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Sustainable and eco-friendly synthesis of zinc oxide nanoparticles using *Kleinia longiflora* and their antibacterial and antioxidant activities

Hanen Sellami^{1,*} , Oluwaseun J Ajala^{2,10}, Maroua Gdoura-Ben Amor^{3,10} , Khaoula Elhade^{4†}, Slim Smaoui⁴, Mercy C Ogwuegbu^{5,6}, Oluwasayo E Ogunjinmi⁷ and Damian C Onwudiwe^{8,9,*}

¹ Laboratory of Treatment and Valorization of Water Rejects, Water Research and Technologies Center (CERTe), Borj-Cedria Technopark, University of Carthage, 8020 Soliman, Tunisia

² Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, Ogbomosho, Nigeria

³ Research Laboratory of Environmental Toxicology, Microbiology and Health (LR17ES06), Sfax University, Faculty of Sciences, BP 1171, Sfax 3000, Tunisia

⁴ Laboratory of Microbial, Enzymatic Biotechnology, and Biomolecules (LBMEB), Center of Biotechnology of Sfax, University of Sfax-Tunisia, PO Box 1177, 3018 Sfax, Tunisia

⁵ Food Security and Safety Focus Area, Faculty of Natural and Agricultural Science, North-West University (Mafikeng Campus), Private Bag X2046, Mmabatho, South Africa

⁶ Department of Animal Science, Faculty of Agriculture, University of Nigeria, Nsukka 410001, Enugu State, Nigeria

⁷ Department of Industrial Chemistry, Abiola Ajimobi Technical University, Ibadan 200243, Oyo State, Nigeria

⁸ Department of Chemistry, School of Physical and Chemical Sciences, Faculty of Natural and Agricultural Sciences, North-West University, Mafikeng Campus, Private Bag X2046, Mmabatho 2735, South Africa

⁹ Materials Science Innovation and Modelling (MaSIM) Research Focus Area, Faculty of Agriculture, Science and Technology, North-West University (Mafikeng Campus), Private Bag X2046, Mmabatho, South Africa

¹⁰ These authors contributed equally to this work.

* Authors to whom any correspondence should be addressed.

E-mail: hanen.sallami@certe.ucar.tn and Damian.Onwudiwe@nwu.ac.za

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Abstract

Zinc oxide nanoparticles (ZnO-NPs) are highly versatile materials with a broad range of applications, including nanomedicine, environmental remediation, cancer therapy, and drug delivery. However, conventional synthesis methods for ZnO-NPs often rely on hazardous chemicals, high energy consumption, and costly procedures, which limit their sustainability, biocompatibility, and large-scale biomedical applicability. Furthermore, there is a critical need to develop eco-friendly synthetic routes that produce nanoparticles with enhanced, tunable antibacterial and antioxidant activity against clinically relevant pathogens. This study reports the synthesis of ZnO-NPs using *Kleinia longiflora* extracts and zinc nitrate hexahydrate in volume ratios of 1:1, 1:2, and 1:3, yielding ZnO₁₁, ZnO₁₂, and ZnO₁₃ nanoparticles, respectively. X-ray diffraction analysis confirmed their hexagonal wurtzite crystal structure, while transmission electron microscopy revealed spherical morphology with average particle sizes of 15.67 nm, 21.59 nm, and 26.92 nm for ZnO₁₁, ZnO₁₂, and ZnO₁₃, respectively. The nanoparticles exhibited ultraviolet-visible absorption peaks between 360 and 376 nm. Antibacterial tests against Gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*) and Gram-negative bacteria (*Escherichia coli* and *Salmonella enterica*) demonstrated greater efficacy against Gram-positive strains. The anti-*S. aureus* activity ranged from 0.195 to 1.56 mg ml⁻¹ and was significantly affected ($P < 0.05$) by the ZnO-NPs concentration prepared with varying extract volumes. ZnO₁₁ and ZnO₁₂ showed comparable antibacterial effects against *S. aureus* ($P > 0.05$), with ZnO₁₁ achieving the lowest minimum inhibitory concentration of 0.39 mg ml⁻¹ against *L. monocytogenes* ($P < 0.05$). Additionally, antioxidant activity, assessed via the 1, 1-diphenyl-2-picrylhydrazyl assay, showed ZnO₁₂ had the highest inhibition (69.63%) and lowest IC₅₀ (32.44 µg ml⁻¹), outperforming other nanoparticle formulations

(ZnO₁₁ and ZnO₁₃). In conclusion, the plant-mediated synthesis of ZnO-NPs generated nanoparticles with robust antioxidant and antibacterial properties, underscoring their promising potential for biomedical applications.

1. Introduction

Nanotechnology has emerged as one of the most dynamic fields in modern materials science, enabling precise manipulation of materials at the atomic and molecular levels to tailor their physical, chemical, and biological properties. Its applications span diverse sectors, including healthcare, catalysis, food technology, biomedical applications, pharmaceuticals, and environmental remediation [1]. The nanoscale dimension confers a high surface-to-volume ratio, enhancing reactivity, stability, and functionality compared to bulk materials. Among various metal oxide nanoparticles, zinc oxide (ZnO) is particularly attractive due to its simplicity in synthesis, biocompatibility, non-toxicity, strong catalytic and optoelectronic properties, and a wide band gap of approximately 3.37 eV, and a high exciton binding energy of 60 meV [2].

ZnO is classified by the U.S. FDA as ‘Generally Recognized as Safe’, and it exhibits antibacterial, antifungal, antioxidant, and anticancer properties [3]. These biological effects arise mainly from its nanoscale size, which enables efficient cellular interaction and reactive oxygen species (ROS) generation, leading to membrane disruption and oxidative stress in microbial and cancer cells [4]. Despite its wide potential, conventional chemical and physical methods for ZnO nanoparticle synthesis, such as sol-gel, chemical vapour deposition, and hydrothermal routes, are energy-intensive, costly, and often involve hazardous chemicals that raise environmental and safety concerns [5].

To address these challenges, green synthesis using plant extracts has emerged as an eco-friendly and sustainable alternative. Plant-mediated synthesis leverages natural phytochemicals such as flavonoids, phenolics, alkaloids, terpenoids, and proteins as both reducing and stabilizing agents, eliminating the need for toxic reagents. This approach is cost-effective, reproducible, and compatible with large-scale production under ambient conditions. In this context, different plants have been utilized for the green synthesis of ZnO. A green route to different shapes of wurtzite-structured ZnO nanoparticles (ZnO-NPs) was reported, in which zinc nitrate hexahydrate was used as the precursor and extract of *Melia azedarach* plant leaves was utilized as the capping and reducing agent [6]. Similarly, Gawade *et al* used the peels of *Punica granatum* for the successful synthesis of ZnO-NPs [7], while Gawade *et al* reported the green synthesis of ZnO-NPs using extracts of *Calotropis Gigantea* [7]. A highly crystalline ZnO, whose dimension was 7.0 nm and of pure wurtzite phase, has been reported using *Dysphania ambrosioides* [8]. The aqueous extract of seeds of *Eriobotria japonica* has been utilized as a mediator in the green synthesis of ZnO-NPs by Shabaani *et al* [9]. Using extracts from both *Lupinus albus* and *Lupinus pilosus* plants, ZnO-NPs were prepared from a solution of zinc nitrate and NaOH reagents [10]. Pristine ZnO-NPs have also been reported by Alvarado *et al* using lemon juice with less solvent and without the use of a strong base as a chemical precursor [11].

However, many plant-based syntheses reported to date suffer from limitations such as poor control over particle size and shape, aggregation, and low yield due to variability in plant composition and reaction conditions [12–14]. Thus, optimizing synthesis parameters and identifying suitable plant species with rich and stable phytochemical profiles remain critical research needs.

Kleinia longiflora, a medicinal succulent belonging to the Asteraceae family, is native to arid regions of Africa, including Kenya, Somalia, Tanzania, and South Africa [15]. It is known to contain diverse bioactive compounds such as tannins, phenolics, alkaloids, flavonoids, glycosides, and thiols, which can serve as natural reducing and capping agents in nanoparticle synthesis [16]. Despite its therapeutic potential and phytochemical richness, *K. longiflora* has not previously been explored for the biosynthesis of ZnO-NPs.

