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# GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES WITH BIOLOGICAL ACTIVITY AND THEIR CHARACTERIZATION BY USING BANANA PEEL EXTRACT

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## Abstract

The anti-bacterial performance of ZnO nanoparticles, which have been effectively synthesised utilising banana feel extract, has been studied. To verify the synthesis, the structural, compositional, and morphological characteristics of the NPs were meticulously documented and examined. An UV-Vis absorption peak of 302 nm was noticed in the aqueous suspension of NPs, which essentially indicated their production. By using X-ray diffraction analysis, it was discovered that ZnO-NPs had a hexagonal wurtzite structure with an average particle size of 16.5nm. Some biomolecules and functional groups in the leaf extract were discovered using FTIR analysis to be in charge of the stability and encapsulation of ZnO-NPs. The material has the necessary constituent compositions, according to energy-dispersive X-ray analyses. At a grain size of about 15 nm, scanning electron microscopy revealed a flower-like morphology of ZnO nanoparticles. By using UV visible spectrophotometer to examine the NPs' optical characteristics, the band gap was determined to be 3.37 eV. The prepared ZnO-NPs have demonstrated antibacterial activity against gram negative bacteria *V. cholerae,* (MTCC-3906), *S. flexneri* (ATCC-12022), *Enterobacter aerogenes* (MTCC-241) and gram-positive bacteria *Staphylococcus aureus* (MTCC-3160), *C. glutamicum* (MTCC-2745), *Clostridium perfringens* (ATCC 13124). The maximum zone of inhibition 24.82±0.75mm (Fig.) against *C. perfringens* at the concentration of 200µg/mL followed by 21.86±0.35mm against *S. flexneri* and 20.46±0.50mm against *V. chloreae* at the concentration of 200µg/mL. It is clear from the creation of an inhibitory zone that membrane disruption is a

necessary component of nanoparticles' biocidal effect. It is believed that ZnO's antibacterial ability is primarily caused by the generation of hydrogen peroxide on its surface.

**Key words:** Banana peel extract, ZnO-NPs, Microorganisms, Antibacterial activity

## Introduction

Nanotechnology is today regarded as a proven cutting-edge technology with extensive applications in pharmaceutical, chemical, food processing and mechanical sectors. Nanotechnology also has applications in computers, power generation, optics, medicinal delivery, and environmental sciences (Albrecht *et al.,* 2006). Many nanoscale devices have been produced since the emergence of nanotechnology, employing a variety of technologies such as chemical, physical and green approaches. Green nanoparticle synthesis, on the other hand, is a versatile tool that can be simply generated and modified (Herlekar *et al.,* 2014). Conventional approaches to nanoparticle synthesis have several drawbacks, including lengthy processing, high costs, arduous operations, and, in particular, the use of toxic compounds. Due to these constraints, the majority of relevant research has focused on eco-friendly and rapid synthesis procedures for the creation of nanoparticles (Simonis and Schilthuizen, 2006). The development of environmentally benign technologies for manufacturing nanoscale materials has been a key priority for material scientists in recent years. In this regard, green synthesis of nanoparticles, particularly employing extracts from various plants, is an emerging trend in green chemistry since it is easy, inexpensive, and safe (Bala *et al.,* 2015).

Metallic oxide nanoparticles have received a lot of attention in the last decade due to their numerous uses in several technical domains (Altavilla *et al.,* 2011). ZnO-NPs are an intriguing inorganic material with several advantages. ZnO-NPs have a wide range of applications, including energy conservation, electronics, textiles, catalysis, healthcare, cosmetics, semiconductors, and chemical sensing (Al-Naamani *et al.,* 2016). The NPs are nontoxic and biocompatible, and they have a wide range of medicinal uses, including anticancer (Mishra *et al.,* 2017), anti-inflammatory and antibacterial characteristics, targeted drug delivery (Cai *et al.,* 2016), wound healing, and bioimaging (Lai *et al.,* 2016). Nanoproducts with a wide range of attributes and uses may be created using a variety of processes (chemical, physical, and biosynthetic). Although plant-based synthesis of ZnO nanoparticles has previously been described, there is little research on their different biological features such as antibacterial, ant larvicidal, protein kinase, and anticancer activity (Lai *et al.,* 2016).

