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Antibacterial and seed germination activities of biosynthesized zinc oxide nanoparticles using aqueous leaf and fruit extract of *Annona reticulata*

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Abstract

In the present work, the zinc oxide nanoparticles were synthesized using an environmentally friendly green synthesis method utilizing *Annona reticulata* leaf and fruit extracts and compared with the ZnO prepared by the chemical route method. The Rietveld refinement X-ray diffraction (XRD) pattern revealed the formation of wurtzite-structured ZnO. The presence of abundant hydroxyl functional groups with Zn-O stretching vibrations was corroborated using Fourier Transform Infrared (FTIR) spectroscopic analysis. The particle size distribution and the morphological analysis were inferred through the Field emission scanning electron microscopic (FESEM) images. The antibacterial potential of the prepared ZnO NPs was evaluated against pathogenic strains of *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Based on the zone of inhibition, the fruit-derived ZnO NPs showed higher antibacterial activity, followed by leaf-derived and chemically synthesized nanoparticles. Notably, fruit-derived ZnO NPs exhibited an almost equal zone of inhibition of 21.1 mm at 100 µg and a greater zone of inhibition of 16.9 mm at 50 µg against *Klebsiella pneumoniae* compared to antibiotic streptomycin. Finally, the germination of *Vigna radiata* seeds under the influence of ZnO NPs demonstrated that the nanoparticles favoured seed germination at a lower concentration of 2.5 mg. The highest seed germination was observed in green synthesized ZnO NPs (26 cm), with both leaf and fruit-derived nanoparticles exhibiting almost similar impacts on seed germination. The findings indicate that *Annona reticulata*-derived ZnO NPs could be utilized as potential antibacterial agents.

Keywords: Zinc Oxide; *Annona reticulata*; Antibacterial

1. Introduction

Amid various metal oxide nanomaterials, Zinc oxide nanoparticles (ZnO NPs) have attracted considerable attention for the last two decades in multiple applications due to their versatile characteristics, like biocompatibility, lower costs, biodegradability, solubility in alkaline medium, peculiar semiconducting, optical as well as piezoelectric features [1, 2]. The ZnO NPs are inexpensive, less toxic than metal oxide NPs, and have outstanding biomedical applications [3]. Recently, there has been a rising concern regarding the escalating microbial resistance observed toward numerous antimicrobial drugs [4]. Antimicrobial-resistant bacteria are identified to possess resistance traits to methicillin, penicillin, sulfonamide, vancomycin, etc., and many antibiotics struggle to combat these multidrug-resistant mutants [5]. In this line, there has been an increasing interest in the antibacterial potential of metal and metal oxide-based nanomaterials as a highly potential strategy for reducing or preventing microbial drug resistance [4]. In particular, ZnO nanomaterials possess remarkable antibacterial efficiency at low doses against a broad spectrum of bacterial strains owing to producing reactive oxygen species (ROS), electrostatic interactions, and creating antimicrobial ions, etc. [6,7]. Further, the nanoparticle size, buffer type, surface chemistry of NPs like surface charge, and functional groups enhance nanomaterials' antibacterial effects [8]. Several studies have reported that the antibacterial properties of ZnO NPs against gram-negative bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, and gram-positive bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas vulgaris*, *Klebsiella pneumoniae*, and *Candida albicans*, etc. [9-12]. Furthermore, various investigations have inferred the biocompatibility of ZnO nanomaterials to human cells, rendering their suitability as antibacterial agents that are harmful to microbes while remaining non-toxic to human tissues [1].

Among various plant enzymes and essential proteins, zinc is a vital plant micronutrient and is significant in their overall growth [13, 14]. Photosynthesis, antioxidant function, preserving the structural and functional integrity of biological membranes, expression of genes under regular and hassle conditions, and growth regulation are just some of the many processes that rely on zinc [15]. Zinc deficiency on leaves could be visible as chlorosis and white necrotic areas that hinder plant growth and photosynthesis in numerous plant varieties [16]. Zinc produces proteins, nucleic acids, carbohydrates, chlorophyll production, and energy generation. Additionally, zinc plays a role in metabolizing carbohydrates, proteins, phosphates, and lipids [17, 18]. Zinc improves germination rates, stimulates rapid seedling development, modifies seedling water intake and transport capacities, and mitigates the damaging impacts of heat, drought, and salt stresses [18]. However, prominent zinc levels can affect cell function and interrupt various vital functions in a plant owing to replacing other elements with a comparable size and charge [19].

Green synthesis has also received immense popularity in nanotechnology in the last decade as a sustainable and environmentally friendly strategy for synthesizing various metal oxide nanomaterials [4]. Among the various sources, plant extracts is the most convenient and accessible approach for large-scale production of nanomaterials compared to other sources like bacteria and fungi [4]. Plants are known to produce more stable forms of nanomaterials than microorganisms [20]. Hence, biosynthesis approaches based on plant extracts are attracting more attention as a simple, economical, and feasible choice [4]. Moreover, plants possess a rich range of biomolecules such as carbohydrates and proteins and numerous phytoconstituents/secondary metabolites like ketones, terpenoids, aldehydes, flavones, carboxylic acids, and amides, etc., which demonstrate excellent abilities to reduce metal salts into nanomaterials [4, 21]. These molecules serve a dual purpose in forming nanomaterials by functioning as reducing and capping agents to ensure the NP's stability and

biocompatibility, thereby preventing the need for additional chemical reducing and capping agents [22]. Although the exact underlying process for the formation of metal oxide nanomaterials from plant extracts is not yet completely understood, the process typically involves the bio-reduction of metal ions/salts, followed by nucleation, Ostwald ripening, and ultimately influencing the eventual form of nanomaterial during the termination phase [4].

