

Research

Green synthesis, characterization and antimicrobial activity of ZnO and Co-doped ZnO nanoparticles obtained using aqueous extracts of *Platycladus orientalis* leaves

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Abstract

This study investigates the synthesis, structural characteristics, and antimicrobial applications of zinc oxide (ZnO) and cobalt-doped ZnO (Co-doped ZnO) nanoparticles synthesized via a green method utilizing *Platycladus orientalis* aqueous extract. Comprehensive characterization techniques, including x-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), UV–vis spectroscopy, and Fourier transformed infra-red (FTIR) spectroscopy, were employed to evaluate the structural, morphological, and optical properties. XRD analysis confirmed the hexagonal wurtzite structure of ZnO with successful Co-doping, evidenced by lattice distortion and reduced crystallite size. The estimated crystallite size and lattice microstrain of the ZnO and Co-doped ZnO nanoparticles were 26.9 and 19.0 nm, and 0.16% and 0.2%, respectively. Furthermore, the lattice parameters showed a significant distortion of the lattice along the c-axis in the Co-doped ZnO nanoparticles. SEM and TEM revealed improved particle uniformity and decreased agglomeration in Co-doped ZnO nanoparticles. UV–Vis spectroscopy demonstrated a narrowing of the optical band gap, enhancing visible light absorption. Antimicrobial studies showed significant concentration-dependent activity, with Co-doped ZnO nanoparticles exhibiting superior antibacterial and antifungal properties compared to pristine ZnO. Co-doped ZnO demonstrated enhanced inhibition zones against *Listeria monocytogenes* (13.50 mm), *Escherichia coli* (13.65 mm), and *Enterococcus faecalis* (14.05 mm), and also showed better minimum inhibitory concentrations (MICs) against fungal strains such as *Mucor mucedo* (0.05 mg/mL), *Penicillium chrysogenum* (0.05 mg/mL), and *Aspergillus niger* (0.03 mg/mL). The superior antimicrobial performance is attributed to modifications in particle size, morphology, and lattice defects induced by cobalt doping. These findings highlight the potential of Co-doped ZnO nanoparticles as advanced antimicrobial agents, suitable for applications in biomedical fields.

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1 Introduction

Antimicrobial resistance is a serious global health threat that has necessitated the exploration of novel approaches. Nanoparticles with antimicrobial activity have emerged as a promising strategy, with their unique ability to generate hydroxyl radicals and superoxide ions, with high efficiency in destroying microbial cells [1]. Nanoparticles are increasingly being explored to control microbial infections as alternatives to antibiotics. They are currently being used as coatings for implantable devices, infection prevention, detection and diagnostic purposes and in delivery systems [2]. The mechanism of action of nanoparticles is poorly understood, however, metal ion release oxidative stress induction and non-oxidative mechanisms are widely proposed. Furthermore, the ability of the microbes to develop resistance to nanoparticles is limited as it would require multiple simultaneous gene mutations in the microbe cells to outwit the multiple simultaneous mechanism actions of nanoparticles [3]. Nanoparticles possess the intrinsic ability to penetrate cell membrane barriers and reach target sites with a higher level of accuracy compared to traditional antibiotics [1]. To further enhance antimicrobial activity, nanoparticles can be engineered to incorporate different components or surface functionalities.

In the past few decades, metal oxides nanoparticles have emerged as a promising antimicrobial agent against broad spectrum of micro-organisms [4]. The excellent activity of this class of material is often attributed to their high surface area-to-volume ratio. Modification of this characteristic often result in materials with unique chemical, mechanical, electrical and optical properties [5]. These materials offer a high therapeutic activity, drug release control, and lower microbial resistance [6]. Several metal oxide nanoparticles such as AgO, CuO, ZnO, MgO have been explore for their activity against different classes of microbes. Furthermore, in the quest for improved activity, metal ion doping has been observed to significantly alter the physicochemical properties of metal oxide nanoparticles, which alters their antimicrobial activity.

Due to stoichiometric deviation and the presence of intrinsic defects such as vacancies, ZnO exhibit a n-type semi-conductivity [7]. ZnO doping often aims to enhance its optical, structural and electrical properties, which often leads to improved activity in numerous applications [8–11]. Doping of zinc with transition metals often changes its crystal size and morphology. Several reports have shown that transition metal doping often results in materials with smaller crystallite sizes and enhanced surface area [12]. Furthermore, metal doping modifies the crystal lattice and alters the electronic band structure, while introducing several crystal defects in the ZnO structure. In addition to the improved antimicrobial activity offered by doped metal oxides, the facile synthesis offered by green synthesis have made them desirable materials for biological activities.

Cobalt is often considered as an effective doping specie because of its abundant electronic states compared to other transition metals such as iron and nickel. Cobalt is also well suited for electronic state modification by substituting Co^{2+} into the lattice of the host, without inducing significant distortion [13]. Furthermore, its ability to stabilize desired defects and increase the surface hydroxyl groups in metal oxides increases the functionality of the host material [14]. Green synthesis of nanoparticles offers numerous advantages over traditional methods such as environmental friendliness and sustainability and reduced toxicity, making them suitable for large-scale synthesis [15]. Green synthesis uses plant constituents such as enzymes, fats, flavonoids, alkaloids and polyphenols as reducing agents [16]. Additionally, they provide a facile route in tailoring nanoparticles to varying applications by modifying their morphology and surface properties [17, 18].