Green manufacturing of metal oxide nanoparticles is an intriguing subject in the realms of nanoscience and nanobiotechnology (Muthu *et al.,* 2016). Conventional techniques are rapid and create a huge number of nanoparticles at once, but they contribute to hazardous levels of toxicity in the environment since poisonous compounds are used as capping agents (Parthasarathy *et al.,* 2016). Both microbes and plants may absorb and accumulate inorganic metallic ions from their surroundings, allowing them to drastically reduce pollution of environment and recover metals from industrial waste. Leaf extracts nanoparticles are very effective, economical, and green catalysts that help reduce pollution (Abdol Aziz *et al.,* 2019). Since different fruit peel extracts

have characteristics with plant extracts, like being abundant in bio-components that are crucial for the biological synthesis of nanoparticles and being easily accessible, they are of considerable interest (Kumar *et al.,* 2017). Bananas are used for a variety of reasons in India, resulting in a considerable quantity of trash in the form of banana peels, which are sometimes tossed without further regard to their potential. Banana peels contain antifungal and antibiotic components that have been successfully used in the agriculture industry to treat tomato fungus (Kumar *et al.,* 2012).

Under this study, Nano - particles were created from plantain peel extract. The nanomaterials were then characterised and tested for antibacterial potency against Gram-positive and Gram-negative human pathogenic pathogens. SEM was used to measure the nanoparticles' size and shape, and UV-vis spectroscopy was used to investigate how the particles were made (SEM).

**Materials and Methods**

$Zn(CH_3COO)_2$ in powder form and NaOH in pellet form were obtained from Bio Enviro Chemical Suppliers for use in this investigation. Fresh banana peels were purchased from local food stall merchants in and around Visakhapatnam**.**

**Banana Peel Extract (BPE) preparation**

The steps for creating banana peel extract are the same as those that Agarwal mentioned (2017). The peels were thoroughly cleansed with water before being allowed to dry at room temperature for an overnight period. In a 600-milliliter beaker, Fifty gm of the peels were put to 500 ml of water after being chopped into little pieces. After 30 minutes of heating at a steady 70°C, the mixture was wrapped in aluminium foil and stirred with a magnetic stirrer at 1000 rpm. The liquid was then strained to remove the banana skins. The final extract was then stored for later use at 4°C.

**ZnO nanoparticle synthesis**

A 0.1M solution of $Zn(CH_3COO)_2$ was first put into a 1L conical flask. Thereafter, 20 ml of banana peel extract and 180 ml of 0.2M $Zn(CH_3COO)_2$ solution were mixed in a 1:9 ratio and 1M sodium hydroxide (NaOH) solution was then added. After that, the mixture was boiled at 70°C for an hour with a magnetic stirrer, constant stirring at 1000 rpm, until the liquid turned pale yellow and a white precipitate formed at the bottom of the beaker. The mixture was then boiled and filtered using Whatman No. 1 filter paper, which was weighed beforehand. The leftover material was then crushed and dried at 40°C.

**Characterization of ZnO nanoparticles**

**SEM analysis**

Scanners of the Carl Zeiss Japan model were used to do the SEM analysis. A thin film of powder sample was created on carbon covered film by connecting a little quantity of dried, finely ground material to the grid. Extra sample was then removed with blotting paper. The material on the SEM plate was permitted to air out for five minutes under a mercury lamp. The characterization of ZnO nanoparticles generated biologically was determined by SEM examination.

**UV–Vis spectroscopy**

After dilute small dilutions of the mixture ten times with distilled water and transferring to

a cuvette, the transmittance of the reaction mixture was measured using UV-Vis spectroscopy to track the deduction of zinc ions (Ocean Optics, USA).

