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### Research Article

## Green Synthesis of Zinc Oxide Nanoparticles using *Peristrophe bicalyculata* (Retz.) Nees Leaf Extract: Characterization and Antibacterial Activity

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### ABSTRACT

Nanotechnology has developed as a key part of investigation in modern-day material science in recent decades. Biosynthesized nanoparticles garnered substantial significance due to their accelerated synthesis, economic viability and environmentally benign characteristics. The current study uses *Peristrophe bicalyculata* (Retz.) Nees leaf extract to produce ZNO NPs in an ecologically safe manner. These nanoparticles were created using zinc acetate dihydrate, utilizing the extract of leaves as a biological reductant. Created nanoparticles were evaluated using UV-Visible (UV-VIS) spectroscopy. Characteristics of produced nanoparticles and their constituent elements were analysed through XRD (X-ray diffraction). The ZNO NPs exhibited crystalline morphology. According to SEM imaging, the ZNO NPs appeared to possess a non-uniform spheroid morphology. The functional groups involved in capping, along with reduction processes, were identified using Fourier transform infrared (FTIR) spectroscopy. Efficacy of ZNO-NPs may be associated with their size and shape as observed through scanning electron microscope (SEM) as well as transmission electron microscope (TEM). Our investigation assessed the efficiency of ZNO-NPs as potent bactericidal agents against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus albus* and *Streptococcus pneumoniae*. This study demonstrates that zinc oxide nanoparticles synthesized via a green approach possess intrinsic antibacterial efficacy, supporting their potential application in the development of antimicrobial therapeutics. Additional studies are essential to explore the mechanistic pathways and determine the cytotoxicity of zinc oxide nanoparticles.

## INTRODUCTION

Nanotechnology is a rapidly evolving field focused on creating new materials at the nanoscale level. This field involves the synthesis, characterization and manipulation of matter at a size of 1 to 100 nm.<sup>[1]</sup> High surface-to-volume proportion is a special characteristic of NPs that makes them especially intriguing. This characteristic makes nanoparticles more sensitive as compared with bulk substances since atoms in the center are often less active as compared with atoms on the surface.<sup>[2]</sup> Research has indicated that a nanoparticle's surface-to-volume ratio increases with declining size, leading to

increased antimicrobial potency.<sup>[3]</sup> Biological, physical, along chemical approaches can be utilized to fabricate nanoparticles.<sup>[4]</sup> Despite their effectiveness, these Approaches are resource-intensive, time-intensive and environmentally hazardous because they employ hazardous chemicals as reductants and stabilizing agents during the nanoparticle formation process, which has a number of negative consequences on the surroundings and living things.<sup>[5-7]</sup> Compared to conventional chemical or physical processes, green chemistry approaches for producing nanoparticles from plant extracts are more financially sustainable and environmentally benign

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because they are carried out at room temperature and physiological pH.<sup>[8]</sup> Various biological sources, including yeast, bacteria, actinobacteria, algae and plants, are utilized for NP synthesis. Among these sources, plants are regarded as the most suitable candidates for the synthesis of metal nanoparticles.<sup>[9]</sup> The phytochemicals, especially secondary metabolites such as alkaloids, tannins, terpenoids, and flavonoids of the plant extract, can function as reductants, facilitating the conversion of metal salts into nanoscale metallic particles.<sup>[10,11]</sup> This method also contributes to extending the life span of nanoparticles.<sup>[12]</sup>

The utilization of metallic/metal oxide nanoscale particles, including silver, aluminum oxide, iron oxide and zinc oxide, is steadily increasing over time.<sup>[13]</sup> Researchers are particularly interested in zinc oxide nanoparticles amongst various metal oxide NPs due to their many uses in solar cells, electronics, communications, sensors, photocatalytic degradation, cosmetics, ecosystem preservation and biological and medical fields. These applications are made possible by their advantageous properties, which include minimal cell damage, biological safety and economic efficiency.<sup>[14-17]</sup> Furthermore, there are numerous biological uses for zinc oxide nanoparticles, such as biosensing, bio-labelling, gene and medication delivery, cancer therapy advancements, the agricultural sector, and nanomedicines.<sup>[18-20]</sup> Furthermore, the USFDA has acknowledged ZNO as a highly stable material.<sup>[21]</sup> Zinc oxide exhibits an energy band gap of 3.3 eV and generates OH ions at elevated concentrations than many other nanoparticles upon reaction, contributing to its bioactivity.<sup>[22]</sup> Factors including particle diameter, concentration, morphological characteristics, as well as specific surface area, substantially impact the antimicrobial effectiveness of zinc oxide NPs. Research has indicated that antimicrobial activity increases as particle size decreases from bulk zinc oxide to smaller zinc oxide particles. High concentration of H<sub>2</sub>O<sub>2</sub> produced on the surface of ZNO NPs is the main cause of their antibacterial potency.<sup>[23]</sup> ZNO NPs kill bacteria by binding to their cell surfaces and interfering with membrane permeability. Once they get into the cell, they trigger oxidative stress, which stops the cell proliferation thus eventually kill them.<sup>[24]</sup>