In this regard, *Annona reticulata* leaf and fruit are used to prepare ZnO NPs. *Annona reticulata* (family: Annonaceae), also called bullock's heart, is a tropical plant widely found in India [23,24]. It is prominent in Ayurvedic medicine due to its therapeutic benefits [25]. Moreover, the pharmacological evaluation of *A. reticulata* leaves demonstrates antioxidative and antimicrobial properties against various human pathogenic strains [26]. Numerous phytochemicals like phenolics, flavonoids, and flavanols in *A. reticulata* leaves and fruits constitute an excellent source of natural antioxidants as an efficient free radical scavenger [27,28]. In particular, investigations have shown the antibacterial activity of *A. reticulata* leaves against gram-positive bacteria like *Bacillus subtilis*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* as well as gram-negative bacteria like *E. coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* etc. [29]. In addition, fruit extracts were found to have antibacterial properties against *E. coli*, *S. aureus*, and *K. pneumoniae* [30]. These findings indicate the possibility of ZnO NPs synthesized from *A. reticulata* leaf and fruit to possess antibacterial and antioxidant properties. Therefore, the present work aims to prepare ZnO NPs by the green synthesis method using the aqueous solutions of *A. reticulata* leaf (L-ZO) and fruit (F-ZO) extracts as reducing agents and compared with the pristine ZnO (ZO) NPs. Further, the antibacterial effects and the *Vigna radiata* seed germination potential of the synthesized ZnO NPs are also analyzed.

2. Experimental Procedure

2.1. Sample collection and preparation:

Annona reticulata leaves and fruits were collected from the local area of Nagercoil (8° 11' 11.328" N 77° 25' 51.3228" E), Kanyakumari district, Tamil Nadu. The fresh fruit and leaves were cleaned with distilled water and dried under sunlight for 20 days. Subsequently, they ground with a mixer grinder individually to make a fine powder and kept in an air-tight container for further analysis.

2.2. Preparation of Zinc Oxide (ZnO):

Initially, 5 g of *Annona reticulata* leaf powder was added to 100 ml of double distilled water and boiled at 100°C till the amount was reduced to 50 ml. The product is then filtered using Whatman filter paper 1. Simultaneously, 8.26 gm of zinc sulphate is mixed in 75 ml of double distilled water and stirred for 3 hours. Then, drop the leaf extract solution into the above solution. Further, 3.5 ml of ammonia solution is added to the above mixture and centrifuged at 15000 rpm for 15 minutes. The resultant slurry was allowed to dry at 50°C and calcinated at 400°C for 2 hours under an N₂ atmosphere to get the ZnO-NPs named L-ZO. The same experiment was repeated using fruit extract (F-ZO) of *Annona reticulata* and without biomass (ZO).

2.3 Characterization of synthesized Zinc Oxide (ZnO) nanoparticles:

The synthesized Zinc oxide (ZO), *A. reticulata* leaf (L-ZO), and fruit-assisted Zinc Oxide (F-ZO) nanoparticles were characterized by X-ray diffraction (XRD) with Cu K α ($\lambda=1.5406$ Å) in D8 Advance diffractometer, BRUKER, Fourier Transformation Infrared Spectroscopy (FTIR) was done in JASCO FT-IR- 6600 instruments and Field Emission Scanning Electron Microscopy (FESEM) was performed with help of Carl Zeiss- Sigma 300 microscope. The crystalline nature of nanoparticles was carried out using X-ray diffraction (XRD). The

binding energy and stretching vibrations of prepared ZnO-NPs were carried out by FTIR spectrum analysis. The size and morphologies of the formed nanoparticle were analyzed using Field Emission Scanning Electron microscopy (FESEM).

2.4. Antimicrobial activity:

Antimicrobial activity of the green (Fruit and Leaf mediated) and chemically synthesized ZnO NPs was carried out by agar well diffusion method[31] against the bacterial strains, namely *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* followed by Muller-Hinton agar (MHA), according to the guidelines of Clinical and Laboratory Standards Institute (CLSI). The MHA agar plates were evenly swabbed with overnight cultures of respective bacterial strains by rotating the plate at a 60° angle to ensure a homogeneous distribution of the inoculums. Using a sterile cork borer, wells (6 mm) were created in each petri plate. The 6 mm wells were made with a sterile cork borer. Then varied concentrations (25, 50, 75, 100 µg) of various concentrations of ZnO (leaf-mediated ZnO NPs and Fruit-mediated ZnO NPs) were loaded in the well with standard antibiotics streptomycin (20 µg/ml) as control. Then, the agar plates were kept in an incubator at 37 °C for 18 hrs. After incubation, a clear zone around the wells was observed and measured.

2.5. Seed germination activity:

Mung seeds(*Vigna radiata*) were sterilized using double distilled water for 10 min to ensure surface sterility. Mung seeds were allowed to germinate in a petri dish using different concentrations of ZnO-NPs (2.5 mg, 25 mg, 50 mg) for all ZO, L-ZO, and F-ZO samples. The seeds were checked for germination, and shoot lengths were calculated for all the days and all the concentrations.