**XRD (X-ray diffraction) analysis**

The XRD (X-ray diffraction) method was used to examine the production and quality of compounds. To do this, produced ZnO nanoparticles were centrifuged (1400 rpm) for 15 min before being rinsed 3 times with ethanol and 3 times with sterile distilled water. The dried, purified ZnO nanoparticles precipitate was ground using a ceramic mortar and pestle after being baked in an oven at 60 degrees Celsius. To assess the powdered material, an XRD (X-ray diffraction) (X'Pert PROPAN Analytical, Europe) was employed. The 20 to 80 scans took around 2 hr to complete.

**FTIR (Fourier-Transform Infrared) analysis**

The FTIR analysis (Perkin Elmer Spectrum One FT-IR Spectrometer) was performed at room temperature with a scan range of 500-4000 cm-1. The functional group of the produced ZnO-NPs was determined using this technique.

**Antibacterial assay**

The antibacterial activity of the ZnO-NPs on selected microorganisms was assayed by well diffusion method. Minimum inhibitory concentration of the compound to inhibit the bacterial growth was also determined by well diffusion method.

**Test Microorganisms**

The Bacterial cultures of, *Staphylococcus aureus* (MTCC-3160), *Corynibacterium glutamicum* (MTCC-2745), *Vibrio chloreae,* (MTCC-3906), *Shigella flexneri* (ATCC-12022), *Enterobacter aerogenes* (MTCC-241), *Clostridium perfringens* (ATCC 13124) grown overnight at 37°C were used for testing the antibacterial activity.

**Well diffusion method**

**Muller Hinton Agar media preparation**

The media was made by dissolving 33.9 g of medium, a product, in 1000 mL of distilled water. The dissolved media was thoroughly mixed, autoclaved at 15 lbs of pressure for 15 minutes at 121°C, and then poured while still molten onto 100mm petri plates (25–30 mL/plate).

**Nutrient broth preparation**

To make one litre of nutrient broth, 13 gramme of commercial nutrient medium (Hi-Media) was dissolved in 1000 mL of purified water and then heated until thoroughly dissolved. The medium was administered as requested and sterilised in an autoclave for 15 minutes at 121°C under 15 lbs of pressure.

1. Standard Antibiotic (Chloramphenicol)

In a 100 mL conical flask, Muller Hinton agar medium (High-Media) was mixed with water, sterilised in an autoclave at 121°C, 15 pounds for 15 min., and then placed into sanitised petri plates. For antibacterial activity, chloramphenicol was used as the positive control and DMSO as the negative control. By using the agar well diffusion method, the isolated compound's anti - bacterial activity was assessed (Perez et al., 1990). Using a sterilised glass spreader, inocula were

applied to the surface of agar plates. Using a sterile cork borer, five equal-sized wells were created. The purified chemical was produced to a final concentration of 100 mg/mL to assess its antibacterial efficacy. The drug was placed in aliquots at different concentrations (40, 60, 80, and 100 µg/mL) into each well. The plates were then incubated for 24 hours at 37ºC in an incubator, and the diameter (mm) of the clear inhibitory zone that developed around each well was recorded.

## Results and Discussion

### Synthesis of Zn-NPs

Plantain extract includes phytochemicals that change depending on the section of the fruit used. As a result, the composition has a substantial impact on nanoparticle creation. These phytochemicals are antioxidants as well as non-toxic compounds. As a result, they operate as stabilising agents and may also convert metal precursors to metal oxide nanoparticles, acting as both reducing and stabilising agents (Jiang *et al.,* 2005). It takes two steps to make ZnO nanoparticles: first, the zinc salt and plantain peel extract combine to form the hydroxide, which is then converted into ZnO in a 350 °C calcination reaction. Due to Zn's attachment to the acetate anion, the precursor salt has a high reduction potential and a tendency to give electrons. Due to the ease with which the Zn cation may couple to the different antioxidants in the PPE through the OH group, Zn $(OH)_2$ is produced, which causes an off-white precipitate to form.

