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# Green Synthesis of Antibacterial Zinc Oxide Nanoparticles Using Biopolymer *Azadirachta indica* Gum

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**ABSTRACT**

The progress of green chemistry in the synthesis of nanoparticles with use of plants has engrossed a great attention nowadays due to its inexpensive, simple, non-toxic and environmental-friendly nature. The present study has been undertaken to prepare *Azadirachta indica* gum stabilized ZnO nanoparticles with multifunctional properties. The prepared nanoparticles were characterized by FT-IR, XRD, FE-SEM and UV-Vis absorption studies. It was clear from XRD pattern that nanoparticles were crystallized in hexagonal wurtzite structure. The average size of the nanoparticles was found to be 30-60nm. The synthesized nanoparticles exhibited potent antibacterial activity against *E. coli* and *S. aureus*.

**Keywords:** Zinc Oxide Nanoparticles, *Azadirachta indica*(AI), Green Synthesis, Antibacterial activity.

## INTRODUCTION

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new material at the nanoscale level[1]. Nanotechnology involves synthesis, characterization and application of nanoparticles by controlling shape and size. In the field of nanotechnology, metal nanoparticles have attained a great importance due to their unique optical, catalytic, electronic, magnetic and antimicrobial properties owing to their small size [2-4]. Among the metal oxide nanoparticles, zinc oxide is interesting because of its physical and chemical properties such as stability, high catalytic activity, luminous transmittance, effective antibacterial property, intensive IR and UV absorption etc[5,6]. Interestingly, ZnO nanoparticles are reported by several studies as non-toxic to human cells[7,8].This aspect necessitatedtheir usage as antibacterial agents, noxious to microorganisms and hold good biocompatibility to human cells[9]. Synthesis of nanoparticles can be performed using a number of routinely used chemical methods such as chemical

precipitation[10], sonochemical, solovo thermal, sol-gel process, hydrothermal decomposition etc[11]. The biological method of the synthesis of ZnO nanoparticles is gaining importance due to its simplicity, eco-friendliness and extensive antimicrobial activity [12].

The use of plant materials has been considered as a green route for the biosynthesis of nanoparticles owing to their environmental friendly nature. Plant based gum is a heteropolysaccharide of natural gum and its morphological, thermal, compositional, physico-chemical properties have been studied well [13-15]. The biopolymer of *Azadirachta indica* gum has the highest amount of proteins found amongst all plant gums[16]. Mukherjee and Srivastava reported that, the major constituents of neem gum were found to be polysaccharides such as D-galactose-glucuronic acid, D-xylose, L-arabinose and L-fucose. The naturally occurring *Azadirachta indica* gum exudates is a easily available, non-toxic, glassy transparent brown colour, water soluble complex polysaccharide which has various applications in paper, cosmetic, textile and pharmaceutical industries.

The presence of acetyl, carboxyl, hydroxyl and carbonyl functional groups and metal- biosorption properties of the neem gum encouraged us to use this AI gum exudates as a biotemplate/stabilizer for the green synthesis of ZnO nanoparticles. The present investigation describes the advantages of ZnO nanoparticles synthesized by the green biological method using AI gum, their characterization and the determination of antibacterial activity.

## MATERIALS AND METHODS

### Chemicals

All the chemical such as zinc nitrate hexahydrate ($ZnNO_3.6H_2O$) and sodium hydroxide (NaOH) was of analytical grade and used without further purification. All the synthesis was carried out using double distilled water.

### Synthesis of zinc oxide nanoparticles

### Chemical method

The bulk zinc oxide particles were prepared by using wet chemical method. In this method zinc nitrate (0.1 M) was prepared in distilled water under constant stirring for an hour using magnetic stirrer. Stirring was continued for another 2 hours with the drop by drop addition of 0.2 M sodium hydroxide solution. The content was allowed to settle for overnight and the supernatant was then discarded carefully. The impurities were removed by washing and centrifuging several times with distilled water. The pure nanoparticles were dried at 80°C for overnight in a hot air oven. The complete conversion of zinc hydroxide into zinc oxide takes place during drying.

### Green method

### Collection and purification of *Azadirachta indica* gum

Dried gum exudates of *Azadirachta indica* were collected from *Neem* trees in the campus of PSG College of Arts and Science (Coimbatore, India). The collected gum was dissolved using double distilled water, filtered and kept in dessicator to get a glassy mass of purified gum.

