

Research

Antibacterial and antifungal activities of *Platycladus orientalis* leaf extract-mediated Fe₂O₃ and Ce-doped Fe₂O₃ nanoparticles

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Abstract

Green synthesis of nanomaterials harnesses naturally occurring materials, including plant extracts, to offer environmentally friendly alternatives to conventional biomedicine, agriculture, and other field applications. This study explores the green route to Fe₂O₃ and cerium-doped Fe₂O₃ (Ce-doped Fe₂O₃) nanoparticles synthesized for the first time using the leaf extract of *Platycladus orientalis*. The synthesized nanoparticles were characterized for their structural, morphological, chemical, and optical properties. The hematite phase of Fe₂O₃ nanoparticles with spherical morphology was obtained. The introduction of Ce as a dopant into Fe₂O₃ increased the lattice strain of Ce-doped Fe₂O₃ nanoparticles (0.51%) compared to pristine Fe₂O₃ (0.46%) even though the size of both nanomaterials was similar. Compared to pristine Fe₂O₃ nanoparticles, Ce-doped Fe₂O₃ nanoparticles also demonstrated enhanced antimicrobial and antifungal activities against *Escherichia coli*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Penicillium chrysogenum*, *Aspergillus niger*, and *Mucor mucedo*. The green-synthesized Ce-doped Fe₂O₃ nanoparticles possess potential for application in biomedical and environmental fields based on their relevance to human health and food safety, diversity in microbial characteristics, and potential for resistance to conventional treatments.

Keywords Green synthesis · Hematite · Plant extracts · Antibacterial · Antifungal

1 Introduction

Green-synthesized nanomaterials have gained relevance in several biomedical, agricultural, and environmental applications [1–3]. Their use of natural materials, including sugars, vitamins, polymers, and plant extracts in nano-synthesis is considerably attractive for materials scientists [4, 5]. Compared to the use of microorganisms, plant-based materials have been the most explored because of their environmental safety, cost-effectiveness, and amenability to large-

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scale synthesis of nanoparticles [6]. Different plant parts (seeds, roots, stems, latexes, and leaves) have been used in phyto-nanosynthesis to exploit their rich phytochemical composition for reducing and stabilizing nanoparticles, circumventing the hazards of toxicity and the exorbitant costs of conventionally used chemicals [7–9].

Oxides of iron are widely used oxide because they are inexpensive and play significant roles in many biological processes. The most common forms of iron oxide in nature are hematite ($\alpha\text{-Fe}_2\text{O}_3$), magnetite (Fe_3O_4) and maghemite ($\gamma\text{-Fe}_2\text{O}_3$), and they are of high importance in various fields [10]. Hematite ($\alpha\text{-Fe}_2\text{O}_3$) nanoparticles have gained significant attention in recent years because of their superparamagnetic properties, non-toxicity, high surface area-to-volume ratio, and biocompatibility [11]. It is the most thermodynamically stable phase at ambient temperature and exhibits n-type semiconductivity, with a narrow band gap of 2.2 eV [12, 13]. They have found applications in bio-imaging [14], drug delivery [15, 16], wastewater treatment [17], catalysis [18, 19], and agriculture [20, 21]. However, the application of these nanomaterials in biomedicine and other fields is fraught with several challenges, including poor stability and dispersibility [22] as well as water-solubility and conjugation with biomolecules [23] that ultimately compromise their efficacy in physiological systems.

A growing number of literature reports have recently shown improvements in the bioactivity of Fe_2O_3 nanoparticles due to the introduction of other metals within their lattices. For instance, Al-Zahrani et al. [24] reported enhanced antimicrobial efficacy of Fe_2O_3 against bacterial pathogens *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa* upon its doping with Ag as $\text{Ag}/\text{Fe}_2\text{O}_3$. Furthermore, doped iron oxide nanoparticles ($\text{Co}/\text{Fe}_2\text{O}_3$; $\text{Ni}/\text{Fe}_2\text{O}_3$; and $\text{Zn}/\text{Fe}_2\text{O}_3$) reported by Atul et al. [25] showed broader activity against *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. neoformans* infection, showing the effectiveness of metal ion doping in enhancing the antimicrobial activity of Fe_2O_3 nanoparticles. Similarly, Rahdar et al. [26] exploited the inhibitory activities of cobalt-doped Fe_2O_3 against some pathogenic bacteria (*S. dysenteriae*, *K. pneumoniae*, *A. baumannii*, and *S. pyogenes*) and fungi (*F. oxysporum*, *A. fumigatus*, and *C. albicans*). This shows the potential of phyto-mediated doped- Fe_2O_3 nanoparticles as excellent antibacterial, anticancer, and antifungal agents [27–30]. However, no studies have previously investigated biogenic cerium Ce-doped Fe_2O_3 hybrid nanoparticles synthesized using the extract of any part of the *Platycladus orientalis* plant.

Cerium is well known for its ability to enhance the catalytic and biological activity of nanoparticles. The cerium ion has unique biological activity emanating from its ability to change oxidation state between Ce^{3+} and Ce^{4+} during redox reactions [31, 32]. Because of this versatility, Ce can serve as both an antioxidant (Ce^{3+}) [31, 33] and a pro-oxidant (Ce^{4+}) (Pop et al. 2020). As a pro-oxidant, it can generate reactive oxygen species that damage cells and therefore enhance its antibacterial activity [34]. Therefore, combining the biocompatibility and catalytic activity with the unique redox properties of cerium could yield a material with improved biomedical activity.

Platycladus orientalis (common name: Chinese thuja; *Cupressaceae* family) is a monotypic genus of evergreen coniferous trees. Its leaves have been used in traditional Chinese medicine as blood-cooling and hemostatic herbs for thousands of years [35]. Its leaf and fruit extracts are rich in tannins and flavonoids, while its essential oil has fungistatic and bacteriostatic activities [36]. The extracts of this plant have been explored in the synthesis of metallic nanoparticles [37], however, their use in the synthesis of Fe_2O_3 nanoparticles and Ce-doped Fe_2O_3 nanoparticles has not been investigated. Furthermore, the use of green-synthesized Fe_2O_3 and Ce-doped Fe_2O_3 as antimicrobial agents could be essential in addressing various biomedical issues as they highlight their potential applications in preventing foodborne disease, spoilage, and treating infections caused by these organisms. Therefore, this study investigated the leaf extract of *P. orientalis* in the biosynthesis of Fe_2O_3 and Ce-doped Fe_2O_3 nanoparticles and assessed their antibacterial and antifungal activities.

