

Research article

Antibacterial activity and characterization of ZnO and Ag-doped ZnO nanoparticles fabricated using *Leucophyllum frutescens* leaf extract

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Abstract

Globally, there has been considerable interest in the environmentally benign production of transition-metal nanoparticles. This study describes the synthesis of ZnO and Ag-doped ZnO nanoparticles using the leaf extract of *Leucophyllum frutescens* via a simple green method. The synthesized nanoparticles were characterized by Ultraviolet-Visible (UV-Vis) spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, X-ray diffraction (XRD), Transmission electron microscopy (TEM), and scanning electron microscopy (SEM). TEM analysis showed that the crystallites were spherical and polyhedral, highly agglomerated, and had interplanar diameters ranging from 0.2005 to 0.2625 nm. The antimicrobial efficacy of the developed ZnO and Ag/ZnO nanoparticles at concentrations of 3%, 5%, and 7% was evaluated against human pathogens, including *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*. The synthesized ZnO and Ag-doped ZnO nanoparticles showed a characteristic absorption band in the UV region, confirming successful formation of ZnO-based semiconductor nanostructures and supporting their antibacterial activity against the tested pathogens. The results revealed that at 7% Ag-doping, Ag-doped ZnO nanoparticles exhibited greater antibacterial efficacy than pure ZnO nanoparticles, with the largest inhibition zones observed against *S. aureus* (11 ± 3.0 mm) and *B. cereus* (12.45 ± 0.57 mm). Overall, this plant-mediated synthesis of Ag-doped ZnO nanoparticles offers a promising route for developing enhanced antibacterial agents and highlights a green, cost-effective, and sustainable method for producing ZnO and Ag-doped ZnO nanoparticles, with various applications for future exploration.

Keywords: Ag-doped ZnO, Antibacterial activity, Green synthesis, *Leucophyllum frutescens*, ZnO nanoparticles

Background

Nanoparticles (NPs) have distinct properties, including a high surface-to-volume ratio that depends on the decreasing particle size, distribution, and morphology [1]. This property makes NPs better suited to high-performance applications (e.g., photocatalysis, gas sensors, energy storage, antibacterial activity, and environmental remediation), while also promoting the introduction of NPs into a wide variety of commercial, biotechnological, and biomedical processes [2, 3]. Ultimately, advances in nanotechnology have benefited human society by enabling the synthesis of novel nanoparticles with a wide range of applications [4]. Nanotechnology has emerged as a major platform for developing advanced antimicrobial materials because of its tunable surface chemistry, high surface-to-volume ratio, and interactions with microbial membranes. The potential for a multifaceted mode of action, including disruption of the cell membrane, ion release, and oxidative stress, has led to interest in metal and metal-oxide nanoparticles, such as zinc oxide (ZnO) [5]. The use of

nanoparticles has recently been reviewed as a potential alternative for addressing antimicrobial resistance [6].

ZnO nanoparticles synthesized using acorn fruit extract exhibited a significant, dose-dependent zone of inhibition against β -lactamase-producing *Escherichia coli* (*E. coli*), outperforming amoxicillin under similar conditions and supporting the therapeutic potential of metal-oxide nanoparticles as promising alternatives to classical antibiotics for treating multidrug-resistant bacteria [7]. ZnO nanoparticles possess several beneficial properties, including low toxicity, biocompatibility, and biodegradability, as well as high photostability, thermodynamic stability, and a high electrochemical coupling coefficient [8, 9]. These nanoparticles have a wide range of applications, including Ultraviolet-Visible (UV-Vis) light-emitting devices, photocatalysts, sensors, and use in pharmaceuticals and cosmetics [10]. ZnO nanoparticles can be synthesized via physical, chemical, and biological routes, but green synthesis using plants is increasingly preferred for its simplicity, economic viability, eco-friendliness, and non-toxicity. Plant extracts contain phytochemicals that act as reducing, stabilizing,

and capping agents simultaneously, making them suitable for nanoparticle synthesis [11].

Green synthesis of NPs has gained significant popularity in recent years for producing doped ZnO NPs because it yields NPs with superior morphological, photochemical, photocatalytic, and electrochemical characteristics compared to physicochemical methods [12]. Because plant extracts are inexpensive, easy to use, non-toxic, and eco-friendly, they are a more promising tool for the simple green production of metal oxide and doped metal oxide nanoparticles [13]. Consequently, green synthesis methods provide a sustainable way to generate metallic nanoparticles while maintaining a simple, eco-friendly, low-cost, and repeatable procedure [14].

The antibacterial activity of silver (Ag) and ZnO is well established [15], but limited research has examined the effectiveness of Ag-doped ZnO. Some studies suggest that Ag-doped ZnO nanoparticles exhibit greater antibacterial activity than pure ZnO [16]. These NPs have been biosynthesized using various plant extracts, including *José Persica* for antioxidant properties [17], *Moringa oleifera* for antimicrobial potential [18], and *Azadirachta indica* for antibacterial, antidiabetic, and wound-healing effects [19]. Recently, phytosynthesized silver nanoparticles produced using the extract of *Azadirachta indica* seed have been evaluated for their antidiabetic activity using a variety of complementary *in vitro*, *in vivo*, and *in silico* methods, reinforcing the pharmacological versatility provided by phytochemical-mediated synthesis of nanoparticles and *Hibiscus rosa-sinensis* to enhance structural, optical, thermal, and antibacterial characteristics [20]. The plant used in these studies, *Leucophyllum frutescens* (*L. frutescens*), possesses notable medicinal qualities, making it suitable for a diverse range of applications.

Ag-doped ZnO-NPs synthesized by a green method using *L. frutescens* leaf extract as a reducing agent against human pathogens such as *Staphylococcus* (*S.*) *aureus*, *Bacillus* (*B.*) *cereus*, *E. coli*, and *Salmonella* (*S.*) *typhi* have not yet been reported. Therefore, the present study utilized *L. frutescens* leaf extract to synthesize ZnO-NPs and Ag-doped ZnO-NPs. This green approach is rapid, eco-friendly, and cost-effective compared with chemical or physical methods of NPs synthesis. The present study was designed to synthesize and characterize ZnO and Ag-doped ZnO nanoparticles using *L. frutescens* leaf extract and to elucidate their structural, morphological, and optical properties using a suite of spectroscopic and microscopic techniques. Two Gram-positive bacteria (*S. aureus* and *B. cereus*) and two Gram-negative bacteria (*E. coli* and *S. typhi*) were selected to evaluate the antibacterial performance of the synthesized nanomaterials. These pathogens are clinically and

epidemiologically important and are frequently used in antimicrobial studies. Furthermore, the study aimed to systematically evaluate and compare the antimicrobial efficacy of these green-synthesized nanoparticles against selected human-pathogenic bacteria across different dopant concentrations, thereby assessing their potential as enhanced antibacterial agents.

