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Synthesis and antibacterial activity of ZNO nanoparticles using *Ipomoea Carnea* flower extract

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Abstract
The objective of this study is to synthesize ZnO nanoparticles (ZnO NPs) using *Ipomoea carnea* flower extract for the first time and investigate its antibacterial activity. The study on the antibacterial activity of ZnO NPs against gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), and gram negative bacteria (*Klebsiella pneumoniae*, *Escherichia coli*) reveals that the material prepared has good antibacterial efficacy. The mechanism behind the antibacterial efficiencies have been discussed in detail. The results obtained through UV-Visible spectroscopy, SEM, TEM, FT-IR spectroscopy support well the mechanism proposed.

Keywords: green synthesis, *ipomoea carnea* flower extract, electron microscope, antibacterial activity

Introduction

Nanoparticles are synthesized by various physical, chemical and biological methods for applications like chemical sensing, catalytic activity, biosensing, antimicrobial activity, drug delivery and medical imaging (Hariharan 2006; Siavash Iravan 2011; Happy Agarwal *et al* 2017) [1, 2, 3]. The physical and chemical methods often involve high energy radiations, high temperature and pressure, toxic chemicals that are hazardous to the environment and affect living beings.

Biological techniques using microorganisms and plant mediated sources are considered as safe alternative to synthetic methods. Green synthesis of nanoparticles using plants or their by-products in the form of extracts is consistent with the green chemistry principles. It produces nanoparticles that have appropriate dimensions and higher stability. Green synthesis procedure involves non-toxic, eco-friendly, inexpensive and safe reagents and consumes less energy (Khadeeja Parveen *et al* 2016; Sidra Sabir *et al* 2014) [4, 5].

A number of metal nanoparticles like zinc, gold, silver, palladium, indium and iron oxides have been synthesized through green synthesis Amitkumar Mittal *et al* (2013) [6]. Among these nanoparticles, ZnO NPs are leading semiconductor and found to be one of the multifunctional inorganic materials owing to its optical, magnetic, electrical properties, large excitation binding energy (60 meV) and n-type conductivity. The ZnO NPs are widely used in many industrial applications. Its stable wide band gap at high temperatures and harsh chemical environment allow electronic and opto-electronic devices operate at high temperatures and hostile environments (Fatemeh Davar *et al* 2015; Hong *et al* 2009; Thangavel *et al* 2016) [7, 8, 9].

Effluents released from the textile, leather and food industries contain different organic dyes that affect the human reproductive and immune systems (Mohammod Aminuzzaman *et al* 2018) [10]. For treating these effluents, several physical, chemical and biological methods such as settling and filtration, flocculation, chlorination, aeration, coagulation and photocatalysis are available (Susheela Bai Gajbhiye 2012; Abebe Balcha *et al* 2016) [11, 12]. Among these methods, photocatalysis has gained worldwide attention due to its potential to overcome the pollution issues. The ZnO NPs are well known photocatalyst for their low cost, non-toxicity, stability to high energy radiation and abundance in nature Ravichandran *et al* (2016) [13]. Green synthesized ZnO NPs have effective antimicrobial activity against microorganisms like bacteria and fungi Padmavathy and Vijayaraghavan (2008) [14].

ZnO NPs have been synthesized using the extracts of plants such as *Nephelium lappaceum* L (Karnan and Selvakumar 2016) [15], *Calatropis gigantea* (Vidya *et al* 2013) [16], *Phyllanthus embilica* (Joel and Badhusha 2016) [17] and *Aloe barbadensis miller* Sangeetha *et al* (2011) [18]. However to the best of our knowledge *Ipomoea carnea* (Convolvulaceae family) has not been used for the synthesis of nanoparticles. Hence in the present study, ZnO NPs have been synthesized using *Ipomoea carnea* flower extract and their antibacterial activity have also been investigated.

Materials and methods

Zinc acetate dihydrate (Zn $(CH_3COO)_2.2H_2O$) and glass wares were purchased from Merck Chemical Reagent Co. Ltd. India. All glass wares were washed with sterile distilled water and dried in hot air oven before use.