**Fig. 1 Synthesis of ZnO nanoparticles**

### UV–visible absorption spectrum studies

A UV-Vis spectrophotometer was used for preliminary validation of the generated ZnO nanoparticles. Using ultrasonication, 1 mg of zinc oxide nanoparticles were mixed with 10 ml of distilled water, and the optical density of the mixture was assessed between 280 and 375 nm. The

Zinc oxide nanoparticles absorption peak at 302 nm in the spectra can be attributed to the material's natural band-gap absorption caused by electron transitions. ZnO nanoparticles with an average size of 16 nm were reported to have a blue-shifted absorption peak at 325 nm by Senthil Kumar and Sivakumar (2014).

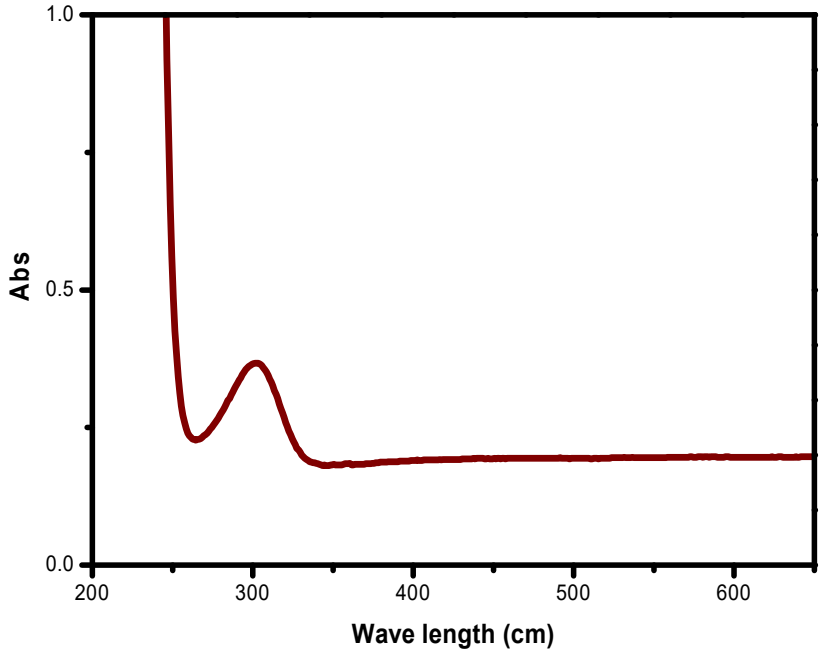


**Fig. 2 UV-Visible spectra**

## X-ray diffraction analysis

Fig. 3 shows the synthesised ZnO-NPs' X-ray diffraction (XRD) pattern. The orientation and crystal structure of zinc oxide nanoparticles were revealed by the XRD pattern. The peak position is indexed as (100), (002), (101), (102), (110), (103), (200), (112), (201), and (202) planes, with 2 values of 31.7°, 34.4°, 36.2°, 47.5°, 56.6°, 62.8°, 65.5°, 67.9°, 68.8°, 72.3°, and 76.7°. These values are roughly consistent with those of powder ZnO obtained from the International Center The absence of additional diffraction peaks from other phases demonstrates the phase purity of ZnO-NPs. The research was carried out in four main processes, including the extraction of three distinct kinds of banana peels, according to Siti Marfu'ah *et al.,* 2020. Using extracts from all three species of banana peels, ZnO-NPs have been effectively synthesised, as shown by the diffractogram, which yielded values of 2 angles at 31.78°, 34.42°, 36.25°, 47.53°, 56.57°, 62.87°, 66.42°, 67.93°, and 69.09°.