*Peristrophe bicalyculata*, belonging to the genus *Peristrophe* in the family Acanthaceae, is an erect shrub with a slender, branched stem, growing to a height of 30 to 70 cm.<sup>[25]</sup> This erect shrub originates from tropical regions of Africa, extending to Nigeria as well as Niger from Mauritania, and is also found in parts of Asia, including India, Burma and Thailand. It is usually denoted to as the "Goddess of mercy".<sup>[26]</sup> In traditional medicine, it is used as an antivenom, applied for the treatment of skeletal fractures, ligament injuries, fever, cold, respiratory irritation, bronchial asthma, cardiovascular diseases and for treating various skin problems, as well as eye and ear infections.<sup>[27-29]</sup> The plant's leaves are applied for pain relief, fever

suppression, inflammation reduction, cancer prevention, fertility-promoting and preventing diarrhea.<sup>[30]</sup> The herb also exhibits antibacterial properties (tuberculostatic).<sup>[31]</sup> The investigation was designed to synthesize zinc oxide nanoparticles employing *P. bicalyculata* (Retz.) Nees leaf extract characterizes the fabricated nanoparticles and evaluates antimicrobial efficacy targeting some gram-negative, in addition to gram-positive pathogenic microbes affecting humans. Synthesized zinc oxide NPs were investigated through UV-visible spectroscopy (UV-vis), Fourier transform infrared (FTIR), transmission electron microscopy (TEM), X-ray diffraction (XRD), and scanning electron microscopy (SEM).

## MATERIALS AND METHODS

*Peristrophe bicalyculata* (Retz.) Nees (Acanthaceae) was collected from Sankleshwari hill, Banaskantha. HiMedia Laboratories Pvt. Ltd. (Mumbai) supplied the sodium hydroxide, zinc acetate dihydrate, and other chemicals. Bacterial strains were purchased from IMTECH, Chandigarh, India.

### Plant Extract Preparation

The leaves were properly cleaned multiple times using distilled H<sub>2</sub>O to eliminate dust as well as debris. Leaves were permitted to dry for 15 to 20 days at ambient temperature in the shade. Dried leaves were then crushed to yield coarse powder. About 5 g of powder was weighed, then boiled using 50 mL of double-distilled H<sub>2</sub>O for 15 minutes. Once cooled, this aqueous extract was subjected to filtration using filter paper (Whatman #1). This extract was preserved at 4°C for later examination. For future use, the dried powder was kept at ambient temperature in a hermetically sealed container.

### Biogenic Synthesis of ZNO NPs

15ml of extract of the plant was heated for 10 min at 50°C and 50 mL of 50 mM zinc acetate dihydrate solution was gradually added drop by drop with constant stirring. The mixture was held at a stable pH 9. The mixture was reduced completely by heating it to 95°C for 30 minutes. A cream-colored, yellow precipitate of zinc hydroxide was generated from the reaction solution. After centrifuging the precipitates for 20 minutes at 4°C at 13,000 rpm, it was twice cleaned with sterile de-ionized water. Using a hot air oven, the zinc oxide nanoparticle powder was dried for 24 hours at 70°C.

### Evaluation of zinc oxide nanoparticles

A Shimadzu UV-1800 UV-vis spectrophotometer (Shimadzu Corporation, Japan) was utilized to record UV-VIS light spectra of ZNO NPs at diverse wavelengths between 300 and 700 nm.

FTIR was performed on dried ZNO NP powder with the help of Nicolet 6700 Fourier Transform Infrared

Spectrophotometer (Thermo Fisher Scientific) in the 4000 to 400  $\text{cm}^{-1}$  range.

XRD of ZNO nanoparticles was performed with the help of an X-ray diffractometer (model D8 Advance, Bruker, Germany), in the  $2\theta$  range from 20 to  $80^\circ$ .