3. Results and Discussion

3.1. X-ray Diffraction Analysis (XRD)

The powder XRD pattern of Zinc Oxide particles prepared with and without *Annona Reticulata* fruit and leaf extract is shown in Figure 1. The XRD pattern shows the sharp and intense peaks around the mean position 31.7°, 34.4°, 36.2°, 47.5°, 52.76°, 56.5°, 59.5°, 62.9°, 66.3°, 67.9°, 69°, 72.6°, and 76.9° corresponds to (010), (002), (011), (012), (003), (110), (111), (013), (020), (112), (021), (004) and (022) respectively. These peaks are indexed to a hexagonal Wurtzite structure that agrees with JCPDS 89-1397. The unidentified phases are denoted with (*) at 37.2°, 39.5°, 42.80°, 49.5°, 52.42°, 54° and 54.8°. The prepared extract shows the secondary phase formation in ZO and FZO. The leaf-derived extract revealed the pure Zinc oxide particles.

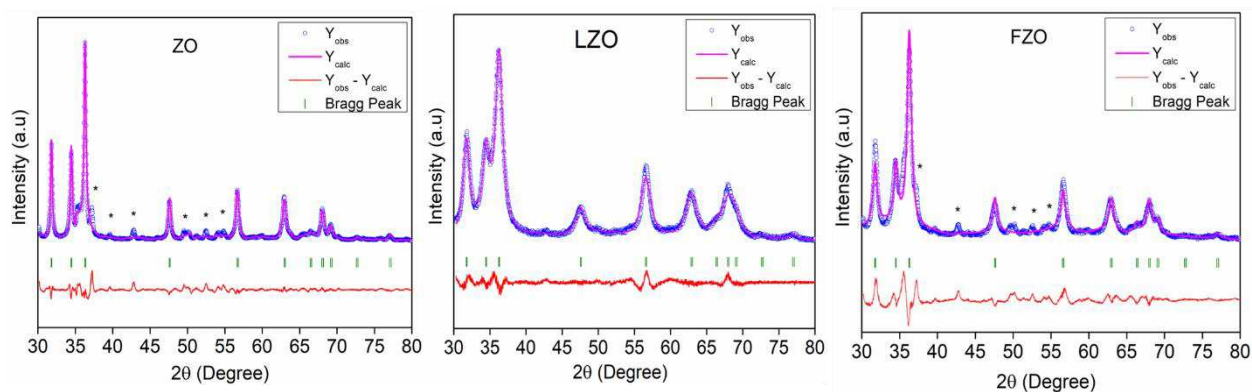


Figure 1: Rietveld Refinement of the prepared ZnO nanoparticles (a) ZO (b) LZO (c) FZO using the Thompson-Cox-Hastings pseudo-Voigt - Axial divergence asymmetry.

It is evident from the figure that ZO represents relatively sharp and distinguished peaks for (100), (002), and (101) planes. In addition to extract, the presence of functional groups has restricted the growth of crystallites on annealing. This shows the order of crystallinity decreased severely in L-ZO, leading to the pure ZnO phase. Unlike Leaf extract, the ZnO nanoparticles prepared without extract and fruit-derived extract exhibited a minor secondary

phase indicated with (*). Rietveld refinement was performed to understand the structural changes in the prepared ZnO particles and the lattice constants tabulated in Table 1.

Table 1: Rietveld refinement lattice parameters for the prepared samples

Sample	a Å	b Å	c Å	$\alpha=\beta$ (degree)	γ (degree)	Volume (Å ³)	R _p	R _{wp}	χ^2
ZO	3.2478	3.2478	5.2040	90.0	120	47.539	9.10	13.2	15.8
FZO	3.2524	3.2524	5.1996	90.0	120	47.6335	11.4	14.4	16.3
LZO	3.2529	3.2529	5.2046	90.0	120	47.6943	4.57	6.02	3.12

Furthermore, in the indexed ZnO phase, the structural parameters are calculated using equations (1-7)

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

$$n = \frac{4}{3\pi \left(\frac{D}{2V} \right)} \quad \text{----- (2)}$$

$$D = \frac{16 M}{N a^3} \quad \text{----- (3)}$$

$$\delta = \frac{1}{D^2} \quad \text{----- (4)}$$

$$\varepsilon = \frac{\beta \cos \theta}{4} \quad \text{----- (5)}$$

$$\sigma_{stress} = \varepsilon E \quad \text{----- (6)}$$

$$SF = \left[\frac{2\pi^2}{45\sqrt{(3 \tan \theta)}} \right] \quad \text{----- (7)}$$

Where, M is the molar mass of ZnO, N is the Avogadro number (6.023×10^{23} particle /mol), a is lattice constant, θ is the Bragg angle, β is FWHM and E denotes the Young's Modulus of Zinc Oxide (128 GPa).

Table 2: Estimated Structural Parameters of the prepared ZnO particles.