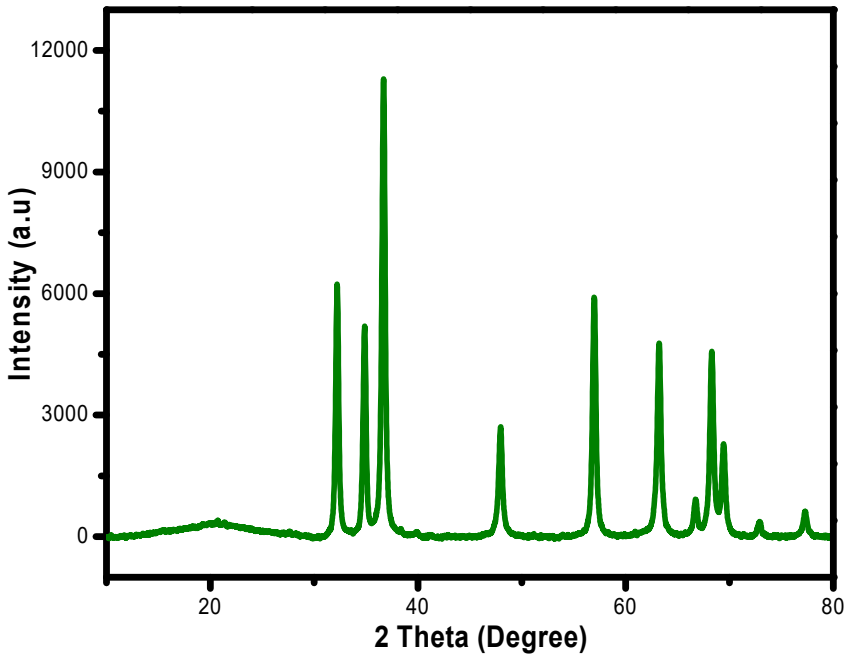


**Fig. 3: XRD pattern of the ZnO-NPs**

**FTIR (Fourier transform infrared spectroscopy) analysis**

Previously, the plantain peel was shown to contain biogenic amines, phenolic compounds, and carotenoids (Pereira *et al.,* 2015). These chemicals gave rise to some of the functional groups found in plantain peel extract. On the sample was taken, a little amount of material was placed, and spectra were taken in the 4000-400 cm-1 wavelength range. This FT-IR identifies the likely structural features involved in the production of ZnO-NPs and provides information about the molecules' vibrational and rotational modes of motion. Figure 4 shows the FTIR analysis of the ZnO nanoparticles made with banana peel extract. In order to identify the functional groups of the prospective biomolecules that cap the ZnO-NPs and efficiently stabilise them, a spectroscopic test was conducted. The literature (Rad *et al.,* 2019) states that the O-H stretching alcohols, primary and secondary amine stretching vibrations, and C-H stretching of alkanes can all support the peaks that emerged at 3300-3500 $cm^{-1}$ in the FTIR. The peaks at 1658, 1421, and 1100 cm-1 were caused by C = C stretching in the aromatic ring in polyphenols and aliphatic amines, whereas the peaks at 2300 $cm^{-1}$ and 550 $cm^{-1}$ were caused by the hexagonal phase of zinc oxide, respectively (Selvarajan *et al.,* 2013).

