Research Article

Antimicrobial activity of zinc oxide nanoparticles synthesized from *Aloe vera* peel extract

Asha Chaudhary[1] · Naresh Kumar[1] · Ravinder Kumar[1] · Raj Kumar Salar[1]

Abstract
This study was aimed to synthesize zinc oxide nanoparticles from the aqueous peel extract of *Aloe vera* and assess their antimicrobial activity against pathogenic bacteria and fungi. The nanoparticles were characterized by UV–Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Scanning electron microscopy (SEM) and transmission electron microscopy (TEM). UV–Vis spectroscopic analysis confirmed the synthesis of zinc oxide nanoparticles. Fourier transform infrared spectroscopy (FTIR) depicted functional groups associated with the formation of zinc oxide nanoparticles, whereas XRD showed their crystalline nature. Scanning electron microscopy (SEM) showed that nanoparticles were rough in appearance with agglomeration. Transmission electron microscopy (TEM) confirmed the size of nanoparticles from 50 to 220 nm with hexagonal shape. The antimicrobial activity of zinc oxide nanoparticles was assessed against pathogenic bacteria, *Staphylococcus epidermidis* (MTCC-3382), *S. epidermidis* (MTCC-3382), *Klebsiella pneumoniae* (MTCC-3384), *Escherichia coli* (MTCC-41) and fungi, *Aspergillus niger* (MTCC-404) and *Aspergillus oryzae* (MTCC-3107). The results showed the effectiveness of zinc oxide nanoparticles against *E. coli* (MTCC-41) and *A. niger* (MTCC-404). However, in combination with antibiotic, there was a decrease in the antimicrobial activity against bacteria and fungi as compared to antibiotics. Hence, a molecular research is needed to check the effect of zinc oxide nanoparticles on antibiotics.

Keywords Antibiotics · Bacteria · Fungi · Nanoparticles

1 Introduction

Nanotechnology deals with the study and applications of particles with dimension from 1 to 1000 nm. Due to small size from bulk equivalent, nanoparticles have unique properties, which make them ideal for applications in different fields such as electronics, energy, environment and health etc. [2, 10, 23]. Nanoparticles can be metallic, polymeric and lipophilic in nature. They can be synthesized by physical, chemical and biological methods with desired specifications such as size and shape [11]. However, physical and chemical methods are not much explored due to expensive and toxic chemicals used for synthesis. Hence, extensive studies have been made in the biological synthesis of metallic nanoparticles like silver, gold, titanium dioxide, magnesium oxide, copper oxide, iron oxide, aluminium oxide, zinc oxide [31]. Among these, zinc oxide nanoparticles (ZnONPs) have attracted attention of scientists worldwide due to its medicinal values.

Zinc oxide nanoparticles have unique ultraviolet filtration, semiconducting and catalytic activity, which fascinated scientific community all over the world [16]. Moreover, these nanoparticles have been reported to be non-toxic, biologically safe and bio-compatible. The zinc oxide nanoparticles also find applications in cosmetics and sunscreen lotions as they can absorb harmful radiations such as UV-A and UV-B [23]. According to United States Food and Drug Administration, zinc oxide is safe (21 CFR 182.8991) and can be used as medicine [14]. Zinc oxide nanoparticle can be used as an antimicrobial

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agent to kill pathogenic microorganisms. Depending upon their particle size, shape, concentration and exposure time to the bacterial cell, they first damage the cell wall, penetrate, accumulate in the cell membrane and ultimately cause death by interfering with metabolic functions [34]. Several methods have been developed for the synthesis of zinc oxide nanoparticles such as wet chemical [17], chemical micro emulsion [18], hydrothermal [32], vapor phase transparent [19], solvothermal [8] etc. But these methods are expensive and use toxic chemicals. Hence, biological synthesis of zinc oxide nanoparticles from plant extracts and microorganisms is an alternative to physical and chemical methods [6]. Among these, plants have been extensively explored due to rapid synthesis of zinc oxide nanoparticles and being cost effective.