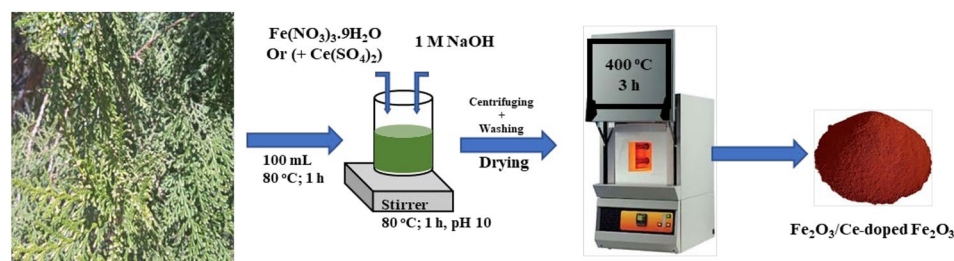
2 Methods and methods

2.1 Materials

2.1.1 Materials

Cerium(IV) sulphate ($\text{Ce}(\text{SO}_4)_2$), and iron(III) nitrate nonahydrate ($\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$) were obtained from Merck, South Africa, and used without further purification. Potato Dextrose Broth (PDB), Muller-Hinton Broth (MHB), and Muller-Hinton Agar (MHA) were purchased from Sisco Research Laboratories (Mumbai, India).

Fig. 1 Schematic representation of the synthesis route for Fe_2O_3 and Ce- Fe_2O_3 NPs



2.1.2 *Platycladus orientalis* extract preparation

The *Platycladus orientalis* plant (Genus: *Platycladus*; species: *orientalis*) was obtained from Molelwane Experimental Farm, located (Coordinates: 25°40.459'S, 26°10.563'E) approximately 8 km outside Mahikeng City in North-West Province, South Africa, and was identified by Dr. Madeleen Struwig at the Botany Department of the North-West University. A voucher specie was deposited at S.D. Phalatshe herbarium (UNWH), with voucher number Ogwuegbu-1. The leaves were harvested and washed four times with distilled water to remove dirt. Thereafter, 20 g of the macerated leaves were weighed into a 200 mL beaker, and 100 mL of distilled water was added. The mixture was stirred for 1 h at 80 °C and was filtered to obtain the aqueous extract, which was stored in a sealed container at 4 °C for future use.

2.1.3 Synthesis of Fe_2O_3 and Ce- Fe_2O_3 NPs

The synthesis of Fe_2O_3 and Ce-doped Fe_2O_3 NPs followed the method reported by Ogwuegbu et al. [38] with slight modification. The synthesis procedure is presented in Fig. 1. In a typical synthesis, 5 g of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ was dissolved in 10 mL of distilled water, and to this solution was added 20 mL of *P. orientalis* leaf extract. The pH of the mixture was adjusted to 10 by the dropwise addition of 1 M NaOH. The solution was further stirred for 2 h at 80 °C to afford a deep red precipitate. The precipitate was recovered from the solution by centrifugation at 5000 rpm for 10 min, followed by washing with water/acetone mixture (50:50) to remove any unreacted $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$. The precipitate was dried overnight under a vacuum, and subsequently calcined at 400 °C for 3 h. A similar procedure was followed for the synthesis of Ce-doped Fe_2O_3 nanoparticles, however, 0.02 g of $\text{Ce}(\text{SO}_4)_2$ was introduced into the solution of the $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ and the subsequent procedures were as described for Fe_2O_3 .

2.1.4 Characterization of Fe_2O_3 and Ce-doped Fe_2O_3 NPs

The crystalline properties of the nanomaterials were investigated using a D8 advance diffractometer (BRUKER AXS, Germany), which uses a secondary graphite monochromated $\text{CuK}\alpha$ radiation ($\lambda = 1.54060 \text{ \AA}$) at 40 kV/50 mA, covering a range of 20–80°. Fourier transform infrared spectroscopy (FTIR) studies were performed with a Spectroquant® 300 spectrophotometer within a wavenumber range of 400–5000 cm^{-1} . The morphology of the nanoparticles was examined by using an FE-SEM FEI 430 Nova NanoSEM system for scanning electron microscopy and an FEI Tecnai G2 Twin system for transmission electron microscopy. The optical properties of the nanoparticles were studied with a Spectroquant® Prove 300 spectrophotometer.

2.1.5 Microbial strains

The bacterial strains used in this study include one Gram-negative strain namely, *Escherichia coli* (ATCC 25922) and two Gram-positive strains: *Enterococcus faecalis* (ATCC 29212) and *Listeria monocytogenes* (ATCC 7644). These were maintained on Mueller Hinton Agar (MHA), with subculturing performed on the same media at 37 °C. The fungal species: *Penicillium sp.*, *Aspergillus niger*, and *Mucor sp.* were isolated from decayed strawberry fruit surfaces. The fungal species were isolated and those showing fast growth were then consistently isolated and identified according to procedures described by Campbell et al. [39] and López et al. [40]. The fungal pathogens were maintained on potato dextrose agar (PDA) medium, which was also used for subculturing at 25 °C.

2.1.6 Antibacterial activity evaluation

In vitro antibacterial efficacy of the Fe₂O₃ and Ce-doped Fe₂O₃ NPs was evaluated using the agar well diffusion method as described by [41] with slight modification. The inoculum for each of the bacterial strains (*Escherichia coli*, *Enterococcus faecalis*, and *Listeria monocytogenes*) was prepared using the direct suspension method. Overnight cultures of each bacterium were made on MHA medium after which colonies were suspended into Mueller Hinton Broth (MHB). This was then adjusted to an optical density (600 nm) of 0.08 – 0.1, to match the turbidity of the 0.5 McFarland standard. Working volumes of each suspension were then made by preparing 1:100 dilutions of the adjusted suspension in sterile MHB to give a final inoculum density of approximately 1.5×10^6 colony-forming units (CFU)/mL. A 100 µL volume of each inoculum was then pipetted into MHA plates in triplicates and spread evenly on the surface using a metal spreader. Plates were allowed to dry after which 6 mm holes were made on each agar plate using a sterile borer. For each of the Fe₂O₃ and Ce-doped Fe₂O₃NPs, solutions were prepared to give concentrations of 6.4, 3.2, 1.6, and 0.8 mg/mL. Neomycin (3.2 mg/mL) was prepared as the positive control with sterile distilled water as the negative control. About 80 µL volume of each test solution was then pipetted into each well. Plates were incubated at 36 ± 1 °C for 24 h under aerobic conditions. After incubation, the zones of inhibition were observed and measured in mm. All analyses were done in triplicates.

2.1.7 Antifungal activity evaluation

The antifungal efficacy of the nanoparticles was evaluated using the broth microdilution method as described by [42] with slight modification. The preparation of the inoculum for *Penicillium sp.*, *Aspergillus niger*, and *Mucor sp* was done by firstly sectioning a 5 × 5 mm agar block from 5-day-old fungal cultures. This was then transferred into McCartney bottles containing 15 mL of potato dextrose broth (PDB) with 0.1% (v/v) Tween 80 for 72 h. After incubation, the cell suspensions were vortexed and adjusted to match the turbidity of a 0.5 McFarland standard corresponding to $1-5 \times 10^6$ CFU/mL. A working suspension was then prepared by making a 1:10 dilution of each adjusted inoculum in sterile PDB to yield a final inoculum size of approximately $1-5 \times 10$ CFU/mL. The Fe₂O₃ and Ce-doped Fe₂O₃ nanoparticles were dissolved in PDB to make stock solutions. Two-fold serial dilutions of these nanoparticles were then made in 96-well plates at double the final desired strength for each well with a volume of 100 µL. Each of the wells with test substances was then inoculated with 100 µL of the adjusted fungal suspension. The nanoparticles' concentration ranged from 3.2 to 0.025 mg/mL after the addition of the inoculum. The positive control well contained amphotericin B (1.6 mg/mL) while the sterility and growth control wells contained PDB and the inoculum, respectively. After closing the lids, the plates were sealed with parafilm and incubated at 37 °C for 48 h. After incubation, growth in the plates was visually observed with Minimum Inhibitory Concentrations (MIC) being defined as the lowest concentration of each nanoparticle which completely inhibited fungal growth and expressed as mg/mL.