Experimental details

Preparation of plant extract

Fresh *Ipomoea carnea* flowers were cut and washed with water. The extraction procedure was as follows: 20g of fresh

flower was added to 100ml of ethanol and soaked for 24 hours. The obtained extract was filtered using Whatman No.1 filter paper and the filtrate was collected and stored at room temperature for further usage Is Fatimah *et al* (2016) [19].

Synthesis of nanoparticles

For the preparation of pure ZnO nanoparticles, 100 ml of 0.1M Zinc acetate dihydrate (Zn $(CH_3COO)_2.2H_2O$) solution was added with 100 ml of *Ipomoea carnea* flower extract. The obtained yellow coloured solution was stirred constantly at room temperature for 3 hours. The colloidal particles obtained were dried in hot air oven at 85^0C for one hour. The precipitate was calcinated at 350^0C for 3 hours.

Results and discussion

UV-Visible spectroscopy

Optical properties of green synthesized ZnO NPs were studied by UV Visible absorption spectroscopy. Fig.1 shows the UV - Visible absorption spectrum of green synthesized ZnO NPs. A strong and clear absorption peak was observed at the wavelength 373 nm. From the wavelength value obtained, band gap energy can be calculated using the equation Ramesh *et al* (2015) [20].

$$E_g = \frac{hc}{\lambda} eV$$

where, E_g is the band gap energy (eV), h is the Planck's constant (6.626×10^{-34}Js), c is the light velocity (3×10^8 m/s) and λ is the wavelength (nm). The band gap value was found to be 3.32eV corresponding to the wavelength observed from the spectrum. It is known that UV–Vis spectroscopy is the most widely used technique for the structural characterization of nanoparticles. Fig. 1 shows the UV–Vis absorption spectrum of green synthesized ZnO nanoparticles. Typical exciton absorption at 199.01, 222.44 and 373.91nm was observed at room temperature. The blue shift in the ZnO nanostructures, comparing to bulk ZnO (380 nm) is observed due to the size quantization effect Koch *et al* (1985) [21]. It is clear that the absorption edge systematically shifts to the lower wavelength or higher energy with decreasing size of the nanoparticle. This definite and orderly shift in the absorption edge is due to the quantumsize effect. Present study is in agreement with Gupta *et al* (2015) [22] studies.