Biosynthesis of zinc oxide nanoparticles have been reported from different plants such as *Aloe barbadensis* [29], *Aeromonas hydrophila* [12], *Parthenium hysterophorus* [21], Trifoliate orange (*Poncirus trifoliata*) [16], *Ocimum basilicum* [25], *Borassus flabellifer* fruit [39], *Tamarindus indica* [9], *Solanum nigrum* [24] and *Plectranthus amboinicus* [40], *Caltropis procera* [28], *Moringa oleifera* [35], *Passiflora caerulea* [30], *Garcinia mangostana* [1]. *Aloe vera* (L.) Burm. f. is a perennial succulent cactus like plant which belongs to Liliaceae family and grow in hot and dry climates [13]. It is stem less plant with 60–100 cm in length, with thick and fleshy leaves with green to gray-green appearance [22]. *A. vera* gel contains different types of vitamins such as A (beta-carotene), C, and E, which acts as antioxidants. The gel has been reported to possess immunomodulatory, anti-inflammatory, UV protective, wound & burn-healing promoting properties. The peel extract contains reducing agents, which can be used to synthesize nanoparticles with good crystalline structure and optical properties [3, 13]. Keeping in view the importance of *A. vera* in medicine, the present study was aimed to synthesize, characterize and check antimicrobial activity of zinc oxide nanoparticles synthesized from peel extract for use in the healthcare industry.

2 Materials and methods

2.1 Plant material

Fresh and healthy leaves of *A. vera* were collected from the campus of Chaudhary Devi Lal University, Sirsa, Haryana. The leaves were washed twice with distilled water, followed by double distilled water to remove the dust and other contaminants.

2.2 Preparation of leaf extracts

Peeled off *A. vera* leaves carefully and discarded gel portion. Small pieces of peel was cut with a knife and grounded with pestle mortar in distilled water to make an aqueous solution of peel extract. Aqueous solution was filtered with Whatman filter paper no. 1 to remove debris.

2.3 Microorganisms

Pathogenic microorganisms, bacteria and fungi, used to check the antimicrobial activity of zinc oxide nanoparticles were purchased from Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh and listed in Tables 1 and 2, respectively. These cultures were revived as per instruction given in the catalogue.

2.4 Biosynthesis of zinc oxide nanoparticles

Zinc oxide nanoparticles were synthesized from aqueous extract of *A. vera* as per method given by Awwad et al. [4] with slight modifications. Prepared 10 mM solution of zinc sulphate ($ZnSO_4 \cdot 7H_2O$) and sodium hydroxide (NaOH)

Table 2 Fungi used for testing antifungal activity of zinc oxide nanoparticles

S. no.	Fungi	Strain no.	Growth media
1.	*Aspergillus niger*	MTCC-404	Czapek yeast extract agar
2.	*Aspergillus oryzae*	MTCC-3107	Czapek yeast extract agar

Table 1 Bacteria used for testing antibacterial activity of zinc oxide nanoparticles

S. no.	Bacteria	Strain no.	Gram stain	Growth media
1.	*Staphylococcus epidermidis*	MTCC-3382	+ve	Nutrient agar
2.	*Staphylococcus aureus*	MTCC-6908	+ve	Nutrient agar
3.	*Klebsiella pneumoniae*	MTCC-3384	−ve	Nutrient agar
4.	*Escherichia coli*	MTCC-41	−ve	Nutrient agar

in distilled water. Added 15 ml of *A. vera* peel extract in 100 ml of zinc sulphate solution, followed by dropwise addition of sodium hydroxide till white suspension of nanoparticles were not produced. Nanoparticles were centrifuged at 10,000 rpm for 10 min and stored in a refrigerator for further use.

3 Characterization

3.1 UV–Vis spectroscopy

UV–Vis spectroscopy is widely used to examine the optical properties of nanoparticles [36]. In the present study, UV–Vis absorption spectrum of zinc oxide nanoparticles was recorded from 200 to 300 nm with NanoDrop 2000c spectrophotometer (Thermo Scientific, Waltham, MA, USA) at initial (0 min) and after 24 h to confirm the synthesis of nanoparticles.

3.2 Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) confirms different functional groups associated with the synthesis of zinc oxide nanoparticles. For FTIR analysis, zinc oxide nanoparticles were dried to make powder. The powder was mixed with potassium bromide (KBr) (2:98 ratios by weight) and pressed at 11,000 psi to make the disc. The detector was purged carefully using clean dry nitrogen gas to increase the signal level and reduce moisture. The discs were then introduced in the spectrophotometer and the spectrum was recorded in scan range from 4000 to 400 cm^{-1}. The FTIR spectra were analyzed using online spectroscopic analysis.