FESEM combined with X-ray spectroscopy (EDAX) was utilized to observe the surface features of produced ZNO NPs utilizing a Nova Nano FEG-SEM 450 (FEI Ltd, Netherlands). HRTEM was utilized to examine morphological characteristics and size dispersion of ZNO NPs using a Talos F200i S/TEM (HRTEM-200KV) (Thermo Fisher Scientific).

### Antimicrobial Assay

Antibacterial potential of produced ZNO NPs was assessed with the help of agar well diffusion approach, with minor modifications.<sup>[32]</sup> The test microorganisms were *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, *Acinetobacter baumannii* and *Streptococcus pneumoniae*. With a sterilized glass spreader, 100  $\mu\text{L}$  aliquots of bacterial suspension at a concentration of 106 CFU per mL were evenly distributed on nutrient agar plates. Each plate had three wells 3 mm in radius, formed with a sterile cork borer. Aliquots of 100  $\mu\text{L}$  of multiple concentrations (1, 3, 5, 10, 20 and 30 mg per mL) of ZNO NPs solutions were poured into corresponding wells. The positive control was ciprofloxacin (5 mg/mL), while the negative control was sterile distilled water. Also, aqueous plant extract was poured to compare the antibacterial activity. To make sure the samples were homogeneously dispersed throughout the nutrient agar, plates were incubated for 24 hours at  $37^\circ\text{C}$  after being refrigerated for 30 minutes. Measurements and records were made of the radius of the bacterial growth inhibition zone. Three separate runs of the experiment were made.

## RESULTS AND DISCUSSION

### Structural Analysis

Biofabricated ZNO NPs were investigated through XRD analysis in the  $2\theta$  range of 20 to  $80^\circ$ . The XRD spectrum of green synthesized ZNO NPs is displayed in Fig. 1. Lattice planes (202), (004), (201), (112), (200), (103), (110), (102), (101), (002), and (100) were represented by diffraction peaks that were found at  $2\theta$  values of  $76.94^\circ$ ,  $72.52^\circ$ ,  $69.07^\circ$ ,  $67.94^\circ$ ,  $66.38^\circ$ ,  $62.85^\circ$ ,  $56.59^\circ$ ,  $47.52^\circ$ ,  $36.25^\circ$ ,  $34.40^\circ$ , and  $31.75^\circ$  sequentially. According to the Joint Committee on Powder Diffraction Standards (JCPDS file: 36-1451), this peak displays the wurtzite hexagonal structure of ZNO and verifies the presence of ZNO Nps.<sup>[33]</sup> Narrow and sharp XRD peaks validated the great crystallinity of the produced zinc oxide NPs.<sup>[34]</sup> Demissie *et al.*, achieved similar results by synthesizing ZNO NPs utilizing leaf extract from *Lippia adoensis*.<sup>[35]</sup> They observed diffraction peaks at  $69.96^\circ$ ,  $67.97^\circ$ ,  $66.41^\circ$ ,  $62.86^\circ$ ,  $56.59^\circ$ ,  $47.54^\circ$ ,  $36.24^\circ$ ,

$34.42^\circ$ , and  $31.76^\circ$ , which correspond to (201), (112), (200), (103), (110), (102), (101), (002), and (100). The computed size and lattice strain values for ZNO NPs are accessible in Table 1. Dimensions of nanoparticles were determined by applying Debye-Scherrer's equation, which is expressed as

$$D = \frac{0.94\lambda}{\beta \cos \theta}$$

Where  $\beta$  is for full width at half maximum (FWHM),  $\theta$  stands for Bragg's angle,  $\lambda$  for X-ray wavelength, and  $D$  for crystalline size. The intense plane of (101) showed that ZNO NPs produced showed 41.13 nm average crystalline size.

### Optical Characterization

#### UV – visible absorption

To authenticate the generation of the ZNO NPs, UV-vis spectroscopy was conducted within the 300 to 700 nm spectral range. An absorbance peak was noticed at 375 nm in the UV-vis spectral analysis of produced ZNO NPs (Fig. 2). The Detected peak value falls within the 360 to 380 nm range, which is indicative of zinc oxide nanoparticle absorption. Rahaiee *et al.* reported a similar outcome, with a significant peak discovered at 370 nm.<sup>[36]</sup> An absorption peak of 375 nm was found as per an investigation led by Mahalaxmi *et al.*, of the formation of ZNO NPs mediated by *Sesbania grandiflora*.<sup>[37]</sup> Using the following formula, the ZNO energy gap at the highest absorption peak was determined to be 3.3 eV.<sup>[38]</sup>