Platycladus orientalis (also known as Chinese Arborvitae) is an evergreen tree belonging to the Cupressaceae family commonly found in China, Japan and Korea. Its rich pharmacological properties make it significant in traditional medicine as an antioxidant, anti-inflammatory and antibacterial agent and application in hair-growth treatment [19, 20]. The presence of bioactive compounds such as tannins, flavonoids and polysaccharides in the leaves makes *Platycladus orientalis* a suitable candidate for green synthesis. Extracts of *Platycladus orientalis* have been reported for the synthesis of metallic nanoparticles such Pd NPs [21], and Ag NPs [22, 23]; however, the plant has not been extensively explored in the synthesis of metal oxide and doped metal oxide nanoparticles. Therefore, in this study, we report the synthesis of ZnO and Co-doped ZnO nanoparticles using the extract of *Platycladus orientalis* leaves. The nanoparticles were characterized through XRD, FTIR, UV–visible spectroscopy, SEM and TEM analysis. Furthermore, the influence of metal ion doping on the antibacterial and antifungal activity of the synthesized materials was explored against three bacterial and three fungal strains. The finding of this study contributes to the potential of metal doping in improving the antimicrobial activity of metal oxide nanoparticles obtained through green synthesis.

2 Methodology

2.1 Materials

Zinc sulphate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), cobalt nitrate hexahydrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), and sodium hydroxide (NaOH) were purchased from Sigma Aldrich, South Africa. Potato dextrose broth (PDB), Muller-Hinton Agar (MHA) and Muller-Hinton Broth (MHB) were supplied by Sisco research laboratories (Mumbai, India). All the chemicals were used as purchased without any further purification.

2.2 Preparation of aqueous extract of *Platyclusus orientalis* leaves

The *Platyclusus orientalis* leaves were harvested from Molelwane Experimental Farm of North-West University, Mahikeng, North-West Province, South Africa. The leaves were washed and dried after harvesting to remove dirt. Afterwards, 20 mL of the macerated leaves were weighed into a 200 mL beaker containing 100 mL of distilled water. The mixture was then stirred at 80 °C for 1 h and the leaves were filtered off to obtain the aqueous extract, which was stored for further use in a sealed container at 4 °C.

2.3 Synthesis of ZnO and Co-doped ZnO nanoparticles

The method reported earlier by Faisal, et al. [24] was employed in the synthesis of ZnO and Co-doped ZnO nanoparticles. In a typical synthesis, 5 g of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ was weighed into a beaker containing 10 mL of distilled water, followed by the addition of 20 mL of the extract. Dropwise addition of 1 M NaOH was used to adjust the pH of the mixture to 10 and it was subsequently heated at 80 °C for 2 h, yielding a white precipitate. The precipitate was recovered by centrifugation and washed with water/acetone mixture (50:50) to remove unreacted materials. The obtained precipitate was dried under vacuum overnight and the powder was calcined in a furnace at 400 °C for 3 h. For the synthesis of Co-doped ZnO nanoparticles, a similar method was used; however, 0.02 g of $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ was introduced alongside $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and the subsequent procedure followed that of ZnO nanoparticles. A schematic representation of the synthesis procedure is shown in Fig. 1.

2.4 Characterization of ZnO and Co-doped ZnO nanoparticles

The obtained nanoparticles were characterized for the morphological, chemical and optical properties using Fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and UV–Visible spectroscopy (UV-vis). The FTIR spectra was recorded on a spectroquant® 300 spectrophotometer in the wavenumber range of 400–5000 cm^{-1} . A D8 advance diffractometer (Bruker AXS, Germany) with a secondary graphite monochromated Cu K α radiation at 40 kV/50 mA ($\lambda = 1.54060 \text{ \AA}$) was used for investigating the

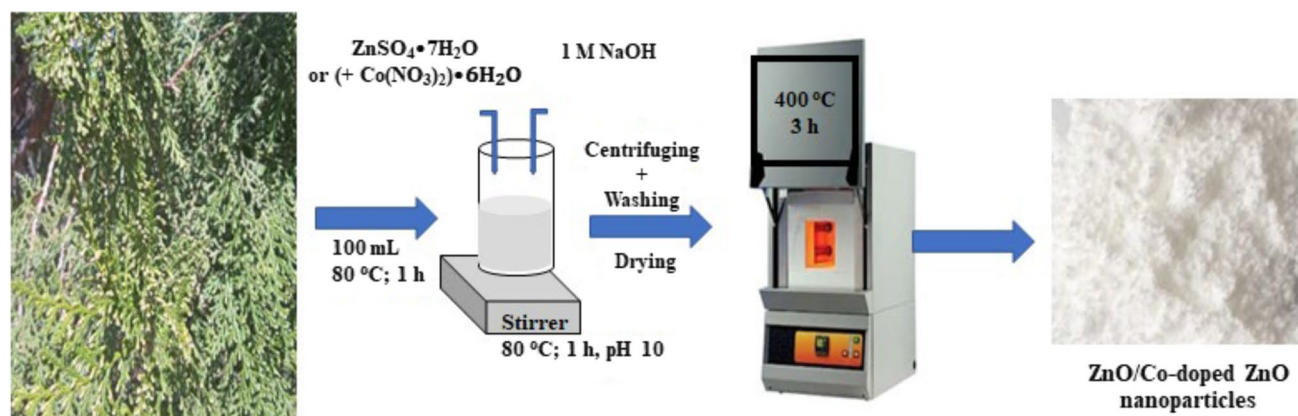


Fig. 1 Schematic representation of the synthesis of ZnO and Co-doped ZnO nanoparticles using aqueous extract of *Platyclusus orientalis* leaves