This study, therefore, aimed to synthesize and characterize ZnO-NPs using *K. longiflora* leaf extract as a green bioreductant, evaluate their antibacterial activity against both Gram-positive and Gram-negative bacteria, and assess their antioxidant potential. The novelty of this work lies in the first-time use of *K. longiflora* for ZnO-NP synthesis, providing a sustainable, low-cost, and non-toxic route for producing nanomaterials with dual antioxidant and antibacterial functions. The findings contribute to the growing body of research on plant-mediated nanotechnology and demonstrate the potential of *K. longiflora*-derived ZnO-NPs for future biomedical and pharmaceutical applications.

2. Materials and methods

2.1. Materials

Zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6 \text{H}_2\text{O}$), sodium hydroxide (NaOH) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Merck (Pty) Ltd. *K. longiflora* leaves were sourced from Mahikeng Local Municipality, North-West Province, South Africa and were properly identified.

2.2. Preparation of the *K. longiflora* extract

Fresh *K. longiflora* leaves were collected and thoroughly washed with distilled water to remove dust and surface impurities. The cleaned leaves were chopped into small pieces, and approximately 50 g was homogenized in 200 ml of distilled water to obtain a fine pulp. The mixture was then heated at 60 °C–70 °C for 30 min, allowed to cool to room temperature, and filtered through a clean cloth sieve to remove particulate matter. The resulting filtrate was collected in an airtight container and stored in the refrigerator until further use.

2.3. Synthesis of ZnO-NPs

Zinc nitrate hexahydrate solution was prepared by dissolving 3.5 g of ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) into 25 ml of distilled water. About 25 ml of *K. longiflora* extract was measured and its pH was adjusted to 8 by the dropwise addition of NaOH solution. To this solution, the prepared solution of ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) was added and stirring was continued at 75 °C–80 °C using a magnetic stirrer for 2 h. Thereafter, the solution was allowed to cool to room temperature and then centrifuged at 5500 rpm for 20 min. The obtained solid was rinsed with a solution of water/ethanol to remove impurities and was calcinated at 350 °C for 2 h. The reaction was repeated using 50 and 75 ml of *K. longiflora* extract to obtain ZnO NPs, which were labelled ZnO_{11} , ZnO_{12} , and ZnO_{13} , representing the 1:1, 1:2, and 1:3 ratios of Zinc nitrate hexahydrate solution to *K. longiflora* extract, respectively [17–19].

2.4. Characterization of ZnO-NPs

The ZnO-NPs synthesized via a plant-mediated method were subjected to comprehensive morphological and structural characterization using several techniques. The successful formation of ZnO-NPs was confirmed by the appearance of a characteristic absorption band in the UV–visible spectrum, measured using a PROVE 300 spectrophotometer (1 nm resolution, 200–800 nm range, in ethanol). The crystalline structure of the nanoparticles was investigated using x-ray diffraction (XRD) analysis performed on a Bruker D8 Advanced diffractometer at room temperature over a 2θ range of 20°–70°, with a step size of 0.05° and a counting time of 5 s per step, employing Cu $K\alpha$ radiation and Bragg–Brentano geometry. The external morphology of the nanoparticles was examined using scanning electron microscopy (SEM) (JSM 6510LV JEOL), while transmission electron microscope (TEM) (JEOL-2100, operating at 200 kV) was employed to assess particle size and morphology. For TEM analysis, the nanoparticles were dispersed in isopropanol, sonicated for 10–15 min, and a drop of the suspension was deposited on a carbon-coated grid. After solvent evaporation, the samples were analysed, and the average particle size was determined using ImageJ software.

2.5. Antibacterial activity of ZnO-NPs

The antibacterial activity of the ZnO NPs and gentamicin, employed as a positive control in antibacterial tests, were assessed on food-borne pathogenic bacteria strains, including Gram-positive bacteria: *Staphylococcus aureus* ATCC 6538 and *Listeria monocytogenes* ATCC 19 117; and Gram-negative bacteria: *Escherichia coli* ATCC 8739 and *Salmonella enterica* ATCC 14 028. Each bacterial inoculum was adjusted to 10^6 CFU ml^{-1} . The minimum inhibitory concentration (MIC) of the ZnO against all test bacteria was determined using the broth micro-dilution method [11, 20]. Precisely, 10 μl of the bacterial suspension (10^6 CFU ml^{-1}) was added to each well containing a varying concentration of NPs. The MIC value (mg ml^{-1}) was recorded as the lowest NPs concentration that perceptibly inhibited bacterial growth. Thiazolyl blue tetrazolium bromide (MTT) was used as an indicator of microbial growth.

2.6. Antioxidant activity of ZnO-NPs

The assessment of the antioxidant activity of the nanoparticles followed an established method [21, 22]. For the preparation of the DPPH solution, 12.8 mg of DPPH was dissolved in 50 ml of MeOH to achieve 0.032 mM conc. and then placed in an incubator for 0.5 h. In 1 ml of MeOH, ZnO solutions with different concentrations of 1.50, 3.00, 6.00, 12.00, 24.00, and 48.00 μg . In each of the solutions, 1 ml of the DPPH solution was added, while a separate container containing just the DPPH solution and MeOH served as the blank (control). At 540 nm, the absorbance was measured against the control,

replicated three times, and the percentage inhibition was calculated as follows (equation (1)):

$$\% \text{ inhibition} = \frac{A_c - A_s}{A_c} \times 100\%, \quad (1)$$

where (A_c) represents the absorbance of the control, while (A_s) is the absorbance of the sample.

2.7. Statistical analysis

For each ZnO-NPs, MICs were determined in triplicate. A one-way analysis of variance with two factors was applied by using the Statistical Package for the Social Sciences (SPSS Ltd, Woking, UK). Means and standard errors were assessed, and a probability level of $P < 0.05$ was utilized in testing the statistical significance of all experimental data. Tukey's post hoc test was employed to determine the significance of mean values for multiple comparisons at $P < 0.05$.

