A Single pot Green synthesis of ZnO nanoparticles using aqueous gum exudates of *Azadirachta indica* and its antifungal activity

A. Geetha[1], R. Sakthivel[2], J. Mallika[1*]

[1]Research scholar, Department of Chemistry, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India.
[1*]Assistant professor, Department of Chemistry, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India.
[2]Associate professor, Department of Electronics, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India.

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Abstract - *The present work focus on Green synthesis of zinc oxide nanoparticles using zinc nitrate and sodium hydroxide as precursors through a simple wet chemical method using aqueous gum exudates of Azadirachta indica a biopolymer, as stabilizing agent. The particle size and morphology of the synthesized ZnO nanoparticles by green method were characterized by using UV-Visible Spectroscopy (UV), X-ray Diffraction (XRD), Field Emission Scanning Electron Microscopy (FE-SEM), and Fourier Transform Infrared Spectroscopy (FT-IR) studies. The average size of the green synthesized ZnO nanoparticles were predominantly found within the range 10-25 nm. Antifungal activity of the prepared nanoparticles towards Aspergillus fumigatus, Candida albicans, and Penicillum varians was also investigated.*

***Key Words*: Zinc oxide nanoparticles, *Azadirachta indica* (AI), XRD, UV-Vis, FE-SEM, antifungal activity**

1. INTRODUCTION

The field of nanotechnology and bionanotechnology are the most active areas of research in modern material science. Nowadays, nanoparticles have been the subject of focused research due to their unique properties that are considerably different from their bulk materials. Nanoparticles are particles that have at least one dimension with 100 nm or less in size.[1,2] Nanotechnology is especially concerned with the synthesis of nanoparticles of variable sizes, shapes, chemical composition and controlled dispersity for their potential use for human benefit.[3] Nanoparticles of noble metal such as platinum, gold, silver and zinc oxide are widely applied in products that are directly come in contact with the human body, such as detergent, cosmetic products and tooth paste, besides medical and pharmaceutical application. Nanoparticles synthesis has been reported using physical and chemical methods. There are various methods for the formation of nanoparticles such as sol-gel method, chemical precipitation, wet chemical method hydrothermal technique, microwave and chemical vapour deposition.[4-6] Among these, the wet-chemical method is the best method for the production of huge amount of nanoparticles at low cost.[7] Biosynthesis of metal nanoparticles is currently under development. The synthesis of metal nanoparticles using inactivated plant tissue, plant extracts, exudates and other parts of living plants is a modern alternative for their production. Gums are naturally occurring polysaccharide components in plants, which are economical and easily available. They have assorted applications such as thickeners, food emulsifiers, sweeteners, binders and drug release modifiers in pharmaceutical industries. Gum from plants may act both as reducing and capping agents in nanoparticles synthesis. In the present study, green synthesis of nanoparticles using neem gum is having advantages such as easy availability, safety, nontoxic and environmentally acceptable "green chemistry" procedure. The gum exudates from the stem of *Azadirachta indica* tree (Neem tree) is a mixture of proteins and complex polysaccharides and it is used in paper industry (adhesive), cosmetics (lotions, face powder),textile industry (dyeing and printing), food industry (stabilizing agent, gels and thickening agent) and pharmaceutical industry (antiseptic creams, coater and tablet binder).[8] Such interesting features of neem gum encouraged us to use this biopolymer as stabilizing agent for the present study. The inorganic materials, metal oxides such as TiO_2, ZnO, MgO are of particular interest as they are stable under harsh process conditions and safe to human beings and animals. Among the metal oxide nanoparticles, ZnO is interesting because it has vast applications in various areas such as optical, electrical and pharmaceutical and gas sensing. ZnO nanostructures exhibits high catalytic efficiency, strong absorption ability and are used more frequently in sunscreens, ceramics, rubber processing, waste water treatment and as a fungicide. Green synthesis of silver nanoparticles using aqueous solution of neem gum have been reported recently.[9] Therefore, the present research work is focused on the synthesis of zinc oxide nanoparticles using the gum exudates of *Azadirachta indica* a biopolymer, as stabilizing agent. The characterization of the synthesized nanoparticles and its antifungal activity of the prepared ZnO nanoparticles towards *Aspergillus fumigatus, Candida albicans,* and *Penicillum varians* were also investigated.