3.3 X-ray diffraction (XRD)

X-ray diffraction (XRD) confirms the nature of zinc oxide nanoparticles. XRD measurement were recorded at voltage of 40 kV and a current of 30 mA, with Cu Kα radiation in a θ–2θ configuration using PANalytical X'Pert Pro (Malvern, U.K.)

3.4 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) help in the confirmation of morphology of nanoparticles. For SEM analysis, few millilitres of zinc oxide nanoparticles were placed in aluminum stubs and then coated with platinum. The scanning electron microscopic images were taken at an acceleration voltage of 15 kV using JEOL Model JSM—6390LV (Akishima, Tokyo, Japan).

3.5 Transmission electron microscopy (TEM)

Size and shape of zinc oxide nanoparticles were measured by TEM using Jeol/JEM 2100 (Akishima, Tokyo, Japan) at an operating voltage of 200 kV.

3.6 Antimicrobial activity

The antimicrobial activity of synthesized zinc oxide nanoparticles were evaluated against pathogenic bacteria and fungi as per agar well diffusion method given by Salar and Suchitra [27]. Each bacterial and fungal strain was swabbed uniformly onto the individual plates using sterile cotton swabs and wells of 8 mm diameter were made on agar plates using gel puncture. Penicillin and ampicilin were used as control. Penicillin was used for all bacteria and fungi except *K. pneumoniae*, where ampicillin was used. The concentration of samples (penicillin, ampicillin, ZnONPs and antibiotic + ZnONPs) used in wells was kept at 1 mg/ml; 50 µl of penicillin and ampicillin and zinc oxide nanoparticles was inoculated in the different wells in a agar plate. A combined formulation of penicillin and ampicillin (25 µl) and zinc oxide nanoparticles (25 µl) was also added in the wells to assess the antimicrobial effect of nanoparticles. The plates were allowed to remain undisturbed for 1 h to ensure even diffusion of samples into agar. The plates were incubated at 37 °C for 18–24 h for bacteria and at 30 °C for 48 h for fungi. At the end of incubation, a zone of inhibition formed around the wells was measured with the help of antibiotic measurement scale and expressed in millimeters. Negative growth zones were measured only after 24 h to avoid misleading results. All experiments were performed in triplicates.

4 Results and discussion

4.1 Synthesis of zinc oxide nanoparticles

Synthesis of zinc oxide nanoparticles from aqueous *A. vera* peel extract was confirmed by visual observation. When colourless zinc suphate was mixed with greenish peel extract, a yellowish-white suspension was produced, which confirmed the synthesis of zinc oxide nanoparticles. Our results were in accordance with previously published reports. Earlier, Poovizhi and Krishnaveni [20] also observed deep yellow colour while synthesizing zinc oxide

nanoparticles from the leaves of *C. procera*. In another study, Mishra and Sharma [15] observed yellow colour while synthesizing zinc oxide nanoparticles from *Punica granatum* peel. Similarly, Shekhawat et al. [33] observed a change in the colour to pale yellow, while synthesizing zinc oxide nanoparticles from leaf, stem and root extracts of *Hybanthus enneaspermus*.

4.2 Characterization of zinc oxide nanoparticles

4.2.1 UV–Visible spectroscopy

The synthesis of zinc oxide nanoparticles was confirmed by UV–Vis spectroscopy, when the colour of zinc sulphate changed from colourless to yellowish-white suspension as discussed in the previous section, due to excitation of the surface plasma vibrations. As shown in Fig. 1a, initially at 0 min, a number of peaks were observed from 200 to 300 nm with maximum absorbance at 240 nm. After 24 h, the intensity of peaks gets increased, with maximum absorbance at 240 nm (Fig. 1b). This confirmed that even after 24 h, synthesis of zinc oxide naoparticles occurred. In earlier study, Rao et al. [22] synthesized zinc nanorods from *A. vera*, which showed a broad absorption peak at 378 nm. Similarly, Divya et al. [7] synthesized zinc oxide nanoparticles from *Hibiscus rosa-sinensis* and observed UV–Visible absorption spectra from 358 to 375 nm due to surface plasmon resonance. Shekhawat et al. [33] synthesized zinc oxide nanoparticles from *H. enneaspermus* and observed absorption peak at 300 nm from leaf extract, stem extract at 290 nm and the root extract at 288 nm. The difference in the maximum absorption from our study might be due to different methods used for synthesis of zinc oxide nanoparticles.