Sample Name	Plane	d-spacing (Å)	D (nm)	$n \times 10^{-22}$	$\delta \times 10^{-15}$ Kg/m ³	$\epsilon \times 10^{-3}$ (Kg/m ³) ⁻²	σ stress ($\times 10^8$ Pa)	SF
ZO	100	2.8127	23	1.74	1.87	1.499	1.918	0.4741
	002	2.6020	14	2.87	5.07	2.471	3.162	0.4544
	101	2.4744	19	2.07	2.62	1.777	2.274	0.4420
LZO	100	2.8171	7	5.19	16.46	4.448	5.693	0.4745
	002	2.6023	6	6.51	25.83	5.572	7.132	0.4544
	101	2.4774	6	6.68	27.20	5.717	7.318	0.4423
FZO	100	2.8166	11	3.50	7.52	3.006	3.848	0.4744
	002	2.5998	6	6.07	22.53	5.204	6.660	0.4542
	101	2.4766	7	5.31	17.27	4.556	5.832	0.4422

$$D_x = 6.31 \times 10^4 \text{ kg/m}^3$$

The above parameters determine the structural characteristics like micro strain(ϵ) , stress(SF), dislocations density (δ) and the X-ray density (D_x) of ZnO. Therefore, solving the equation 1, gives about the crystallite size of the material. Even though there were no significant changes observed in d-spacing, the crystallite size of LZO (7.79 nm) and FZO (11.53 nm) are restricted to less than 50% of ZO (23.12 nm) in (100) plane. A peak broadening along all planes is indicative of decreased crystallite size in LZO and FZO. Therefore, it is evident that the functional groups in the extract acted as both a capping agent and an agent controlling crystallinity. It is also inferred from the tabulated values that the σ stress of LZO is

relatively more stress with 5.693×10^8 Pa whereas 1.918×10^8 Pa on the (100) plane. Correspondingly, the increased microstrain for LZO could be observed, further confirming the stress the particles experienced from the extract. Similarly, for the (002) and (101) planes, the increasing order of σ insists that the extract executes the residual stress along the respective planes. The value n represents the number of unit cells present, considering the shape of the particles is spherical. Since the severity of the fruit extract inflicts stress on the crystallite of ZnO, it is restricted and minimal. Hence, there is a similar formation of secondary phases in ZnO without extract. The Rietveld method utilized the least square refinement for the experimental and calculated pattern, which shows a good fit in LZO. The X^2 of the ZO and FZO is high due to the unidentified secondary phases.

3.2. Fourier Transform Infrared (FTIR) Spectroscopic analysis

The Fourier Transform Infrared (FT-IR) spectroscopy was used to spot the functional groups in the synthesized ZnO nanoparticles. FTIR spectra of F-ZO (Fig.2a) exhibited a strong, broad peak with a maximum at 3412 and 3329 cm^{-1} , indicating the O-H stretching modes of vibration in the hydroxyl functional groups in alcohols and phenolic compounds [32]. The peak at 2349 cm^{-1} is attributed to the free -C=O group [33], while the peak at 1624 cm^{-1} denotes C=C stretching [34]. The band at 617 cm^{-1} is due to the bending vibration of the O-H group [35]. The spectra of L-ZO (Fig. 2.b) had strong absorption peaks at 3309 cm^{-1} , indicating O-H stretching vibrations [36] and 1620 cm^{-1} denoting the C=O stretching vibration [37]. The band at 2347 cm^{-1} indicates alkanes [38] and the peaks at 738 cm^{-1} and 619 cm^{-1} infer Zn-O stretching [39-41]. Spectra of ZO (Fig. 2.c) have prominent peaks at 3444 cm^{-1} and 617 cm^{-1} , denoting stretching and bending vibrations of the O-H group [42,35]. The band at 1622 cm^{-1} corresponds to the primary amide bond of a protein due to carbonyl stretching [43], and the peak at 1130 cm^{-1} denotes the C=O stretching of the alcohol

group [44]. The peak at 1056 cm^{-1} is attributed to the C-NH_2 primary aliphatic amines [45]. The peak at 962 cm^{-1} is assigned to vibrations of the C-O group [46], while the peak at 617 cm^{-1} is attributed to the bending vibration of the O-H group [35]. These hydroxyl, carbonyl, and amine groups act as powerful natural reducing, capping, and stabilizing agents, which play a vital role in the reduction of the Zn^+ ions [47-49].