2. Materials and Methods

Chemicals used in the present study such as zinc nitrate hexahydrate and sodium hydroxide were purchased from Sigma-Aldrich (99.9% purity).

2.1 Collection and purification of *Azadirachta indica* gum

Azadirachta indica gum exudates were collected from the campus of PSG college of Arts and science (Coimbatore, Tamil Nadu and India) and authenticated by Botanical Survey of India (BSI), Coimbatore, Tamil Nadu and India. The collected gum samples were washed with double distilled water to remove impurities. After complete dissolution of gum, it was filtered through Whatman filter paper (no: 40) and kept in dessicator to get a glassy mass.

2.2 Synthesis of zinc oxide nanoparticles

The zinc oxide nanoparticles were synthesized by following the wet chemical method. Zinc nitrate (0.1M) was dissolved in 0.1% solution of AI gum and stirred for 2 hours. It is followed by the addition of 0.2 M sodium hydroxide to the prepared solution of zinc nitrate. The mixture was stirred continuously for 2 hours on magnetic stirrer resulting in the formation of white dispersion. The prepared solution of ZnO nanoparticles was allowed to settle for overnight and the supernatant solution was then discarded. The obtained ZnO nanoparticles were washed with a 3:1 mixture of alcohol and water. Washing was carried out to remove the by-products and the excessive AI gum that were adsorbed on the surface of the nanoparticles. The nanoparticles were dried in a hot air oven at 80°C for 3 hours, where the conversion of zinc hydroxide into zinc oxide takes place.

2.3. Characterization techniques

The UV-Vis spectrum of ZnO nanoparticles synthesized by green method was recorded using JASCO Corp., V-570 spectrophotometer within the range of 200-800 nm. The presence and interaction of chemical functional groups was analyzed using FT-IR spectrophotometer (Perkin Elmer) at the range of 4000-400 cm^{-1}. The X-Ray diffraction pattern of the synthesized ZnO nanoparticles 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 2θ ranging from 20°- 90°. The surface morphology of ZnO nanoparticles were characterized by using FE-SEM. The antifungal activity of the prepared ZnO nanoparticles was investigated using well disc diffusion method.

2.4 Antifungal assay

The activity of the given sample on various fungal strains was assayed by well diffusion method. The fungicidal effect of the given sample can be assessed by the inhibition of mycelia growth of the fungus and was observed as a zone of inhibition near the wells.

Aspergillus fumigatus, Candida albicans, and *Penicillum varians* obtained from PSG IMSR hospital were used to assess the anti-fungal activity of the plant extracts. Potato dextrose agar was prepared and sterilized and poured on to the petriplates. In each plate different test fungal cultures were swabbed over the agar surface using the sterile cotton swab. Wells were made on the agar surface using the sterile gel puncture and about 100 µl of the given samples were loaded onto the wells. The plates were incubated at room temperature for three days and the antifungal effect was seen as crescent shaped zones of inhibition.

3. Results and discussion

3.1 UV- Absorption studies

UV-Vis spectroscopy is one of the important techniques for analyzing the formation nanoparticles in aqueous medium. UV-Visible absorption of spectrum of AI gum was shown in Fig-1. The maximum absorbance of the AI gum was found to be in the range of 250-360 nm, which is due to the presence of complex organic molecules in AI gum carrying different charge centers. For bulk ZnO the absorption peak appears at 380 nm. In the present study, an absorption peak appears at 298 nm for ZnO synthesized by using AI gum as stabilized agent. In general the absorption shift to the lower wavelength is an indication of decrease in the size of the particles. [10,11] UV absorption spectrum of ZnO nanoparticles stabilized using AI gum was shown in Fig-2.