2.1.8 Statistical analysis

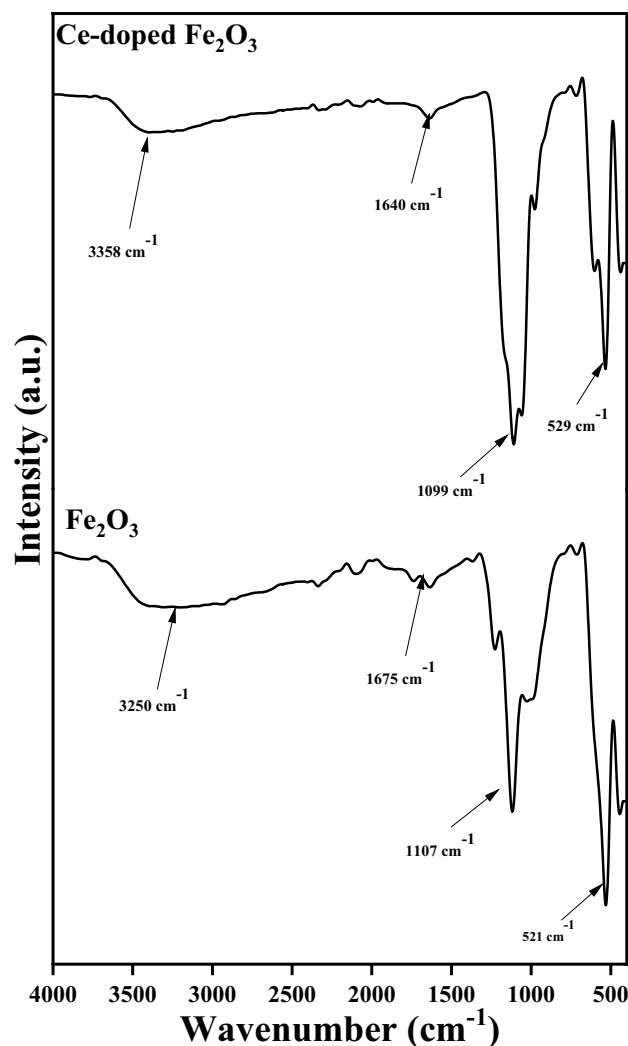
Data were subjected to One-Way Analysis of Variance using the general linear model (GLM) procedure of SAS [43]. Mean differences were tested at 5% probability level ($P < 0.05$) while significantly different means were separated using Tukey's test.

3 Results and discussion

3.1 Structural and functional groups analysis

Fourier transform infrared spectroscopy was used to explore the surface functional groups of the Fe₂O₃ and Ce-doped Fe₂O₃ and the spectra are presented in Fig. 2. Both nanomaterials showed a broad absorption peak around $3310-3500\text{ cm}^{-1}$, which could be ascribed to the O–H stretching vibration of surface-adsorbed H₂O molecules [44]. The peak at 1107 cm^{-1} is ascribed to the C–O stretching vibration. The band around 1675 cm^{-1} could be attributed to the asymmetric stretching vibration of Fe–OH [45]. The stretching vibration due to the Fe–O bond was observed

Fig. 2 FTIR spectra of Fe_2O_3 and Ce-doped Fe_2O_3 obtained by using the aqueous extract of *P. orientalis* leaves



at 521 cm^{-1} in the spectrum of Fe_2O_3 , and this was found to shift to 529 cm^{-1} in the spectrum of Ce-doped Fe_2O_3 . Furthermore, the O–H, Fe–OH, and Fe–O bonds in the pristine Fe_2O_3 displayed a higher intensity peak compared to those of Ce-doped Fe_2O_3 , which could be the consequence of the introduction of Ce^{4+} ions within the lattices of Fe_2O_3 [46, 47].

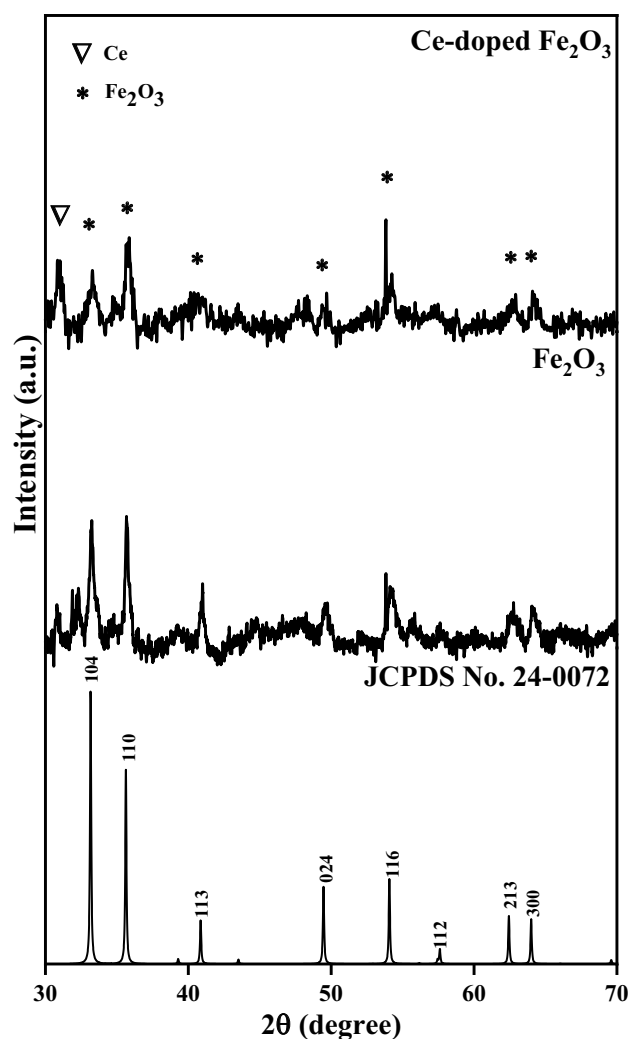
The structure and phase composition of the Fe_2O_3 and Ce-doped Fe_2O_3 were studied by powder X-ray diffraction (XRD). The pristine Fe_2O_3 showed peaks that are indexed to the pure hematite phase (JCPDS No: 24–0072), with a rhombohedral structure [48] (Fig. 3). The diffraction patterns of Ce-doped Fe_2O_3 showed similar peaks to the pristine Fe_2O_3 except for the peak identified at $2\theta = 31$ which is due to Ce. In addition, the Ce-doped Fe_2O_3 showed less crystallinity compared to Fe_2O_3 , which could be due to the creation of defects in the Fe_2O_3 caused by the Cedoping. To determine the crystallite size (D) of the nanoparticles and the microstrain (ϵ) in the crystal, the Scherrer equation (Eq. 1) and Eq. 2 were employed, respectively.