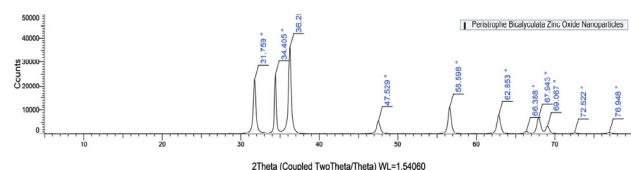
$$E_g = \frac{h\nu}{\lambda} = \frac{1239.83}{\lambda}$$

Here  $\lambda$  denotes the wavelength of maximum absorption;  $E_g$  stands for the bulk band in eV;  $\nu$  is the speed of light;  $h$  denotes Planck's constant.

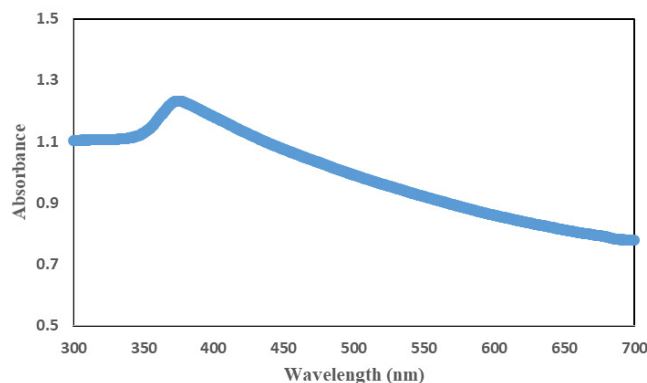
#### Fourier transform infrared spectroscopy (FTIR)

FTIR investigation offers molecular evidence on the chemicals as well as biomolecules in plant extract, which are involved in the manufacturing of NPs by identifying the functional groups on their surface. In this investigation, an FTIR investigation was conducted between 4000 to 400  $\text{cm}^{-1}$ . Fig. 3 displays infrared absorption spectra of ZNO NPs made from the extract of leaves of *P. bicalyculata*. O-H stretching vibration of alcohols involved in hydrogen bonding and the isolated hydroxyl stretch characteristic of the phenol group are characterised by absorption at 3552  $\text{cm}^{-1}$ . IR signal at 3479  $\text{cm}^{-1}$  shows the medium-intensity N-H bond stretch associated with a primary amine and the hydroxyl group vibration characteristic of alcohols. The vibrational stretching of the hydroxyl group in alcohols accounts for the absorption peak at 3414  $\text{cm}^{-1}$ . Peak at 3233