4.2.2 Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy help in the identification of the biomolecules in plant extracts, which play a crucial role in the processes of reduction and stabilization of the nanoparticles [31]. As shown in Fig. 1c, a sharp absorption peak was observed at 3369.34 due to O–H stretching vibration. The absorption peaks at 2136.52 cm^{-1} (CC stretching vibration), 1626.42 cm^{-1} (C= stretching vibration), 1416.48 cm^{-1} (C–H deformation vibration), 1155.47 cm^{-1} (CH_3 deformation vibration), 1077.46 cm^{-1} (C–C stretching vibration), 1044.45 cm^{-1}, 701.50 cm^{-1} (CH_2 deformation vibration) and 472.52 cm^{-1} (C–C skeleton vibration) were also observed, which indicated different functional groups involved in the synthesis of zinc oxide nanoparticles. Earlier, Azizi et al. [5] synthesized zinc oxide nanoparticles from brown marine macroalga *Sargasso muticum* and reported FTIR peaks at 3349 cm^{-1} and 2925 cm^{-1} due to stretching vibrations of the primary amine, OH stretching of alcohols and CH stretching vibrations of alkanes. Poovizhi and Krishnaveni [20] observed FTIR peaks of zinc oxide nanoparticles from *C. procera* near 621.08 to 692.44 cm^{-1}, which was attributed to the Zn–O stretching mode. Broad IR bands at 692.44 cm^{-1}, 952.84 cm^{-1}, 1022.27 cm^{-1}, 1411.89 cm^{-1}, 1442.75 cm^{-1}, 1562.34 cm^{-1}, 3116.97 cm^{-1} indicated the presence of the hydroxyl group, an aromatic group, amine group, saturated primary alcohol and carbonate group.

4.2.3 X-ray diffraction (XRD)

X-ray diffraction was used to confirm phase of zinc oxide nanoparticles. XRD diffractogram of synthesized zinc oxide nanoparticles is shown in Fig. 1d. Two peaks were observed in diffractogram at 33.2542 and 59.0524 (2θ) with Miller indices value of 100, 111, respectively. These strong peaks indicated the crystalline nature of zinc oxide nanoparticles. Miller indices values indicated face centered cubic symmetry of synthesized zinc oxide nanoparticles. In previous study, Rajiv et al. [21] synthesized zinc oxide nanoparticles from leaf extract of *P. hysterophorus* L. and reported Miller indices values at (100), (002), (101), (102), (110), (112) and (202), which also confirmed the crystalline nature of nanoparticles. Gnanasangeetha and Thambavani [10] obtained XRD peaks of zinc oxide nanoparticles synthesized from *Azadirachita indica* and *Emblica Officinalis* at (100), (002), (101), (102), (110), (103), (200), (112) and (201) planes, which confirmed hexagonal phase of nanoparticles. In another study, Jayarambabu and Siva Kumari [11] reported crystalline nature of zinc oxide nanoparticles from green crops with XRD peaks and Miller indices values at 31.7° (100), 34.5° (002), 36.2° (101), 47.7° (102), 56.6° (110), 62.2° (103) and 68.4° (112). Devi and Gayathri [6] synthesized zinc oxide nanoparticles from *H. rosa-sinensis* and reported XRD peaks at 2θ value ranging from 31.73°, 34.38°, 36.22°, 47.50°, 56.56°, 62.81°, 66.34°, 67.91°, 69.03°, 72.6° and 76.90°, indicated the crystalline nature of zinc oxide nanoparticles.

4.2.4 Scanning electron microscopy (SEM)

Scanning electron microscopic images confirmed the surface morphology of zinc oxide nanoparticles at different magnifications. As shown in Fig. 2a–d, nanoparticles were agglomerated in clusters with rough in appearance. Earlier, Vanathi et al. [37] observed spherical shaped zinc oxide nanoparticles from *Eichhornia crassipes* leaf extract. In another report, Divya et al. [7] reported spherical and hexagonal shape of zinc oxide nanoparticles synthesized from *H. rosa-sinensis*. Vidya et al. [38] synthesized zinc oxide nanoparticles from *Calotropis gigantean* and