Materials and methods

Preparation of leaf extract of *L. frutescens* and phytochemical screening

Fresh green leaves of *L. frutescens* were collected from New Bolan Nursery, Dera Ghazi Khan, Pakistan. The plant material was taxonomically authenticated by a botanist at the Department of Botany, Ghazi University, Dera Ghazi Khan. The leaves of *L. frutescens* were air-dried under ambient conditions and then oven-dried at 100°C for 12 h to ensure that all moisture had been removed and that nothing would clog during grinding, as described in the previously reported method [21]. The dried leaves were ground into a fine powder using a laboratory grinder. For aqueous extraction, 10 g of the powdered leaf material was accurately weighed and transferred to a beaker containing 250 mL of distilled water. The mixture was heated in a thermostatically controlled water bath at 80°C for 30 min with intermittent stirring to enhance extraction efficiency. After heating, the extract was allowed to cool to room temperature and was then filtered through Whatman No. 1 filter paper. The resulting filtrate was collected and stored at 4°C for further analysis. Qualitative phytochemical screening of the aqueous crude extract was performed to qualitatively assess the presence of major classes of secondary metabolites, including flavonoids, saponins, alkaloids, carbohydrates, steroids, phenolic compounds, and tannins. Standard phytochemical procedures described in the literature were followed for all assays as previously described [22]. Plant collection complied with institutional and national guidelines, and permission to collect *L. frutescens* leaves from New Bolan Nursery was obtained; no protected or endangered species were involved.

Green synthesis of zinc oxide nanoparticles (ZnO-NPs)

This study used the green synthesis technique reported in a previous study [23], with some modifications. Briefly, 25 mL of *L. frutescens* leaf extract was gradually introduced into 200 mL of 0.1 M zinc acetate solution ($\text{Zn}(\text{CH}_3\text{CO}_2)_2$) under continuous stirring. The reaction mixture was stirred for 2 h, during which a dark green coloration was observed, indicating nanoparticle formation. The mixture was then centrifuged at 8,000 rpm for 20 min to obtain the pellet. After centrifugation, the supernatant was removed, and the pellet was repeatedly washed with distilled water,

then methanol, to remove unreacted constituents and impurities. The purified precipitates were dried in an oven at 100°C for 12 h and subsequently calcined at 900°C to obtain ZnO nanoparticles. This high calcination temperature was selected to ensure complete removal of organic residues from the plant extract and to promote the formation of phase-pure, highly crystalline ZnO, in line with previous plant-mediated ZnO synthesis protocols. The synthesized ZnO-NPs were further characterized and evaluated for antimicrobial activity.

Green synthesis of silver-doped zinc oxide nanoparticles (Ag-doped ZnO-NPs)

Ag-doped ZnO-NPs were synthesized using a green chemistry approach, following the same method used to prepare ZnO-NPs, by adding a silver nitrate (AgNO_3) solution with minor modifications [24, 25]. Briefly, 200 mL of 0.1 M zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{CO}_2)_2$) was

mixed with 25 mL of an aqueous *L. frutescens* leaf extract under continuous stirring. AgNO_3 was then added at varying concentrations (3%, 5%, and 7%) to achieve controlled silver doping. These percentages represent the molar fraction of Ag^+ relative to the total metal cations in the reaction mixture, as shown in Table 1. The reaction mixtures were stirred for two hours at room temperature, during which a visible color change from pale green to dark brownish gray was observed, indicating the formation of Ag-doped ZnO-NPs. The resulting precipitates were collected by centrifugation at 8,000 rpm for 20 min, then washed successively with distilled water and methanol to remove unreacted residues and impurities. The purified nanoparticles were oven-dried and finely ground into powder for subsequent characterization and antibacterial evaluation. A schematic representation of the green synthesis process for ZnO-NPs and Ag-doped ZnO-NPs is illustrated in Figure 1.

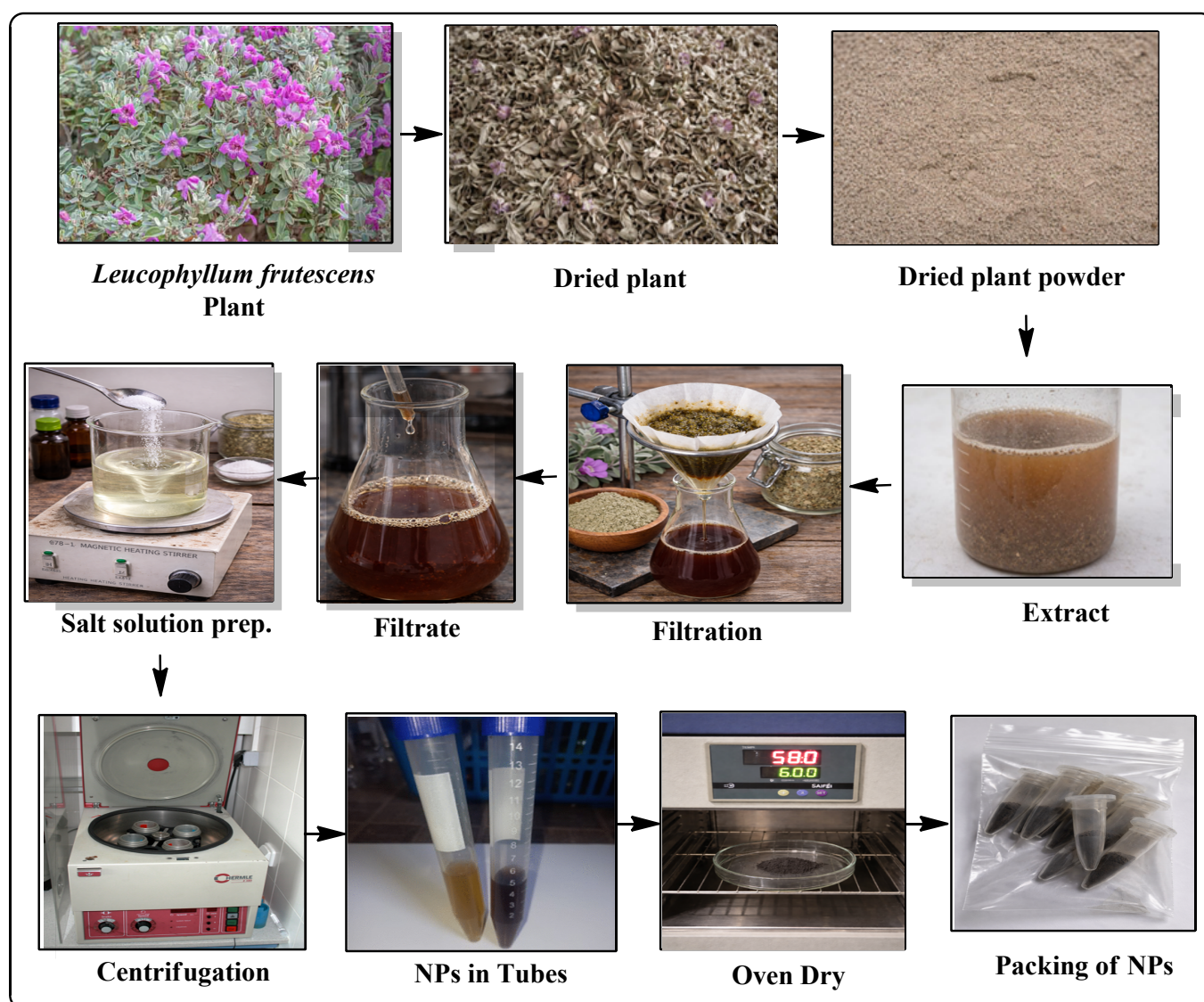


Figure 1: A schematic representation of the green synthesis process of ZnO-NPs and Ag-doped ZnO-NPs.