crystalline properties of the nanomaterial in the 2theta range of 20–70°. For SEM and TEM analysis the FE-SEM FEI 430 Nova NanoSEM system and the FEI Tecnai G2 Twin system was used respectively. For the UV–Vis analysis a spectroquant® Prove 300 spectrophotometer was used. For SEM, TEM and UV–Visible analysis methanol was used as solvent.

2.5 Antimicrobial evaluation

One Gram-negative bacterial strain *Escherichia coli* (ATCC 25922) and two Gram-positive strains *Enterococcus faecalis* (ATCC 29212) and *Listeria monocytogenes* (ATCC 7644) were used for evaluating the antibacterial activity of the nanoparticles. The bacterial strains were maintained and sub cultured on MHA at 37 °C, while colony suspension was in the MHB. For the fungal strains, *Mucor mucedo*, *Penicillium chrysogenum*, and *Aspergillus niger*, isolated from decayed strawberry fruit surfaces were used. The viable fungal strains were consistently isolated and identified using procedures described by López et al. [25] and Campbell, et al. [26].

2.6 Evaluation of antibacterial activity

The in vitro antibacterial activity of ZnO and Co-doped ZnO nanoparticles was evaluated using the agar well diffusion method described by Valgas et al. [27]. Furthermore, the inoculum of the bacterial strains was prepared through the direct suspension method. The suspended colonies were adjusted to the optical density of the 0.5 McFarland standard, subsequently, the density of the inoculum was adjusted to approximately 1.5×10^6 by preparing 1:100 dilutions of the MHB suspension. Afterwards, 100 µL of each inoculum was pipetted into the MHA plate in triplicates and evenly spread on the surface. A 6 mm hole was drilled in each agar plate using a sterile borer, and 80 µL of the nanoparticle solution with concentrations 6.4, 3.2, 1.6 and 0.8 mg/ml were pipetted into the wells and the plates were incubated under aerobic conditions for 24 h at 36 ± 1 °C. The positive and negative controls were made up of Neomycin (3.2 mg/mL) and distilled water, respectively. The analyses were done in triplicates using the zone of inhibition measured in mm.

2.7 Evaluation of antifungal activity

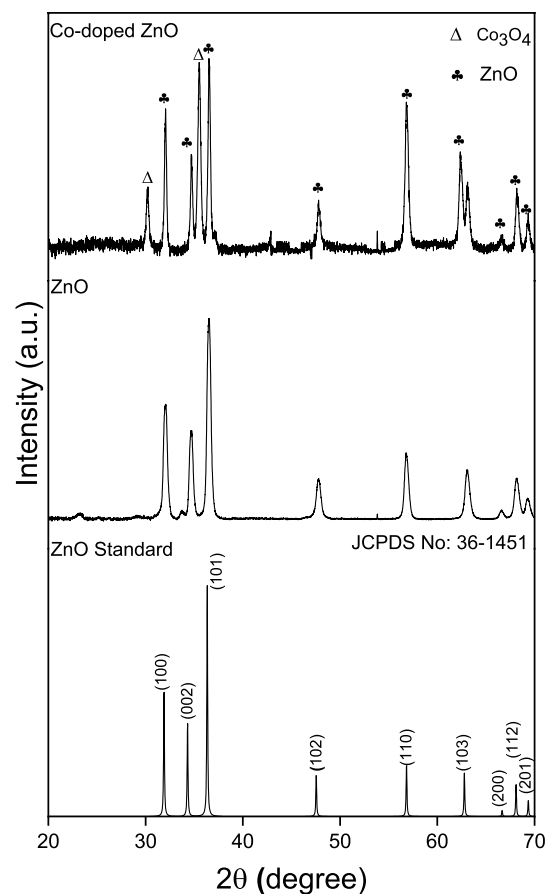
The broth microdilution method reported by Berkow et al. [28] was used in evaluating the antifungal properties activity of ZnO and Co-doped ZnO nanoparticles. The inoculum of *Mucor mucedo*, *Penicillium chrysogenum* and *Aspergillus niger* was prepared by incubating a 5 × 5 mm agar section from a 5-day-old fungal culture in McCartney bottles containing 15 mL of PDB with 0.1%(v/v) Tween 80 for 72 h. Afterwards, the cell suspensions were vortexed, and their concentration adjusted to the 0.5 McFarland standard equivalent to 1.5×10^6 CFU/mL. The working suspension was prepared by adjusting the inoculum concentration to 1.5×10^5 CFU/mL. Stock solutions of ZnO and Co-doped ZnO nanoparticles were prepared by dissolving the nanoparticles in PDB, followed by two-fold serial dilutions in 96 well plates to attain nanoparticle concentrations in the 3.2 to 0.0225 mg/mL range. The positive and sterile control plates contained amphotericin B (1.6 mg/mL) and PDB. Subsequently, each well was inoculated with 100 µL of the fungal suspension. The plates were sealed with paraffin and incubated at 37 °C for 48 h. The minimum inhibitory concentration (MIC) was estimated as the lowest nanoparticle concentration required for complete inhibition of fungal growth.