3. Results and discussion

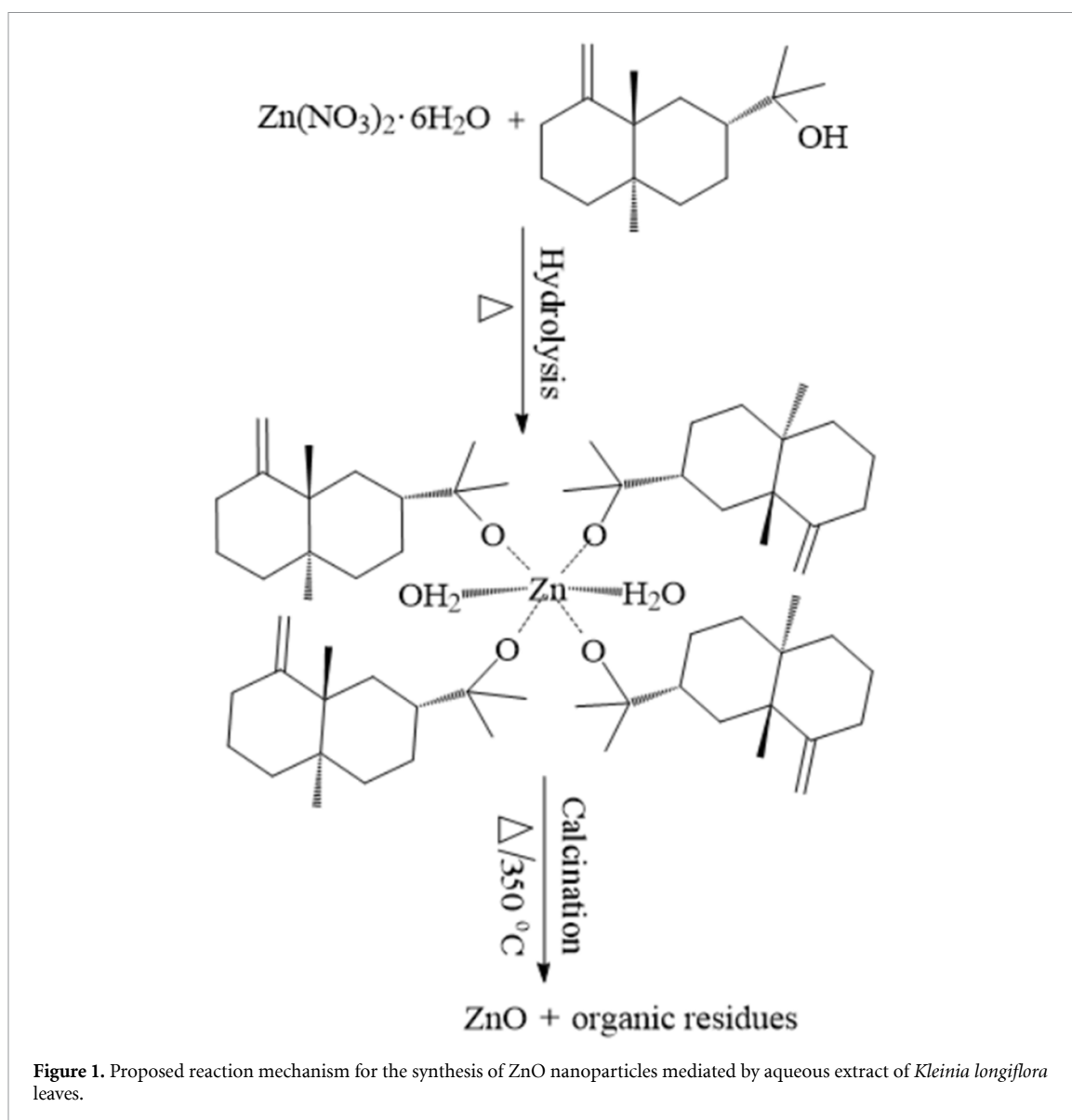
3.1. Synthesis process

The hexane extract of *K. longiflora* leaves has been analysed using gas chromatography-mass spectrometry, which revealed the presence of about 21 different compositions. Apart from β -eudesmol, identified as the main component (80.6%), other primary phytochemicals were lupeol (53.1%), ambrosin (48.4%), and (-)- α -panasinsen (43.6%) [16]. It has been well documented that phytochemicals mediate the production of nanoparticles. It remains unclear exactly what reaction mechanism is involved in the green synthesis of nanoparticles. Nonetheless, several studies have investigated the creation of nanoparticles using plant extracts. The conversion of metal ions to Zn^0 is coordinated by a repeated redox reaction, which is a possible mechanism of interaction between phytochemicals and metal ions [23]. Due to its high concentration in the leaf extract of *K. longiflora*, β -eudesmol was selected as the model phytochemical in this case. The aromatic hydroxyl groups of β -eudesmol bind to the Zn^{2+} ions from $\text{Zn}(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ to form a stable complex system, which is the process by which ZnO NPs are formed. In this instance, however, ZnO NPs are liberated from the heat treatment after the Zn complex system is calcined [24–26]. Figure 1 shows these likely reaction pathways for the synthesis of ZnO NPs.

3.2. XRD analysis

XRD analysis was conducted to examine the crystalline nature and structural characteristics of conventionally and green-synthesized ZnO NPs. The results are shown in figures 2(a) and (b), respectively, and their structural parameters are summarized in table 1. The diffraction patterns of the conventionally synthesized ZnO NPs show distinct peaks at 2θ values of 31.94, 34.50, 36.48, 47.57, 56.55, 62.88, 66.37, 68.03, 69.10, 72.82 and 77.01. These correspond to the crystal planes (001), (002), (101), (102), (110), (103), (200), (112), (201), (204), and (202), respectively. Comparing these patterns with the green synthesized ZnO NPs revealed similar diffraction patterns with peaks at 2θ values of approximately 31.60, 34.25, 36.07, 47.37, 56.44, 62.71, 66.36, 67.82, 68.97, 72.50, and 77.50, consistent with previous reports [14, 27, 28]. The observed patterns correspond to a hexagonal wurtzite structure of ZnO, with lattice parameters $a = 3.26 \text{ \AA}$ and $c = 5.23 \text{ \AA}$, in accordance with JCPDS card no. 36–1451. Notably, the absence of additional peaks across all samples indicates a high degree of purity, with no detectable secondary or impurity phases arising from the plant biomolecules or residual precursors.

Furthermore, the broadening of the diffraction peaks observed in all the samples is characteristic of nanocrystalline materials, signifying the nanoscale nature of the synthesized ZnO NPs. Table 1 summarizes the structural parameters of ZnO-NPs by both processes, as derived from the XRD analysis. The crystallite size (D) was calculated using the Debye–Scherrer equation (equation (3)), whereas the microstrain (ϵ) and crystallinity (%), and Interplanar spacing (d) were determined using X'Pert HighScore Plus software. It was observed that the conventional method yielded the largest crystalline size of 63 nm and highest percentage crystallinity of 96.8% compared to the green synthesized ZnO_{11} (26.45 nm, 93.4%), ZnO_{12} (32.25 nm, 95.6%) and ZnO_{13} (38.20 nm, 96.6%). ZnO_{11} NPs have the smallest crystalline size and the highest percentage crystallinity, which could be attributed to the effect of the volume ratio of the plant extract used in the synthesis process. An increase in the volume of plant extract enhances the crystalline size, which also appears to improve the crystallinity of the nanoparticles, thereby confirming that plant extract influences the structural parameters of the ZnO. However, the diffraction patterns of all green synthesized nanoparticles remained highly similar, suggesting that the



extract volume had minimal influence on the phase formation and integrity of the ZnO-NPs. This uniformity across samples implies reproducibility of the synthesis approach and the robustness of the plant-mediated method in producing phase-pure ZnO nanomaterials.

3.3. SEM analysis

SEM provides valuable information about surface morphology, aggregation, size, and distribution of nanoparticles [29]. Figures 3(a) and (b) present the SEM and TEM images, respectively, of the ZnO NPs prepared by the conventional route. The micrographs show irregularly shaped nanoparticles with obvious agglomeration, an indication that the growth direction of the nanoparticles was anisotropic under the thermal conditions. The energy-dispersive x-ray spectroscopy (EDX) result of figure 3(c) confirms Zn and O as the major components, with a carbon peak ascribed to the carbon tape. Elemental mapping (figures 3(d)–(f)) shows that the elements are uniformly distributed across the surface of the ZnO-NPs. The SEM micrographs of ZnO₁₁, ZnO₁₂, and ZnO₁₃ nanoparticles at low magnification (figures 4(a)–(c)) and at high magnification (figures 5(d)–(f)), respectively, show that the images are spherical in shape, and present obvious agglomeration which is characteristic of nanoparticles whose synthesis process involved a thermal process. Related studies have also ascribed the agglomeration of ZnO NPs to the effect of high surface area, polarity, and electrostatic attraction of the NPs, or from experimental parameters during synthesis, such as changes in temperature and pH [30].

Figure 4(g) presents a representative EDX and figures 4(h) and (i) show representative elemental mapping results of the green-synthesized ZnO-NPs prepared using different volume ratios of the plant's