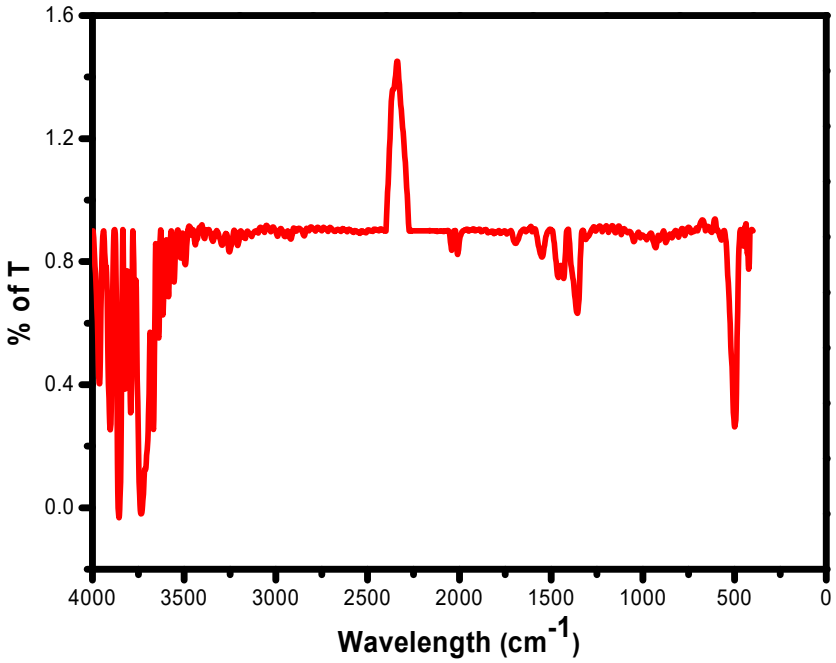


**Fig. 4 FTIR analysis of ZnO nanoparticles**

**SEM analysis of Zinc Oxide nanoparticles**

Scanning electron microscope investigation was used to examine the produced ZnO-NPs' surface morphology. Figure 5 displays the SEM picture of ZnO-NPs, which reveals a regular distribution of ZnO molecules in the form of flowers. Using ImageJ software, the particle size of ZnO nanoparticles was estimated from the SEM picture to be around 15 nm, which is consistent with the predicted particle size (16.5nm) from XRD data. Bioorganic capping molecules and NPs have formed hydrogen bonds and electrostatic interactions that have caused them to collect together (Jiang *et al.,* 2020). Additionally, the SEM picture of ZnO nanoparticles showed that they are not in direct touch with one another, indicating that capping agents have stabilised the NPs (Kadam *et al.,* 2020).