An important application of UV-Visible spectrum is band gap energy calculation. The band gap energy of ZnO nanoparticles synthesized using AI gum as stabilizing agent is calculated using the following relationship [12]

Band gap energy $E = hc/\lambda$

Where, $h = 6.626\times10^{-34}$ Joule sec (Planck's constant),
$c = 3\times 10^{8}$ $msec^{-1}$ (speed of light)

$\lambda = 298 \times 10^{-9}$ (wavelength of nanoparticles),
1 eV= 1.6×10^{-19} Joule (conversion factor)

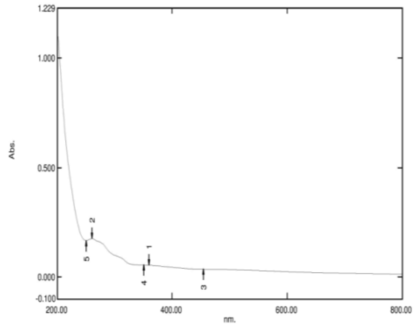


Fig -1: UV spectrum of AI gum

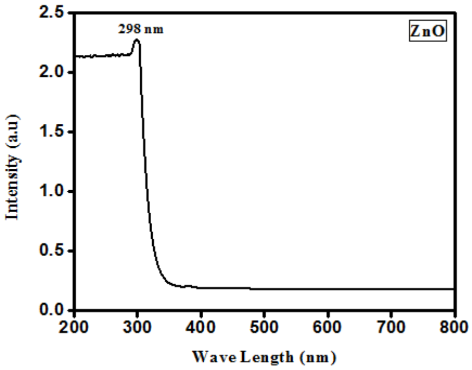


Fig -2: UV spectrum of ZnO nanoparticles using 0.1% AI gum as stabilizing agent

The band gap energy for ZnO nanoparticles in powder form was found to be 4.1 eV. The higher value of ZnO band gap energy has been attributed to the quantum confinement effects in nano region.

3.2. FT-IR Spectroscopic analysis

The interaction and the presence of chemical functional groups in the green synthesized ZnO nanoparticles were analyzed using FT-IR spectrophotometer (Perkin Elmer) in the range of 4000 - 400 cm^{-1} at the resolution of 4 cm^{-1}. The role of AI gum in stabilizing the formation of ZnO nanoparticles is identified by comparing the FT-IR spectrum of ZnO (Fig-3), AI gum (Fig-4), and ZnO synthesized using AI gum as stabilizing agent (Fig-5). In the FT-IR spectrum of ZnO (Fig-3), the broad band between 3200-3600 cm^{-1} centered at 3441 cm^{-1} corresponds to the stretching vibration of intermolecular O-H bond existing between the adsorbed water molecule and oxygen of ZnO nanoparticles. The peak appeared at 1627 cm^{-1} corresponds to O-H bending vibration and the peaks at 439 cm^{-1} and 408 cm^{-1} indicates the stretching vibrations of Zn-O bond.

In the FT-IR spectrum of AI gum (Fig-4) the peaks in between 3600-3200 cm^{-1} indicate the presence of higher amount of hydroxyl groups in the polysaccharides of AI gum. The observed peak at 1728 cm^{-1} indicates the >C=O stretching frequency. The absorption band at 1317 cm^{-1} may arise due to the stretching vibrations of C-N aromatic functional group of protein. The strong broad band at 1033 cm^{-1} indicates presence of amine group. The peaks present at 1612 cm^{-1} and 1550 cm^{-1} represents the C-O stretching vibration and N-H stretching vibration of amine groups in peptides. The peaks at 1458 cm^{-1} and 1319 cm^{-1} show the presence of C-H bond.