3 Results and discussion

3.1 Structural and morphological properties of ZnO and Co-doped ZnO nanoparticles

Figure 2 shows the XRD pattern of ZnO and Co-doped ZnO obtained using the aqueous extract of *Platyclus orientalis* leaf. The observed diffraction peaks in the diffraction pattern of ZnO agreed well with the hexagonal wurtzite ZnO structure with JCPDS card no 36–1451 [29]. The major peaks corresponding to (100), (002) and (101) planes of ZnO were observed at 2θ 32.0°, 34.6°, and 36.5°, respectively. Furthermore, the absence of extra peaks suggests pure ZnO nanoparticles were obtained. For Co-doped ZnO peaks, peaks due to the (220) and (311) planes of Co_3O_4 nanoparticles were

Fig. 2 XRD pattern of ZnO and Co-doped ZnO obtained using the aqueous extract of *Platyclusus orientalis* leaves



observed alongside the characteristic peaks of ZnO, which confirms the formation of solid solution in the Co-doped ZnO nanoparticles.

Using the Scherrer equation (Eq. 1), the crystallite size of the nanoparticles was calculated.

$$D = \frac{k\lambda}{\beta \cos \theta} \quad (1)$$

where k is a constant (0.94), λ is the wavelength of the X-ray, β is the full width at half maximum (FWHM) of the peak, and θ is the diffraction angle. The crystallite size for ZnO and Co-doped ZnO was estimated to be 26.9 and 19.0 nm, respectively. The presence of dopant atoms can influence the growth kinetics of nanoparticles, either by modifying the surface energy or providing alternative diffusion pathways. Furthermore, the presence of dopant atoms also leads to an increase in nucleation centres, resulting in smaller grains [30]. This could account for the reduction in the particle size of the Co-doped ZnO nanoparticles compared to the pristine ZnO NPs.

Furthermore, to understand the influence of Co-doping on the lattice strain of the nanoparticle, the induced lattice strain was estimated using Eq. 2

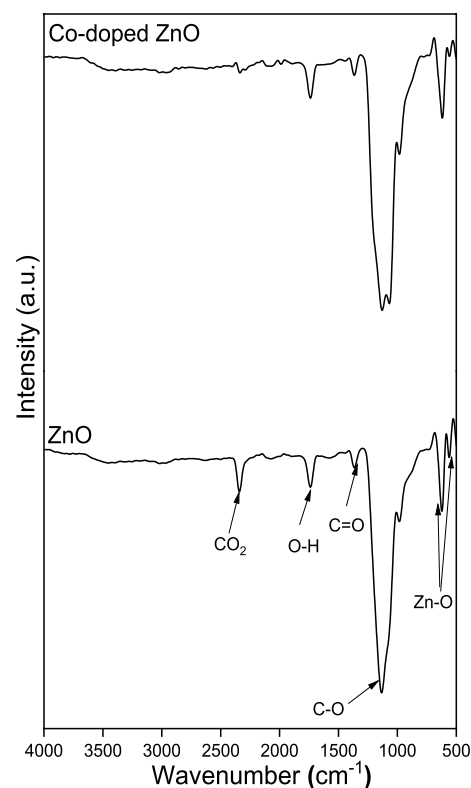
$$\epsilon = \frac{\beta}{4 \tan \theta} \quad (2)$$

The microstrain in ZnO and Co-doped ZnO nanoparticles was estimated to be 0.16 and 0.2% respectively. The introduction of dopants into a crystalline material can induce strain into the system, which alters the lattice periodicity and decreases crystal symmetry [31]. Also, the presence of dopant atoms can hinder the growth rate of nanoparticles leading to smaller crystallites and increased microstrain [32].

Metal ion doping has been reported to significantly influence the lattice parameters of crystals due to lattice distortion and structural changes caused by differences in charge, size and electronic properties between the dopant

Table 1 Lattice parameters of ZnO and Co-doped ZnO nanoparticles

	A (Å)	C (Å)	c/a (Å)	L (Å)	V (Å ³)	Δ (× 10 ⁻²) (nm ⁻²)
ZnO	3.226	5.708	1.769	2.03	51.44	5.89
Co doped-ZnO	3.225	4.916	1.524	1.93	44.27	3.63

Fig. 3 FTIR spectra of ZnO and Co-doped nanoparticles obtained from the aqueous extract of *Platyclusus orientalis* leaves

atoms and the host. To estimate the influence of Co-doping on the lattice parameters of Co-doped ZnO NPs, the lattice parameters were calculated from the XRD patterns using Eq. 3:

$$\frac{1}{d_{hkl}^2} = \frac{4}{3a^2} \left[\frac{h^2 + k^2 + hk}{l} \right] + \frac{l^2}{c^2} \quad (3)$$

where (hkl) are the Miller indices with interplanar spacing d , while a and c represent the lattice parameters. The lattice parameters (Table 1) a and c for the ZnO nanoparticles were close to the reported value for ZnO single crystal, which shows the high-quality crystalline material obtained. A significant change in the c -axis was observed in the calculated lattice parameter of Co-doped ZnO and this suggests the substitution of Zn^{2+} with $\text{Co}^{3+}/\text{Co}^{2+}$ ions. A similar change in the c -axis was reported for Er, Ce and Sm doped ZnO nanoparticles by Sagheer et al. [33]. The parameters a and c depend on several factors, such as impurity concentration, defects, difference in ionic radii of Zn^{2+} and dopant ion, and external strains arising from pressures and temperature. The change in lattice parameters could be associated with strain and lattice distortion induced in ZnO by the Co-doping.