The zinc oxide nanoparticles were prepared by green method using zinc nitrate and sodium hydroxide as precursors and AI gum as stabilizing agent. Different concentrations of AI gum 0.1%, 0.3% and 0.5% were prepared in 100 ml. To this solution 2.97g of zinc nitrate was added to get 0.1 M solution. The contents were kept under constant stirring using magnetic stirrer to dissolve the zinc nitrate. Sodium hydroxide (0.2 M) was added drop wise and stirring was continued for 2 hours. The solution was allowed to settle for overnight, centrifuged and the supernatant was discarded. Thus obtained nanoparticles were washed several times with distilledwater to remove the impurities. The nanoparticles were dried at 80°C for overnight in hot air oven where the complete conversion of zinc hydroxide into zinc oxide takes place.

### Characterization techniques

UV-Visible absorption spectroscopy is widely being used technique to examine the optical properties of nanosized particles and band gap is the major factor determining the electrical conductivity. The UV-Vis spectrums of ZnO nanoparticles synthesized by chemical and green method were recorded using JASCO Corp., V-570 spectrophotometer over a range of 200-800 nm. The presence and interaction of chemical

functional groupswere analyzed using FT-IR spectrophotometer (Perkin Elmer) at the scanning range of 4000-400 $cm^{-1}$. The X-ray powder diffraction pattern were recorded on an X-Ray diffractometer (XRD, PW 3040/60 Philips) with Cu (kα) radiation (λ=1.5406 A°) operating at 40 kV and 30 mA with 2èranging from 20°- 90°. The surface morphology of ZnO nanoparticles were characterized by FE-SEM using Carl Zeiss Supra 55 (Germany) microscope.

**Antibacterial activity of ZnO nanoparticles**

The agar well diffusion method was used to screen the antibacterial activity of bulk ZnO and Al gum stabilized ZnO nanoparticles. For this purpose, *Gram-negative* bacteria: *Escherichia coli* and *Gram-positive* bacteria: *Staphylococcus aureus* were employed in the present study.

Muller Hinton agar was prepared and sterilized. Log phase culture of the test specimens were swabbed over the agar surface using the sterile cotton swab. Wells were made on the agar surface using sterile gel puncture and about 10µl of thesample were loaded onto the wells and the plates were incubated at 37° C for 24 hours. The clear zones were used to determine the efficiency of the samples.

## RESULTS AND DISCUSSION

**UV-Vis absorption studies**

The UV-Vis absorption spectra of ZnO nanoparticles obtained by green method are compared with ZnO nanoparticles prepared using chemical method. The absorption spectra of bulk ZnO nanoparticles and Al gum stabilized ZnO nanoparticles were shown in Fig.1.The absorption wavelength and band gap energy of bulk ZnO nanoparticles and different concentrations of Al gum stabilized ZnO nanoparticles were shown in Table .1. Analysis of the data shows the absorption wavelength obtained for ZnO prepared by chemical method is higher than greener method. ZnO nanoparticles prepared using greener method with various concentration of Al gum shift the surface Plasmon resonance band to lower wavelength side (blue shift). This blue shift in the Al gum stabilized ZnO nanoparticles is due to the presence of complex organic molecules carrying different charge centers in Al gum.

**Table 1: Absorption wavelength and band gap energy of bulk ZnO and Al gum stabilized ZnO nanoparticles**

| Particulars of ZnO | Wavelength ( nm) | Band Gap energy (eV) |
|---|---|---|
| Bulk ZnO | 372 | 3.33 |
| 0.1 % Al gum stabilized ZnO | 362 | 3.43 |
| 0.3 % Al gum stabilized ZnO | 359 | 3.46 |
| 0.5 % Al gum stabilized ZnO | 355 | 3.49 |

The absorption wavelength and the size of the different concentrations of Al gum stabilized ZnO nanoparticles decreases as the concentration of Al gum increases. The concentration of Al gum increases, band gap energy also increases.

**FT-IR Spectra**

The role of varying Al gum concentration in the formation of ZnO nanoparticles were identified by comparing the FT-IR spectrum of Al gum (Figure.2), and FT-IR spectra of bulk ZnO and ZnO nanoparticles synthesized using various concentrations of Al gum as stabilizing agent (Figure.3).