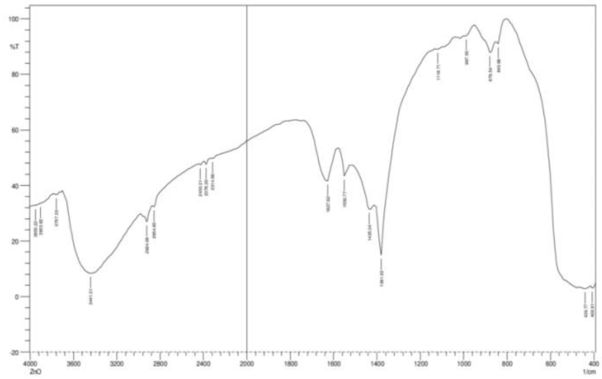


Fig -3: FT-IR spectrum of ZnO nanoparticles

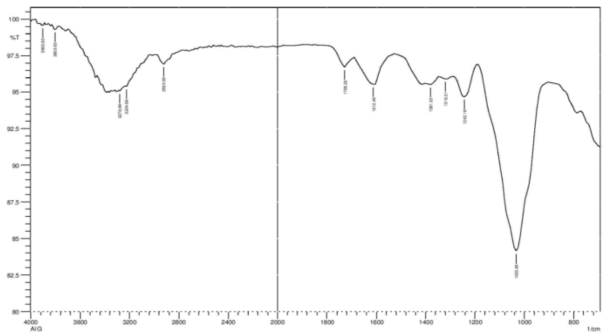


Fig -4: FT-IR spectrum of AI gum

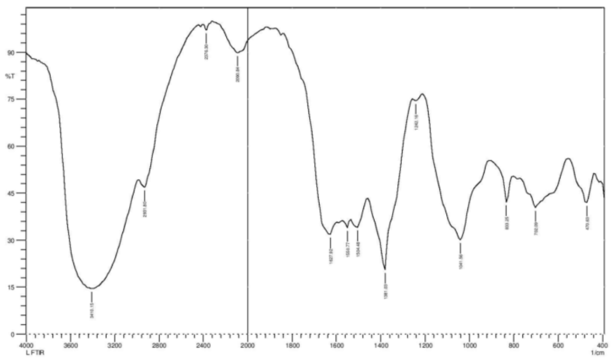


Fig -5: FT-IR spectrum of 0.1% AI gum stabilized ZnO nanoparticles

The FT-IR spectrum of ZnO nanoparticles synthesized using AI gum as stabilizing agent is given in Fig-5. A broad band appeared at 3600-3200 cm^{-1} in the AI gum and a same type of band appeared in ZnO nanoparticles centered at 3441 cm^{-1} were shifted to 3410

cm^{-1} in the spectrum of synthesized ZnO nanoparticles using AI gum as stabilizing agent. The lowering of frequency from 3441 cm^{-1} to 3410 cm^{-1} may be due to the participation of –OH group from the molecule of AI gum through some alternative force such as hydrogen bonding. The peak at 2931 cm^{-1} is due to C-H stretching vibration. The N-H stretching vibration of AI gum appeared at 1612 cm^{-1} is shifted to 1627 cm^{-1} in the spectrum of synthesized ZnO nanoparticles using AI gum which may due to some conformational change in the bulk AI Gum molecule. [13-15] Thus, there is a possibility of interaction between the proteins present in AI gum and ZnO nanoparticles.

The stretching of vibration of C-O at 1033 cm^{-1} in AI gum is shifted to 1041 cm^{-1} in the ZnO nanoparticles prepared using AI gum as stabilizing agent indicates the formation of new bond and the peak at 470 cm^{-1} represents the stretching vibration of Zn-O nanoparticles. The peaks appeared at 439 cm^{-1} and 408 cm^{-1} in the ordinary ZnO nanoparticles are shifted to 470 cm^{-1} in FT-IR spectrum of ZnO synthesized using AI gum. The higher frequency shift may be an indication of some structural changes in the ZnO nanoparticles in presence of AI gum.

3.3 X-Ray diffraction

X-Ray diffraction is a well known technique for the structural identification and determination of crystalline size of the synthesized ZnO nanoparticles. The X-Ray powder diffraction pattern of the synthesized ZnO nanoparticles by using AI gum was shown in Fig-6. The X-ray diffraction peaks with high intensity are observed at 2θ values of 31.31, 34.01, 35.83, 47.17, 56.01, 62.5, 67.4, and 68.5° corresponding to lattice planes (100), (002), (101), (102), (110), (103), (112) and (201) respectively. The comparison of observed XRD pattern with the standard JCPDS data, all the peaks are matched with standard JCPDS card no. 76-0704, confirms that the ZnO nanoparticles are of wurtzite hexagonal type structure.[16,17] Crystallite size (D) was calculated using Debye-Scherrer׳s formula [18,19]

$$D = K\lambda/\beta\ Cos\theta$$

D - Crystalline size of zinc oxide
λ - Wavelength of X- ray source 0.15406 nm in (XRD),
β - Full width at half maximum of the diffraction peak
K - Scherer׳ s constant (0.94)
θ - Bragg angle