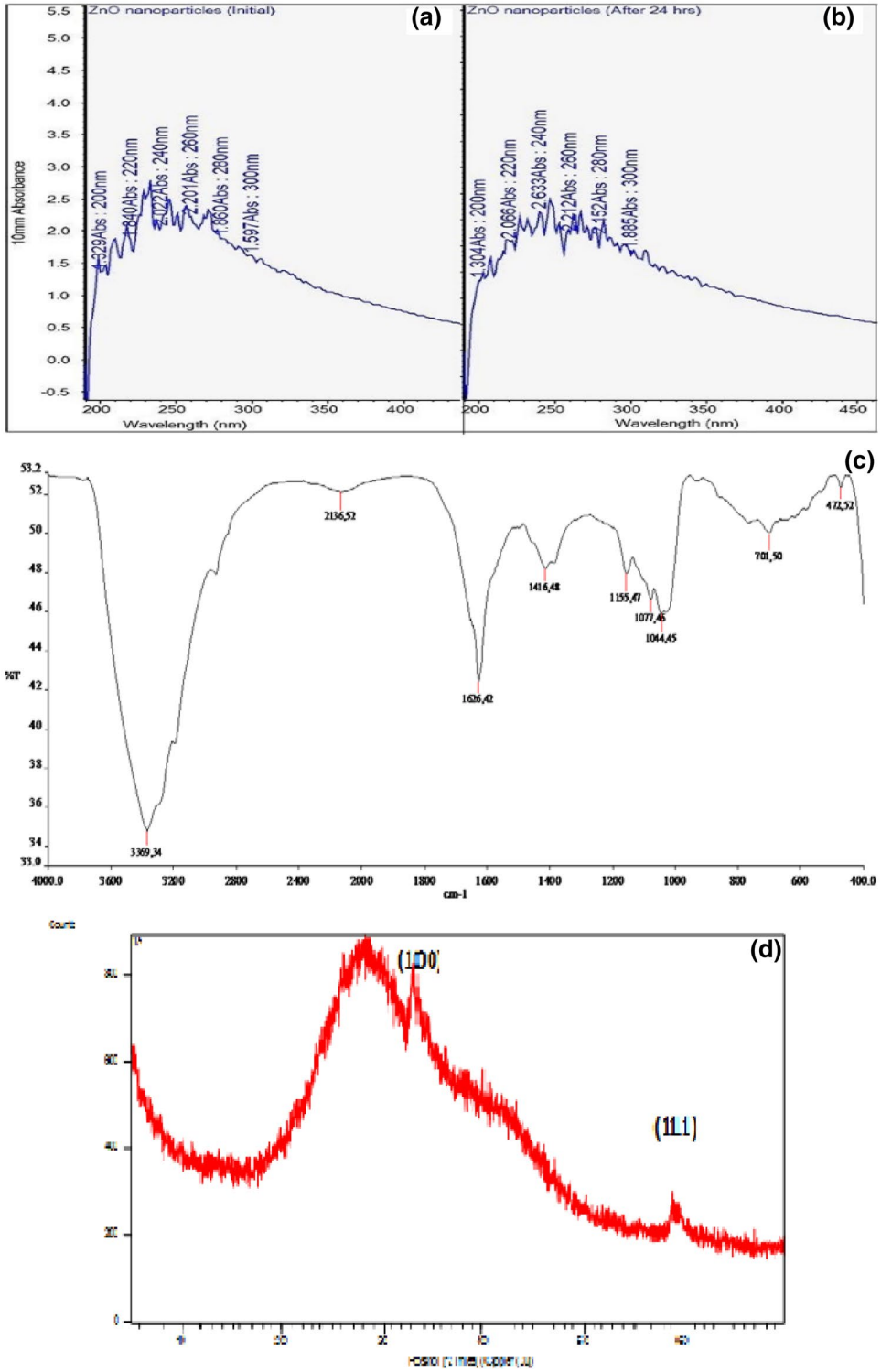

Fig. 1 Zinc oxide nanoparticles showing UV–Vis spectrum absoprtion peaks **a** initial, **b** after 24 h; **c** FTIR spectrum of zinc oxide nanoparticles; **d** XRD peaks confirming crystalline nature

reported spherical shape with diameter range from 11 to 25 nm. Anand Raj and Jayalakshmy [2] reported spherical shape of zinc oxide nanoparticles synthesized from *Zingiber officinale* with the average size from 30 to 50 nm.