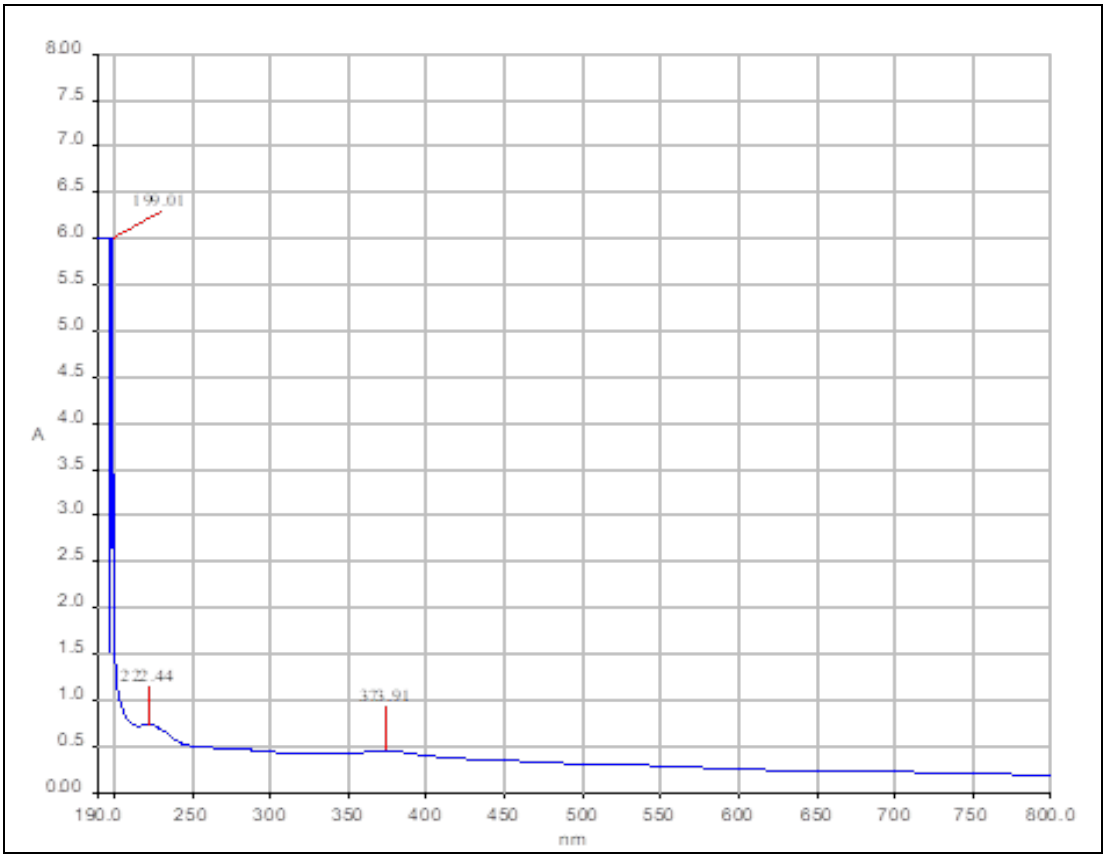

Fig 1: UV- Visible spectrum of green synthesized ZnO nanoparticles

FT-IR spectroscopy

The results were further reinforced by FT-IR analysis, which showed the shifts and difference in areas of the peaks. FT-IR spectroscopy consists in measuring the absorption of IR radiations by a sample, and the results of such measurement are shown by means of a wavelength. The reading of the IR spectrum includes the interpretation of the interdependence between the absorption bands (vibrational bands) and the chemical compounds in the sample. By means of this technique, it is possible to identify the biomolecules in plant extracts which play the crucial role in the processes of reduction and stabilisation of the green synthesis of nanoparticles Senthilkumar and Sivakumar (2014) [23].

Fig.2 shows the IR spectrum of green synthesized ZnO NPs recorded in the wavelength ranging from 4000 to 400 cm^{-1}. IR spectrum shows various peaks at 3419.57 cm^{-1}, 2925. 84 cm^{-1},1619.14 cm^{-1},1385.30 cm^{-1},1124.05 cm^{-1},1100.12 cm^{-}

[1],938.87 cm-1,773.14 cm-1,679.40 cm-1,493.00 cm-1,433.47 cm-1. The broad peak at 3419.57 cm-1 indicates O-H stretching vibrations. The peak at 2925.8 cm-1 shows the presence of stretching mode of alkyl C-H. The sharp peak at 433.47 cm-1 indicates Zn-O stretching vibration and confirmed that ZnO is base material. The peak at 1385.30 cm-1 shows the presence of C-O stretching mode.The finger print region of green synthesized ZnO NPs is exhibited in the region from 1500 cm to 600 cm-1.