Figure 3 shows the FTIR spectra of ZnO and Co-doped ZnO nanoparticle. The spectra of pristine ZnO showed the Zn–O stretching vibration peaks were observed at 555 and 620 cm^{-1} [34]. The peaks at 1738 cm^{-1} could be attributed to the O–H bending vibration of adsorbed water, while the peaks around 1130, 1375 and 2350 cm^{-1} , could be attributed to adsorbed CO_2 [35]. In the Co-doped ZnO, a shift in the Zn–O stretching vibration peaks to a higher wavenumber (563 and 612 cm^{-1}) was observed. This shift could be attributed to the introduction of dopant atoms in the crystal lattice of ZnO. The smaller ionic radius of cobalt could lead to a reduction in bond length, which resulted in the shift of the Zn–O

band to a higher wavenumber [36]. Furthermore, lattice strain, defects and oxygen vacancies occasioned by Co-doping could also account for the peak shifts [37]. The O–H and CO₂ peaks were also observed in Co-doped ZnO, however with reduced intensity.

3.2 Morphological properties of ZnO and Co-doped ZnO nanoparticles

Figure 4a–d shows the surface morphology of ZnO and Co-doped ZnO obtained from the SEM and TEM micrograph. The SEM image of ZnO nanoparticles exhibited irregularly shaped particles with closely packed particles, suggesting a high degree of agglomeration arising from their nanoscale size and surface energy. For Co-doped ZnO, the SEM showed less aggregated morphology, suggesting Co-doping influenced the growth dynamics leading to a change in the surface characteristic of the material. The TEM image of ZnO nanoparticle (Fig. 4c) shows porous and clustered structures, with irregular shapes as suggested by the SEM image. This shows the ZnO nanoparticles were polycrystalline and aggregated. Furthermore, the Co-doped ZnO nanoparticles possessed more uniform, spherical nanoparticles with fewer agglomerations compared to pure ZnO. This change in morphology suggests that Co-doping modified particle formation during the synthesis. The energy dispersive spectroscopy (EDS) spectra of ZnO nanoparticles (Fig. 4c) confirms the elemental composition of ZnO, predominantly showing peaks for Zn and O. However, traces of Na, K and Ca were observed, which might be from the plant extract. The presence of Co in the EDX spectra of Co-doped ZnO confirms the doping of the ZnO nanoparticles.

3.3 Optical properties

The absorption spectra and Tauc plot of ZnO and Co-doped ZnO are presented in Fig. 5. Figure 5a shows that ZnO exhibited significant absorption in the ultraviolet region, which is typical of wide band gap semiconductors. For Co-doped