4.2.5 Transmission electron microscopy (TEM)

Transmission electron microscopy gives information about particle size and shape of nanoparticles. The zinc oxide nanoparticles synthesized from aqueous peel extract were hexagonal in shape, with different sizes

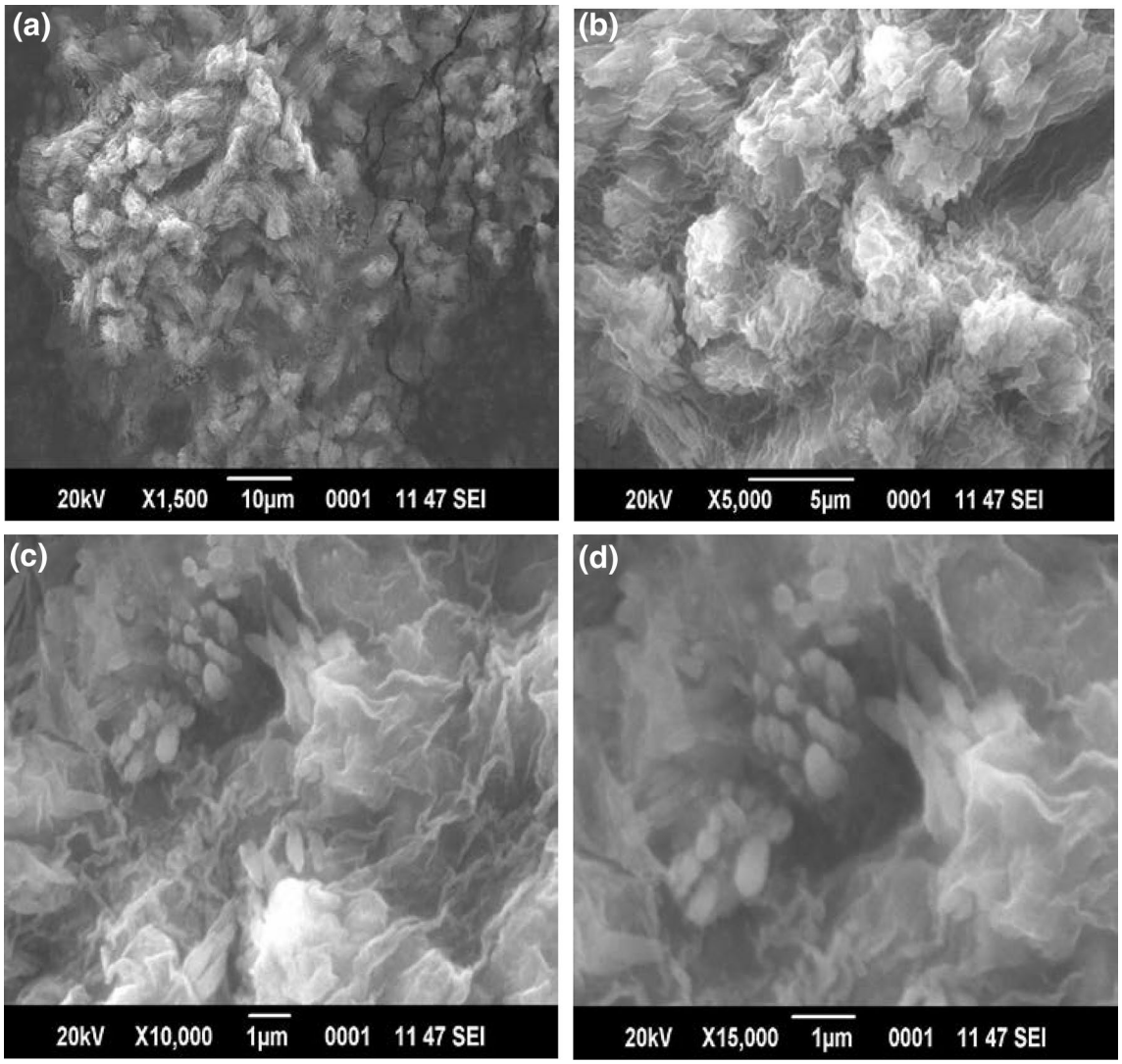

Fig. 2 Agglomerated zinc oxide nanoparticles as visualised through SEM at different magnifications **a** × 1500, **b** × 5000, **c** × 10,000, **d** × 15,000

from 50 to 220 nm (Fig. 3). Earlier, Salam et al. [25] synthesized zinc oxide nanoparticles from *O. basilicum* L. var. purpura scens Benth.-Lamiaceae leaf extract and observed hexagonal (wurtzite) shape with the size than 50 nm.