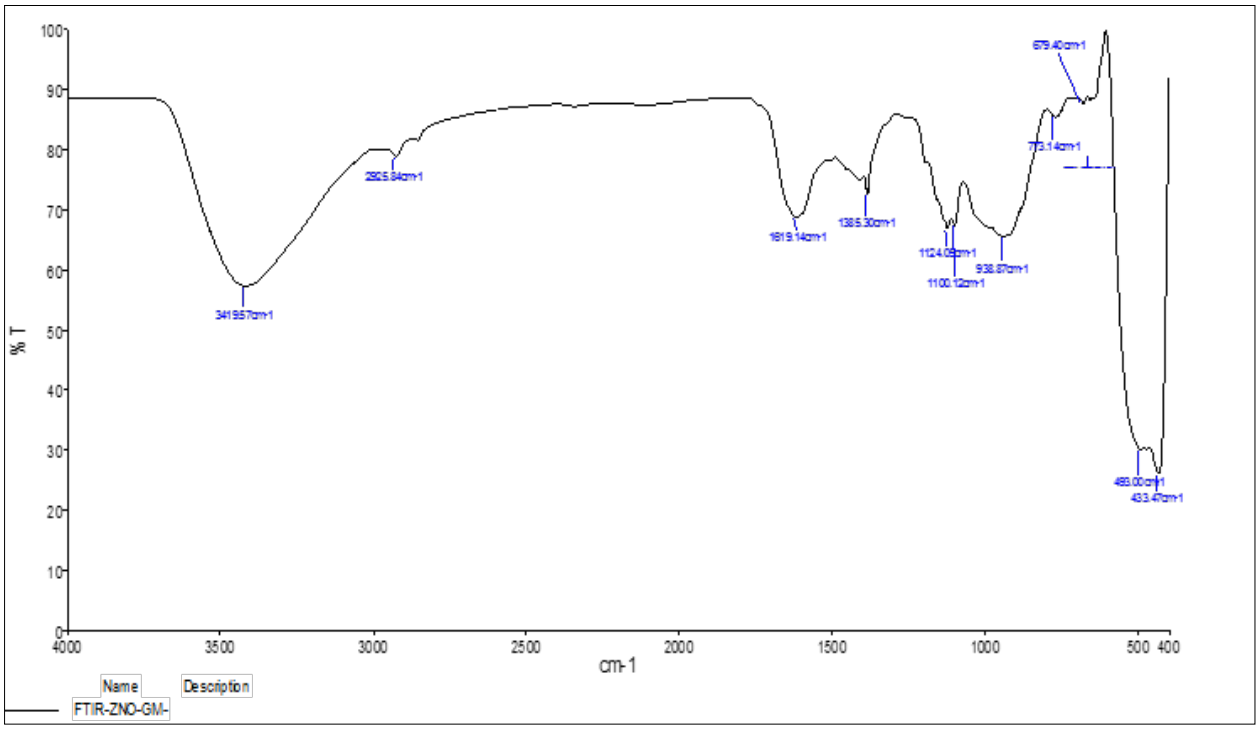

Fig 2: FT-IR analysis of green synthesized ZnO nanoparticles

Surface morphological studies

SEM and TEM Analysis

The surface morphology and crystallographic information about green synthesized ZnO NPs was studied by SEM analysis. Fig. 3 represent the SEM images, which clearly show the shape and size of green synthesized ZnO NPs. It shows that, green synthesized ZnO NPs are agglomerated and spherical shaped. Usually particles with smaller size have higher relative surface area and higher relative number of surface atoms. High surface area to volume ratio of green synthesized ZnO NPs provides a very high surface energy. Therefore to minimize its surface energy, green synthesized ZnO NPs create agglomeration. Due to agglomeration of smaller ZnO NPs, size of the sample is large. The average size of green synthesized ZnONPs studied by SEM was in the nanometres range of approximately about 200nm.

Fig.4 represent TEM image that provides topographical, compositional, morphological information. The average particle size of the green synthesized ZnO NPs calculated from the TEM image. From the morphological studies it was confirmed that the *I. carnea* fresh flower extract has changed the size and morphology of the green synthesized ZnO NPs.

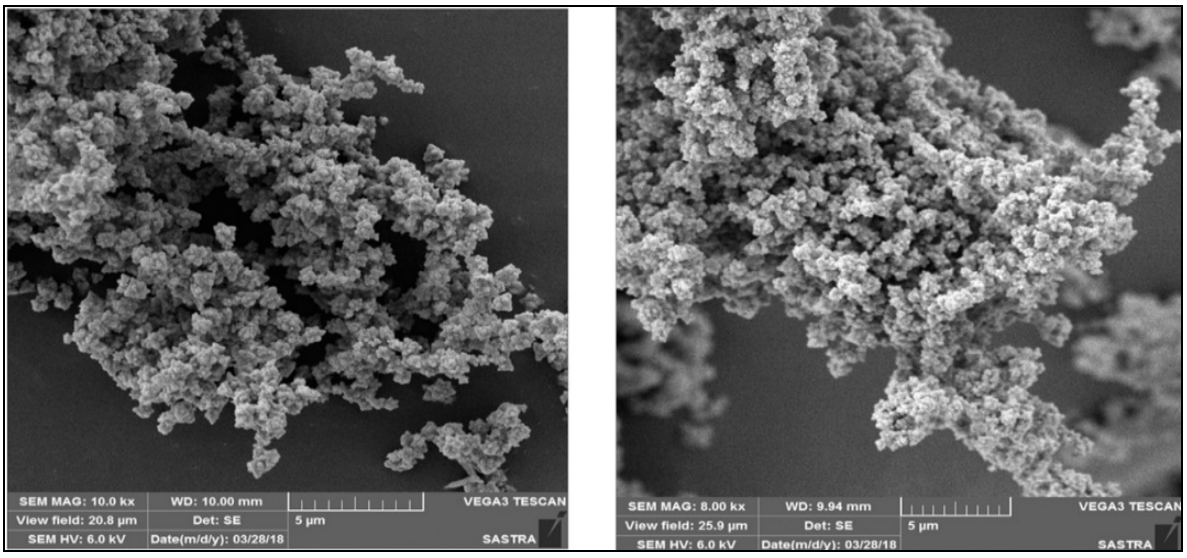

Fig 3: SEM images of green synthesized ZnO nanoparticles at different magnifications