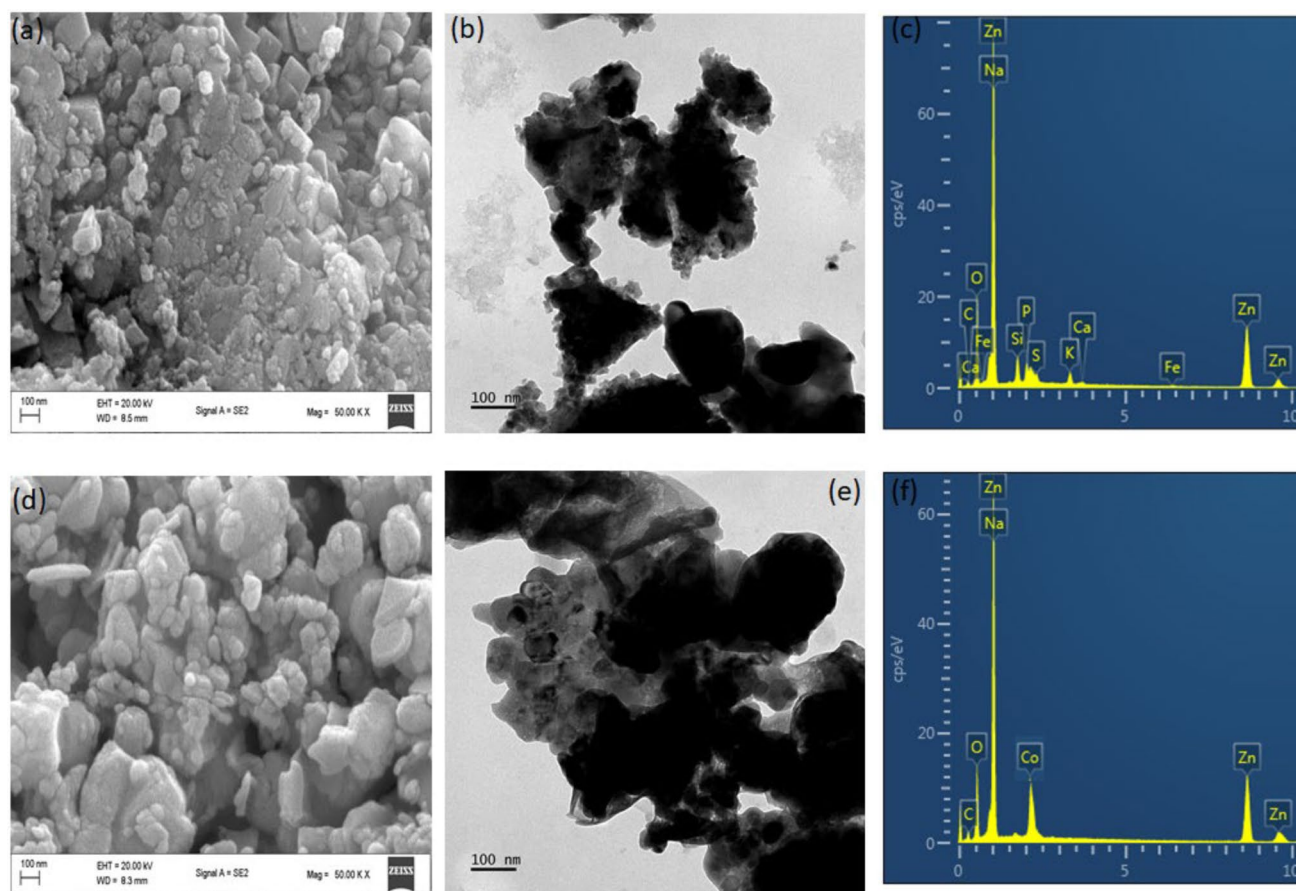


Fig. 4 **a–c** SEM, TEM and EDS spectra of ZnO nanoparticles, **d–f** SEM, TEM and EDS spectra of Co-doped ZnO

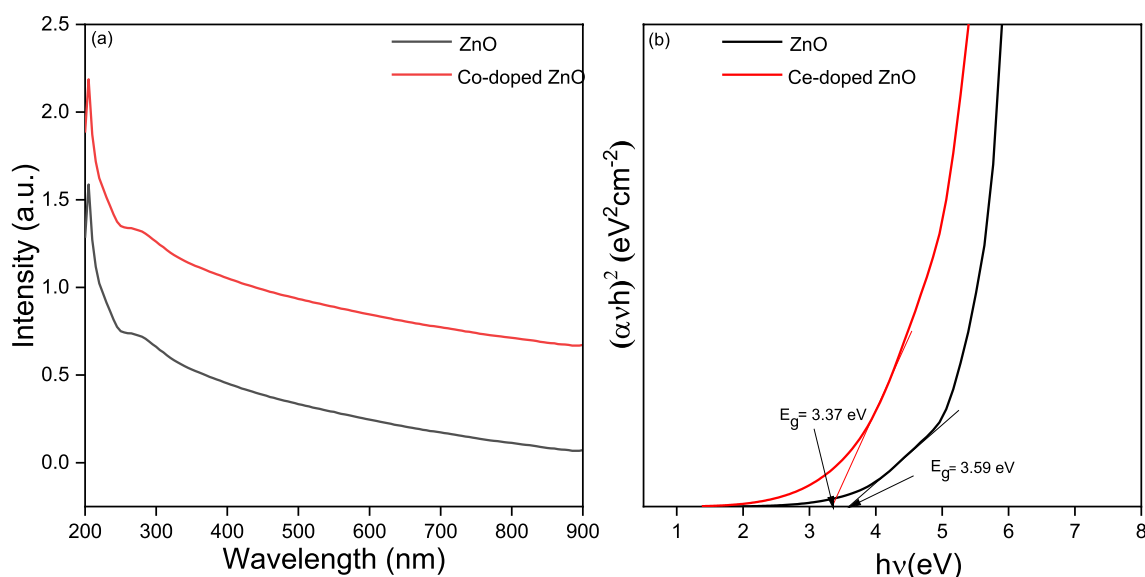


Fig. 5 Absorption spectra (a) and Tauc plots (b) of ZnO and Co-doped ZnO nanoparticles obtained from the aqueous extract of *Platycladus orientalis* leaves

ZnO, the absorption is slightly enhanced, which is attributed to changes in the electronic structure due to Co-doping. The wavelength of maximum absorption (λ_{max}) was also observed to move to a higher wavelength in Co-doped ZnO (206.6 nm) compared to ZnO (205.7 nm). This suggests a reduction in the band gap energy arising from Co-doping. Figure 5b shows the Tauc plot obtained from the absorption spectra by plotting $(\alpha h\nu)^2$ against $h\nu$ (photon energy). The optical bandgap is determined by extrapolating the linear portion of the curve to intersect the X-axis. The estimated band gap energy was 3.59 and 3.37 eV for ZnO and Co-doped ZnO, respectively. The lowering of the bandgap energy could be a result of additional electronic states introduced by Co within the band gap of ZnO.