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

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

where K is the crystallite-shape factor, λ is the wavelength of the X-rays, β is the width (full-width at half-maximum) of the X-ray diffraction peak in radians and θ is the Bragg angle [49]. The crystallite size and microstrain of the Fe_2O_3 were

Fig. 3 XRD pattern of Fe_2O_3 and Ce-doped Fe_2O_3 obtained from the aqueous extract of *P. orientalis* leaf



obtained to be 25.7 nm and 0.457%, respectively, while the crystallite size and microstrain were 27.8 nm and 0.505% for the Ce-doped Fe_2O_3 nanoparticles. The difference in the microstrain confirms the earlier assertion of induced defects arising from Ce doping.

Furthermore, to estimate the effect of Ce doping on the induced defect in the material, the dislocation density, which is a measure of the line defect in the material was evaluated. The dislocation density was calculated using the expression (Eq. 3)[50]:

$$\rho_d = \frac{1}{D^2} \quad (3)$$

The dislocation density for Fe_2O_3 and Ce-doped Fe_2O_3 was estimated to be 1.5×10^{-3} and 1.3×10^{-3} line/nm². This shows that although Fe_2O_3 had a lower amount of microstrain, its line defects are higher than those of Ce-doped Fe_2O_3 . This further affirms the significant role played by Ce-doping on the structural properties of Ce-doped Fe_2O_3 .

The level of distortion in the crystal was evaluated by calculating the lattice constant of Fe_2O_3 and Ce-doped Fe_2O_3 . Using Bragg's law, the interplanar spacing (d) for the materials was calculated from the expression (Eq. 4):

$$n\lambda = 2d\sin\theta \quad (4)$$

where n is the order of reflection, λ is the X-ray wavelength, and θ is the diffraction angle. For the rhombohedral system, the interplanar spacing is related to the lattice parameter (a) by the expression (Eq. 5):

$$\frac{1}{d^2} = \frac{h^2 + k^2 + l^2 + 2(hk + kl + lh), \cos(\alpha)}{a^2 \sin^2(\alpha)} \quad (5)$$

where h, k, l are the Miller indices, and α for hematite is typically 55° . The calculated value of lattice constant (a) was $1.528 \text{ \AA}$ for both materials, which showed there was no distortion in the crystal of the Ce-doped Fe_2O_3 . Table 1 shows a summary of the structural properties of Fe_2O_3 and Ce-doped Fe_2O_3 .

3.2 Surface morphology and elemental composition

The SEM and TEM images of the synthesized Fe_2O_3 and Ce-doped Fe_2O_3 nanoparticles are shown in Fig. 4. The SEM images of both nanomaterials (Fig. 4a, b) show highly agglomerated particles with irregular shapes; however, Fe_2O_3 showed a higher level of agglomeration compared to Ce-doped Fe_2O_3 . The observed difference in the aggregation behaviour of the materials could be attributed to the modification of the functional groups and surface charge on Fe_2O_3 induced by Ce doping, which alters the inter-particle interactions [51]. The EDX image of Fe_2O_3 and Ce-doped Fe_2O_3 (Fig. 4c, d), confirmed the presence of Fe and O as the main constituents of Fe_2O_3 and Fe, O and Ce as the main constituents of Ce-doped Fe_2O_3 . This confirms the doping of Fe_2O_3 with Ce. The presence of carbon, potassium, and sulphur in the EDX spectra could have originated from the biomolecule of the *P. orientalis* leaf extract [52, 53].

The TEM images of Fe_2O_3 and Ce-doped Fe_2O_3 shown in Fig. 5a, b confirmed the formation of mostly rectangular-shaped materials; however, the Ce-doped Fe_2O_3 displayed more dense particles compared to Fe_2O_3 . In addition, the TEM images of the materials showed a wide particle size distribution. The particle size of the Fe_2O_3 and Ce-doped Fe_2O_3 was estimated to be $132.8 \pm 15.8 \text{ nm}$ and $21.7 \pm 6.7 \text{ nm}$, respectively. This confirms the high level of agglomeration and particle size distribution in Fe_2O_3 compared to Ce-doped Fe_2O_3 . The growth kinetics and nucleation rates of nanoparticles are often influenced by doping, which affects the morphology of the nanomaterial [54].

3.3 Optical properties

Figure 6 presents the optical absorption spectra of Fe_2O_3 and Ce-doped Fe_2O_3 . Two transitions are observed: in the 500–600 nm range, which corresponds to the indirect band edge, and the 400–450 nm range which could be ascribed to the direct band edge [55]. The indirect band edge corresponds to the transition from the $\text{Fe } e_g$ state to the empty t_{2g}^* state, while the direct band transition corresponds to the transition from occupied O 2p to the empty $\text{Fe } t_{2g}^*$ state. The absorption spectrum of Ce-doped Fe_2O_3 -NPs displayed similar features to that of Fe_2O_3 ; however, its absorption edge appears less steep than that of the pristine Fe_2O_3 , which may arise from the presence of defect states, as earlier confirmed in the diffraction patterns of the materials. Furthermore, a red shift in the band edges was observed in the spectrum of Ce-doped Fe_2O_3 NPs, indicating a reduction in the band gap energy. Figure 4b shows the Tauc plot obtained from Eq. 6:

$$\alpha h\nu = A(h\nu - E_g)^n \quad (6)$$

The band gap energy was obtained from a plot of $(\alpha h\nu)^2$ against $h\nu$ by extrapolating the linear portion of the plot to $h\nu=0$. The direct bandgap energy for Fe_2O_3 and Ce-doped Fe_2O_3 was evaluated to be 3.23 and 3.05 eV, respectively. The introduction of dopants has been reported to create localized energy states, which can facilitate electronic transitions that require lower energy than band-to-band transitions, leading to an overall reduction in the band gap [56, 57].