Table 1: Different compositions of Ag-doped ZnO nanoparticles.

Sample	Amount of AgNO ₃	Amount of (Zn(CH ₃ CO ₂) ₂)
3% Ag/ZnO-NPs	0.236 g	13.081 g
5% Ag/ZnO-NPs	0.393 g	12.812 g
7% Ag/ZnO-NPs	0.551 g	12.542 g

Characterization of ZnO-NPs and Ag-doped ZnO-NPs

ZnO-NPs and Ag-doped ZnO-NPs were characterized using various analytical techniques. Fourier Transform Infrared (FTIR) spectroscopy with Attenuated Total Reflectance (ATR) (Perkin-Elmer Spectrum BX, Waltham, Massachusetts, USA) was used, along with Scanning Electron Microscopy (SEM) (TESCAN VEGA) equipped with Bruker Quantax Compact and XFlash 600Mini. Ultraviolet-Visible (UV-Vis) spectroscopy (UV-1900i Fiber Optic, Shimadzu) and X-ray Diffractometer (XRD) (Rigaku SmartLab, Tokyo, Japan) were employed. For FTIR, samples dissolved in distilled water were placed on the ATR plate, and spectra were recorded over 400–4,000 cm⁻¹ to identify functional groups in both the plant extract and the green-synthesized nanoparticles. UV-Vis spectra, obtained by scanning from 200 to 800 nm using glass cuvettes, confirmed nanoparticle formation. SEM analysis of particle size was performed at 10,000X magnification, with 15 kV acceleration and a 15 mm working distance. About half of a small spatula was dissolved in deionized water for the TEM measurement. The sample was placed in an ultrasonic bath for 30 min to ensure that the crystallites were evenly dispersed. The resulting liquid was then placed on the TEM grid and heated in an oven for 1 h at 80°C. TEM measurements were performed at 200 kV using a field-emission electron gun (FEG). XRD analysis, using Cu Kα radiation at a scan rate of 2°/min over 20°–80°C, determined the sample purity and phase characteristics as previously described [26].

Antibacterial activity

The antibacterial activity of the synthesized ZnO and Ag-doped ZnO nanoparticles was evaluated against both Gram-positive bacteria (*S. aureus* and *B. cereus*) and Gram-negative bacteria (*E. coli* and *S. typhi*) using the Kirby-Bauer disc diffusion assay [27]. 15–20 mL of nutritional agar medium was prepared, sterilized, and then added to the Petri plates, where it was allowed to solidify. Once the nutritional agar medium solidified, each bacterial strain was uniformly transferred to a different agar plate using a sterile swab. ZnO and Ag-doped ZnO nanoparticles (3%, 5%, and 7%) were prepared as colloidal solutions at 50 mg/mL. Sterile 6-mm absorbent discs were saturated with nanoparticle suspensions and placed onto inoculated agar plates. Gentamicin discs served as the positive control, and DMSO served as the negative control.

Plates were incubated for 24 h at 37 °C to allow bacterial growth and nanoparticle interaction. Antibacterial activity was quantified by measuring the zones of inhibition (ZOI) surrounding the discs. The presence and size of these inhibition zones indicated the effectiveness of ZnO and Ag-doped ZnO nanoparticles against the tested bacterial strains, with Ag doping expected to enhance antimicrobial efficacy compared to undoped ZnO. All antibacterial tests were performed in triplicate for each bacterial strain and nanoparticle formulation. Zones of inhibition were measured using a transparent ruler and are reported as mean ± standard deviation (n = 3). Statistical analysis was carried out using one-way ANOVA followed by Tukey's post hoc test, and differences were considered significant at *p* < 0.05.

Results

Phytochemical analysis of *L. frutescens*

A phytochemical assay of the aqueous leaf extract of *L. frutescens* was conducted for seven major classes of bioactive compounds, including alkaloids, flavonoids, saponins, carbohydrates, steroids, phenolic compounds, and tannins, following the literature [28]. Phytochemical tests were conducted three times, where the symbol [+] indicates the presence of a phytochemical, and the symbol [–] indicates the absence of phytochemicals (Table 2).

Table 2: Phytochemical profile of *L. frutescens*.

Phytochemical test	Result		
	1	2	3
Alkaloid	-	+	-
Flavonoid	+	+	+
Saponin	+	+	+
Carbohydrate	-	+	-
Steroid	-	-	-
Phenolic Compounds	+	+	+
Tannin	+	+	+

(+) = presence of bioactive compounds in *L. frutescens*, (–) = absence of bioactive compounds in *L. frutescens*.

Ultraviolet-visible spectroscopy (UV-Vis) of ZnO-NPs and Ag-doped ZnO-NPs

The UV–Visible absorption spectra of the synthesized ZnO-NPs and Ag-doped ZnO-NPs showed strong absorption bands at approximately 365 nm and 390–410 nm in the UV region, respectively. These features are characteristic of ZnO-based semiconductor nanostructures and arise from bandgap-related electronic transitions rather than surface plasmon resonance (Figure 2). This confirms the successful formation of ZnO-based nanomaterials and collectively verifies the high purity and uniformity of the synthesized nanoparticles.

Fourier transform infrared spectroscopy (FTIR) of ZnO-NPs and Ag-doped ZnO-NPs

FTIR spectroscopy, employing the ATR technique, was used to identify and characterize the active functional groups and their vibrational modes in the synthesized ZnO-NPs and Ag-doped ZnO-NPs over the spectral range

of 400–4,000 cm^{-1} . The FTIR spectra of ZnO-NPs and Ag/ZnO-NPs (3%, 5%, and 7%) are presented in Figure 3, and Table 3 summarizes the detailed assignment of FTIR absorption peaks to their respective functional groups for ZnO-NPs and Ag-doped ZnO-NPs.