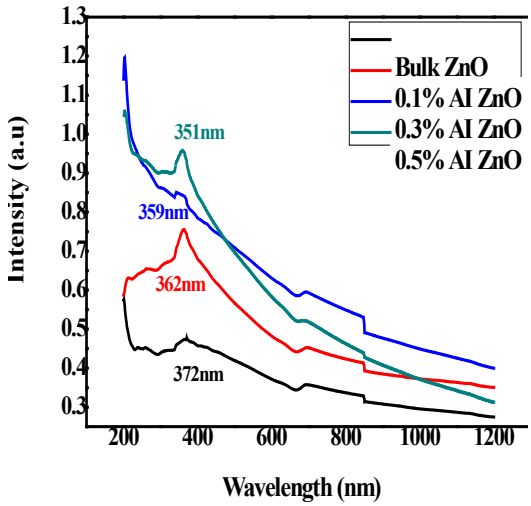


**Fig. 1: UV absorption spectra of bulk ZnO and Al gum stabilized ZnO nanoparticles**

The peak in between 3600-3200 $cm^{-1}$ in the FT-IR spectrum of AI gum (Figure.2) indicates the presence of higher amount of hydroxyl groups in the polysaccharides of gum. The observed peak at 1705 $cm^{-1}$ indicates the >C=O stretching frequency. The peaks at 1658 $cm^{-1}$ and 1550 $cm^{-1}$represents the carbonyl stretching vibration and N-H stretching vibration of amine groups in peptides.

The peaks at 1458 $cm^{-1}$ and 1311 $cm^{-1}$ show the presence of C-H bond. A medium stretch at 1080 $cm^{-1}$represents the presence of C-O bond. From the FT-IR spectra, for bulk ZnO (Figure.3), the broad band between 3200-3600 $cm^{-1}$ centered at 3402 $cm^{-1}$ corresponds to the stretching vibration of intermolecular hydrogen bond (O-H) existing between the adsorbed water molecule and oxygen of zinc oxide. The peak at 1627 $cm^{-1}$ corresponds