Table 1 Structural properties of Fe_2O_3 and Ce-doped Fe_2O_3

	Crystallite size (nm)	Microstrain (%)	Dislocation density (line/ nm^2)	Lattice constant ($\text{\AA}$)
Fe_2O_3	25.7	0.457	1.5×10^{-3}	1.528
Ce- Fe_2O_3	27.8	0.505	1.3×10^{-3}	1.528

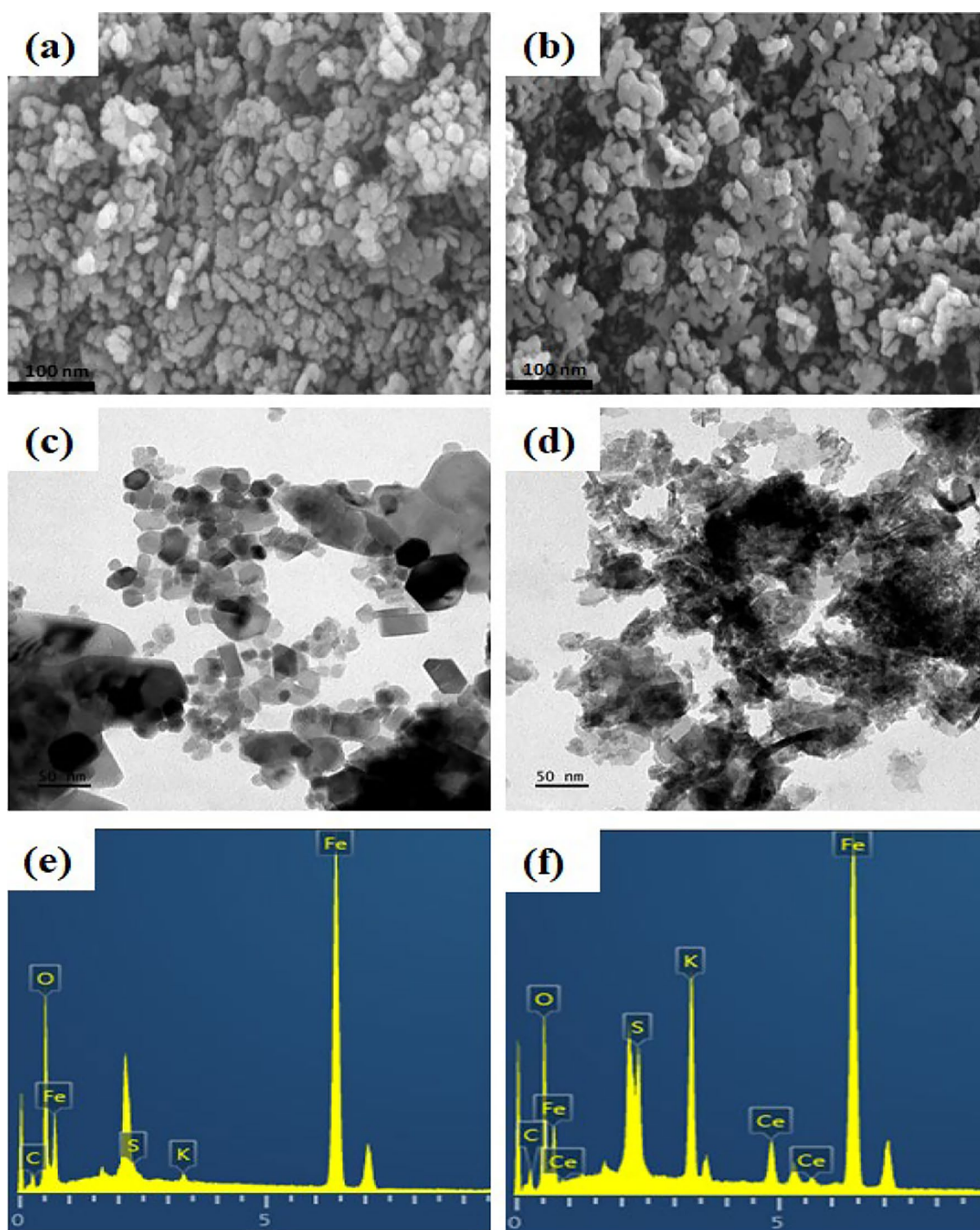


Fig. 4 a, b SEM, and c, d EDX spectra of Fe₂O₃ and Ce-doped Fe₂O₃ obtained from the aqueous extract of *P. orientalis* leaf

3.4 Antibacterial activity of Fe₂O₃ and Ce-doped Fe₂O₃NPs

Table 2 and Fig. 7 present the antibacterial properties of Fe₂O₃ and Ce-doped Fe₂O₃NPs. The results were presented as zones of inhibition in millimetres (mm) at different concentrations (6.4, 3.2, 1.6, and 0.8 mg/mL) and compared with Neomycin (3.2 mg/mL). The result indicates statistically significant differences among treatments with neomycin (antibacterial standard drug) significantly inhibiting the growth of all tested bacterial pathogens,

Fig. 5 TEM images of (a) Fe_2O_3 and (b) Ce-doped Fe_2O_3 obtained from the aqueous extract of *P. orientalis* leaf

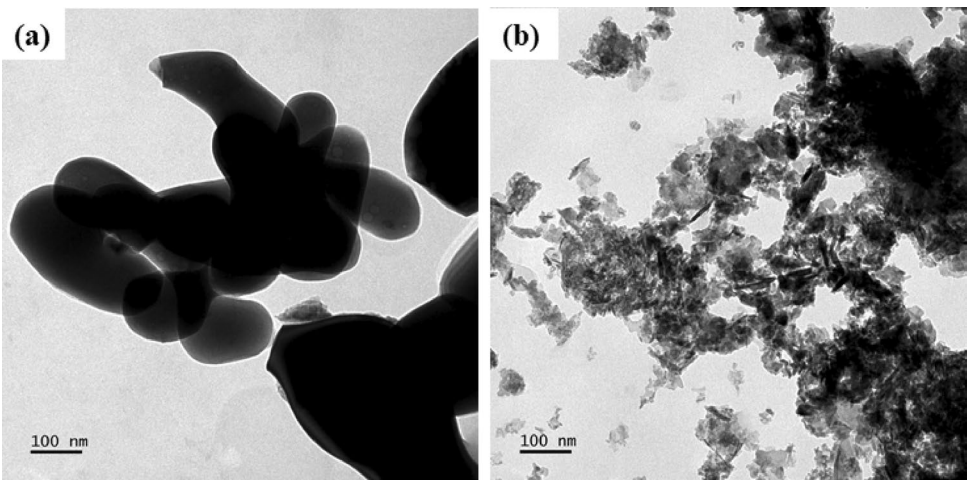


Fig. 6 a UV-vis spectra and b Tauc plot of Fe_2O_3 and Ce-doped Fe_2O_3 obtained from the aqueous extract of *P. orientalis* leaves

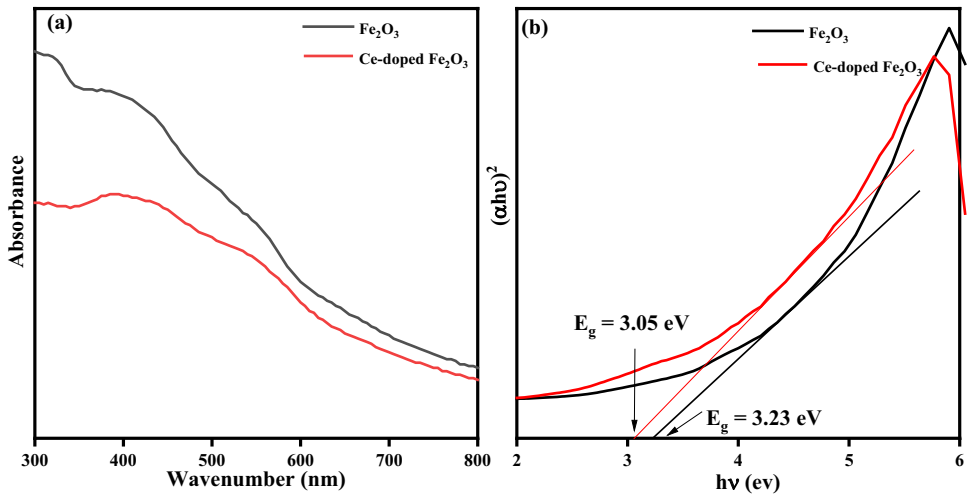


Table 2 Effect of concentration of Fe_2O_3 and Ce- Fe_2O_3 NPs zone of inhibition on the growth of some pathogenic bacterial