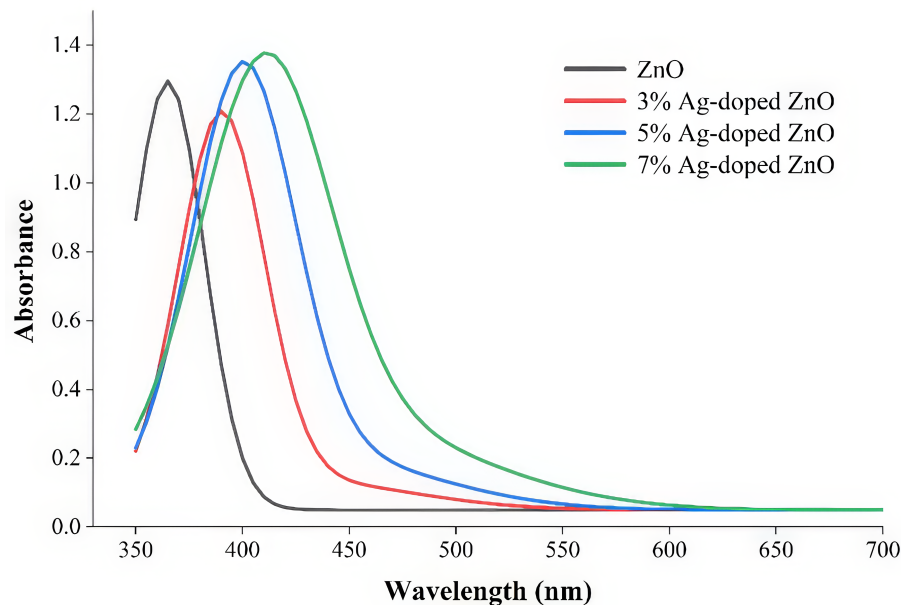


Figure 2: Comparison of UV-Vis spectra of ZnO and (3%, 5%, and 7%) Ag-doped ZnO nanoparticles.

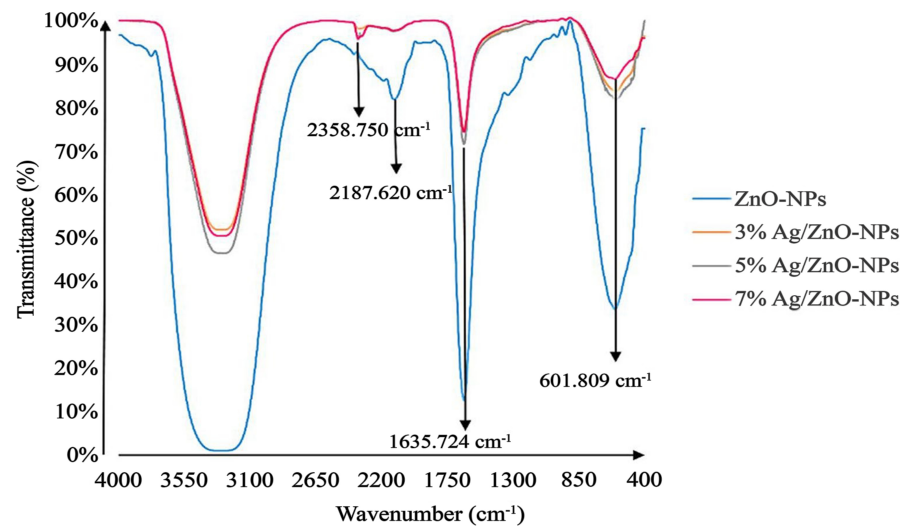


Figure 3: FTIR spectra of ZnO and (3%, 5%, and 7%) Ag-doped ZnO nanoparticles.

Table 3: The peak assignment for FTIR of ZnO-NPs and Ag-doped ZnO-NPs.

Wavenumber (cm^{-1})	Functional group
3,550–3100	O-H stretching mode
2,187.62	C-H stretching mode
2,358.75	Uncommon CO_2 peak from the measurement conditions
1,635.72	C = O stretching mode
601.81	Zn-O stretching mode

O–H = hydroxyl group; C–H = carbon–hydrogen group; C=O = carbonyl group; Zn–O = zinc–oxygen bond; CO_2 = carbon dioxide.

Scanning electron microscopy (SEM)

SEM images of ZnO-NPs and (3%, 5%, and 7%) Ag-doped ZnO-NPs are shown in Figure 4. All the samples displayed irregular, quasi-spherical to ellipsoidal particles with a relatively high degree of aggregation. These agglomerates consist of smaller nanocrystallites, as confirmed by TEM and XRD analyses. Thus, although the primary ZnO and Ag-doped ZnO crystallites are nanoscale, the SEM images highlight their tendency to aggregate into larger secondary particles due to surface energy and calcination conditions.

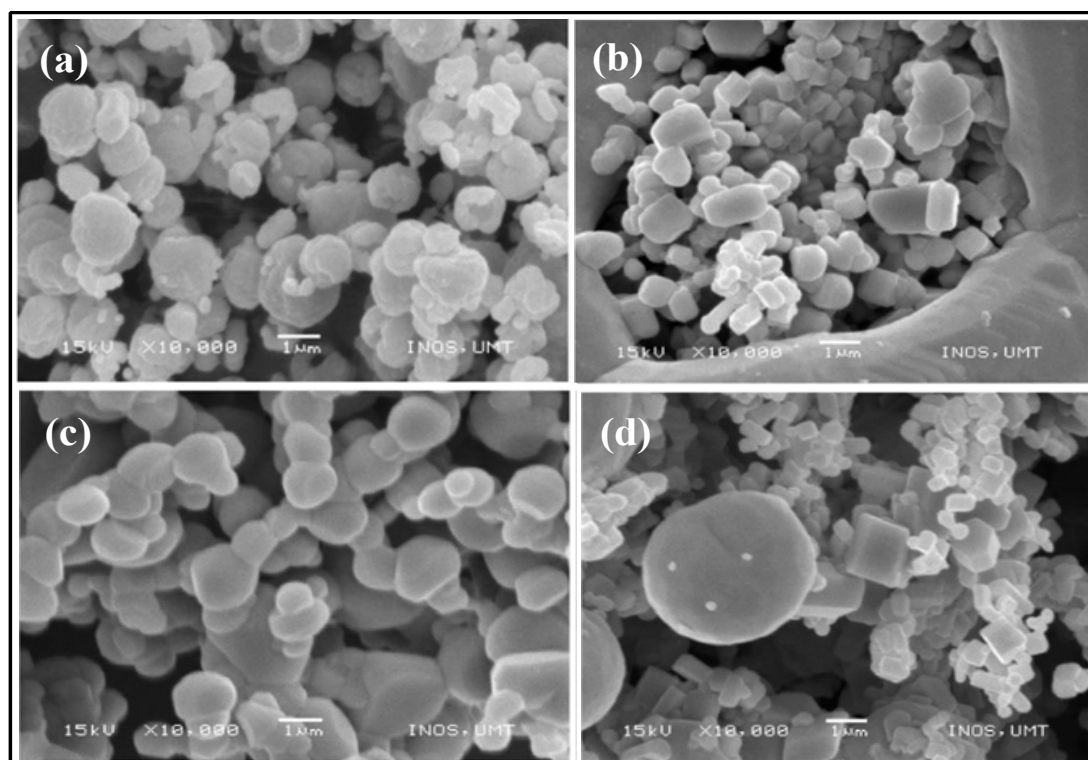


Figure 4: SEM images of (a) ZnO nanoparticles, (b) 3% Ag-doped ZnO nanoparticles, (c) 5% Ag-doped ZnO nanoparticles, and (d) 7% Ag-doped ZnO nanoparticles.