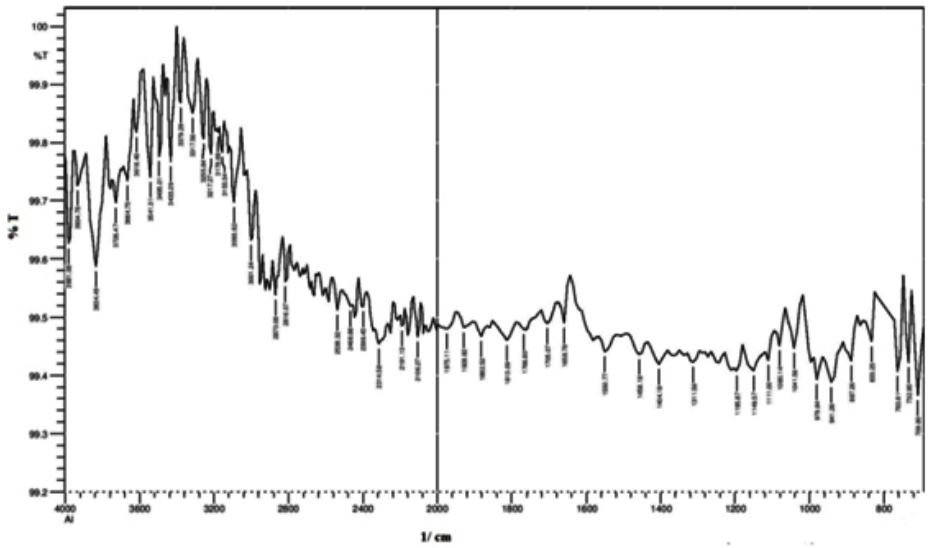


**Fig. 2: FT-IR spectrum of AI gum**

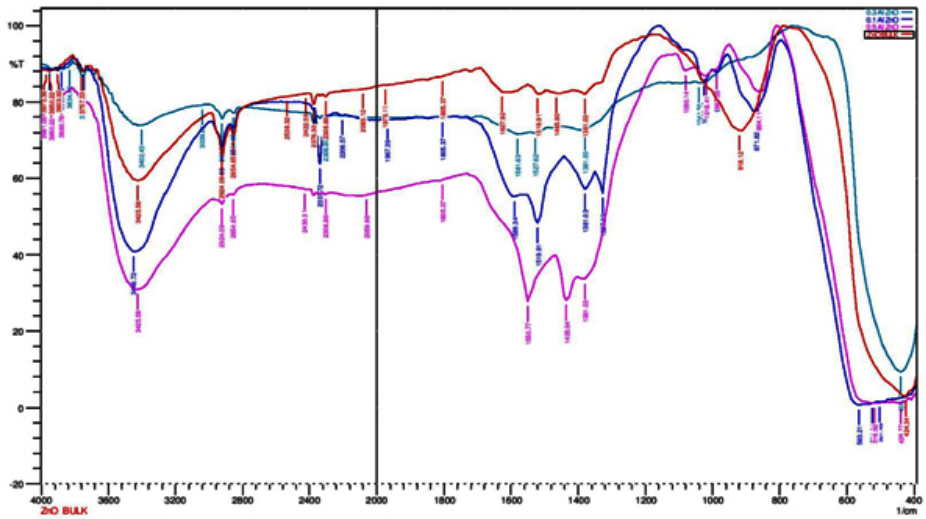


**Fig. 3: FT-IR spectra of bulk ZnO and 0.1%, 0.3% and 0.5% AI gum stabilized ZnO nanoparticles**

to O-H bending vibration. The peaks at 424 $cm^{-1}$ indicates the stretching vibration of Zn-O bond.

The FT-IR spectra of ZnO nanoparticles synthesized using 0.1%, 0.3% and 0.5% AI gum as stabilizing agent is given in Fig.3. A broad band between 3600-3200 $cm^{-1}$ centered at 3402 $cm^{-1}$ in ZnO nanoparticles were shifted to 3425, 3448 $cm^{-1}$ in the spectrum of ZnO nanoparticles synthesized using AI gum as stabilizing agent. The higher shift frequency from 3402 $cm^{-1}$ to 3425, 3448 $cm^{-1}$ may be due to the participation of –OH group from the bulk molecule of AI gum. The peaks at 2924 and 2854 $cm^{-1}$ are due to C-H stretching vibration. The N-H stretching vibration of AI gum appeared at 1658 $cm^{-1}$ is lowered to 1589, 1581 and 1550 $cm^{-1}$ in the spectra of ZnO nanoparticles synthesized using AI gum.[17-19] Thus there is a possibility of interaction between the proteins of AI gum and ZnO nanoparticles.

The stretching of vibration of C-O at 1085 $cm^{-1}$ in AI gum is lowered to 1018, 1026 and 1041 $cm^{-1}$ in the ZnO nanoparticles synthesized using AI gum as stabilizing agent indicating the formation of new bond. The peak appeared for bulk ZnO nanoparticles at 424 $cm^{-1}$ is shifted to higher frequency side in the presence of AI gum stabilized ZnO nanoparticles. The shift depends on the concentration of AI gum used. The values are 563, 524, 501 and 439 $cm^{-1}$ for 0.1%, 0.3% and 0.5% AI gum stabilized ZnO respectively. The higher frequency shift may be an indication of structural changes in the ZnO nanoparticles in presence of AI gum.

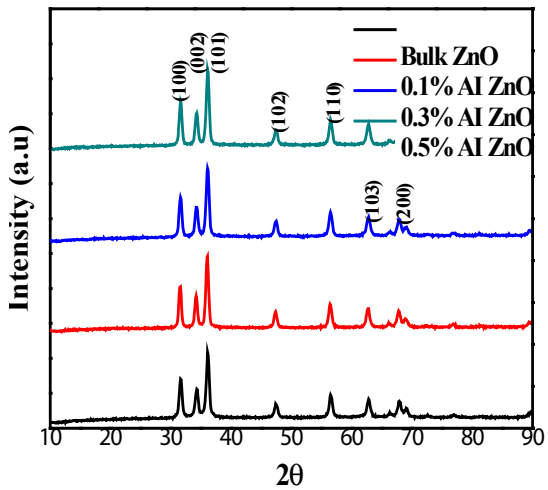


**Fig. 4: XRD pattern of bulk ZnO and AI gum stabilized ZnO nanoparticles**

**X-ray diffraction studies**

X-Ray diffraction is a well known technique for the structural identification and determination of crystalline size.