4.3 Antimicrobial activity

The antibacterial activity of zinc oxide nanoparticles was investigated against Gram-positive (*Staphylococcus epidermidis*, *Staphylococcus aureus*) and Gram-negative (*K. pneumoniae* and *Escherichia coli*) bacteria grown on nutrient agar medium. The antifungal activity was checked against *Aspergillus niger* and *Aspergillus oryzae* grown on Czapek yeast extract agar using the agar well diffusion method. The antibacterial and antifungal activity of zinc oxide nanoparticles, antibiotics (positive control) and antibiotic supplemented with zinc oxide nanoparticles is shown in Tables 3 and 4, respectively. The diameter of zone of inhibition was measured and expressed as millimetres. The results of present investigation revealed the effect of zinc oxide nanoparticles on the antibacterial and antifungal activity in combination with antibiotics. It was observed that zinc oxide nanoparticles did not show any antibacterial effect against Gram-positive bacteria, which might be due to the presence of thick layers of peptidoglycans present on the cell membrane [26]. For Gram-negative bacteria, zinc oxide nanoparticles did not show any effect against *Klebsiella pneumonie*, and a zone of inhibition was observed against *E. coli*. Penicillin showed its effect against *S. epidermidis* and *S. aureus*, but no effect was observed against *E. coli*. Ampicillin showed its effect against *K. pneumoniae*. However, when zinc oxide nanoparticles