Transmission electron microscopy

TEM images of ZnO-NPs and (3%, 5%, and 7%) Ag-doped ZnO-NPs recorded at different magnifications are shown in Figure 5. A comparative examination of Figure 5 (a, d, g, and j) and Figure 5 (b, e, h, and k) reveals a progressive reduction in crystallite size with increasing Ag concentration in the ZnO lattice. We further investigated the nanoparticles' crystallographic structure and orientation using selected area electron diffraction (SAED). The corresponding SAED patterns, shown as insets in the HRTEM images (Figure 5c, f, i, and l), display intense, spotty fringes, confirming the polycrystalline nature of all samples.

X-Ray diffraction (XRD)

XRD analysis was carried out to investigate the phase composition, crystallinity, and lattice structure of the synthesized ZnO-NPs and (3%, 5%, and 7%) Ag-doped ZnO-NPs. The measurements were performed over the 2θ range of 20° – 80° at a scanning rate of 2° min^{-1} and a step size of 0.02° , as shown in Figure 6. XRD analysis also facilitated the determination of lattice parameters and phase purity. Finally, the XRD results show that all synthesized samples are highly crystalline and phase-pure, with no detectable impurity phases or foreign contaminants, confirming the effectiveness of the green synthesis and silver doping processes. XRD parameters of ZnO nanoparticles before and after Ag-doping are shown in Table 4.

Antibacterial activities

The antibacterial activity of the synthesized pure ZnO-NPs and (3%, 5%, and 7%) Ag-doped ZnO-NPs was examined through the Kirby-Bauer disc diffusion assay against a range of species, such as *S. aureus*, *B. cereus*, *E. coli*, and *S. typhi*, as shown in Figure 7. The results demonstrated that increasing the Ag doping concentration enhanced the antibacterial efficacy, as summarized in Table 5. Nutrient broth was used to keep the bacteria at 37°C as previously recommended [29]. ZnO-NPs proved that (3%, 5%, and 7%) Ag-doped ZnO-NPs showed significant antibacterial effects against the specified species. With respective zones of inhibition of 10.57 ± 0.56 and 11 ± 5.0 mm, 7% Ag-doped ZnO-NPs were demonstrated to be noteworthy in working against *S. typhi* and *E. coli* using the Kirby-Bauer disc diffusion technique. Since Gram-positive bacteria are effective targets, the larger zones of inhibition against *S. aureus* and *B. cereus* by 7% Ag-doped ZnO-NPs (11 ± 3.0 and 12.45 ± 0.57 mm, respectively) suggest their potential as antibacterial agents. One-way ANOVA indicated that Ag doping level significantly affected the zone of inhibition ($p < 0.05$) for all tested bacteria. Tukey's post hoc analysis showed that 7% Ag-doped ZnO-NPs produced significantly larger inhibition zones than pure ZnO-NPs and the lower Ag-doping levels.

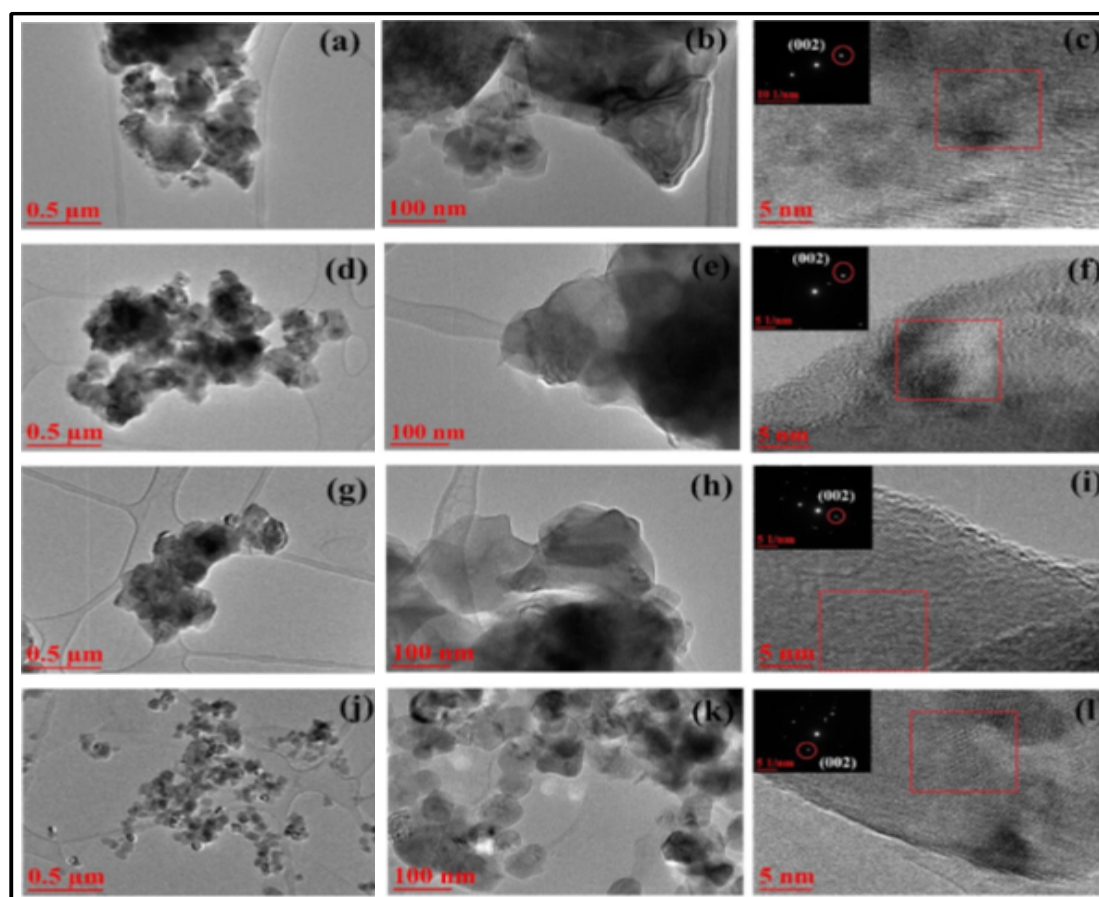


Figure 5: TEM image for the synthesized ZnO-NPs with magnification of (a) 0.5 μm , (b) 100nm, and (c) 5nm; TEM image for the synthesized 3% Ag-doped ZnO-NPs with magnification of (d) 0.5 μm , (e) 100nm, and (f) 5nm; TEM image for the synthesized 5% Ag-doped ZnO-NPs with magnification of (g) 0.5 μm , (h) 100nm, and (i) 5nm; TEM image for the synthesized 7% Ag-doped ZnO-NPs with magnification of (j) 0.5 μm , (k) 100nm, and (l) 5nm.