	<i>Escherichia coli</i>	<i>Enterococcus faecalis</i>	<i>Listeria monocytogens</i>
Fe_2O_3NPs			
6.4 mg/mL	15.00 ± 0.58 ^a	11.00 ± 0.72 ^b	11.00 ± 0.59 ^b
3.2 mg/mL	11.00 ± 1.15 ^b	9.00 ± 1.15 ^{bc}	9.50 ± 0.29 ^b
1.6 mg/mL	8.50 ± 0.87 ^{bc}	6.42 ± 0.39 ^c	7.00 ± 0.58 ^c
0.8 mg/mL	7.00 ± 0.58 ^c	0.00 ± 0.00 ^d	0.00 ± 0.00 ^d
Neomycin (3.2 mg/mL)	16.00 ± 1.15 ^a	17.00 ± 1.73 ^a	20.50 ± 0.29 ^a
Ce-doped Fe_2O_3 NPs			
6.4 mg/mL	19.00 ± 1.05 ^a	14.50 ± 0.29 ^b	15.00 ± 2.89 ^b
3.2 mg/mL	16.00 ± 0.67 ^{ab}	13.00 ± 1.73 ^{bc}	13.55 ± 1.03 ^b
1.6 mg/mL	14.00 ± 0.57 ^b	11.00 ± 1.15 ^c	12.00 ± 0.58 ^b
0.8 mg/mL	10.00 ± 1.16 ^c	5.05 ± 0.33 ^d	5.50 ± 0.26 ^c
Neomycin (3.2 mg/mL)	17.00 ± 1.73 ^{ab}	18.50 ± 0.28 ^a	22.00 ± 1.14 ^a

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

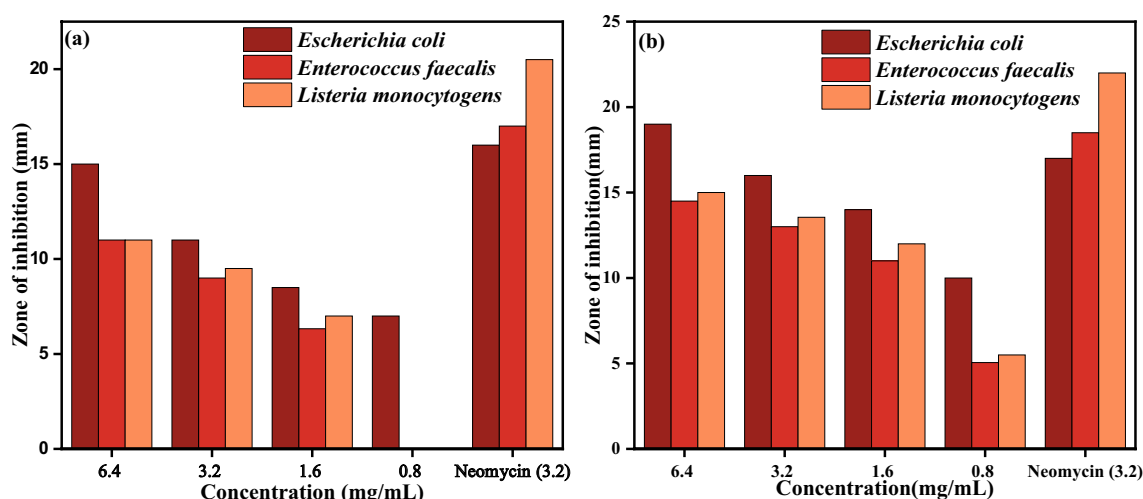


Fig. 7 Antibacterial activity of **a** Fe₂O₃ and **b** Ce-doped Fe₂O₃ nanoparticles obtained using the aqueous extract of *P. orientalis* leaves against *Escherichia coli*, *Enterococcus faecalis*, and *Listeria monocytogenes*

compared to the Fe₂O₃ and Ce-doped Fe₂O₃ NPs. However, with respect to the nanoparticles, the highest zone of inhibition was exhibited at 6.4 mg/mL ($P < 0.01$) against *Escherichia coli*, *Enterococcus faecalis*, and *Listeria monocytogenes* (Fig. 8). The Fe₂O₃ NPs showed moderate antibacterial activity against *Escherichia coli* (zone of inhibition of 7.00 ± 0.58 , 8.50 ± 0.87 , 11.00 ± 1.15 and 15.00 ± 0.58 mm), *Enterococcus faecalis* (zone of inhibition of 0.00 ± 0.00 , 6.42 ± 0.39 , 9.00 ± 1.15 and 11.00 ± 0.72 mm) and *Listeria monocytogenes* (zone of inhibition of 0.00 ± 0.00 , 7.00 ± 0.58 , 9.50 ± 0.29 and 11.00 ± 0.59 mm). In comparing the means across the inhibition zones, *Enterococcus faecalis*, *Enterococcus faecalis* and *Listeria monocytogenes* were significantly higher ($P < 0.05$) at the neomycin and 6.4 mg/mL concentrations compared to other concentrations. In contrast, the 0.8 mg/mL concentration exhibited the lowest ($P < 0.05$) zone of inhibition. Whereas Ce-doped Fe₂O₃ NPs had higher antibacterial potency against *Escherichia coli* (zone of inhibition of 10.00 ± 1.16 , 14.00 ± 0.57 , 16.00 ± 0.67 and 19.00 ± 1.05 mm), *Enterococcus faecalis* (zone of inhibition of 5.05 ± 0.33 , 11.00 ± 1.15 , 13.00 ± 1.73 and 14.50 ± 0.29 mm) and *Listeria monocytogenes* (zone of inhibition of 5.50 ± 0.26 , 12.00 ± 0.58 , 13.55 ± 1.03 and 15.00 ± 2.89 mm). Additionally, for *Enterococcus faecalis*, the neomycin, 6.4 mg/mL, 3.2 mg/mL concentrations showed the highest ($P < 0.05$) zone of inhibition, while the 0.8 mg/mL concentration exhibited the lowest ($P < 0.05$) inhibition zone. In all cases, the doped nanoparticles (Ce-doped Fe₂O₃ NPs) showed more antibacterial potency than the pristine nanoparticles (Fe₂O₃), which confirmed an enhancement of biological activity due to the dopant ion. Our findings align with previous results indicating that the incorporation of cerium