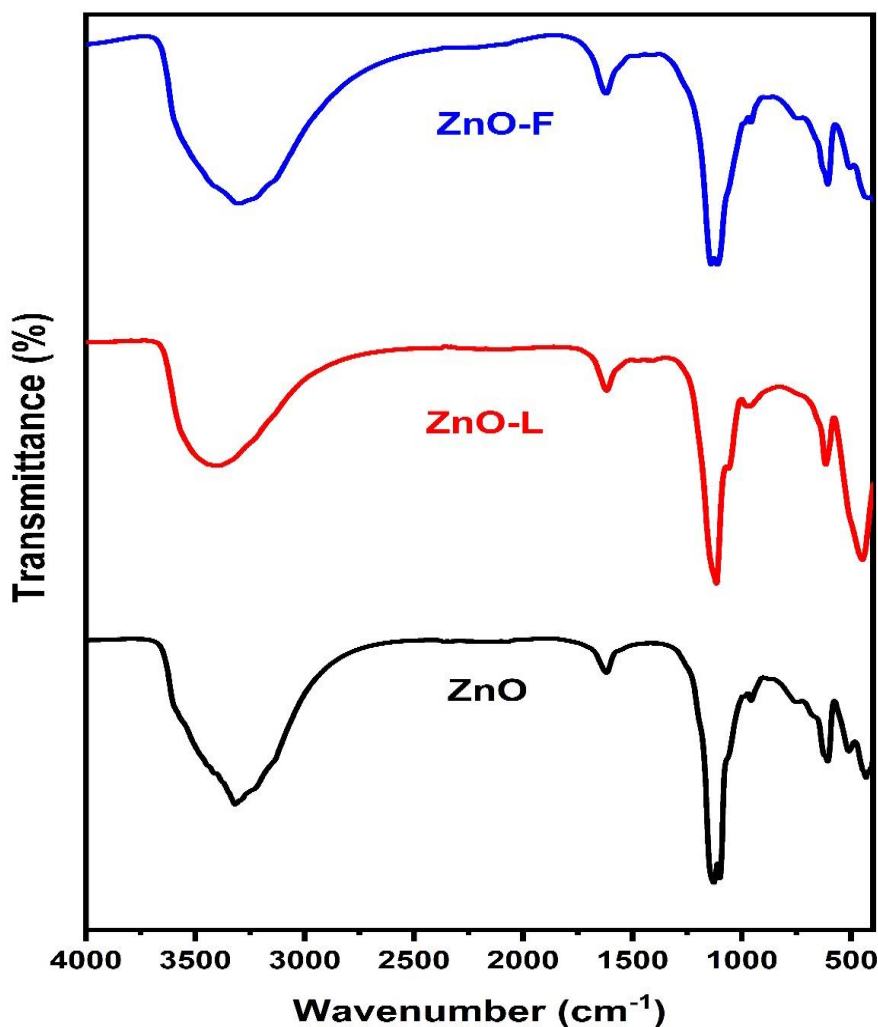


Figure 2. FTIR spectra of Zinc oxide (ZnO), *A. reticulata*-assisted Zinc Oxide (ZnO-L), and *A. reticulata* fruit-assisted Zinc Oxide (ZnO-F)

The functional groups on ZnO NPs surface have been shown to influence their antibacterial properties [1]. The hydroxyl groups were more prominent in all three kinds of ZnO NPs. Various reports have indicated that hydroxyl groups in ZnO NPs modulate the

photocatalytic processes in zinc oxide by producing antimicrobial superoxide radicals [1,50,51]. Moreover, carbonyl groups have been found to have a greater capacity to bind the surface of nanomaterials to produce a layer of covering and to avoid agglomeration [52].

3.3. Field Emission Scanning Electron Microscopy (FESEM) analysis

The FESEM images of three different zinc oxide nanoparticles with different magnifications are shown in Figure 3-5. The obtained FESEM images of ZO (Figure 3) indicated an irregular morphology characterized by plate-like structures and a few sharp-edged needle-like particles. The NPs exhibited a broad range of particle sizes spanning from 200 nm – 2µm with significant aggregation and non-uniform distribution. The FESEM images of L-ZO showed an agglomerated morphology with a size distribution ranging between 100 – 200 nm. The nanoparticles displayed aggregation and formed clusters characterized by a relatively spherical morphology. FESEM images of F-ZO showed particles with a size range between 300-500 nm with no distinct boundaries between individual particles. The particles were agglomerated and formed large clusters with irregular shapes and sizes. The increase in size may be due to the overlapping of particles on each other. Moreover, our results also corroborate with other reports [53].