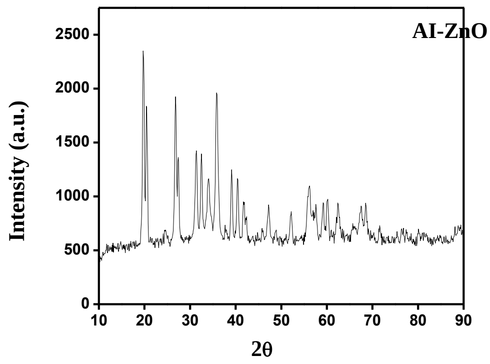
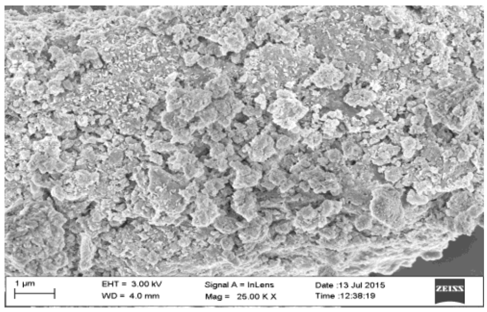


Fig – 6: XRD Spectra of 0.1% AI gum stabilized ZnO nanoparticles

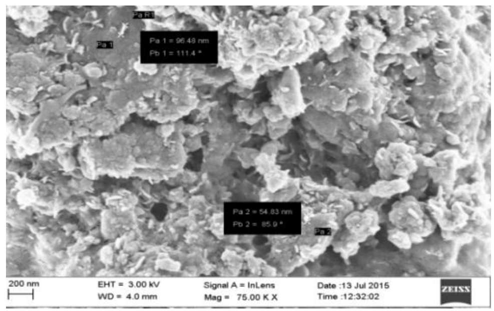
The average crystalline size (D) is found to be increased from 10 to 25 nm. Similar observations are reported by several authors. [20-22]

3.4 FE-SEM analysis

The surface morphology of ZnO nanoparticles were characterized by using FE-SEM.

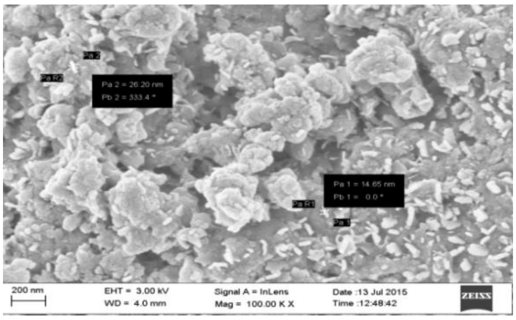


7a

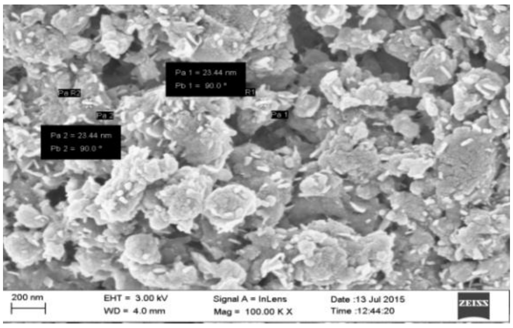


7b

Fig -7a, 7b: FE-SEM images of ZnO nanoparticles



7c



7d

Fig- 7c, 7d: FE-SEM images of ZnO nanoparticles using AI gum as stabilizing agent

FE-SEM images in Fig- (7a and7b) show the particle size and external morphology of the ZnO nanoparticles. FE-SEM images in Fig (7c and 7d) show the particle size and external morphology of the AI gum stabilized ZnO nanoparticles. It can be seen from the images 7a, 7b shows that the zinc oxide nanoparticles range from 53-96 nm and 7c, 7d shows the particle size of AI gum stabilized ZnO nanoparticles are 14-26 nm with nearly flower shape morphology. The particle size reduced from 53 nm to 14 nm confirms the ability of the AI gum as an excellent stabilizing agent for the synthesis of ZnO nanoparticles.