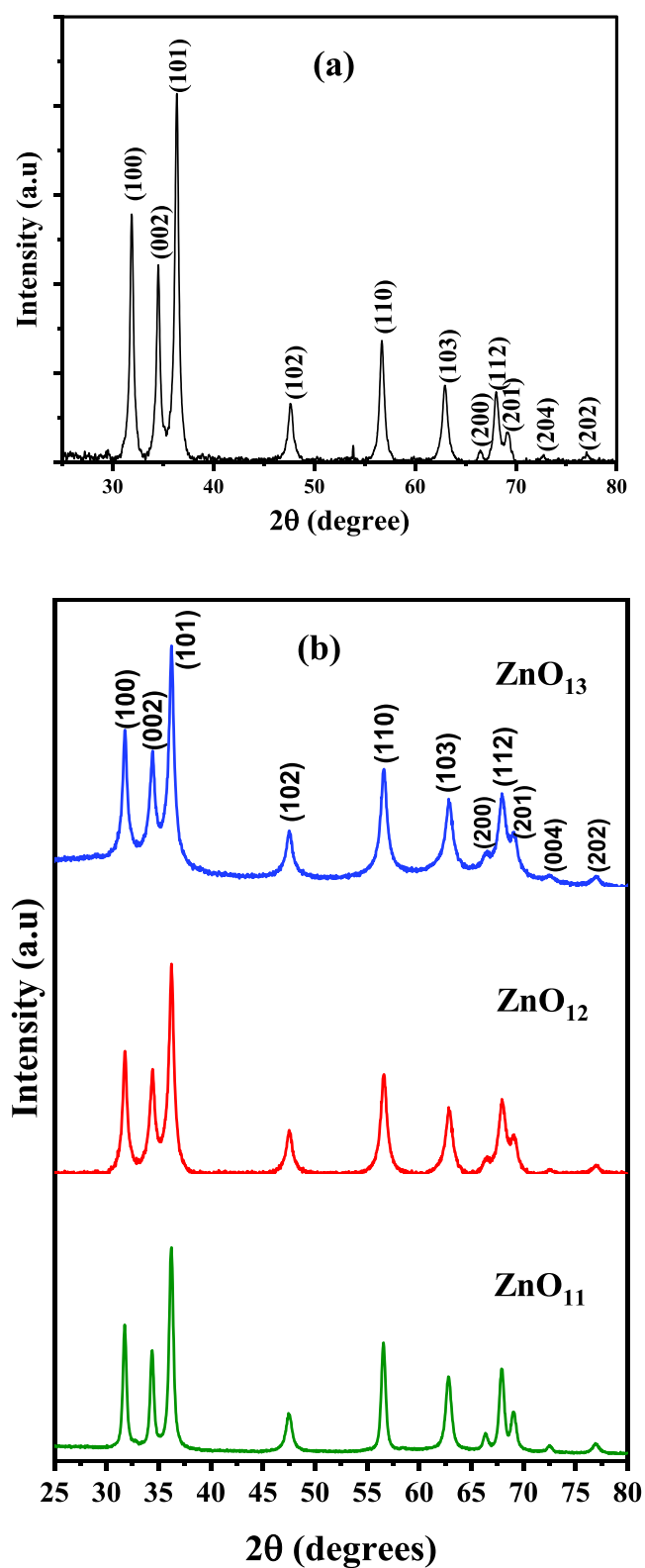


Figure 2. XRD of (a) ZnO by conventional method, and (b) ZnO₁₁, ZnO₁₂, and ZnO₁₃ nanoparticles prepared using 25, 50, and 75 ml of the *Kleinia longiflora* plant extract, respectively.

extract. The EDX confirms the purity of the nanoparticles with Zn and O as main composition, while the elemental mapping micrographs reveal well-defined morphologies with uniformly distributed grains and no detectable impurities. A homogeneous elemental distribution is observed throughout the samples, confirming the isotropic nature of the synthesized nanoparticles.

Table 1. A summary of the structural parameters of the ZnO prepared by the conventional route and ZnO₁₁, ZnO₁₂, and ZnO₁₃ nanoparticles prepared using 25, 50, and 75 ml of the *Kleinia longiflora* plant extract, respectively.

Samples	Crystallite size (<i>D</i>) (nm)	Interplanar spacing (<i>d</i>) Å	Strain (ε)	Crystallinity (%)
ZnO	63.32	2.521	0.028	96.8
ZnO ₁₁	26.45	2.431	0.013	93.4
ZnO ₁₂	32.25	2.452	0.016	95.6
ZnO ₁₃	38.20	2.468	0.020	96.5

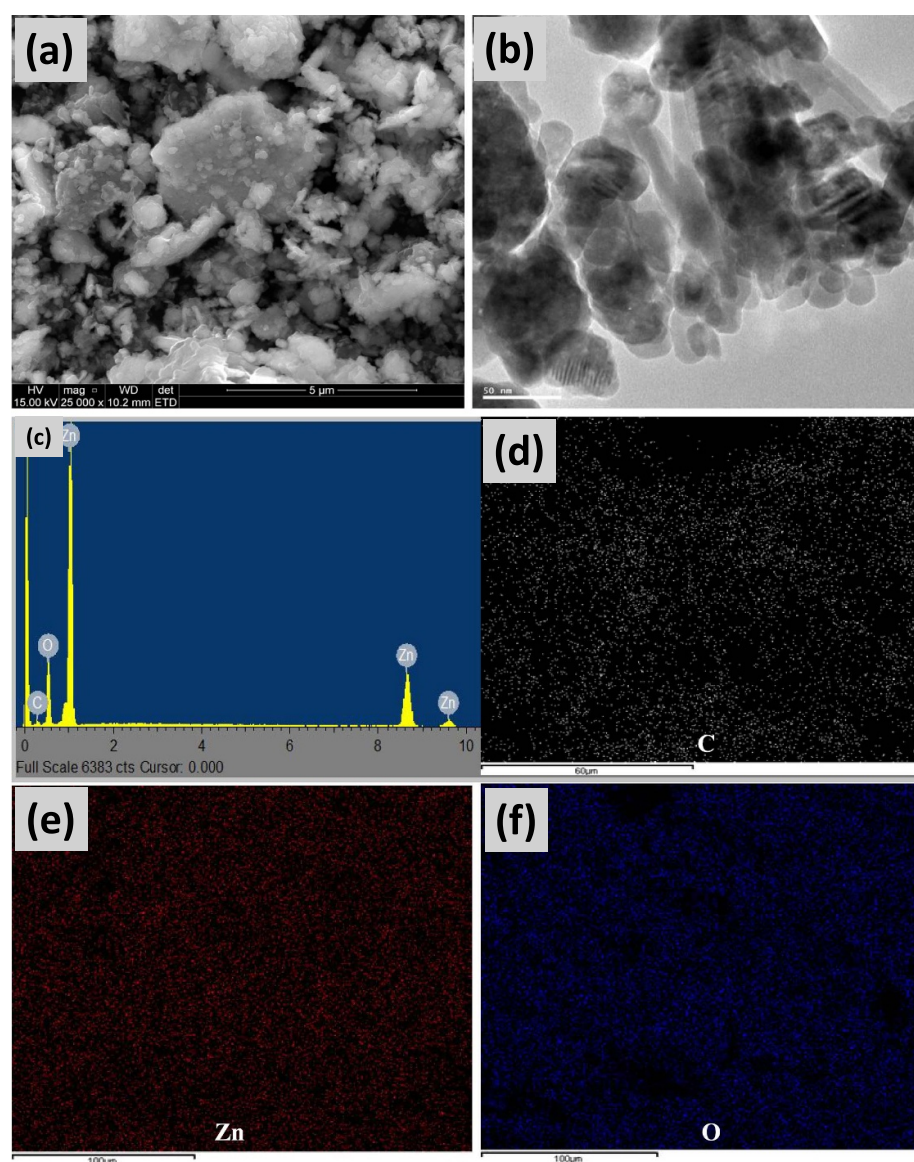


Figure 3. (a) and (b) SEM and TEM images, (c) EDX spectrum, and elemental mapping showing (d) C, (e) Zn, and (f) O elements of ZnO nanoparticles prepared using the conventional method.