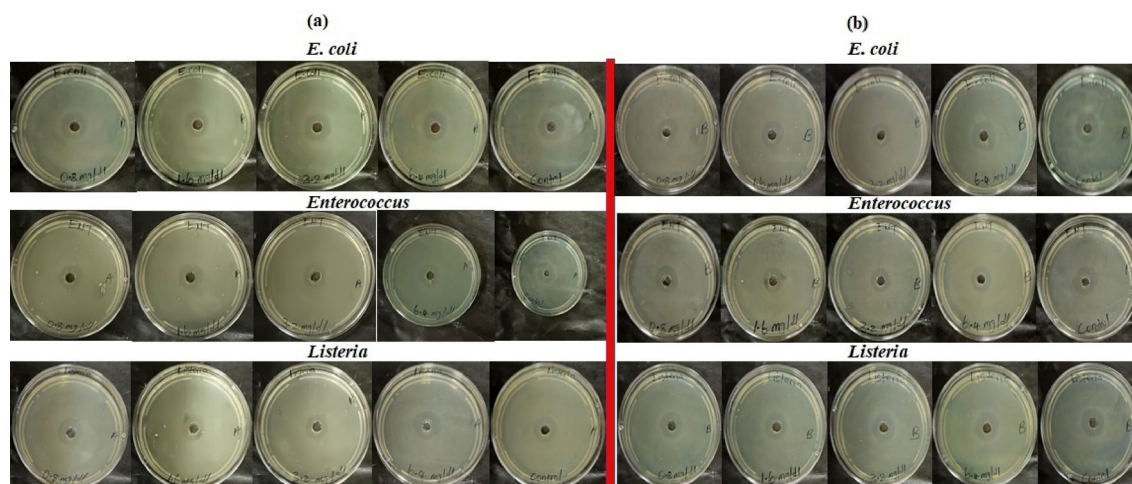


Fig. 8 Antibacterial activity (inhibition zones) of **a** Fe₂O₃ and **b** Ce-doped Fe₂O₃ obtained using the aqueous extract of *P. orientalis* leaves against *Escherichia coli*, *Enterococcus faecalis*, and *Listeria monocytogenes*

Table 3 Comparison of the inhibition activity of Fe₂O₃ and Ce-doped Fe₂O₃ obtained from *P. orientalis* with other reported green synthesized Fe₂O₃ nanoparticles

Nanoparticle	Plant extract	Inoculum density	Nanoparticle concentration	MIC	Refs.
Fe ₂ O ₃ (IONPs)	<i>Salvia officinalis</i> L	1.0 × 10 ⁸	100 ug	8.78%	[66]
Ag/Fe ₂ O ₃	<i>Buddleja lindleyana</i>	–	5 mg/mL	20.8 mm	[24]
Fe ₂ O ₃	<i>Orange peel</i>		0.5 mg/L	3 mm	[67]
A-Fe ₂ O ₃	<i>Moltkia ciliata</i>	1.0 × 10 ⁸	100 mg/mL	11.7 mm	[68]
Fe ₂ O ₃	<i>Platycladus Orientalis</i>	1.5 × 10 ⁶	6.4 mg/mL	15.0%	This study
Ce-doped -Fe ₂ O ₃	<i>Platycladus Orientalis</i>	1.5 × 10 ⁶	6.4 mg/mL	19.0%	This study

Table 4 Minimum Inhibitory Concentration (MIC) of Fe₂O₃ and Ce-doped Fe₂O₃NPs on the growth of some pathogenic fungi using broth dilution assay

Organism	MIC value (mg/mL)		
	Fe ₂ O ₃ -NPs	Ce-doped Fe ₂ O ₃ -NPs	Amphotericin (1.6 mg/mL)
<i>Penicillium sp</i>	0.6 ± 0.20 ^a	0.07 ± 0.03 ^a	0.04 ± 0.01 ^a
<i>Aspergillus niger</i>	0.15 ± 0.05 ^b	0.04 ± 0.01 ^b	0.04 ± 0.01 ^a
<i>Mucor sp</i>	0.2 ± 0.00 ^b	0.04 ± 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 ($n = 2$)

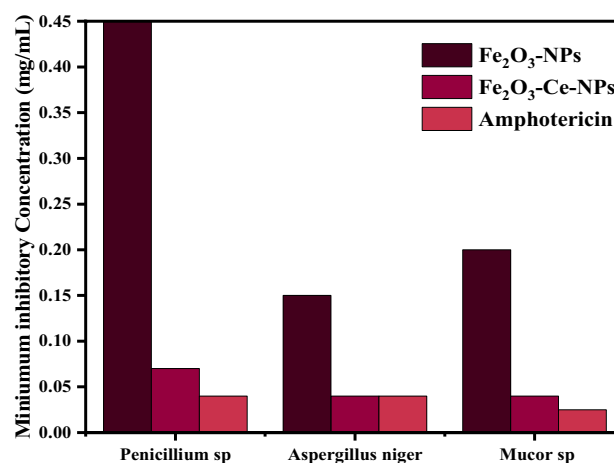
(Ce) into ZnO nanoparticles enhances their antibacterial activity due to the synergistic effects of Ce and ZnO [58]. The enhanced antibacterial activities can be attributed to the fact that cerium ions can generate reactive oxygen species (ROS) more efficiently within the cell wall, thereby leading to apoptosis [59]. Also, Ce doping can alter the surface properties of Fe₂O₃ NPs, thereby improving their permeability in bacterial cell membranes, leading to distortion and destruction of the membranes [60]. Our results also showed that Fe₂O₃-NPs showed no inhibition potency against *Enterococcus faecalis*, and *Listeria monocytogenes* at the lowest concentration (0.8 mg/mL). In contrast, the doped nanoparticles exhibited better antibacterial activity at the lowest concentration, especially against *E.coli*, in comparison to other bacterial strains. This implies that Ce-doped Fe₂O₃NPs were better able to permeate the cell walls of the *E.coli* strain. Doping has been reported to enhance the generation of reactive oxygen species, which are highly effective in damaging microbial cell membranes, proteins and DNA [61]. Also, the release of metal ions such as Ce⁴⁺ can disrupt microbial cell membranes, cause oxidative stress, and interrupt enzymatic functions [62, 63]. Based on previous literature, the ability of doped metal oxides to compromise the cell wall of Gram-negative bacteria such as *E.coli* despite the presence of lipopolysaccharides, proteins, and lipids which typically play a role in preventing bacterial cell death may contribute to Ce-doped Fe₂O₃'s antibacterial activity. The phospholipids-rich structure of cell membranes of Gram-negative bacteria has been reported to restrict the movement of positively charged metal ions, however, studies reported that these ions can enter bacteria and cause cell death by utilizing ionic channels such as voltage-gated and ligand-dependent channels [64, 65]. This result shows that Ce-doped Fe₂O₃ NPs could be developed as effective antibacterial agents for use in medical applications formulation, such as coatings for medical devices, wound dressings, or as part of antimicrobial formulations especially for Gram-negative bacteria. The enhanced activity due to the smaller size and improved permeability indicates that these nanoparticles could provide a more effective means of controlling bacterial infections, particularly those caused by resistant strains.

Table 3 shows the comparison of the antibacterial activity of the *P. orientalis* synthesised Fe₂O₃ and Ce-doped Fe₂O₃ nanoparticles with other green-synthesized nanoparticles. The Fe₂O₃ and Ce-doped Fe₂O₃ reported in this study showed comparable activity against *E. coli*.