3.5 Antifungal studies

The antifungal activities of the ZnO nanoparticles prepared by chemical method and prepared using AI Gum as stabilizing agent were investigated against *Aspergillus fumigatus, Candida albicans,* and *Penicillum varians*. The zone of inhibition (mm) of these ZnO nanoparticles was determined using 100 µl concentration and tabulated. (Table.1)

The zone of inhibition (mm) of these ZnO nanoparticles was determined using 100 µl concentration and tabulated (Table.1) and the antifungal activities of the prepared ZnO nanoparticles by chemical and green method against the studied pathogenic strains are shown in fig 8.

S.no	Test fungal Culture	Antifungal activity (Zone of inhibition in mm)	
		ZnO	0.1%AI-ZnO
1.	*Aspergillus fumigatus*	2	7
2.	*Candida albicans*	10	15
3.	*Penicillum varians*	7	8

Table - 1: Antifungal activity of bulk ZnO and 0.1% AI gum stabilized ZnO nanoparticles

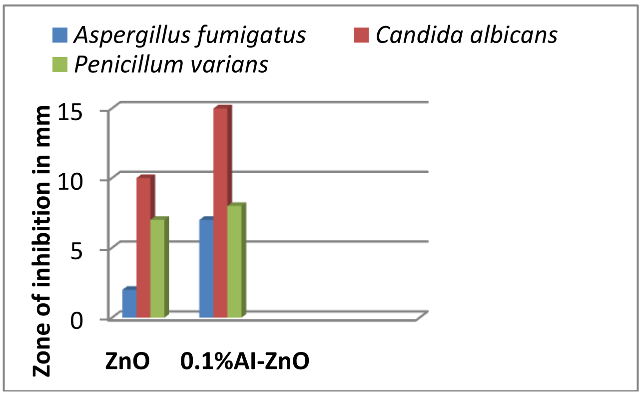


Fig- 8: Growth of fungal strains against *Aspergillus fumigatus, Candida albicans,* and *Penicillum varians* exposed to ZnO nanoparticles

Analysis of the data in the table reveals that the ZnO nanoparticles prepared using AI gum as stabilizing agent shows efficient antifungal activity than ZnO nanoparticles prepared by chemical method. Among the studied fungus *Aspergillus fumigatus, Candida albicans* are greatly inhibited by AI gum stabilized ZnO nanoparticles and the zone of inhibition was found to be 7 mm and 15 mm respectively. This is due to the unique properties such as larger surface area to volume ratio and smaller particle size.

The formation of reactive oxygen species (ROS) from ZnO nanoparticles was responsible for the increase in the permeability of the cell membrane which enables the cell death. The released oxygen species from ZnO nanoparticles react with hydrogen ion to produce molecules of H_2O_2. The generation of H_2O_2 depends strongly on the surface area of ZnO, which results in more oxygen species on the surface and this leads to higher antifungal activity of the smaller nanoparticles. In the present study, the smaller size ZnO (14 nm) synthesized by green method showed enhanced antifungal activity compared to ZnO (53 nm) prepared by chemical method. The DNA code of the micro organism was also affected by the ZnO nanoparticles. [23-26]

Conclusion

ZnO nanoparticles have been successfully synthesized using AI gum as stabilizing agent. UV-Vis, FT-IR, XRD and FE-SEM analysis were used to confirm the formation of ZnO nanoparticles. The synthesized ZnO has an average particle size of about 10-25 nm and particles are nearly uniform with hexagonal wurtzite structure. This conform the ability of the AI gum to stabilize the ZnO nanoparticles. The influence of nanocrystallinity can be seen in the band gap enhancement of 4.1 eV. The AI gum stabilized ZnO nanoparticles was found to be a good antifungal agent against *Aspergillus fumigatus, Candida albicans,* and *Penicillum varians.*

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