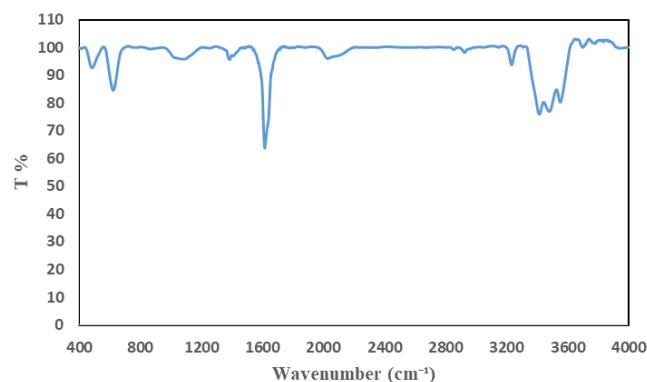




**Fig. 1:** XRD spectra of *Peristrophe bicalyculata* leaf-extract mediated zinc oxide nanoparticles



**Fig. 2:** UV-vis spectral analysis of zinc oxide nanoparticles



**Fig. 3:** FTIR spectra of zinc oxide nanoparticles derived from *P. bicalyculata* leaf extract

$\text{cm}^{-1}$  results from alcoholic O-H stretch as well as carboxyl group vibrations. Absorption bands at 2925 as well as 2853  $\text{cm}^{-1}$  relate to the hydroxyl stretching vibration of carboxyl acid along with alcohol, the stretching vibration of the amino group in amine salts and the C-H stretching of alkanes. The vibrational band that appeared at 2028  $\text{cm}^{-1}$  is attributed to stretching vibrations of the  $\text{N}=\text{C}=\text{S}$  moiety in isothiocyanate and the  $\text{C}=\text{C}=\text{N}$  group in ketenimine. The 1616  $\text{cm}^{-1}$  band is characteristic of the conjugated  $\text{C}=\text{C}$  bond stretch, cyclic alkenes,  $\alpha$ ,  $\beta$ -unsaturated ketones, along with N-H bending vibrations in amine groups. Medium 1385  $\text{cm}^{-1}$  peak arises from C-H bending vibration of aldehyde and O-H bending vibrations of alcohol, as well as phenol. Absorbance at 1074  $\text{cm}^{-1}$  specifies the vibrational stretch of the C-N linkage in amines. The resonance at 624  $\text{cm}^{-1}$  is indicative of the vibrational mode of the C-Br bond in alkyl halides with medium intensity. The absorption band

**Table 1:** Crystalline size and lattice strain of ZNO nanoparticles determined by X-ray diffraction (XRD) analysis

| $2\theta$ ( $^\circ$ ) | Crystalline size (nm) | Lattice strain |
|------------------------|-----------------------|----------------|
| 31.75                  | 48.34 (100)           | 0.0050         |
| 34.40                  | 59.43 (002)           | 0.0035         |
| 36.25                  | 41.13 (101)           | 0.0045         |
| 47.52                  | 25.91 (102)           | 0.0043         |
| 56.59                  | 24.75 (110)           | 0.0032         |
| 62.85                  | 21.23 (103)           | 0.0031         |
| 66.38                  | 20.25 (200)           | 0.0029         |
| 67.94                  | 19.46 (112)           | 0.0029         |
| 69.06                  | 18.58 (201)           | 0.0030         |
| 72.52                  | 25.28 (004)           | 0.0020         |
| 76.94                  | 17.31 (202)           | 0.0027         |

confirms the existence of ZNO at 484  $\text{cm}^{-1}$ .<sup>[39]</sup> Absorption bands between 1066 to 1250  $\text{cm}^{-1}$  correspond to C-N stretching of aliphatic amines, indicative of proteins/enzymes. As reported by Khan *et al.*, the vibrational bands between 3300 to 2500 per cm are attributed to O-H stretching of carboxylic acid.<sup>[40]</sup>

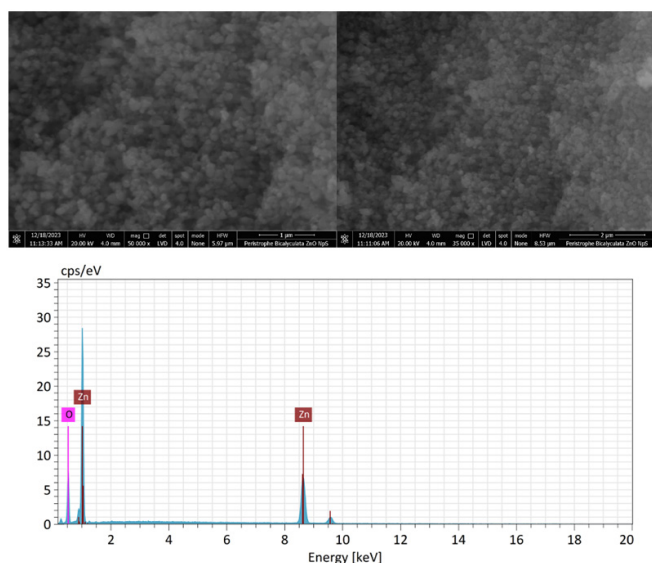
## Microscopic Analysis

### Scanning electron microscopy with energy dispersive X-ray (EDX)

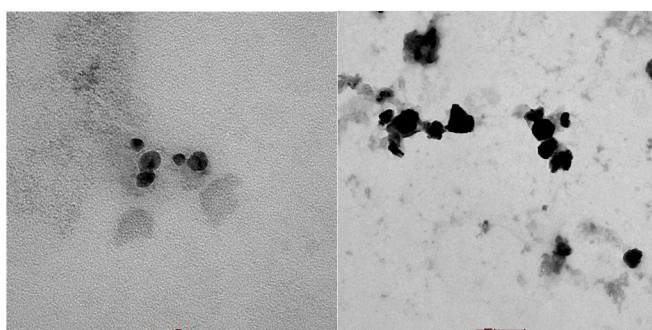
The size, shape and surface area of produced ZNO NPs were investigated through SEM-EDX characterization. The produced ZNO NPs have an asymmetrical spherical structure, exhibiting similarity to that described by Alamdari *et al.*, as illustrated in the SEM micrograph of Fig. 4a.<sup>[41]</sup> The image displays clustered zinc oxide nanoparticles as well as an unevenly formed microstructure that resembles a flower. The enhanced surface area and persistent intermolecular interactions of biomediated nanoparticles contribute to their propensity for agglomeration.<sup>[42]</sup> EDX examination of the produced nanoparticles demonstrates that oxygen and zinc are present as their constituent elements. For the ZNO nanoparticles produced by *P. bicalyculata*, the elemental analysis reveals 78.25% zinc and 21.75% oxygen components (Fig. 4b).