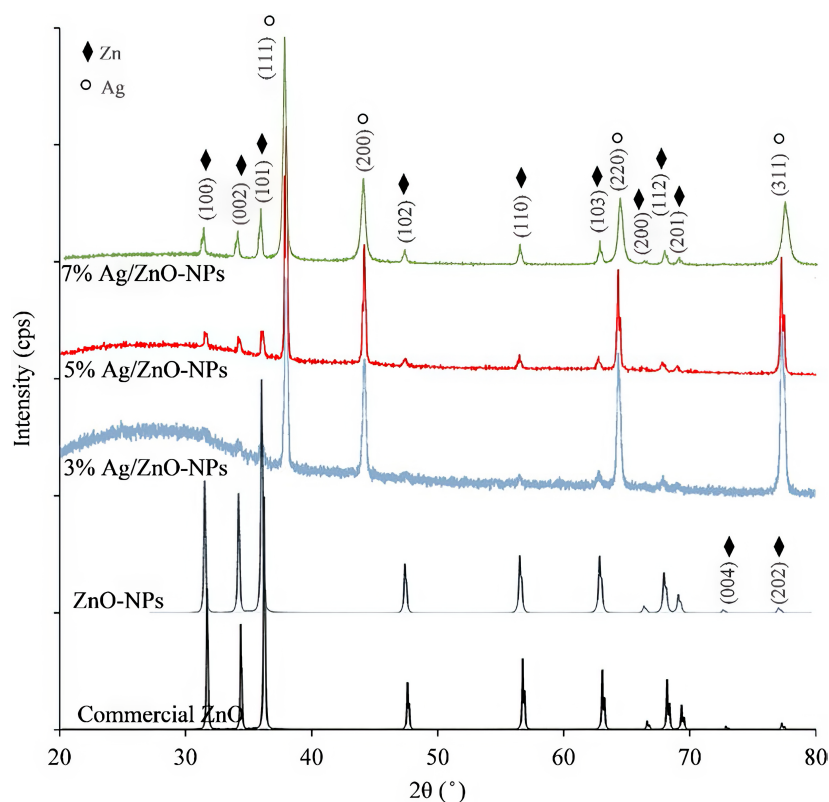


Figure 6: XRD spectra of ZnO and (3%, 5%, and 7%) Ag-doped ZnO nanoparticles.

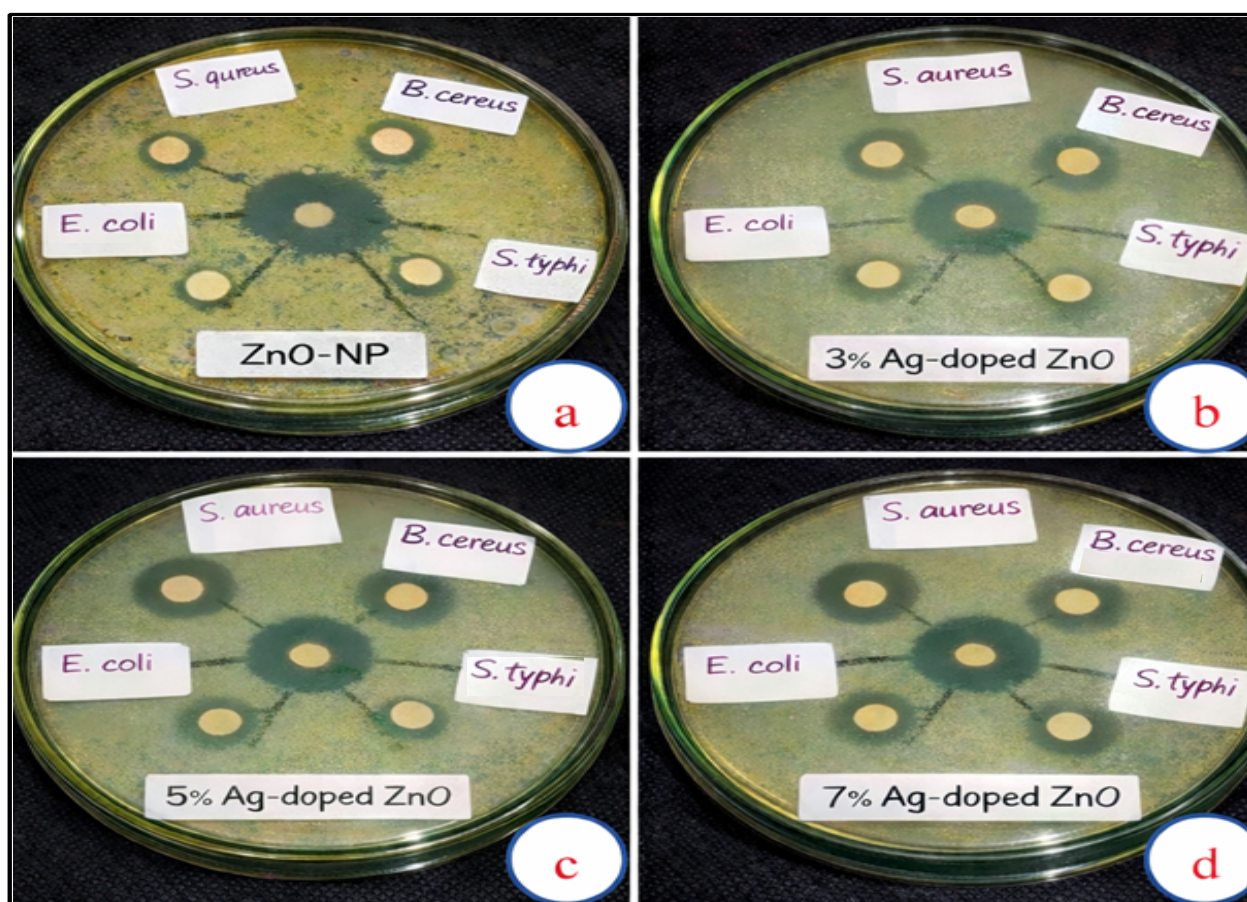


Figure 7: Zone of inhibition of pure ZnO and (3%, 5%, and 7%) Ag-doped ZnO nanoparticles against *S. aureus*, *B. cereus*, *E. coli*, and *S. typhi*.

Table 4: XRD parameters of ZnO nanoparticles before and after Ag-doping.

Material	Plane	Standard 2θ (°)	Experimental 2θ (°)	FWHM (°)	Crystallite size (nm)
Pure ZnO-NPs	(100)	31.77	31.70	0.315	25.92
	(002)	34.42	34.34	0.342	24.10
	(101)	36.25	36.18	0.329	25.40
	(102)	47.54	47.43	0.356	24.05
	(110)	56.60	56.47	0.385	22.70
	(103)	62.86	62.71	0.411	21.80
	(200)	66.38	66.22	0.438	20.85
	(112)	67.96	67.79	0.451	20.35
	(201)	69.10	68.92	0.465	19.90
	(004)	72.58	72.38	0.482	19.35
	(202)	76.98	76.77	0.498	18.90
	(100)	31.77	31.70	0.352	23.20
3% Ag/ZnO-NPs	(002)	34.42	34.34	0.371	22.20
	(101)	36.25	36.18	0.367	22.80
	(102)	47.54	47.43	0.398	21.50
	(110)	56.60	56.47	0.424	20.60
	(103)	62.86	62.71	0.451	19.90
	(200)	66.38	66.22	0.477	19.15
	(112)	67.96	67.79	0.493	18.65
	(201)	69.10	68.92	0.507	18.25
	(004)	72.58	72.38	0.523	17.85
	(202)	76.98	76.77	0.541	17.40
	Ag (111)	38.12	38.00	0.392	21.40
	Ag (200)	44.28	44.17	0.418	20.30
	Ag (220)	64.44	64.25	0.465	19.20
	Ag (311)	77.39	77.15	0.552	17.10