3.4. TEM and particle size distribution analyses

TEM provides valuable insights into the internal morphology, shape, aggregation, size, and distribution of nanoparticles [31]. Figures 5(a)–(c) present the TEM micrographs of ZnO₁₁, ZnO₁₂, and ZnO₁₃ nanoparticles, which confirm the uniform spherical morphology of the nanoparticles, with slight agglomeration that can be attributed to van der Waals forces. Different studies have reported the formation of spherical ZnO NPs at lower precursor concentrations, while higher concentrations lead to a mixture of spherical, hexagonal, and cubical shapes [14]. The average size of the ZnO NPs was determined using ImageJ software, and the results are depicted in figures 6(a)–(c), which yielded mean diameters of

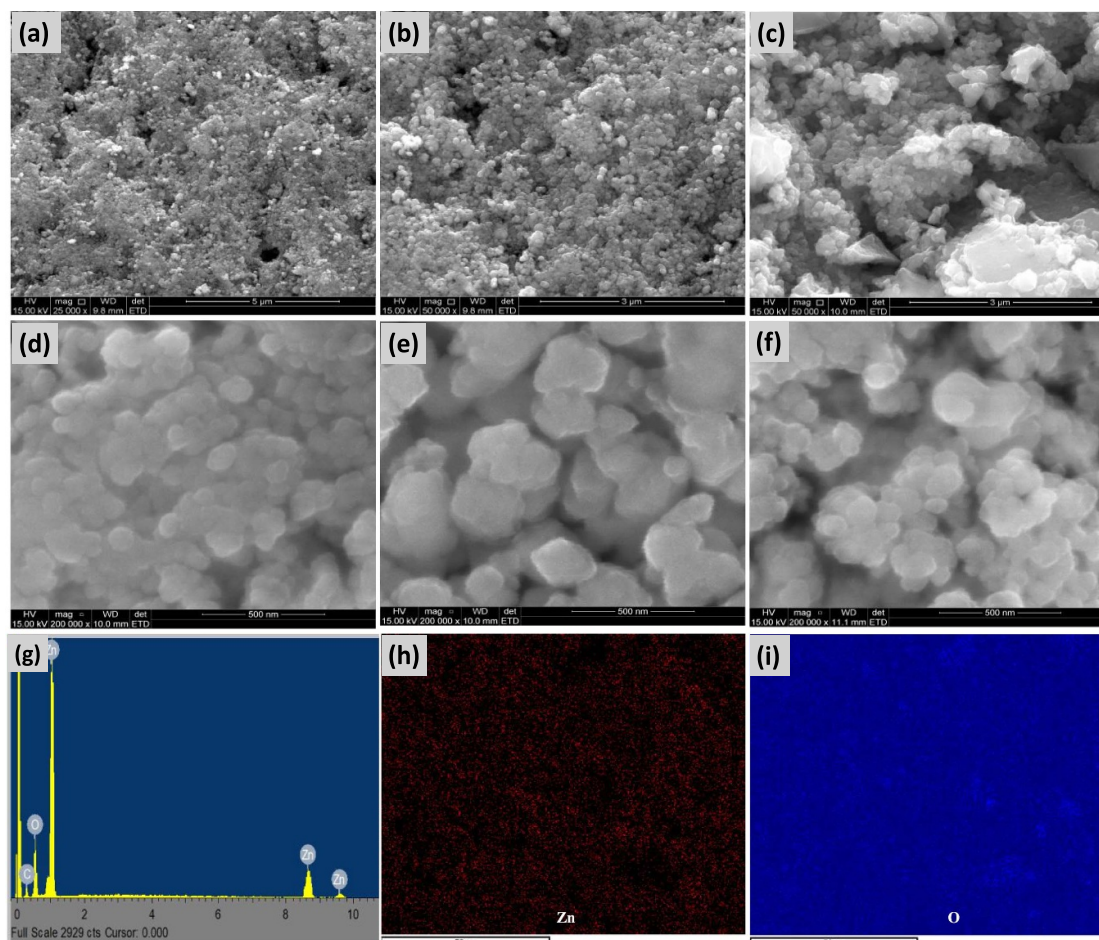


Figure 4. (a)–(c) Low magnification, and (d)–(f) high magnification SEM images of ZnO_{11} , ZnO_{12} , and ZnO_{13} nanoparticles, respectively, prepared using 25, 50, and 75 ml of the *Kleinia longiflora* plant extract. Representative EDX spectrum (g), and elemental mapping showing Zn (h), and O (i).

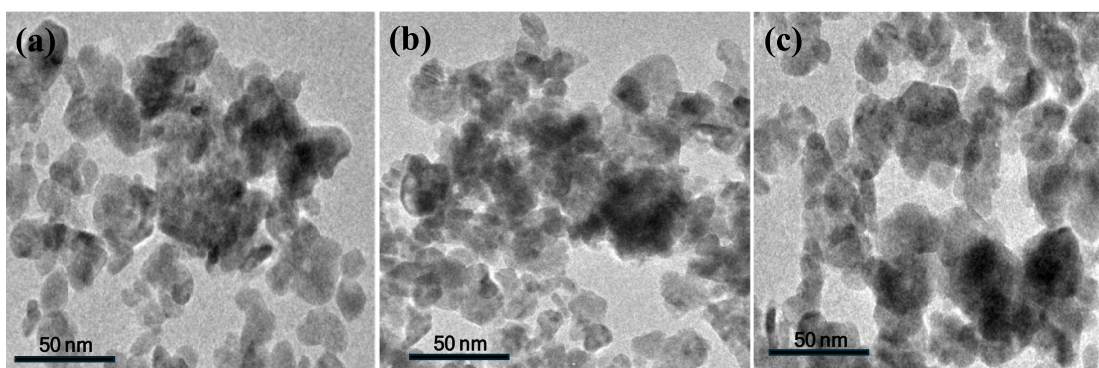


Figure 5. TEM images of (a) ZnO_{11} , (b) ZnO_{12} , and (c) ZnO_{13} nanoparticles prepared using 25, 50, and 75 ml of the *Kleinia longiflora* plant extract, respectively.

15.67 nm, 21.59 nm, and 26.92 nm for ZnO_{11} , ZnO_{12} , and ZnO_{13} , respectively. This varies from the results obtained from the XRD patterns, and the discrepancy may be ascribed to the fact that TEM reflects the physical size of individual particles or agglomerates, whereas XRD estimates the crystallite size corresponding to coherent diffracting domains within the particles.

3.5. UV spectroscopy analysis

The UV–visible spectra of the ZnO NPs, shown in figure 7(a), are typical of the absorption property of ZnO, with peak maxima at 365 nm (ZnO), 372 nm (ZnO_{11}), 370 nm (ZnO_{12}), and 368 nm (ZnO_{13}), consistent with previous studies [14, 27, 29]. The peak in the UV region around 370 nm is typically

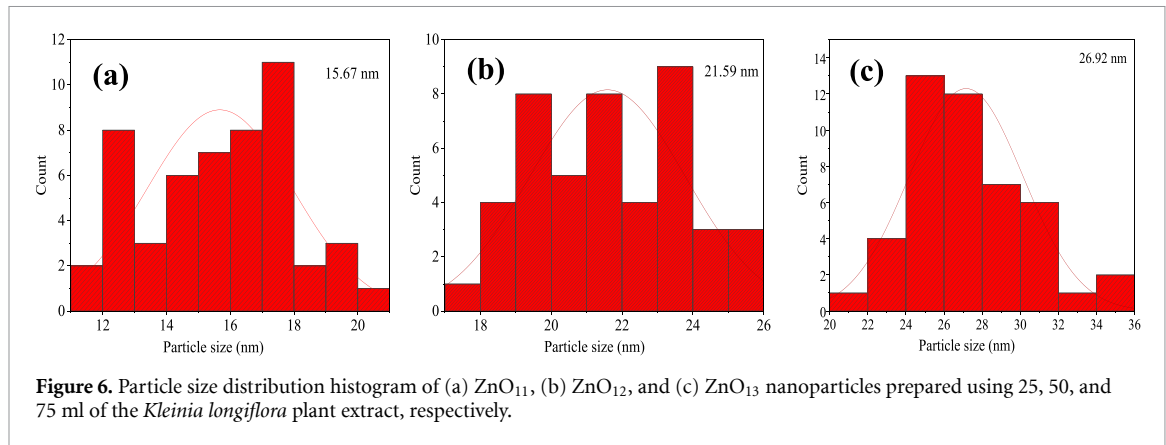


Figure 6. Particle size distribution histogram of (a) ZnO₁₁, (b) ZnO₁₂, and (c) ZnO₁₃ nanoparticles prepared using 25, 50, and 75 ml of the *Kleinia longiflora* plant extract, respectively.