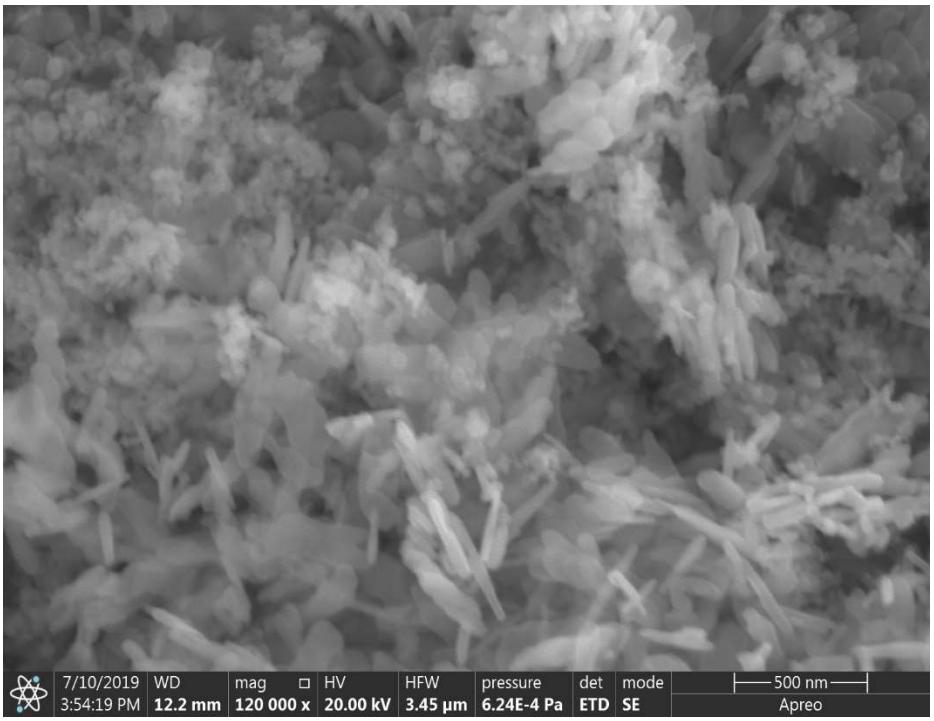


**Fig.5 SEM image of ZnO nanoparticles**

## Anti-bacterial activity of ZnO nanoparticles

The anti-bacterial performance of Zn-oxide nanoparticles was tested against gram negative bacteria *V. cholerae,* (MTCC-3906), *S. flexneri* (ATCC-12022), *Enterobacter aerogenes* (MTCC-241) and gram-positive bacteria *S. aureus* (MTCC-3160), *C. glutamicum* (MTCC-2745), *C. perfringens* (ATCC 13124) by well diffusion method. In fig.6 anti-bacterial performance of Zn-oxide nano particles at different concentrations 100µg/ml, 150µg/ml, 2000µg/ml against both gram- negative and gram-positive bacteria were shown. The diameter of inhibition zones around each well is measured in millimeter and represented in table 1. The maximum zone of inhibition 24.82±0.75mm (Fig.) against *Clostridium perfringens* at the concentration of 200µg/mL followed by 21.86±0.35mm against *Shigella flexneri* and 20.46±0.50mm against *Vibrio chloreae* at the concentration of 200µg/mL. The formation of zone of inhibition states the mechanism of the biocidal action of nano-particles involves disrupting the membrane. It is believed that ZnO's antibacterial ability is primarily caused by the generation of hydrogen peroxide on its surface (Rai *et al.,* 2009)