### Transmission electron microscopy

Fig. 5 illustrates the spherical and flower-shaped structure of ZNO NPs as seen in acquired TEM micrographs. The average size of ZNO nanoparticles was found to be around 30 nm, which is similar to the value described by Dhandapani *et al.*<sup>[43]</sup> Additionally, the TEM scans showed that the nanoparticles were clumped together. Surrounding the ZNO NPs, there are biomolecules in the plant extract that may be responsible for this aggregation.



**Fig. 4:** (a) SEM micrograph of *P. bicalyculata* leaf extract-derived zinc oxide nanoparticles (b) EDX spectral data for *P. bicalyculata* leaf extract-derived zinc oxide nanoparticles



**Fig. 5:** Transmission electron microscopy (TEM) image of synthesized zinc oxide nanoparticles

### Assessment of the Bacterial inhibition capability of biosynthesized zinc oxide nanoparticles

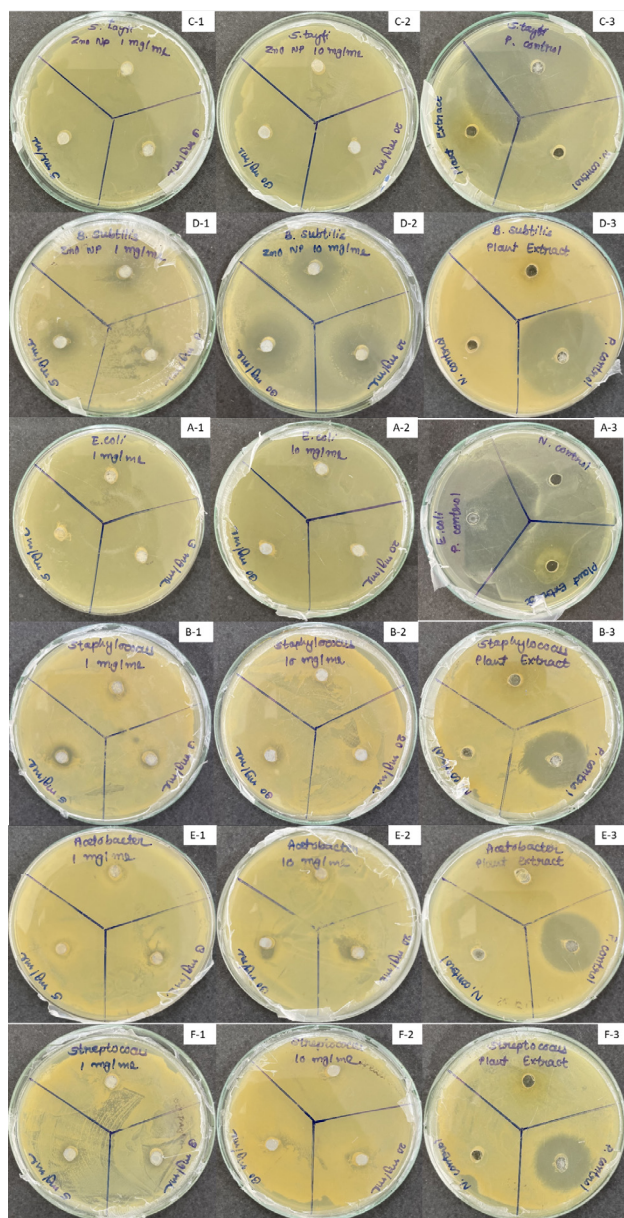
The agar well diffusion assay was utilized to inspect the bactericidal efficacy of the produced zinc oxide nanoparticles targeting six pathogenic microorganisms. A range of nanoparticle concentrations (1, 3, 5, 10, 20 and 30 mg per mL) was poised and used. Antibacterial potency was measured targeting three gram-positive bacteria (*B. subtilis*, *S. aureus*, *S. pneumoniae*) and three gram-negative bacteria (*S. typhi*, *E. coli*, *A. baumannii*). Ciprofloxacin served as the standard reference for each bacterial strain. The antimicrobial properties of the formulated nanoparticles, which inhibit bacterial growth, are observed as a distinct inhibition zone, as represented in Fig. 6. Table 2 presents the inhibition outcomes of zinc oxide nanoparticles. With two exceptions, all bacterial strains were vulnerable to the action of the produced ZNO NPs. Zones of inhibition for *S. aureus* at 3, 5 and 30 mg per mL concentration were  $10.33 \pm 0.57$ ,  $11.66 \pm 0.57$  and  $10.33 \pm 0.57$ , respectively. The zones of inhibition for *B. subtilis* at