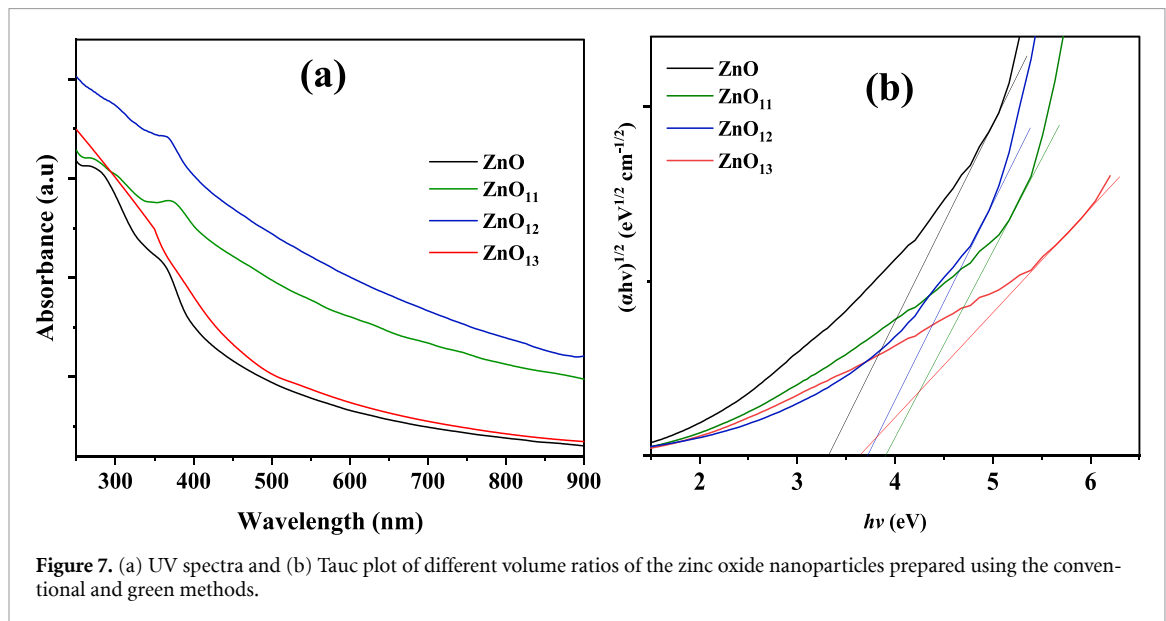


Figure 7. (a) UV spectra and (b) Tauc plot of different volume ratios of the zinc oxide nanoparticles prepared using the conventional and green methods.

associated with ZnO's intrinsic absorption due to electrons transitioning from the valence band to the conduction band ($O\ 2p \rightarrow Zn\ 3d$) [32]. Tauc relation, (given in equation (2)) was used to estimate the intrinsic band gap energies (E_g) of the nanoparticles [33]. Here, a plot of $(\alpha h\nu)^n$ versus $(h\nu)$ is constructed, and the linear portion of the plot is extrapolated to the energy axis (x -axis) (figure 5(b)), where $(\alpha h\nu)^n = 0$, and the intercept gives the optical band gap E_g [33]. From this relation, the values of the E_g were found to be 3.4 eV, 3.90 eV, 3.70 eV, and 3.60 eV, for the ZnO, ZnO₁₁, ZnO₁₂, and ZnO₁₃, respectively

$$(\alpha h\nu)^n = A(h\nu - E_g) \quad (2)$$

where α = absorption coefficient, $h\nu$ denotes photon energy (Planck's constant $\times$ frequency), E_g is the optical band gap energy, and A is a constant.

3.6. Antibacterial susceptibility assay

Overall, the synthesized ZnO-NPs exhibited stronger antibacterial activity against Gram-positive bacteria than Gram-negative strains. This difference is primarily due to structural variations in their cell walls. Gram-positive bacteria possess a thick peptidoglycan layer but lack an outer membrane, which makes them more susceptible to nanoparticle-induced oxidative stress and membrane disruption. In contrast, the outer lipopolysaccharide layer in Gram-negative bacteria acts as a protective barrier, limiting nanoparticle penetration and reducing their susceptibility to antimicrobial agents [34].

As shown in table 2, the lowest MIC of 0.195 mg ml⁻¹ was observed for ZnO₁₁ and ZnO₁₂ against *S. aureus*. The anti-*S. aureus* activity of the ZnO-NPs, which ranged from 0.195 to 1.56 mg ml⁻¹, was significantly ($P < 0.05$) affected by the volume ratio of *K. longiflora* extract used during synthesis.

Table 2. MIC values, expressed by mg ml^{-1} , of three synthesized ZnO by using *Kleinia longiflora* extract and the standard antibiotic gentamicin.

Target microorganisms	ZnO ₁₁	ZnO ₁₂	ZnO ₁₃	Gentamicin
Gram positive bacteria				
<i>Listeria monocytogenes</i>	$0.39 \pm 0.00^{\text{aA}}$	$0.78 \pm 0.00^{\text{bA}}$	$1.56 \pm 0.00^{\text{cA}}$	$0.0125 \pm 0.00^{\text{b}}$
<i>Staphylococcus aureus</i>	$0.195 \pm 0.00^{\text{bA}}$	$0.195 \pm 0.00^{\text{bC}}$	$1.56 \pm 0.00^{\text{aA}}$	$0.0125 \pm 0.00^{\text{b}}$
Gram negative bacteria				
<i>Salmonella enterica</i>	$0.78 \pm 0.00^{\text{aB}}$	$0.78 \pm 0.00^{\text{aB}}$	$1.56 \pm 0.00^{\text{bA}}$	$0.025 \pm 0.00^{\text{a}}$
<i>Escherichia coli</i>	$3.125 \pm 0.00^{\text{aD}}$	$3.125 \pm 0.00^{\text{aC}}$	$3.125 \pm 0.00^{\text{aB}}$	$0.025 \pm 0.00^{\text{a}}$

±Standard deviation of three replicates; values with a different letter (a)–(c) within the same row are significantly different ($P < 0.05$) (Tukey's test); values with a different letter (A)–(D) within the same column are significantly different ($P < 0.05$) (Tukey's test).

ZnO₁₁ and ZnO₁₂ displayed comparable inhibition zones, with no significant difference ($P > 0.05$) in their antibacterial effects, suggesting that extract ratios of 1:1 and 1:2 produce optimally active nanoparticles. Similarly, ZnO₁₁ exhibited the lowest MIC (0.39 mg ml^{-1}) against *L. monocytogenes*, confirming its superior antibacterial potency among the tested formulations.

For Gram-negative bacteria, ZnO-NPs exhibited varying inhibitory effects. ZnO₁₁ and ZnO₁₂ were significantly ($P < 0.05$) more active against *S. enterica* (MIC = 0.78 mg ml^{-1}), whereas all samples exhibited a consistent MIC of 3.125 mg ml^{-1} against *E. coli*, indicating that this concentration was sufficient to suppress bacterial proliferation. These findings align with earlier reports on *Kleinia grandiflora*-derived silver nanoparticles, which also inhibited *E. coli* and *Pseudomonas aeruginosa* [35]. AgNPs synthesis of using *K. grandiflora* leaf extract and its antibacterial activity against *E. coli* was explored by using the standard disc diffusion method and standard antibiotic ciprofloxacin at $10 \mu\text{g disc}^{-1}$, which revealed a strong antibacterial activity with 22 mm inhibition zone. In contrast, the *K. longiflora*-mediated AgNPs ($100 \mu\text{g disc}^{-1}$) produced a moderate inhibitory effect, with a zone of 13 mm [22]. Gentamicin was employed as the standard drug, and the results obviously presented a stronger antibacterial activity against Gram-negative bacteria than Gram-positive strains. Precisely, gentamicin exhibited a MIC value of 0.025 mg ml^{-1} against Gram-negative bacteria, whereas the inhibition zone and MIC against Gram-positive bacteria were $0.0125 \text{ mg ml}^{-1}$ (table 2). It should be noted that gentamicin, a bactericidal aminoglycoside and well-established broad-spectrum antibiotic, displays its greatest efficacy against a wide range of Gram-negative bacterial infections [20]. Although lower than the reference standard drug, the reported results illustrated in table 2 indicate that the biologically synthesized ZnO NPs have measurable activity against Gram-negative and Gram-positive bacteria.