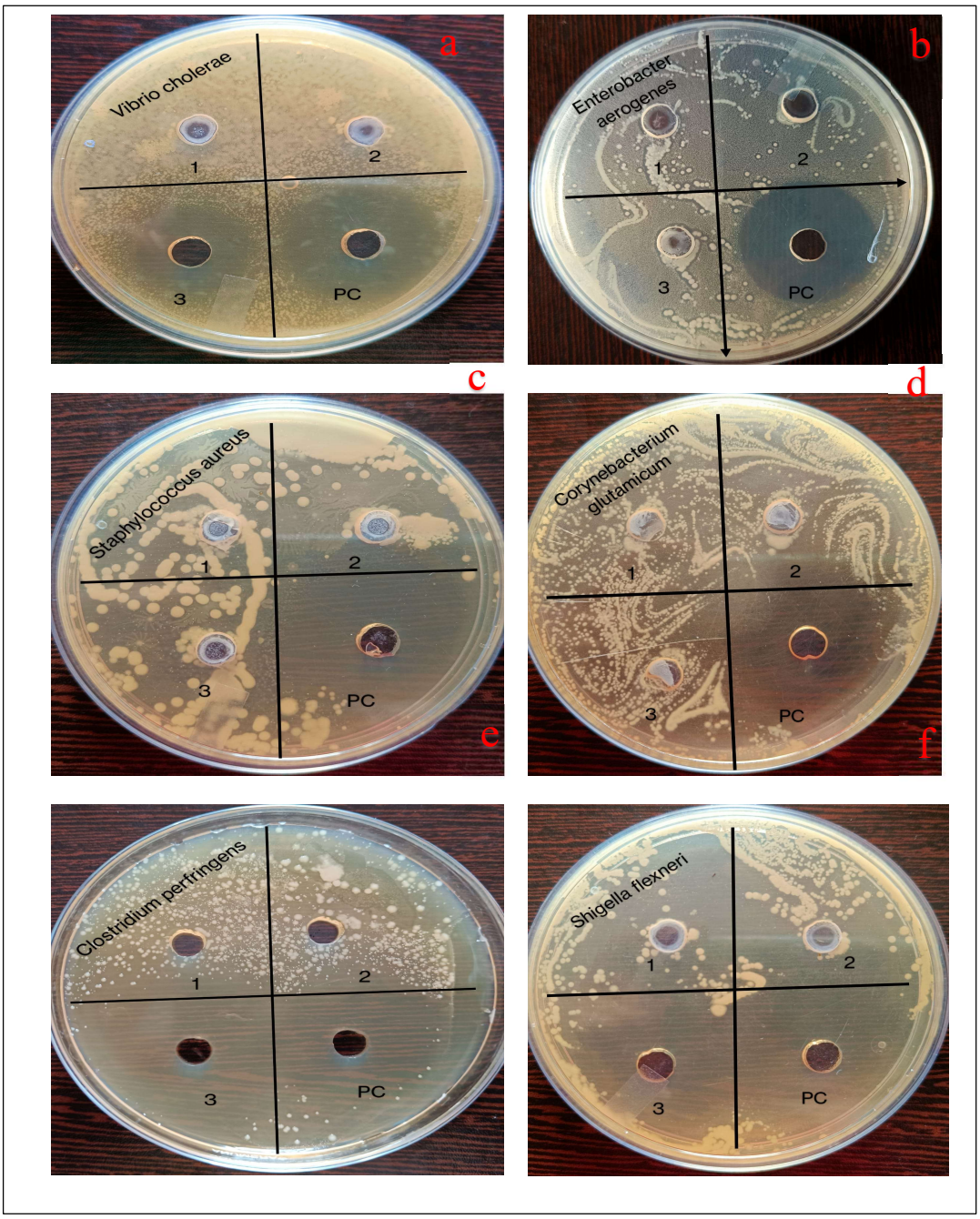


**Fig. 6 Antibacterial activity of ZnO nanoparticles against microorganisms: a) *Vibrio chloreae* b) *Enterobacter aerogenes* c) *Staphylococcus aureus* d) *Corynibacterium glutamicum* e) *Clostridium perfringens* f) *Shigella flexneri***

**Table. 1 Antibacterial activity of ZnO nanoparticles against microorganisms**

| S.No | Test Organism | Zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | ZnO nanoparticles (µg/mL) | | | |
| | | 100 | 150 | 200 | Standard (Chloramphenicol) 30µg/mL |
| 1 | *S. aureus* (MTCC-3160) | - | - | - | 27.36±0.32 |
| 2 | *Corynibacterium glutamicum* (MTCC-2745) | - | - | - | 22.91±0.36 |
| 3 | *Shigella flexneri* (ATCC-12022) | - | - | 21.86±0.35 | 24.93±0.50 |
| 4 | *Vibrio chloreae* (MTCC-3906)) | - | - | 20.46±0.50 | 25.33±0.75 |
| 5 | *Enterobacter aerogenes* (MTCC-241) | - | - | - | 22.13±0.76 |
| 6 | *Clostridium perfringens* (ATCC 13124) | - | - | 24.82±0.75 | 25.36±0.77 |

## Conclusion

Through a straightforward and environmentally benign method involving onion, carrot, cabbage tomato fruit extraction, ZnO nanoparticles were entirely synthesised. Characterization techniques such as XRD, FTIR, SEM, and UV-visible spectroscopy were used to assess the generated nano

particles. From the XDR data, the crystal structures of the produced ZnO nanoparticles with their particle sizes were detected. The generated ZnO nanoparticles had a single-phase hexagonal shape with mean particle sizes of 16 nm, 17 nm, 22 nm, and 15 nm, according to Scherrer's equation. From the SEM data, a little aggregation of the isolated nanoparticles was seen. The vegetable retrieving itself would function as a decreasing or capping agent depending on the scenario because extract capping agents are toxic, thus they were not used to reduce the clustering. As a consequence, generating ZnO nanoparticles via green synthesis is more economical and ecologically responsible than using traditional methods. The dye-sensitive solar cell based on ZnO nanoparticles was successfully fabricated, and its performance was assessed applying current density-voltage behaviour under the impact of artificial sunshine. UV-visible spectroscopy indicated a significant surface plasmon resonance absorption spike. The performance of the created DSSC has significantly improved as a result of a large improvement in dye molecule absorption upon this surface of Zinc oxide nanoparticles. Accordingly, implementing eco-friendly ZnO-NPs in the creation of dye-sensitive solar cells is a straightforward and advantageous plan for the future. ZnO nanoparticles are effective alternatives to antibiotics as well as clever weapons against a variety of drug-resistant microorganisms.

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