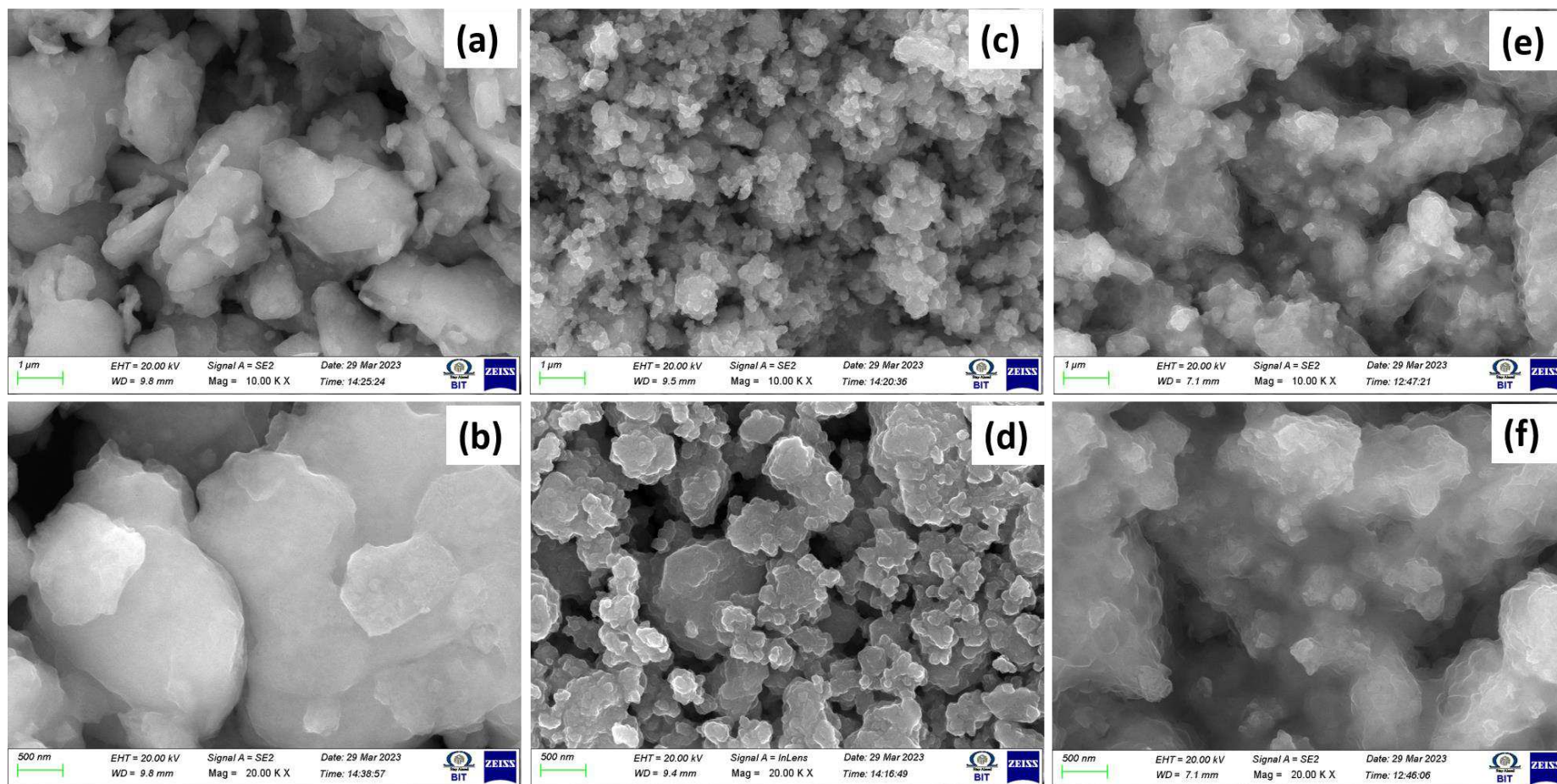


Figure 3. FESEM images of (a, b) Zinc Oxide, (c, d) *A. reticulata* Leaf, and (e, f) *A. reticulata* fruit-assisted Zinc Oxide nanoparticles with different magnifications.

3.4. Antibacterial activity

In recent decades, antibacterial resistance has been a major problem worldwide. The plant-derived nanoparticles may possess more biologically active compounds and therapeutic agents [54]. Besides, green-mediated synthesis is beneficial to the environment and non-toxic to the organisms surrounding us. In the present study, the antibacterial activity of leaf-derived and fruit-derived ZnO NPs and chemically synthesized ZnO NPs was studied against gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*) and gram-negative bacteria (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*). Fig. 4 and Table 1 show the growth inhibition results obtained with L-ZO, F-ZO, and ZO NPs. In general, a rise in nanoparticle concentration increased the zone of inhibition for ZnO NPs tested against the bacterial species, indicating a dose-dependent effect. In this work, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* exposed to ZnO NPs at various concentrations (25-100 µg). The ZnO alone group shows a maximum zone of inhibition observed at 100 µg against *Klebsiella pneumoniae* bacteria with 17.8mm compared to the control group (Antibiotic-Streptomycin). Next, the L-ZnO mediated NPs showed a maximum zone of inhibition (14.7) noted at 100 µg against *Staphylococcus aureus* bacteria and the antimicrobial activity was decreased when compared with the control group. On the other hand, the fruit-mediated F-ZnO NPs have a zone of inhibition noted at 100 µg against *Staphylococcus aureus* bacteria (21.1mm). However, in the same concentration, *Klebsiella pneumoniae* has a maximum zone of inhibition of 18.1mm, compared with the control streptomycin-treated group (Streptomycin 13 mm).

At 100 µg concentration, fruit-derived zinc oxide (F-ZO) nanoparticles exhibited the maximum zone of inhibition compared to leaf-derived zinc oxide (L-ZO) nanoparticles against *E.coli*, *S. aureus*, *K.pneumoniae*, and *P.aeruginosa*. However, against *S. aureus*, F-ZO showed a comparable zone of inhibition at 100 µg (21.1 mm) compared to antibiotic

streptomycin (21.8 mm), and interestingly, for *K. pneumoniae* F-ZO exhibited even a higher zone of inhibition diameter of 16.9 mm at 50 µg surpassing streptomycin's activity value of 13.0 mm. This highlights the potential of fruit-derived ZnO nanoparticles as an alternative or complementary antimicrobial agent.

Nanomaterials seem efficient against various strains, although their efficacy against specific strains might vary considerably [6]. *S. aureus* consistently showed more susceptibility to the ZnO NPs, indicated by larger zones of inhibition. Among the three different kinds, fruit-derived ZnO NPs showed a considerable increase in the zone of inhibition against all bacterial species except *E. faecalis*, for which the zone of inhibition was the lowest and remained the same (9.6 mm) irrespective of NPs concentrations.

The antibacterial activity observed in ZnO NPs by green synthesis could be attributed to the presence of plant constituents possessing antibacterial properties. Reports have shown the superior antimicrobial effectiveness of green synthesized nanoparticles in comparison to their commercially available counterparts [55, 56]. In a study by Aher and team, methanol and chloroform extracts of *Annona reticulata* fruit showed significant antibacterial effects against *S. aureus*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa* strains. This property has been reported due to the occurrence of phytochemicals in the fruit extracts [57]. In another study, alcoholic solvent extracts were used for both dry and fresh *A. reticulata* fruit peel extracts showed noteworthy antibacterial activity against *S. aureus* and *P. aeruginosa* [58].

Likewise, the antibacterial activity of leaf-derived ZnO NPs could be due to phytochemicals. Phytochemical screening of *A. reticulata* leaf extracts has indicated that alkaloids, tannins, steroids, terpenoids, and coumarins can cause antibacterial activity. Moreover, in a previous study, ZnO NP has been proposed as a substitute for antibiotics since it is non-toxic to healthy cells even at 100 µg/ml [12]. Even though the antibacterial property

of ZnO NPs has been linked to various factors, the precise toxicity mechanism is not fully elucidated. The most distinct mechanisms proposed are the straight contact of ZnO NPs with bacterial cell walls, the destruction of bacterial cell integrity, and the release of antimicrobial ions, primarily Zn^{2+} ions. The released Zn^{2+} ions significantly inhibit active transport, disrupt amino acid metabolism, and interfere with enzyme systems. The development of reactive oxygen species has been identified as another prominent factor. Reactive species can penetrate the bacterial cell membrane and cause damage to essential cellular components such as lipids, DNA, and proteins [1].