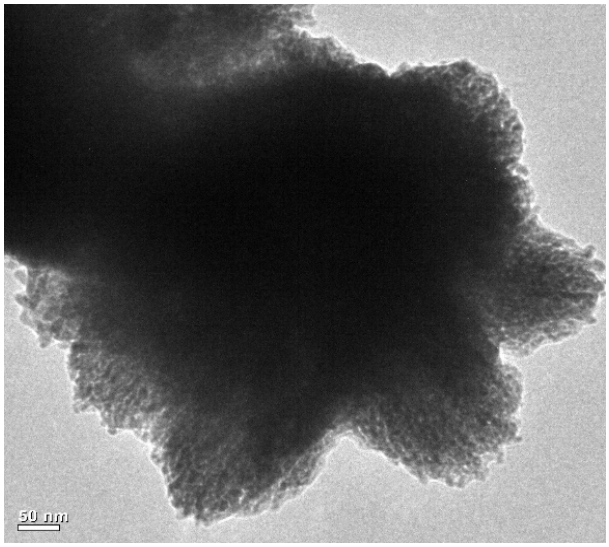

Fig 4: TEM image of green synthesized ZnO nanoparticles at different magnifications

Antibacterial studies

Disk diffusion method was used to investigate the antibacterial activity of green synthesized ZnO NPs, against gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis*), gram negative bacteria (*Klebsiella pneumoniae, Escherichia coli).* Antibacterial activity of green synthesized ZnO NPs was evaluated by measuring the zone of inhibition against the strains of test organism as shown in Table (1). Results clearly demonstrate that green synthesized ZnO NPs have tendency to inhibit the growth of both gram positive and gram negative bacteria. However, maximum zone of inhibition was observed against gram positive bacterium (*Staphylococcus aureus*) than gram negative bacterium (*Escherichia coli, Klebsiella pneumoniae*). A gram positive bacterium is thick, having cytoplasm surrounded by cell wall containing peptidoglycan whereas gram negative bacterium, have both structurally and chemically more complex cell wall. The antibacterial activity of green synthesized ZnO NPs was assumed to be due to the release of reactive oxygen species such as hydrogen peroxide (H_2O_2) which penetrates into the cell wall through the small pores that are present in the cell membrane of microorganisms. It damages the cell membrane and cell organelles that leads to the inhibition of cell growth and kills the microorganisms. It is also due to the interaction of reactive oxygen species, including $OH^{\cdot}$ hydroxyl radicals, and superoxide anions O_2^{-} which slows down the bacterial growth by attacking the carbonyl carbon atom which is present in the cell wall proteins as peptide linkages and leads to the death of microorganisms.

Table 1: Assay of antibacterial activity

S. No.	Microbes	Zone of inhibition (mm in diameter)		
		C	S	Green synthesized Zn O NPs
1	*Bacillus subtilis*	-	17	18
2	*Escherichia coli*	-	16	10
3	*Klebsiella pneumonia*	-	20	19
4	*Staphylococcus aureus*	-	35	27

*Nitrofurantoin (300 µg) for Bacteria;