1, 3, 5, 10, 20 and 30 mg per mL concentration were  $13.66 \pm 0.57$ ,  $11 \pm 1$ ,  $14.33 \pm 1.15$ ,  $26.66 \pm 0.57$ ,  $26.66 \pm 0.57$  and  $29.66 \pm 0.57$ , respectively. The value zone of inhibition for *A. baumannii* at 10, 20 and 30 mg per mL concentration was  $10.33 \pm 0.57$ . The value of zone of inhibition for *S. pneumoniae* at 3, 5 and 20 mg per mL concentration was  $10.33 \pm 0.57$ ,  $10.33 \pm 0.57$  and  $10.66 \pm 0.57$ , respectively. *B. subtilis* had a significant zone of inhibition (mean value) of  $29.66 \pm 0.57$  mm, indicating greater susceptibility. In comparison to *B. subtilis*, *A. baumannii*, *S. aureus*, as well as *S. pneumoniae* demonstrated reduced susceptibility, with zones of inhibition of  $11.66 \pm 0.57$ ,  $10.33 \pm 0.57$ , and  $10.66 \pm 0.57$ , respectively. *S. typhi* as well as *E. coli* did not show any susceptibility, with no zone of inhibition. The antibacterial performance of ZNO nanoparticles is well established in the literature. Farooq et al. assessed the antimicrobial potential of ZNO nanoparticles synthesized by *Calotropis gigantea* flower, stem and leaf extract targeting *B. subtilis*, *P. multocida*, *E. coli*, and *S. aureus*.<sup>[44]</sup> Zinc oxide NPs synthesized using *Crotalaria verrucosa* were shown by Sana et al. to be effective against diverse disease-causing bacterial strains, including *P. vulgaris*, *S. aureus*, *E. coli*, as well as *K. pneumoniae*, with the greatest cytotoxic effects occurring at higher nanoparticle concentrations.<sup>[45]</sup> El-Beley et al. synthesized ZNO nanoparticles and assessed their antimicrobial efficacy targeting *P. aeruginosa*, *E. coli*, *C. albicans*, *B. subtilis*, as well as *S. aureus*.<sup>[46]</sup> Nanoparticles possess antibacterial properties primarily due to their tiny size, which leads to a significantly higher surface area relative to volume. This intensified surface area enhances their interaction with microbial structures, allowing them to exert antibacterial effects. The reactive oxygen species (ROS) formation induced by NPs also contributes to their antibacterial effectiveness by disrupting bacterial cellular proteins and deoxyribonucleic acid. Moreover, bacteria have not yet developed highly effective resistance mechanisms against nanoparticles, further supporting their antibacterial efficacy.<sup>[47]</sup> Production of several reactive species, including hydroxide ion ( $\text{OH}^-$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), and superoxide radical ( $\text{O}_2^-$ ), is responsible for the harmful effects that arise from ROS creation. These reactive species are produced when nanoparticles interact with hydroxyl groups and absorbed water ( $\text{H}_2\text{O}$ ), leading to the formation of hydroxide ions and protons, which subsequently generate  $\text{O}_2^-$ . The reaction of  $\text{O}_2^-$  with  $\text{H}^+$  results in the formation of hydroperoxyl radical ( $\text{HO}_2$ ), which subsequently combines with electrons and protons to generate  $\text{H}_2\text{O}_2$ . The final product,  $\text{H}_2\text{O}_2$ , penetrates the microbial envelope, where it binds to lipids, proteins and nucleic acids, ultimately causing cellular apoptosis.<sup>[48]</sup> Zinc oxide nanoparticles are drawn to the cytoplasmic membrane of bacteria, resulting in damage to the bacterial genome. This ultimately results in microbial cell death due to the rupture of surface of cell.<sup>[36]</sup> Distinct cell wall structures of gram-negative as well as gram-positive