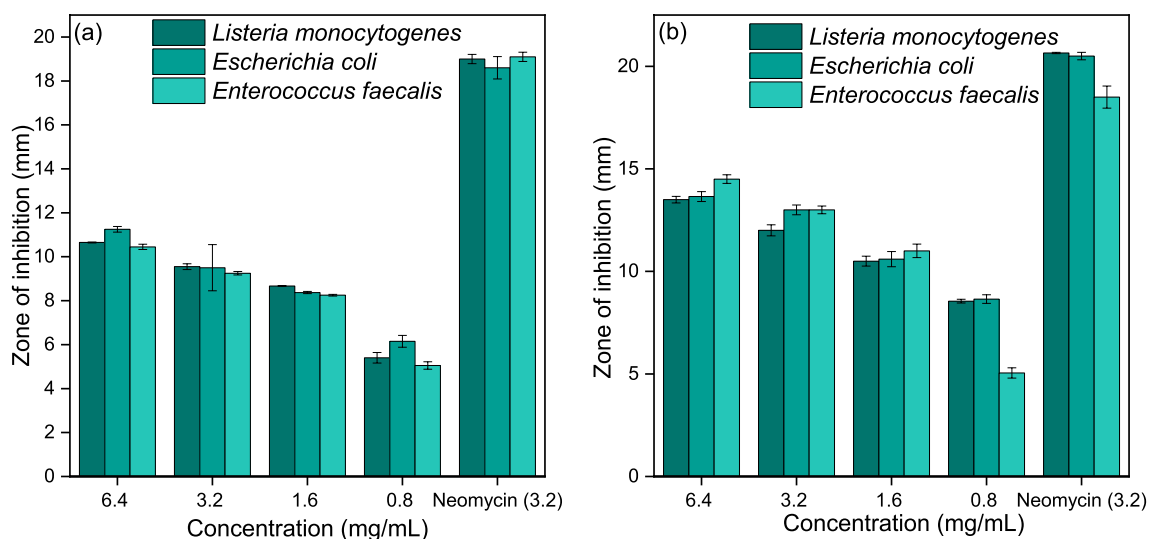


Fig. 6 Zone of inhibition of (a) ZnO and (b) Co-doped ZnO against *Listeria monocytogenes*, *Escherichia coli* and *Enterococcus faecalis*

Table 2 Effect of the concentration of ZnO and Co-doped ZnO NPs zone of inhibition on the growth of some pathogenic bacteria

	<i>Listeria monocytogenes</i>	<i>Escherichia coli</i>	<i>Enterococcus faecalis</i>
ZnO NPs			
6.4 mg/mL	10.65 ± 0.02 ^b	11.25 ± 0.13 ^b	10.45 ± 0.12 ^b
3.2 mg/mL	9.55 ± 0.13 ^c	9.50 ± 1.05 ^c	9.25 ± 0.08 ^c
1.6 mg/mL	8.67 ± 0.02 ^c	8.37 ± 0.05 ^d	8.25 ± 0.04 ^c
0.8 mg/mL	5.40 ± 0.24 ^d	6.15 ± 0.27 ^e	5.05 ± 0.17 ^d
Neomycin (3.2 mg/mL)	19.00 ± 0.21 ^a	18.60 ± 0.51 ^a	19.10 ± 0.21 ^a
Co-doped ZnO NPs			
6.4 mg/mL	13.50 ± 0.16 ^b	13.65 ± 0.24 ^b	14.05 ± 0.21 ^b
3.2 mg/mL	12.00 ± 0.27 ^b	13.00 ± 0.24 ^b	13.05 ± 0.19 ^b
1.6 mg/mL	10.50 ± 0.24 ^d	10.60 ± 0.37 ^c	11.20 ± 0.33 ^c
0.8 mg/mL	8.55 ± 0.09 ^d	8.65 ± 0.21 ^d	9.05 ± 0.25 ^d
Neomycin (3.2 mg/mL)	20.65 ± 0.03 ^a	20.50 ± 0.18 ^a	21.00 ± 0.54 ^a

^{a,b,c,d,e} Columns means with different superscripts for each organism between the different concentrations are significantly ($P < 0.05$) different ($n = 3$)

3.4 Antimicrobial studies

3.4.1 Antibacterial activity

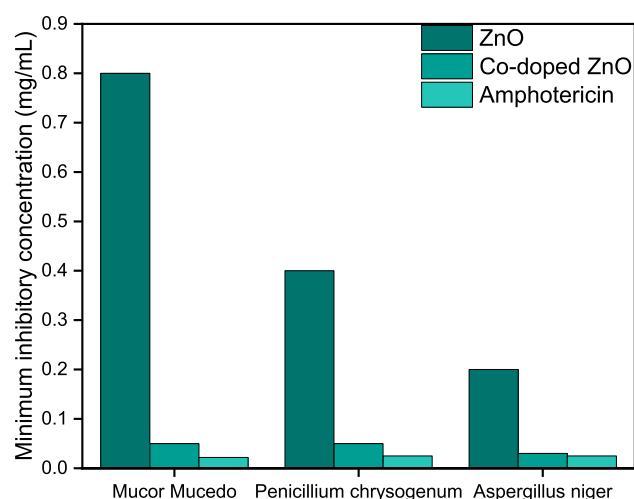
The antibacterial activities of ZnO and Co-doped ZnO nanoparticles were tested against *Listeria monocytogenes*, *Escherichia coli* and *Enterococcus faecalis*. As shown in Fig. 6a and b and Table 2, both ZnO and Co-doped ZnO exhibited significant, concentration-dependent antibacterial activity on the three bacterial species. For pristine ZnO, the highest antibacterial activity was observed for *Escherichia coli*, while *Listeria monocytogenes* was least impacted. However, in Co-doped ZnO nanoparticles, the highest activity was observed for *Enterococcus faecalis*, while *Listeria monocytogenes* was still the least impacted. This suggests a modification in the antibacterial activity of the Co-doped ZnO, which could be attributed to the changes in size and surface properties induced by Co-doping. Furthermore, the Co-doped ZnO nanoparticles exhibited higher activity compared to the pristine ZnO, which was attributed to Co-doping. Generally, the antibacterial activity of nanoparticles is mediated by the interaction between the nanoparticles and bacterial cell walls. Therefore, the decrease in particle size induced by Co-doping enhanced its antibacterial activity by enhancing its penetration into the bacterial cell [38, 39]. Furthermore, the mechanism of action could involve the generation of reactive oxygen species (ROS), notably ·OH radicals, which lead to phospholipid peroxidation and oxidative cell death [40]. Additionally, the lattice defects induced by Co-doping could also contribute to improved activity [41]. The nanoparticles showed considerable activity against both gram-negative and gram-positive bacteria, suggesting a broad-spectrum activity. This further affirms reports in literature that the antimicrobial activity of nanoparticles depends on morphology, size, electrostatic attraction and the release of metal ions [42].