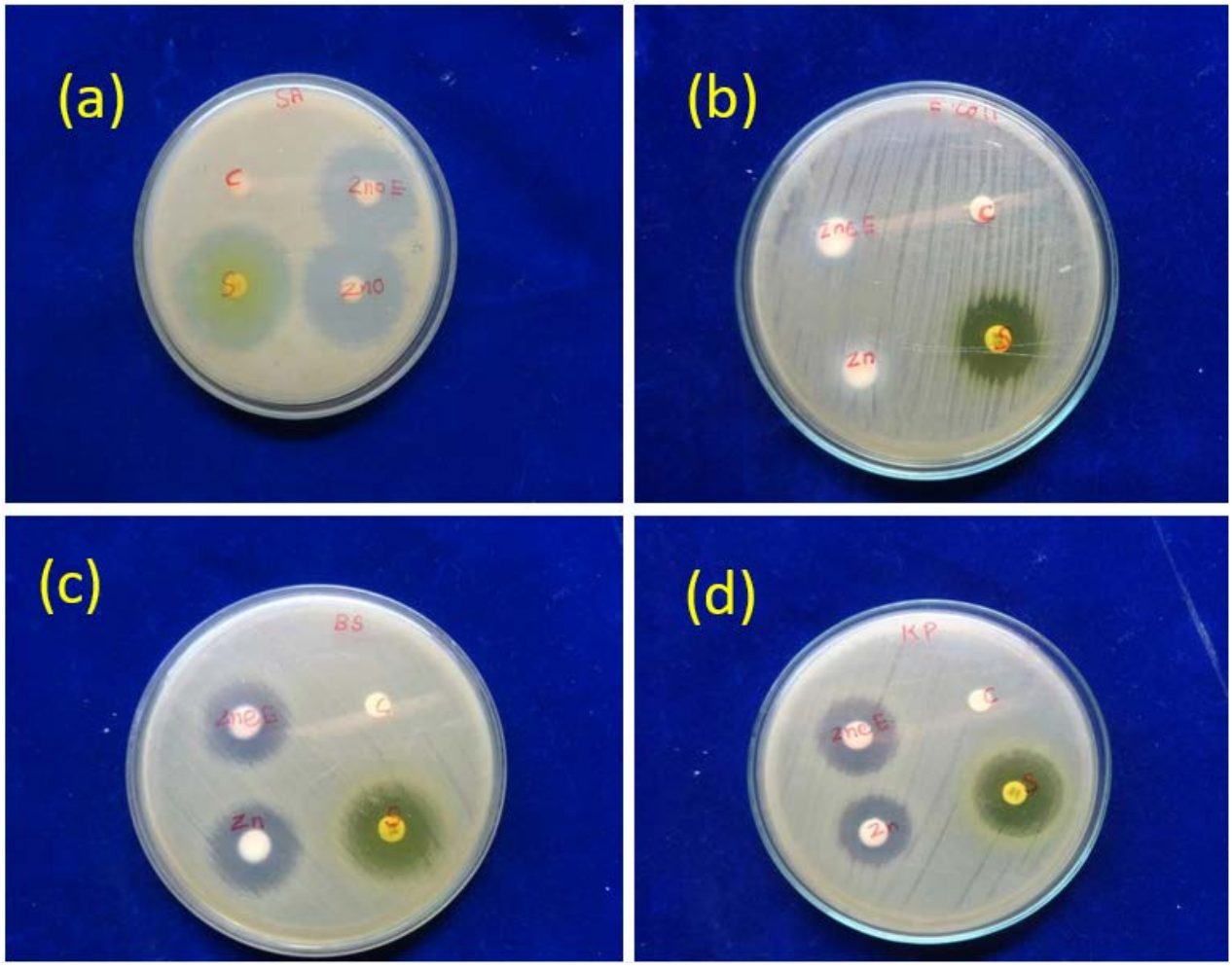

Fig 5: Antibacterial activity of green synthesized ZnO nanoparticles against (a) *S.aureus* (b) *E.coli* (c) *B. subtilis* (d) *K. pneumoniae*

Conclusion

ZnO NPs are synthesized successfully through green method using *Ipomoea carnea* flower extract and characterized by UV-Visible spectroscopy, FT-IR spectroscopy, SEM, TEM. UV-Visible spectrum shows the absorption peak at 373 nm. Morphological studies reveal that the grains are spherical shape as confirmed from SEM study. The green synthesized ZnO NPs exhibit antibacterial activity and has maximum zone of inhibition against (*Staphylococcus aureus*) gram positive bacteria. The present study suggests that ZnO NPs obtained through green synthesis produces ZnO NPs of well-defined morphology. It has reduced impact on the environment without affecting the human and involves simple working conditions, consumes less energy, less time, inexpensive and simple instrumentation.

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