The enhanced antibacterial activity of ZnO₁₁ and ZnO₁₂ can be attributed to their smaller particle size and higher surface reactivity, which facilitate direct interaction with bacterial membranes. Smaller nanoparticles penetrate the cell wall more readily, leading to the leakage of intracellular contents and interference with vital cellular processes [36]. In addition, ZnO-NPs generate ROS such as hydroxyl radicals, hydrogen peroxide, and superoxide ions that induce oxidative stress, damaging lipids, proteins, and DNA, and ultimately leading to cell death [1, 37]. The release of Zn^{2+} ions further enhances antimicrobial efficacy by binding to bacterial enzymes and disrupting metabolic pathways [38].

Moreover, the phytochemicals in *K. longiflora* extract, particularly phenolics and flavonoids, serve as reducing and capping agents, stabilizing the nanoparticles while also contributing intrinsic antimicrobial properties. These bioactive compounds may act synergistically with ZnO to enhance bacterial membrane disruption and ROS generation [39]. Collectively, these mechanisms explain the pronounced antibacterial potency observed in ZnO₁₁ and ZnO₁₂ compared to ZnO₁₃, underscoring the influence of extract concentration on nanoparticle size, stability, and bioactivity [40].

Hence, the *K. longiflora*-mediated ZnO-NPs displayed broad-spectrum antibacterial activity, with greater efficacy against Gram-positive pathogens. The combined effects of small particle size, high surface energy, Zn^{2+} ion release, ROS generation, and phytochemical synergy highlight the potential of these biogenic ZnO-NPs as effective, eco-friendly antimicrobial agents for biomedical and food safety applications. As shown in table 3, variations in antibacterial activity among plant-mediated ZnO-NPs underscore the crucial role of plant phytochemicals and synthesis conditions in determining their antimicrobial efficacy. Optimizing both the synthesis parameters and plant extract selection is therefore essential to maximize antibacterial performance for specific applications.

Table 3. Comparative overview of the antibacterial activities of ZnO NPs synthesized in this study with those of previously reported green-synthesized ZnO nanoparticles.

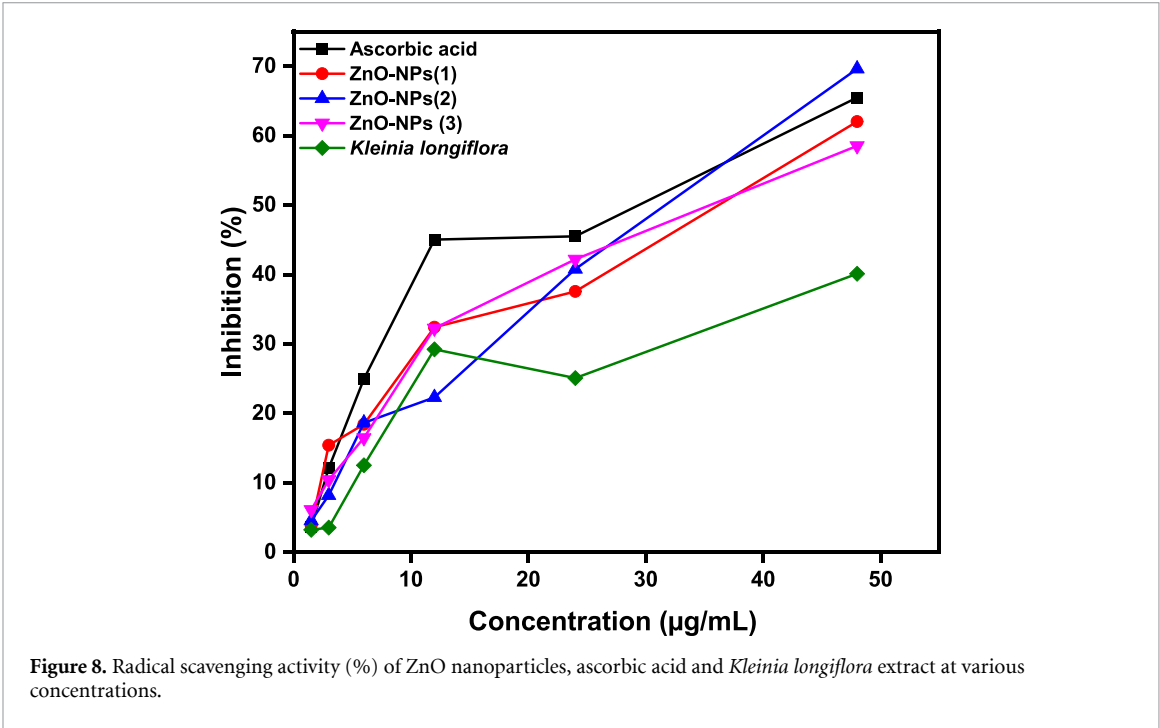
Plant extract	Pathogens	MICs (mg ml ⁻¹)	References
<i>Aloe vera</i>	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Salmonella typhi</i> , <i>Bacillus subtilis</i> , and <i>Klebsiella pneumoniae</i>	Ranged between 0.013 and 0.0625	[41]
<i>Cymbopogon citratus</i>	<i>Escherichia coli</i> <i>Staphylococcus aureus</i>	0.092 07 0.088 13	[42]
<i>Cinnam verum</i>	<i>Escherichia coli</i> <i>Staphylococcus aureus</i>	0.125 0.0625	[43]
<i>Elaeagnus angustifolia</i>	<i>Pseudomonas aeruginosa</i> <i>Klebsiella pneumoniae</i> <i>Escherichia coli</i> <i>Staphylococcus aureus</i> <i>Bacillus subtilis</i>	0.075 0.0375 0.0375 0.0375 0.0375	[44]
Plantain peel extract	<i>Salmonella enterica</i> <i>Klebsiella pneumoniae</i> <i>Bacillus cereus</i> <i>Staphylococcus aureus</i>	0.1	[45]
<i>Euterpe oleracea</i> Mart.	<i>Staphylococcus aureus</i> <i>Listeria monocytogenes</i> <i>Escherichia coli</i> <i>Salmonella enteritidis</i>	0.16 1.25 1.25 2.50	[46]
<i>Capsicum chinense</i>	<i>Escherichia coli</i> <i>Klebsiella oxytoca</i> <i>Staphylococcus aureus</i> <i>Bacillus cereus</i>	4 5 5 5	[47]
<i>Artocarpus hirsutus</i>	<i>Enterobacter aerogenes</i> <i>Listeria monocytogenes</i>	0.125 0.25	[48]
<i>Berberis aristata</i>	<i>Bacillus cereus</i> <i>Bacillus subtilis</i> <i>Escherichia coli</i> <i>Klebsiella pneumoniae</i> <i>Staphylococcus aureus</i> <i>Serratia marcescens</i>	0.128 0.064 0.256 0.256 0.128 0.064	[49]
<i>Ficus palmata</i>	<i>Staphylococcus aureus</i> <i>Bacillus subtilis</i> (<i>Salmonella typhi</i>) <i>Escherichia coli</i> <i>Klebsiella pneumoniae</i>	0.3906 (the lowest MIC reported)	[50]
<i>Kleimia longiflora</i>	<i>Staphylococcus aureus</i> <i>Listeria monocytogenes</i> <i>Salmonella enterica</i> <i>Escherichia coli</i>	0.195 to 1.56 0.39–1.56 0.78–1.56 3.125	This work

On the hand, some investigations have recognized the potential antimicrobial characteristics of NPs synthesized using green methods from the Asteraceae family. In this line, Ag, Cu, Au, ZnO, TiO₂, NiO, CuO, have all been prepared using Asteraceae plant extracts; and many have exposed an aptitude to prevent the growth of several types of food-borne pathogenic bacteria. For example, *Tragopogon collinus* synthesized ZnO-NPs revealed antibacterial properties against *E. coli* PTCC 1270 and *S. aureus* PTCC 1112 [51]. Synthesized *Cynara scolymus* ZnO NPs from leaf extract display an activity against anti-*S. aureus* ATCC 25 923, *E. coli* ATCC 25 922, and *P. aeruginosa* ATCC 27 853 [52]. The AgNPs have been synthesized using *Carthamus tinctorius* and have been shown to inhibit the *Pseudomonas fluorescens* ATCC 13 867 and *S. aureus* ATCC 25 923 growth [53]. Similarly, AgNPs by *Eupatorium odoratum* leaf extracts exhibited antimicrobial activity against *E. coli*, *Bacillus subtilis*, *S. aureus*, and *Salmonella typhi* [54]. Additionally, leaves of *Tagetes erecta* were used to mediate the synthesis of AgNPs, which

Table 4. Antioxidant activity (%) of ZnO nanoparticles and *Kleinia longiflora* extract.