The XRD pattern of synthesized bulk ZnOand AI gum stabilized ZnO were shown in Figure.6. The prominent peaks corresponding to the diffraction planes (100), (002), (101), (102), (110), (103) and (200) were obtained. The comparison of observed XRD pattern with the standard JCPDS card data indicates that, all the peaks are matched with standard JCPDS card no.36-1451, confirms that the bulk ZnO and AI gum stabilized ZnO nanoparticles are of hexagonal wurzite type

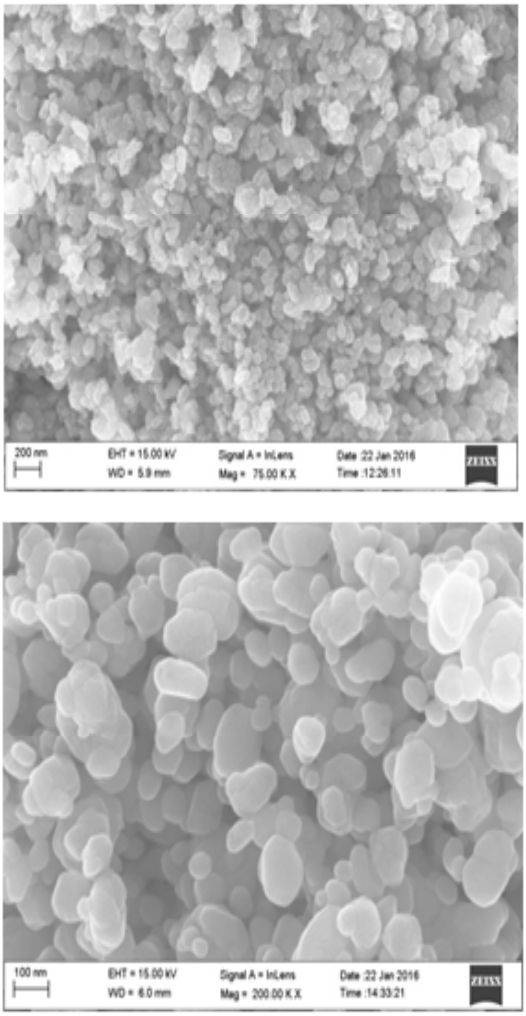

**Fig. 5a, 5b: FE-SEM images of ZnO nanoparticles**

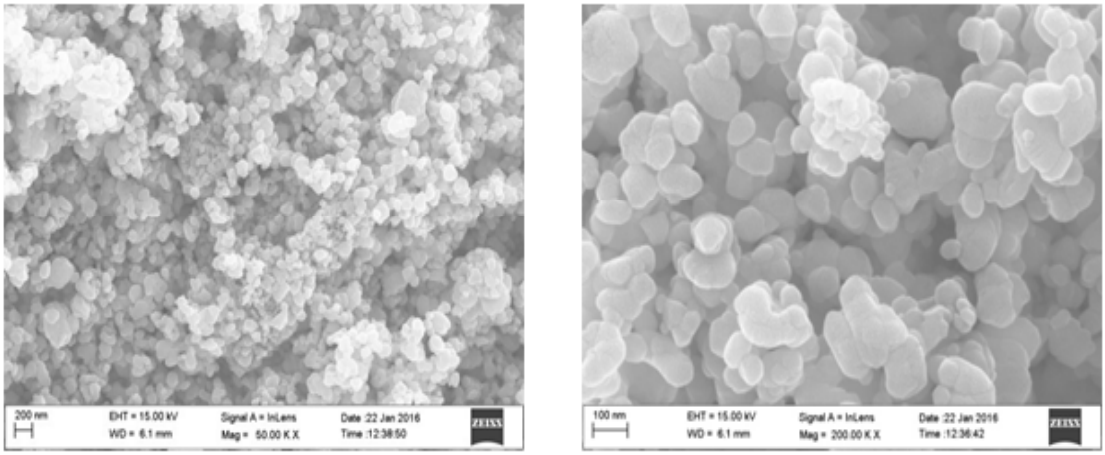


**Fig. 6a, 6b: FE-SEM images of ZnO nanoparticles using 0.1% Al gum**

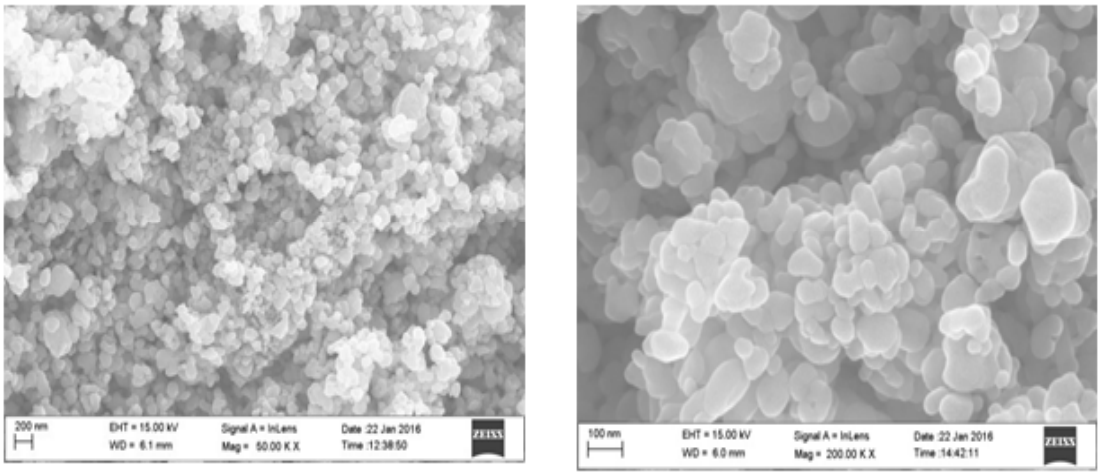


**Fig. 6c, 6d: FE-SEM images of ZnO nanoparticles using 0.3% Al gum**

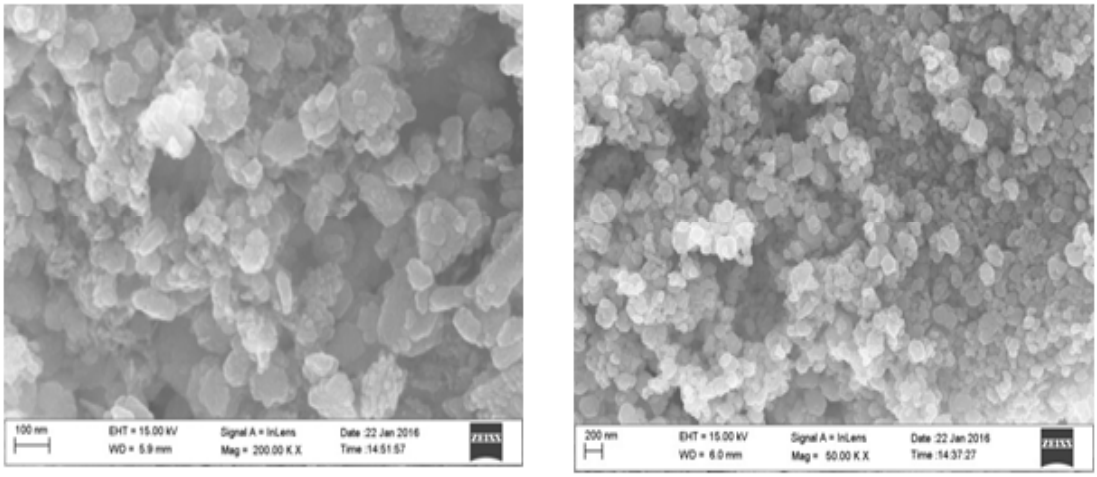


**Fig. 6e, 6f: FE-SEM images of ZnO nanoparticles using 0.5% Al gum**

structure.[20, 21] Crystallite size (D) was calculated using Debye Scherrer s formula[22]

D= 0.9λ/βCosθ where D- crystallinesize of zinc oxide, λ - wavelength of X- raysource 0.15406 nm in (XRD), β- full width at half maximum of the diffraction peak, θ - Bragg angle

The average crystallite size (D) of the prepared ZnO nanoparticles was calculated to be around 13 - 15 nm.

**FE-SEM studies**

FESEM images of ZnO nanoparticles synthesized by chemical method were shown in Figure. 5a, 5b. Analysis of the images shows that formation of zinc oxide nanoparticles ranging from 50-90 nm with flower shape morphology.

ZnO nanoparticles synthesized using Al gum as stabilizing agent was shown in Figure. 6a – 6f.

The images show the same with the average particle size of 30-60 nm and with spherical shape and this also matched with the earlier studies.[3, 23] Thus, the nanoparticles using Al gum as stabilizing agent decreased the particle size.