Usually, the bacteria comprise cell walls, membranes, and inner cytoplasm. The cell wall plays a pivotal role in maintaining the cytoplasm's osmotic pressure and gives the organism shape. The cellular organelles in the bacteria are actively involved in the metabolism, growth, and replication. The cytoplasm of bacteria consists of carbohydrates, proteins, nucleic acids, salts, ions, and water (Fu et al., 2005). The ZnO NPs penetrate the cell wall and produce membrane integrity, which enters into the cytosol of bacteria to produce DNA replication, reduced ATP synthesis, and mitochondrial oxidative stress, leading to cellular death. In the current study, one possible mechanism contributing to the enhanced antibacterial property of ZnO NPs could be the presence of an abundance of O-H (hydroxyl) groups, as indicated by the FTIR spectra. The hydroxyl groups could produce reactive oxygen species through photocatalytic reactions or by interaction with the bacterial cells. The production of hydroxyl radical (OH) and H_2O_2 internalization within the bacterial cell membrane causes lipids, DNA, and protein degradation, contributing to their toxicity [1]. However, further scientific investigation is required to elucidate the precise mechanism involved.

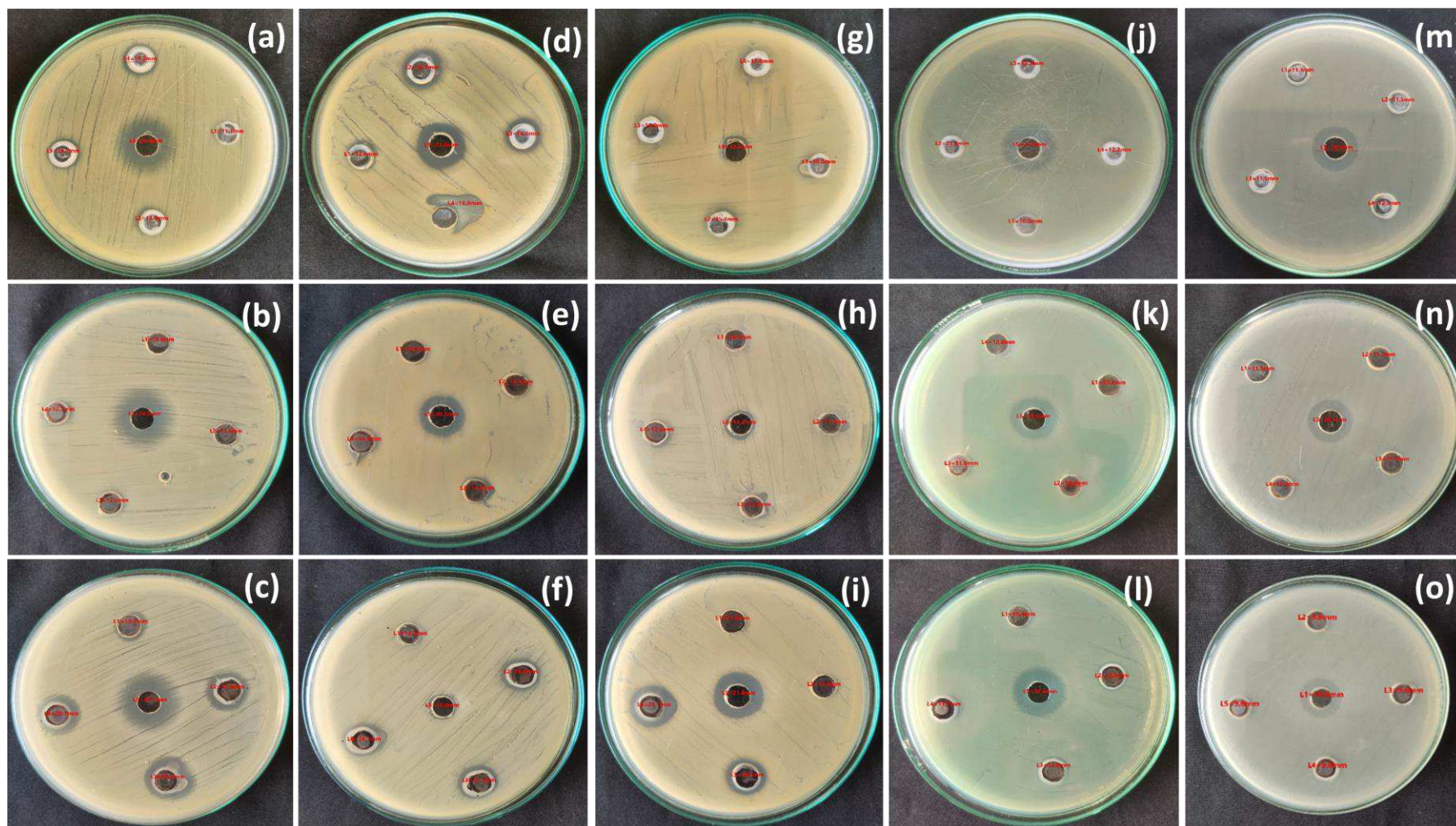


Figure 4. The antibacterial activity studies of ZO, L-ZO, and F-ZO nanoparticles against (a-c) *E. Coli*, (d-f) *Staphylococcus aureus*, (g-i) *Klebsiella pneumoniae*, (j-l) *Pseudomonas aeruginosa*, and (m-o) *Enterococcus faecalis* bacteria.