Test samples	Sample concentrations ($\mu\text{g ml}^{-1}$)						IC_{50} ($\mu\text{g ml}^{-1}$)
	1.50	3.00	6.00	12.00	24.00	48.00	
ZnO-NPs ₁₁	3.82 $\pm$ 0.022	15.41 $\pm$ 0.053	18.43 $\pm$ 0.025	32.41 $\pm$ 0.045	37.56 $\pm$ 0.021	62.04 $\pm$ 0.040	35.30
ZnO-NPs ₁₂	4.55 $\pm$ 0.024	8.20 $\pm$ 0.074	18.63 $\pm$ 0.052	22.30 $\pm$ 0.035	40.80 $\pm$ 0.031	69.63 $\pm$ 0.040	32.44
ZnO-NPs ₁₃	6.11 $\pm$ 0.026	10.41 $\pm$ 0.023	16.47 $\pm$ 0.084	32.25 $\pm$ 0.065	42.19 $\pm$ 0.034	58.53 $\pm$ 0.045	36.13
Ascorbic acid	3.65 $\pm$ 0.052	12.12 $\pm$ 0.032	24.95 $\pm$ 0.041	45.04 $\pm$ 0.030	45.51 $\pm$ 0.042	65.50 $\pm$ 0.034	30.32
<i>Kleinia longiflora</i>	3.22 $\pm$ 0.014	3.54 $\pm$ 0.060	12.52 $\pm$ 0.024	20.23 $\pm$ 0.053	25.09 $\pm$ 0.024	40.11 $\pm$ 0.082	54.28

Values are presented as mean inhibition (%) $\pm$ standard deviation ($n = 3$), with ascorbic acid serving as the reference standard.



showed antibacterial property against *E. coli* and *S. aureus* ATCC 9144 [55]. Equally, *Ageratum conyzoides* can reduce iron metal to FeNPs, which have modest antimicrobial activity toward *E. coli* ATCC25922, *S. aureus* ATCC 25 923 and *P. aeruginosa* ATCC 27 853 [56]. Recent investigations also reported that CuONPs synthesized from *Acanthospermum hispidum* presented antibacterial potency against *E. coli* MTCC 443, *P. aeruginosa* MTCC 1688, and *S. aureus* MTCC 96 [57].

3.7. Antioxidant activity analysis of ZnO-NPs

The antioxidant activity of ZnO-NPs synthesized using *K. longiflora* was investigated at different volume ratios of 1:1, 1:2, and 1:3, producing ZnO₁₁, ZnO₁₂, and ZnO₁₃ nanoparticles, respectively. As shown in table 4 and figure 8, the DPPH scavenging assay was conducted at six concentrations ranging from 1.50 to 48.00 $\mu\text{g ml}^{-1}$. The inhibition percentages for ZnO₁₁, ZnO₁₂, ZnO₁₃, ascorbic acid, and *K. longiflora* extract ranged between 3.82%–62.02%, 4.55%–69.63%, 6.11%–58.53%, 3.65%–65.50%, and 3.22%–40.11%, respectively. At the highest concentration (48.00 $\mu\text{g ml}^{-1}$), ZnO₁₂ showed the greatest antioxidant activity with 69.63% inhibition, outperforming ZnO₁₁ (62.02%), ZnO₁₃ (58.53%), ascorbic acid (65.50%), and the *K. longiflora* extract (40.11%). This indicates that the 1:2 volume ratio used to synthesize ZnO₁₂ NPs is optimal for enhancing antioxidant capacity among these formulations. The IC_{50} values further support these findings. Ascorbic acid exhibited the lowest IC_{50} at 30.32 $\mu\text{g ml}^{-1}$, confirming its strong antioxidant property. Among the nanoparticles, ZnO₁₂ recorded the lowest IC_{50} (32.44 $\mu\text{g ml}^{-1}$), followed by ZnO₁₁ (35.30 $\mu\text{g ml}^{-1}$) and ZnO₁₃ (36.13 $\mu\text{g ml}^{-1}$). In contrast, the aqueous extract of *K. longiflora* showed a much higher IC_{50} of 58.28 $\mu\text{g ml}^{-1}$. Since a lower IC_{50} denotes higher antioxidant activity, these results demonstrate that ZnO-NPs synthesized through the biogenic route with *K. longiflora* possess superior antioxidant potential compared to the plant extract alone. This enhanced antioxidant activity of ZnO-NPs can be attributed to the rich presence of phytochemicals in *K. longiflora*, including steroids, saponins, phenols, glycosides, flavonoids, and alkaloids, which act as

Table 5. Comparison of the antioxidant activity of ZnO-NPs synthesized using *Kleinia longiflora* with that of other green-synthesized ZnO nanoparticles.

Nanoparticle	Plant extract	IC ₅₀ ($\mu\text{g ml}^{-1}$)	Concentration ($\mu\text{g ml}^{-1}$)	Inhibition (%)	References
ZnO	<i>Mallotus philippinensis</i>	66.87	25–125	18.82–84.05	[58]
ZnO	<i>Allium cepa</i> L.	—	50–500	25–95	[60]
ZnO	<i>Allium cepa</i>	—	20–100	45.15–61.41	[61]
ZnO	<i>Calendula officinalis</i>	12.11	2–6	—	[62]
ZnO	<i>Pelargonium odoratissimum</i>	28.11	3.125–100	10.78–76.14	[63]
ZnO	<i>Kleinia longiflora</i>	35.30, 32.44, 36.13	1.50–48.00	3.82–69.63	This study

bio-reducing and capping agents [58]. The hydroxyl (OH) groups in phenolics and flavonoids particularly facilitate the reduction and stabilization of metal oxide nanoparticles, as supported by previous studies [58–60]. Moreover, the phytochemicals that cap the ZnO NPs donate hydrogen atoms, thereby boosting their antioxidant potential. Comparison with other reported ZnO-NPs (table 5) indicates that the nanoparticles synthesized in this study have antioxidant activities comparable to or better than those previously documented, confirming the efficacy of the biogenic synthesis approach using *K. longiflora*.

4. Conclusion

This study demonstrated the successful green synthesis of ZnO-NPs using *K. longiflora* leaf extract, yielding pure hexagonal wurtzite structures with spherical morphology. The particle sizes (15.67–26.92 nm) and optical properties varied slightly with extract volume. Among the samples, ZnO₁₁ showed the smallest size and strongest antibacterial effect, particularly against *L. monocytogenes* (MIC = 0.39 mg ml^{−1}) and *S. aureus* (MIC = 0.195 mg ml^{−1}), while ZnO₁₂ exhibited the highest antioxidant activity (69.63% inhibition; IC₅₀ = 32.44 $\mu\text{g ml}^{-1}$). The enhanced bioactivity of the nanoparticles is attributed to the phytochemical constituents of *K. longiflora* acting as reducing and stabilizing agents. These findings confirm the potential of *K. longiflora*-mediated ZnO-NPs as effective antimicrobial and antioxidant agents suitable for biomedical and pharmaceutical applications.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

Conflict of interest

The authors declare that there is no conflict of interest.

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Author contributions

Hanan Sellami  0000-0001-5479-1707

Formal analysis (equal), Investigation (equal), Writing – original draft (equal)

Oluwaseun J Ajala

Investigation (equal), Writing – original draft (equal)

Maroua Gdoura-Ben Amor  0000-0003-4482-2869

Formal analysis (equal), Investigation (equal)

Khaoula Elhadef

Formal analysis (equal), Investigation (equal)

Slim Smaoui

Formal analysis (equal), Investigation (equal)

Mercy C Ogwuegbu

Formal analysis (equal), Investigation (equal), Writing – original draft (equal)

Oluwasayo E Ogunjinmi

Formal analysis (equal), Investigation (equal), Writing – original draft (equal)

Damian C Onwudiwe  0000-0002-2689-3981

Conceptualization (equal), Resources (equal), Writing – review & editing (equal)

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