**Antibacterial studies**

The nanoparticles of ZnO exhibit attractive antibacterial properties due to increased specific surface area as the reduced particle size leading to enhanced particle surface reactivity and contact with the microbial pathogens. The smaller size of nanoparticles facilitates easy entry into the microbial cell membrane and enables inhibition mechanisms to occur inside the cell. ZnO nanoparticles generate hydrogen peroxides which chemically interact with membrane proteins and lipid bilayers.[24-25] The ZnO nanoparticles may distort and damage the bacterial cell membrane, causing leakage of intracellular contents leading to cell death.

The zone ofinhibition for both Gram-negative bacteria *Escherichia coli* and the Gram-

**Table 2: Antibacterial activity of bulk ZnO and 0.1%, 0.3% and 0.5% Al gum stabilized ZnO nanoparticles**

| Testorganisms | Zone of inhibition in mm | | | |
|---|---|---|---|---|
| | ZnO | 0.1% Al-ZnO | 0.3% Al- ZnO | 0.5% Al- ZnO |
| E. coli | 5 | 5 | 8 | 10 |
| S. aureus | 5 | 8 | 10 | 10 |

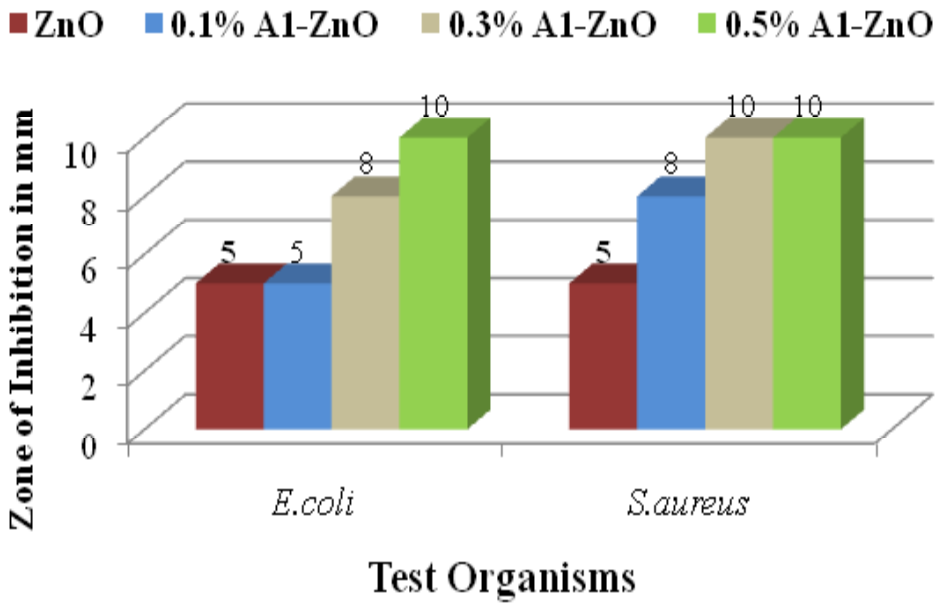


**Fig. 7: Growth of bacterial strains E.coliandS.aureusexposed to ZnO nanoparticles**

positive bacteria *Staphylococcus aureus*were calculated by measuring the diameter of the inhibited growth around the wells. The antibacterial activities of ZnO nanoparticles against the studied pathogenic strains are shown in Fig. 7. The values of zone of inhibition obtained from the assay are presented in Table.2.It is quite interesting to note that all bacterial species tested in this study showed resistance to ZnO nanoparticles synthesized by green method than the bulk ZnO. Both *Gram-negative* and positive bacteria had shown good sensitivity and it is most significant for the 0.5% AI gum green synthesizedZnO nanoparticles for the concentration 10 μg/mL.

## CONCLUSION

The present study demonstrates ZnO nanoparticles have been successfully synthesized using different concentrations of AI gum as stabilizing agent. UV-Vis, FT-IR, XRD and FE-SEM analysis were used to confirm the formation of ZnO nanoparticles by chemical and green method. The synthesized ZnO nanoparticles has an average particle size of about 30-60 nm and particles are nearly uniform with hexagonal wurtzite structure and this conforms the ability of the AI gum to stabilize the ZnO nanoparticles. The synthesized ZnO nanoparticles showed potent antibacterial activity against *Gram-negative* bacteria*Escherichia coli* and *Gram-positive* bacteria *Staphylococcus aureus*. These results concluded that the green synthesized ZnO nanoparticles using *Azadirachta indica* gum extract could be used as a potent antibacterial agent in various biomedical applications.

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