3.5 Antifungal activity of Fe₂O₃ and Ce-doped Fe₂O₃NPs

The antifungal activity of both Fe₂O₃ and Ce-doped Fe₂O₃ nanoparticles was evaluated using the broth dilution method. The results showed that doped nanoparticles (Ce-doped Fe₂O₃) had better ($P < 0.01$) antifungal activity against the *Penicillium sp.*, *Aspergillus niger*, and *Mucor sp.* compared to the pristine Fe₂O₃-NPs. The MIC values indicate

Fig. 9 Antifungal activity of (a) Fe_2O_3 and (b) Ce- Fe_2O_3 NPs obtained from the aqueous extract of *P. orientalis* leaves against *Penicillium sp.*, *Aspergillus niger*, and *Mucor sp.*



the minimum concentration required to inhibit the growth of each organism. For *Penicillium sp.*, the MIC was highest for Fe_2O_3 NPs at 0.6 ± 0.20^a mg/mL, while Ce-doped Fe_2O_3 -NPs and Amphotericin had significantly lower MIC values of 0.07 ± 0.03^a mg/mL and 0.04 ± 0.01^a mg/mL, respectively ($P < 0.05$). For *Aspergillus niger* and *Mucor sp.*, Fe_2O_3 -NPs exhibited higher MIC values of 0.15 ± 0.05^b mg/mL and 0.2 ± 0.00^b mg/mL, respectively, compared to the lower MIC values observed with Ce-doped Fe_2O_3 -NPs and amphotericin ($P < 0.05$). The lower MIC values observed for Ce-doped Fe_2O_3 -NPs and amphotericin indicate greater efficacy in inhibiting the growth of these fungal organisms (Table 4 and Fig. 9). In comparison of the means across the rows, the MIC values for Fe_2O_3 and Ce-doped Fe_2O_3 NPs were significantly higher ($P < 0.05$) for *Penicillium sp.* compared to *Aspergillus niger* and *Mucor sp.* For amphotericin, the MIC values for *Penicillium sp.* and *Aspergillus niger* were significantly higher ($P < 0.05$) than for *Mucor sp.* Our findings are consistent with previous studies that have demonstrated the superior antifungal efficacy of amphotericin compared to ZnO [69]. This can be attributed to differences in their mechanisms of action. Amphotericin specifically targets ergosterol in fungal cell membranes, which is crucial for maintaining membrane structure and function. This targeted action results in high efficacy against fungi, unlike nanoparticles which exhibit general mechanisms such as oxidative stress and disruption of cellular processes. These mechanisms may not be as effective as the specific targeting of ergosterol by amphotericin [69–71].

The superior antifungal properties of Ce-doped Fe_2O_3 nanoparticles compared to Fe_2O_3 can be attributed to the unique properties conferred by the dopant ion. Ce-doping enhances the nanoparticles' physicochemical properties such as increased surface area, improved dispersibility, and enhanced generation of ROS, which facilitate better interaction with fungal cells, leading to more effective disruption of cell membranes and inhibition of fungal growth. Additionally, the doped nanoparticles have an improved ability to generate reactive oxygen species (ROS), which further contributes to their antifungal activity [72, 73]. Nanoparticles exhibit distinct properties not present in the bulk forms of the same materials, owing to their small size and high surface area to volume ratio, which effectively cover the microorganisms and reduce oxygen supply for respiration [74–76]. The enhanced surface reactivity and increased production of ROS by Ce-doped Fe_2O_3 nanoparticles disrupt fungal cell structures and metabolic processes more efficiently than Fe_2O_3 nanoparticles [75]. The enhanced ROS could be leveraged in medical diagnostic imaging, targeted drug delivery systems, crop protection, food preservation and packaging.

Furthermore, the result of the current study indicates that pristine Fe_2O_3 and Ce-doped Fe_2O_3 nanoparticles possess effective antifungal properties against *Penicillium sp.*, *Aspergillus niger*, and *Mucor sp.* even at low concentrations.

Table 5 Comparison of the inhibition activity of Fe_2O_3 and Ce-doped Fe_2O_3 obtained from *P. orientalis* with other reported green synthesized Fe_2O_3 nanoparticles against *A. niger*

Nanoparticle	Plant extract	Nanoparticle concentration	MIC	Refs.
SiO ₂ -doped Fe_2O_3	–		0.21 mg/L	[77]
α - Fe_2O_3 -	Platanus orientalis	0.10 mg/mL	12.0 mm	[78]
Fe_2O_3	Tavernier glabra	100 μ g/L	81%	[79]
Fe_2O_3	Platycladus Orientalis	6.4 mg/mL	0.15 mg/mL	This study
Ce-doped Fe_2O_3	Platycladus Orientalis	6.4 mg/mL	0.04 mg/mL	This study

Their efficacy makes them promising candidates for use in pharmaceutical products aimed at treating fungal infections, which are increasingly resistant to conventional antifungal agents.

Table 5 shows the comparison of the antifungal activity of the *P. orientalis* synthesised Fe₂O₃ and Ce-doped Fe₂O₃ nanoparticles with other Fe₂O₃-based nanoparticles. The Fe₂O₃ and Ce-doped Fe₂O₃ reported in this study showed comparable activity against *E. coli*.

4 Conclusion

In this study, we successfully synthesized iron oxide (Fe₂O₃) and cerium-doped iron oxide (Ce-doped Fe₂O₃) nanoparticles using a green synthesis method involving *Platycladus orientalis* leaf extract. The synthesized nanoparticles were thoroughly characterized, confirming the formation of a hematite phase in Fe₂O₃ nanoparticles and successful doping in the Ce-doped Fe₂O₃ nanoparticles, as evidenced by increased lattice strain. The antimicrobial and antifungal activities of these nanoparticles were evaluated, revealing significant inhibitory effects against various pathogenic bacteria and fungi. Notably, the Ce-doped Fe₂O₃ nanoparticles demonstrated enhanced antimicrobial properties compared to the pristine Fe₂O₃ nanoparticles. The nanoparticles revealed a concentration-dependent activity with the highest inhibition of 19% recorded for *Escherichia coli* by Fe₂O₃. The antifungal activity followed a similar concentration dependent trend, with the highest MIC of 0.6 mg/mL reported for *Penicillium* sp. by Ce-doped Fe₂O₃ compared to 0.07 mg/mL by Fe₂O₃. This enhancement is likely due to the dopant effect of cerium, which may have contributed to the increased activity. These results underscore the potential of green-synthesized Fe₂O₃ nanoparticles as antimicrobial agents for biomedical applications. Further research could explore the detailed mechanisms behind the antimicrobial effects and the potential scalability of this green synthesis method for industrial applications.

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Author contributions M.C.O., O.C.O. and D.C.O. conceptualized and designed the work and reviewed the draft. M.C.O., O.C.O. and T.M.P. carried out the study and were involved in the manuscript preparation. D.M.N.M., O.A.F., and D.C.O. 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 authors.

Declarations

Competing interests The authors declare no competing interests.

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