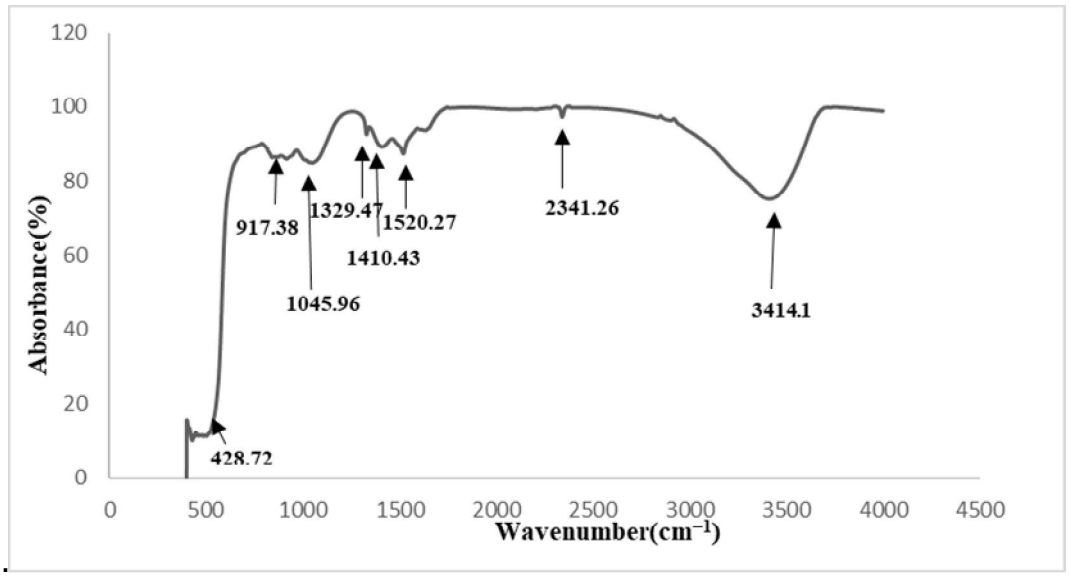


Fig 4. Shows the FTIR spectra of ZnO NPs

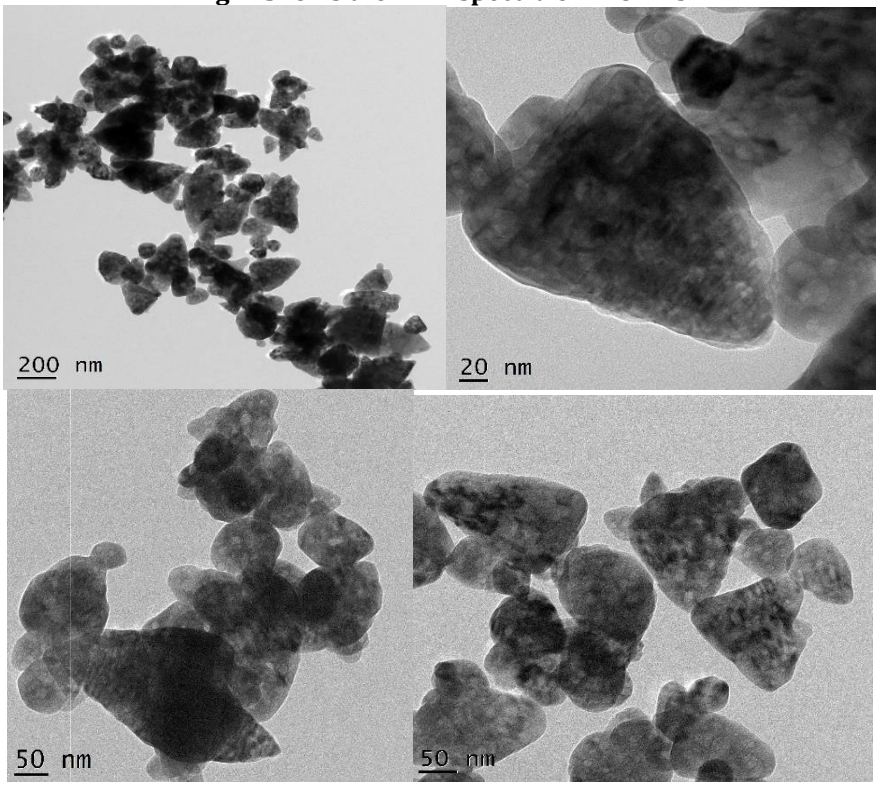


Fig5. TEM image of ZnO NPs

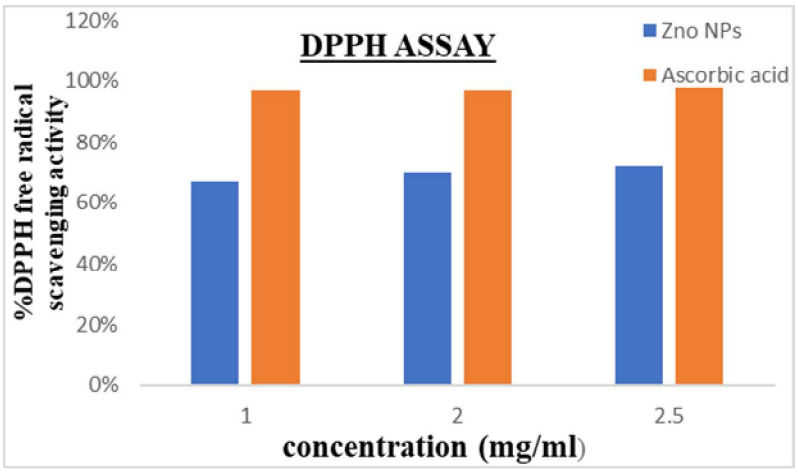
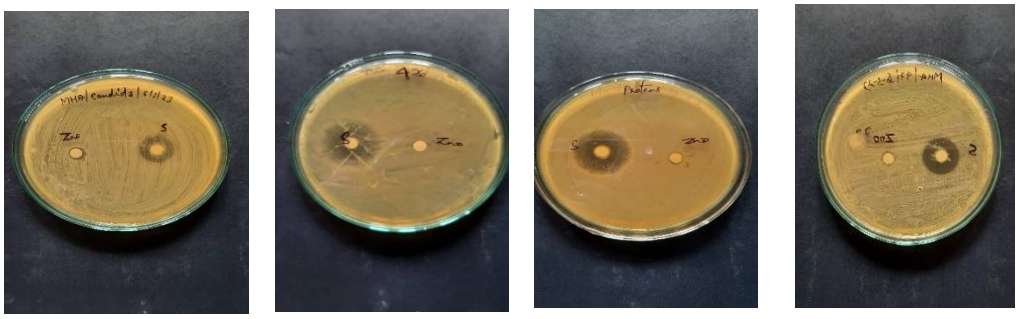


Figure 6: antioxidant activity of the synthesised nanoparticles



1. Candida albicans 2. Proteus vulgaris 3. Serratia marcescens 4. Escherichia coli

Fig 7: Evaluation of antimicrobial activity of biosynthesized ZnO-NPs

1. Candida albicans 2. Proteus vulgaris 3. Serratia marcescens 4. Escherichia coli

Figure 8: Minimum inhibitory Concentration

CONCLUSION

In conclusion, this study successfully synthesized zinc oxide nanoparticles using a green approach, utilizing the leaf extract of *Coleus zeylanicus* (Benth.) L.H. Cramer as a reducing agent. The characterization of the synthesized nanoparticles was performed through various techniques, including visual observation, UV-visible spectroscopy, X-ray diffraction (XRD) analysis, Fourier transform infrared (FT-IR) spectroscopy, and TEM imaging. The results confirmed the formation of crystalline zinc oxide nanoparticles with a hexagonal structure and an average size of 58.49 nm.

The synthesized zinc oxide nanoparticles exhibited promising antimicrobial activity against both bacterial and fungal strains. They showed significant inhibition zones against *Candida albicans*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus vulgaris*, and *Serratia marcescens*. The antimicrobial activity was concentration-dependent, with higher concentrations of nanoparticles resulting in larger inhibition zones. Additionally, the zinc oxide nanoparticles demonstrated considerable antioxidant potential, as indicated by their ability to scavenge free radicals in the DPPH assay. The percentage inhibition increased

with increasing concentrations of the nanoparticles, showcasing their effective free radical scavenging activity.

The findings of this study highlight the potential of biosynthesized zinc oxide nanoparticles as antimicrobial and antioxidant agents. The green synthesis approach offers several advantages over conventional physical and chemical methods, including simplicity, cost-effectiveness, and eco-friendliness. Further research should focus on exploring the biomedical applications of these nanoparticles, such as their anticancer and antidiabetic properties.

Overall, this study contributes to the growing field of nanotechnology and emphasizes the importance of utilizing green synthesis methods for the development of functional nanoparticles with potential applications in medicine and healthcare.

Conflict of interest

There is no conflict of interest from authors

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