5% Ag/ZnO-NPs	(100)	31.77	31.70	0.381	21.45
	(002)	34.42	34.34	0.405	20.35
	(101)	36.25	36.18	0.424	19.70
	(102)	47.54	47.43	0.451	18.95
	(110)	56.60	56.47	0.478	18.25
	(103)	62.86	62.71	0.506	17.70
	(200)	66.38	66.22	0.532	17.15
	(112)	67.96	67.79	0.549	16.75
	(201)	69.10	68.92	0.565	16.40
	(004)	72.58	72.38	0.582	16.05
	(202)	76.98	76.77	0.601	15.70
	Ag (111)	38.12	38.00	0.451	18.60
	Ag (200)	44.28	44.17	0.477	17.80
	Ag (220)	64.44	64.25	0.523	17.05
	Ag (311)	77.39	77.15	0.612	15.45
7% Ag/ZnO-NPs	(100)	31.77	31.70	0.425	19.25
	(002)	34.42	34.34	0.451	18.30
	(101)	36.25	36.18	0.495	16.90
	(102)	47.54	47.43	0.512	16.70
	(110)	56.60	56.47	0.541	16.10
	(103)	62.86	62.71	0.569	15.75
	(200)	66.38	66.22	0.596	15.30
	(112)	67.96	67.79	0.613	15.00
	(201)	69.10	68.92	0.631	14.70
	(004)	72.58	72.38	0.649	14.40
	(202)	76.98	76.77	0.671	14.05
	Ag (111)	38.12	38.00	0.512	16.40
	Ag (200)	44.28	44.17	0.541	15.70
	Ag (220)	64.44	64.25	0.594	14.95
	Ag (311)	77.39	77.15	0.681	13.85

Table 5: Inhibition zone of ZnO and (3%, 5%, and 7%) Ag-doped ZnO nanoparticles against the selected bacterial strains at a concentration of 50 mg/mL.

Inhibition zone (mm)						
Samples					Positive control	Negative control
Bacteria strain	ZnO-NP	3%Ag-doped ZnO	5%Ag-doped ZnO	7%Ag-doped ZnO	Gentamicin	DMSO
<i>S. aureus</i>	9.0 ± 1.0	10.01 ± 0.47	10.57 ± 0.56	11 ± 3.0	28 ± 0.00	No inhibition zone
<i>B. cereus</i>	8 ± 2.0	9.07 ± 0.37	9.47 ± 0.56	12.45 ± 0.57	25 ± 0.00	No inhibition zone
<i>E. coli</i>	7.33 ± 1.00	8.12 ± 0.33	8.97 ± 0.35	11 ± 5.0	26 ± 0.00	No inhibition zone
<i>S. typhi</i>	7.01 ± 0.57	8.12 ± 0.33	8.97 ± 0.35	10.57 ± 0.56	27 ± 0.00	No inhibition zone

Discussion

The current study describes the synthesis of ZnO and Ag-doped ZnO nanoparticles using the leaf extract of *L. frutescens* and reports their antimicrobial effects. Phytochemical analysis identified four bioactive components as flavonoids, phenolic compounds, saponins, and tannins. These findings were consistent with the reference, except for tannins and alkaloids, which might be related to the solvent extraction technique applied. The tannin and alkaloid tests can differ because the production of phytochemicals is affected by environmental factors, such as geographical features and climatic conditions, and by the procedures used to detect them [22, 30].

The peak absorption in the UV-Vis spectra around 365 nm is consistent with previously reported bandgap-related optical responses of ZnO semiconductor nanoparticles and confirms their successful formation [31]. The maximum absorption of 3%, 5%, and 7% Ag-doped ZnO-NPs was observed around 390 nm, 400 nm, and 410 nm, respectively, which aligns with the existing literature [32, 33]. A red shift in the absorption maxima was observed as the Ag concentration increased. This shift may be attributed to electronic interactions between ZnO and Ag, likely enhanced by Ag's surface plasmon effect. While adding Ag modifies ZnO's optical response, XPS, photoluminescence, and diffuse reflectance analyses are needed to confirm Ag's chemical state, lattice incorporation, and defect structure.

The FTIR spectra exhibited absorption peaks at 3,550–3,100 cm^{-1} (broadband), 2,358.50 cm^{-1} , 2,187.62 cm^{-1} , 1,635.72 cm^{-1} , and 601.81 cm^{-1} . The peaks at 3,550–3,100 cm^{-1} can be assigned to hydroxyl group stretching vibrations observed in all samples [34, 35]. For (3%, 5%, and 7%) Ag-doped ZnO-NPs, a common peak at 2,358.750 cm^{-1} is observed, which can be assigned to an uncommon CO_2 signal that may arise from the measurement conditions, whereas 2,187.62 cm^{-1} corresponds to a C-H stretch mode that may originate from *L. frutescens* leaf extract acting as a stabilizing and capping agent. Moreover, all samples show a sharp peak at 1635.72 cm^{-1} with varying intensities, which can be attributed to the carbonyl (C = O) stretch of phytochemicals in *L. frutescens* leaves, further indicating that the extract acts as a capping agent for ZnO-NPs [34]. A characteristic absorption peak at 601.81 cm^{-1} was observed in all samples, corresponding to the Zn-O stretching vibration, which confirms the formation of crystalline ZnO nanostructures. The presence of this band in both undoped and Ag-doped samples indicates that silver incorporation does not disrupt the ZnO lattice framework. The FTIR absorption peaks corresponding to the functional groups of ZnO-NPs and Ag-doped ZnO-NPs are consistent with those reported in previous studies [34, 35, 36].

The SEM images indicate that all samples formed agglomerated structures [37, 38, 39], rather than individual primary particles, which explains why SEM sizes are larger than the crystallite sizes estimated from XRD and TEM. Particle agglomeration can be attributed to the high surface energy of the nanoparticles during synthesis or thermal treatment. The Ag-doped ZnO nanoparticles exhibited more varied morphology than the pure ZnO nanoparticles, which showed rounder, more compact aggregates. The morphology and size varied with increasing Ag content. This morphological diversity may be related to Ag-induced grain growth and changes in ZnO nucleation.