Table 3 Minimum Inhibitory Concentration (MIC) of ZnO and Co-doped ZnO NPs on the growth of some pathogenic fungi using broth dilution assay

Organism	MIC value (mg/mL)		
	ZnO NPs	Co-doped NPs	Amphotericin (1.6 mg/mL)
<i>Mucor mucedo</i>	0.8 ± 0.01 ^a	0.05 ± 0.01 ^a	0.022 ± 0.01 ^a
<i>Penicillium chrysogenum</i>	0.4 ± 0.01 ^b	0.05 ± 0.00 ^a	0.025 ± 0.01 ^a
<i>Aspergillus niger</i>	0.2 ± 0.00 ^b	0.03 ± 0.01 ^b	0.025 ± 0.00 ^b

^{a,b} Row means with different superscripts for each organism between the different concentrations are significantly ($P < 0.05$) different ($n = 2$)

Fig. 7 Minimum inhibitory concentration of ZnO and Co-doped ZnO against *Mucor mucedo*, *Penicillium sp.*, and *Aspergillus niger*



3.4.2 Antifungal activity

The antifungal activity of pristine ZnO and Co-doped ZnO nanoparticles is presented in Table 3 and Fig. 7. The activity of the nanoparticles was evaluated against three fungal strains: *Mucor mucedo*, *Penicillium chrysogenum* and *Aspergillus niger*. ZnO nanoparticles showed the lowest MIC value of 0.2 mg/ml against *Aspergillus niger*, compared to 0.4 mg/mL and 0.8 mg/mL for *Penicillium chrysogenum* and *Mucor mucedo*, respectively. Similarly, for Co-doped ZnO, the lowest MIC value (0.03 mg/mL) was observed against *Aspergillus niger*, with MIC values of 0.05 mg/mL reported for both *Mucor mucedo* and *Penicillium chrysogenum*. Both materials showed lower activity compared to the amphotericin standard; however, Co-doping improved the antifungal activity of ZnO to a comparable level. The antifungal activity of ZnO nanoparticles generally depends on the generation of reactive oxygen species (ROS), which is often influenced by the surface area, metal release, presence of vacancies and crystallite size [43]. The doping of metal ions into metal oxides often alters their physicochemical and biological properties, leading to improved antifungal activity compared to pristine materials [44].

4 Conclusion

The green synthesis of ZnO and Co-doped ZnO nanoparticles using *Platycladus orientalis* extract provides an environmentally friendly approach to producing nanomaterials with enhanced antimicrobial properties. Characterization studies confirmed structural and optical modifications in the doped samples, including reduced particle size, lattice strain, and band gap narrowing contributed to its improved performance. The Co-doped ZnO NPs showed a higher antibacterial efficiency against *Enterococcus faecalis*, with a zone of inhibition of 14.05 ± 0.21 mm. The Co-doped ZnO NPs also showed enhanced antifungal activity, with a minimum inhibitory concentration of 0.05 mg/mL compared to 0.2 mg/mL observed for ZnO NPs. Therefore, Co-doping significantly enhances the nanoparticles' antimicrobial efficacy, making them promising candidates for combating microbial infections. This study underscores the potential of doping and green synthesis techniques in advancing multifunctional nanomaterials for sustainable applications.

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Plant guidelines The authors confirm that the use of plants in this study complies with international, national and/or institutional guidelines.

Author contributions Mercy C Ogwuegbu, Olalekan C Olatunde and Damian C Onwudiwe conceptualized and designed the work and reviewed the draft. Mercy C Ogwuegbu, Olalekan C Olatunde and Trust M Pfukwa carried out the study and were involved in the manuscript preparation. Doctor MN Mthiyane, Olaniyi A Fawole, and Damian C Onwudiwe provided resources for the study and reviewed the final draft. All the authors contributed to the manuscript and agreed to its publication.

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Data availability The data that were used for this study are available upon reasonable request from the corresponding author.

Declarations

Competing interests The authors declare no competing interests.

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