**Table 2:** Mean value of inhibition zone (mm) synthesized by zinc oxide nanoparticles generated by *P. bicalyculata* leaf extract

| S/N | Organism             | Mean width of Inhibition zone (mm) |                       |                       |                        |                        |                        |                                        |
|-----|----------------------|------------------------------------|-----------------------|-----------------------|------------------------|------------------------|------------------------|----------------------------------------|
|     |                      | Concentration of ZNONPs            |                       |                       |                        |                        |                        |                                        |
|     |                      | 1 mg ml <sup>-1</sup>              | 3 mg ml <sup>-1</sup> | 5 mg ml <sup>-1</sup> | 10 mg ml <sup>-1</sup> | 20 mg ml <sup>-1</sup> | 30 mg ml <sup>-1</sup> | Ciprofloxacin<br>5 mg ml <sup>-1</sup> |
| 1   | <i>E. coli</i>       | 0                                  | 0                     | 0                     | 0                      | 0                      | 0                      | 49.66 ± 0.57                           |
| 2   | <i>S. aureus</i>     | 0                                  | 10.33 ± 0.57          | 11.66 ± 0.57          | 0                      | 0                      | 10.33 ± 0.57           | 28.33 ± 0.57                           |
| 3   | <i>S. typhi</i>      | 0                                  | 0                     | 0                     | 0                      | 0                      | 0                      | 39.33 ± 1.15                           |
| 4   | <i>B. subtilis</i>   | 13.66 ± 0.57                       | 11 ± 1                | 14.33 ± 1.15          | 26.66 ± 0.57           | 26.66 ± 0.57           | 29.66 ± 0.57           | 39.66 ± 0.57                           |
| 5   | <i>A. baumannii</i>  | 0                                  | 0                     | 0                     | 10.33 ± 0.57           | 10.33 ± 0.57           | 10.33 ± 0.57           | 29.33 ± 0.57                           |
| 6   | <i>S. pneumoniae</i> | 0                                  | 10.33 ± 0.57          | 10.33 ± 0.57          | 0                      | 10.66 ± 0.57           | 0                      | 27.33 ± 0.57                           |

**Fig. 6:** Zone of inhibition of plant-mediated zinc oxide nanoparticles targeting (A) *E. coli* (B) *S. aureus* (C) *S. typhi* (D) *B. subtilis* (E) *A. baumannii* (F) *S. pneumoniae*

bacteria cause variations in the absorbability of reactive oxygen species (ROS).<sup>[49]</sup> In general, gram-negative species are less sensitive than gram-positive species owing to variations in their cell wall architecture. Gram-negative bacteria possess a thin (<10 nm) layer of peptidoglycan surrounded by an outer membrane that contains multiple pores. In contrast, gram-positive bacteria feature a dense (20–80 nm) peptidoglycan layer that serves as the cell exterior. Compared to gram-positive species, Gram-negative bacteria are less sensitive because they have an extra outer membrane.<sup>[50]</sup> The antibacterial efficiency improved as the particle diameter decreased from macro-scale ZNO to bio-fabricated white zinc oxide nanoparticles, indicating an opposite association amid antibacterial action as well as the size of the metallic oxides.<sup>[51]</sup> Another proposed mechanism highlights the relationship between the structural features of nanoparticles and their association with bacterial organisms. Smaller nanoparticles exhibit higher surface reactivity, allowing them to penetrate cells more easily and release Zn<sup>2+</sup> ions. It is well known that Zn<sup>2+</sup> released from ZNO nanoparticles interferes with bacterial physiological processes, active transport and catalytic efficiency, which in turn causes bacterial cell death.<sup>[39]</sup>

## CONCLUSION

This study demonstrates that a sustainable and economical strategy is ideal for synthesizing spherical ZNO nanoparticles with strong antimicrobial properties using *P. bicalyculata* leaf extract. UV-vis analysis discovered a prominent absorption peak at 375 nm for synthesized NPs. Analysis of the ZNO nanoparticles was done with FTIR, XRD, SEM with EDAX, as well as TEM. The impact of multiple zinc oxide nanoparticle concentrations on infectious bacterial species was assessed. High concentration of zinc oxide nanoparticles (30 mg/ml) successfully inhibited the development of bacteria. A higher concentration resulted in a larger zone of inhibition, indicating an enhanced antibacterial effect. This study holds significant social significance as a consequence of the biocompatible and cost-effective nature of the material,

making it appropriate for the environment and biomedical applications.

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