The TEM observations are consistent with the SEM results, confirming the morphology and nanoscale dimensions of the synthesized crystallites. ZnO-NPs and Ag-doped (3% and 5%) samples exhibit highly agglomerated nanostructures composed of irregular polyhedral-shaped particles. In contrast, the 7% Ag-doped ZnO-NPs display a noticeable morphological evolution, characterized by a mixture of small spherical and polyhedral crystallites within densely agglomerated structures. Current research indicates that Ag incorporation into the ZnO lattice significantly alters nucleation and growth kinetics, inhibiting crystal growth at higher dopant levels. Consequently, the 7% Ag-doped ZnO-NPs, which possess the highest silver content, exhibit the smallest crystallite dimensions. These findings are in good agreement with the work of Kayani *et al.* (2020), who reported a similar decrease in crystallite size with

increasing Ag dopant concentration [40]. Comparable SAED features have been reported previously by Raza *et al.* (2024) for ZnO-based nanostructures [41]. HRTEM analysis revealed interplanar lattice spacings of 0.2497 nm, 0.2005 nm, 0.2194 nm, and 0.2625 nm for ZnO-NPs and (3%, 5%, and 7%) Ag-doped ZnO-NPs, respectively. These lattice spacings correspond to the (002) crystallographic plane, confirming the preservation of ZnO's hexagonal wurtzite structure and agreeing well with the XRD results.

The XRD diffractograms of all samples show well-defined, sharp diffraction peaks, indicating their highly crystalline nature. ZnO-NPs display eleven characteristic diffraction peaks at 2θ values of 31.70°, 34.34°, 36.18°, 47.43°, 56.47°, 62.71°, 66.22°, 67.79°, 68.92°, 72.38°, and 76.77°, corresponding to the (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202) lattice planes, respectively. These reflections match the standard diffraction data for hexagonal ZnO (JCPDS card No. 00-062-0414), confirming the formation of a polycrystalline hexagonal wurtzite crystal structure. The presence of prominent planes such as (100), (002), (101), (102), (110), (103), (200), (112), and (201) further supports this structural assignment and agrees with previously reported studies [42]. The hexagonal wurtzite phase of ZnO was retained even after incorporation of Ag. All the Ag-doped ZnO nanoparticles were composed of two distinct phases: the hexagonal wurtzite phase of ZnO and the face-centered cubic phase of Ag as a secondary phase. Additional peaks in the three diffractograms for the (3%, 5%, and 7%) Ag-doped ZnO-NPs appear at 2θ values of 38.00°, 44.17°, 64.25°, and 77.15°, corresponding to the crystal lattice planes (111), (200), (220), and (311), respectively. Earlier studies on Ag-doped zinc oxide nanoparticles reported similar diffraction features, confirming the formation of Ag-doped ZnO-NPs [37]. Although the additional XRD peaks support the presence of Ag in the doped samples, elemental analysis (e.g., EDX/EDS) was not performed and should be included in future studies to directly confirm Ag content and distribution.

Ag-doped ZnO-NPs synthesized through a straightforward green synthesis approach can be useful for studying antibacterial activity. The nanomaterials were generally more effective against Gram-positive bacteria than Gram-negative bacteria, except for 7% Ag/ZnO-NPs, which exhibited notable activity against both *S. aureus* and *E. coli*. This difference is attributed to structural variations between Gram-positive and Gram-negative bacteria. Gram-negative bacteria possess a thin cell wall surrounded by an outer membrane, which often acts as a barrier to certain drugs and nanoparticles, preventing their penetration into the cell. In contrast, Gram-positive bacteria have a thick peptidoglycan layer and a cytoplasmic membrane, making them more susceptible to

antibacterial agents [17, 43]. ZnO-NPs alone showed limited inhibition of *S. aureus* and *B. cereus*, while (3%, 5%) Ag-doped ZnO-NPs had minimal effects on the growth of *E. coli*, *S. typhi*, and *S. aureus*. In the present study, the zone of inhibition against *E. coli* was 7.33 ± 1.00 mm for pure ZnO-NPs and 11 ± 5.0 mm for 7% Ag-doped ZnO-NPs, representing almost a 50% improvement with increased Ag content. This increasing trend agrees with Umar et al. (2026), which reported that acorn-fruit-derived ZnO nanoparticles exhibited a similar concentration-dependent increase in the inhibition zone against *E. coli*, 15 ± 02 mm at $10 \mu\text{g/mL}$ and 24 ± 05 mm at $80 \mu\text{g/mL}$, better than amoxicillin at a similar concentration [7]. The inhibition zones generated in the present study were comparatively weaker against *E. coli* than those obtained by Umar et al. (2026), whereby *L. frutescens*-mediated Ag-doped ZnO-NPs also showed an increasing trend but with comparatively lesser efficacy against *E. coli* [7].

Both ZnO and Ag-doped ZnO-NPs have been shown in the literature to exhibit cytotoxic effects on bacteria by disrupting membrane integrity, interfering with protein function, and inducing oxidative stress. In the present study, we did not directly quantify reactive oxygen species ROS; therefore, we infer the proposed ROS-based mechanisms from published work rather than direct experimental measurements [13]. If hybrid nanoparticles are doped with silver, they might perform better than a single material on their own. In this research, zinc precursors were used with the leaf extract of *L. frutescens* to form Ag-doped ZnO-NPs (3%, 5%, and 7%), which showed greater antibacterial activity than pure ZnO nanoparticles. This enhanced efficacy is attributed to synergistic interactions among metal oxide nanoparticles within the hybrid system. Overall, the results indicate that the synthesized nanoparticles are more effective against Gram-positive than Gram-negative bacteria. The present study is limited to disc diffusion assays; minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were not determined and should be addressed in future work to provide more quantitative antibacterial potency.

Conclusions

This study successfully synthesized ZnO-NPs and Ag-doped ZnO-NPs using *L. frutescens* leaf extract as a reducing, stabilizing, and capping agent. The characterization results confirmed the formation of crystalline ZnO-based nanostructures, while XRD and TEM revealed that Ag incorporation affected the crystallite size and preserved the hexagonal wurtzite structure. FTIR analysis confirmed Zn–O vibrations and phytochemical-derived functional groups, indicating the plant extract's active role in nanoparticle formation. UV–Vis spectra confirmed successful synthesis of ZnO-based

nanomaterials, and antibacterial testing demonstrated that 7% Ag-doped ZnO-NPs exhibited the strongest inhibition against the tested Gram-positive and Gram-negative bacteria. Overall, the results suggest that Ag doping improves the antibacterial performance of green-synthesized ZnO nanoparticles.

Limitations of the study

This study has several limitations. First, antibacterial activity was assessed only by disc diffusion assays; MIC and MBC values were not determined, limiting quantitative comparisons with other antimicrobial agents. Second, elemental analysis (e.g., EDX/EDS) and surface area measurements (BET) were not performed, so Ag content and specific surface area were inferred indirectly from XRD and morphology rather than measured directly. Third, the experiments were conducted *in vitro* with a limited number of bacterial strains; potential cytotoxicity, environmental behavior, and *in vivo* efficacy of the nanoparticles were not evaluated. Future work should therefore include MIC/MBC testing, comprehensive physicochemical characterization, and *in vivo* or application-specific assessments to fully establish the suitability of these green-synthesized ZnO and Ag-doped ZnO nanoparticles for biomedical or environmental applications.

Declarations and supplementary information

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