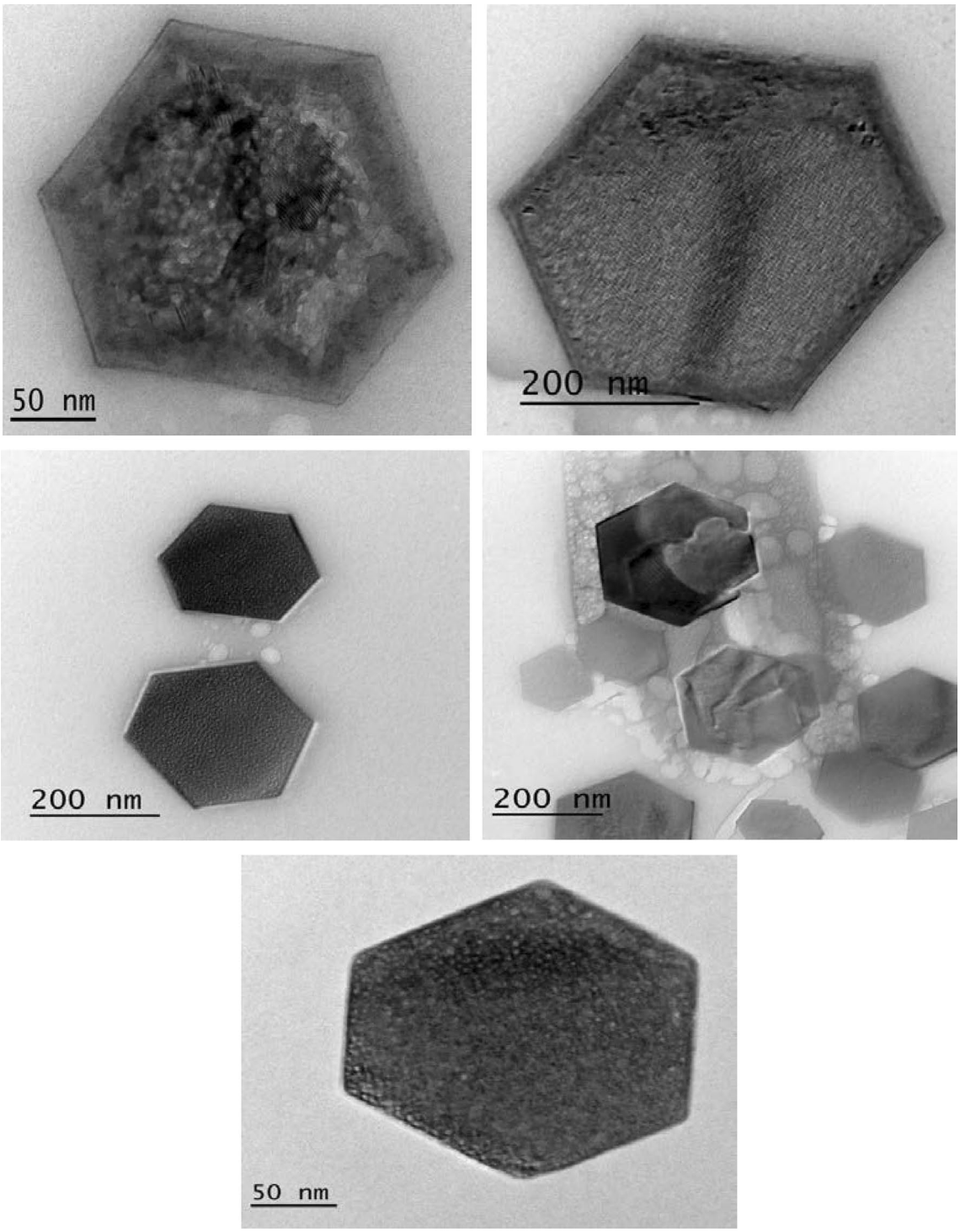

Fig. 3 Hexagonal shapes of zinc oxide nanoparticles as visualized using TEM

were mixed with antibiotics, there was a decrease in the antibacterial activity (Table 3). This may be due to modification in the structure of antibiotics after interaction with zinc oxide nanoparticles. In case of fungi, zinc oxide nanoparticles showed antifungal effect against *A. niger*, whereas, no effect was observed against *A. oryzae*. Similar to bacteria, penicillin showed antifungal effect and there was a decrease in the antifungal activity when zinc oxide nanoparticles were mixed with penicillin (Table 4). From these results, it can be concluded that

Table 3 Zone of inhibition of zinc oxide nanoparticles against bacterial strains

Bacteria	Zone of inhibition (mm)		
	ZnONPs	Antibiotics	Antibiotics + ZnONPs
Staphylococcus aureus	–	28 ± 1.15	24 ± 1.73
Staphylococcus epidermidis	–	22 ± 1.52	17 ± 1.53
Klebsiella pneumoniae	–	23 ± 1.53	17 ± 0.58
Escherichia coli	14 ± 0.58	–	10 ± 1.54

± Standard deviation

ZnONPs Zinc oxide nanoparticles

Table 4 Zone of inhibition of zinc oxide nanoparticles against fungal strains

Fungi	Zone of inhibition (mm)		
	ZnONPs	Antibiotics	Antibiotics + ZnONPs
Aspergillus niger	15 ± 1.53	19 ± 0.58	16 ± 0.58
Aspergillu oryzae	–	25 ± 1.15	23 ± 1.53

± Standard deviation

ZnONPs Zinc oxide nanoparticles

zinc oxide nanoparticles affect the structure of atibiotics and diminished its activity. Further, a molecular study is needed to check the effect of zinc oxide nanoparticles on activity of antibiotics.

5 Conclusion

The present study was aimed to synthesize zinc oxide nanoparticles from aqueous peel extract of *A. vera*. Biosynthesis of nanoparticles was confirmed by a change in colour of zinc sulphate from colourless to yellowish-white suspension. Fourier transform infrared spectroscopic spectrum of synthesized zinc oxide nanoparticles showed the fundamental mode of vibration of O–H stretching and deformation, C–H stretching vibration, C=O asymmetric and C = O stretching vibration. XRD diffractogram showed crystalline nature and face centered cubic symmetry of zinc oxide nanoparticles. Scanning electron microscopy (SEM) showed agglomerated zinc oxide nanoparticles with rough surface. TEM image confirmed hexagonal shape and and size of nanoparticles from 50 to 220 nm. Antimicrobial activity of zinc oxide nanoparticles, antibiotics (penicillin, ampicillin) and zinc oxide nanoparticles + antibiotics was checked against pathogenic bacteria and fungi. Results showed that zinc oxide nanoparticls had antibacterial effect against *E. coli* and antifungal effect was observed for *A. niger*. However, zinc oxide nanoparticles in combination with antibiotics showed lesser effect as compared to antibiotics. Hence, a molecular research is needed to study the effect of zinc oxide nanoparticles on activity of antibiotics.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animals rights No human participant and animal were involved in this study.

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