Table 1: Zone of inhibition parameters against various bacteria

Sample Name	Concentration (µg)	Zone of inhibition (mm)				
		<i>E.coli</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Enterococcus faecalis</i>
ZO	25	11.1	12.4	10.0	10.0	11.3
	50	13.6	16.5	15.2	11.3	11.5
	75	14.7	16.5	15.0	12.2	11.5
	100	15.2	16.8	17.8	12.2	12.5
	Streptomycin 20µg/ml	25.8	22.0	12.0	22.9	18.9
L-ZO	25	10.6	12.5	10.9	10.4	11.5
	50	11.4	13.9	11.7	10.4	11.7
	75	12.3	14.4	12.8	11.8	11.7
	100	12.7	14.7	13.6	12.8	12.2
	Streptomycin 20µg/ml	24.5	20.5	14.3	19.5	20.1
F-ZO	25	13.3	11.6	12.2	10.4	9.6
	50	17.8	13.4	16.9	12.6	9.6
	75	20.4	18.5	17.7	13.6	9.6
	100	20.7	21.1	18.1	13.7	9.6
	Streptomycin 20µg/ml	25.3	21.8	13.0	22.4	18.6

3.6. Effect of ZnO NPs on seed germination

The germination of *Vigna radiata* seeds under the influence of ZO, F-ZO, and L-ZO nanoparticles and the observed shoot length of the plant is given in Table 2 and Fig. 5. Three kinds of NPs favoured seed germination at lesser concentrations but reduced seedling growth at elevated concentrations. A concentration of 2.5 mg showed a higher seed growth, whereas 50 mg showed the lowest growth in measured shoot length. Similar trends in seed germinations have been observed in other investigations. For instance, in a study by De la Rosa and team [59], ZnO NPs positively influenced tomato and wheat seed germination and growth parameters at lower concentrations. However, increased concentrations resulted in a decrease in these parameters. Similarly, Khanm et al. (2018) highlighted that treatment with

lower concentrations of nano-ZnO yielded better results [15]. ZnO NPs in comparatively low concentrations enhanced germination and seedling growth in most species studied, whereas high concentrations of these nanoparticles adversely impacted seedlings [60].

The highest seed germination was observed in green synthesized ZnO NPs (26cm). However, L-ZO and F-ZO exhibited almost similar impacts on seed germination. From Day 4 to Day 6, regardless of the nanoparticle type or concentration, there is typically an increase in seed germination and shoot length. Overall, these findings indicate that ZO nanoparticles, as well as L-ZO and F-ZO nanoparticles to some extent, can influence seed germination and plant growth. The effects vary depending on the concentration of nanoparticles, with lower concentrations potentially stimulating growth.

Zinc has a role in initiating various biochemical modifications in seeds to begin germination, including breaking dormancy, hydrolysis, imbibition, and enzyme activation [61-63]. ZnO NPs have been shown to improve and synchronize germination, and it has previously been established that zinc plays a crucial role in the metabolism of carbohydrates and proteins [64]. Previous findings have shown that ZnO NPs can enhance the radical and plumule length of seeds since zinc is essential for the biosynthesis of endogenous hormones, including auxins and gibberellins [63,65,66]. In another study, Nano priming ZnO NPs for wheat seed germination improved seed water assimilation, increasing α -amylase activity. Significant increases were seen in the concentration of photosynthetic pigments in ZnO nanoprimed plants [63]. However, further investigation is required to determine whether ZnO NPs derived from *A. reticulata* extracts have any specific influence on seed germination.



Figure 5. The representative photographic images of *Vigna radiata* seed germination on various days.

Table -2. The measured shoot length of *Vigna radiata*

Sample	Concentration (μg)	Length (cm)		
		Day 4	Day 5	Day 6
ZO	2.5	16	18	24.5
	25	13	15	20
	50	7	14	21
L-ZO	2.5	15	20	26
	25	13	17	19
	50	5	11	15
F-ZO	2.5	16	18	26
	25	10	12	17
	50	6	8	15

4. Conclusion

In this article, we have investigated the antibacterial, antioxidant, and seed germination perspective of zinc oxide nanoparticles synthesized using a green strategy from *Annona reticulata* leaf and fruit extracts, as well as chemical synthesis. The FESEM analysis of ZnO NPs synthesized using the chemical approach revealed an irregular morphology characterized by the presence of plate-like and needle-like structures. The leaf-derived ZnO NPs demonstrated a relatively spherical morphology, while the fruit-derived ZnO NPs displayed an irregular morphology. Aggregation of nanoparticles was observed in all three kinds of nanoparticles. The three kinds of NPs favoured seed germination at lesser concentrations, but the germination rate decreased at higher concentrations. The green synthesized ZnO NPs exhibited the highest seed germination rate, demonstrating their influence on plant growth. The results of the antibacterial study exhibited a considerable broad-spectrum inhibition effect against five clinical bacterial pathogens, with the highest zone of inhibition exhibited by fruit-derived ZnO NPs. Furthermore, both fruit and leaf-derived ZnO NPs demonstrated enhanced antioxidant properties. Hence, these ZnO NPs obtained by a simple and cost-effective strategy could be used as promising candidates for enhanced antibacterial, antioxidant, and seed germination effects.

Ethics declarations

Ethical Approval

Not applicable.

Competing Interests

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