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Green Synthesis of ZnO Nanoparticles using *Trachyspermum ammi* Seed Extract for Antibacterial Investigation

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ABSTRACT

This research work describes a novel green approach for the synthesis of ZnO nanoparticles at ambient/low temperature, where Ajwain (Carom – Trachyspermum ammi) seed extract functions as the zinc salt reducing agent. Different synthesis conditions provide control of particle size and size-distribution of particle formation. X-ray diffraction patterns confirm that ZnO nanoparticles synthesized with carom seed extract is a potential candidate for future experimentation and implementation in the biomedical field. The experimentally synthesized ZnO nanostructures were characterized using the field emission scanning electron microscopy (FE-SEM) and Powder X-ray diffraction (PXRD) pattern measured with Cu Kα radiation. Moreover the photoluminescence (PL) measurements excited by the 380 nm line from the He–Cd laser were done at room temperature. The UV-Vis spectrum shows high absorption in the ultra-violet region at about 383.5 nm which makes the material to be suitable for UV filters. The average direct energy bandgap is determined as 3.490 eV. The scanning electron microscope study reveals that the morphology of the crystal is in the hexagonal shape. The average crystallite size is determined as 34.27 nm. The analysis of EDAX has shown the presence of zinc and oxygen. The FTIR study has shown the presence of Zn-O bond, C-O bond, C-H bond and metal oxygen bond.

Keywords: Antibacterial study, Coprecipitation, Optical properties, Structural properties, *Trachyspermum ammi*.

INTRODUCTION

Preparation of metal oxide nano particles employing herbal constituents is important in the field of nanotechnology which is one of the most vibrant areas of research in material science [1]. The synthesis and characterization of noble metal oxide nanoparticles such as ZnO, SnO_2, MgO is an emerging field of research due to their important applications in the fields of biotechnology, bio-engineering, textile engineering, water treatment, metal-based consumer products and other areas such as electronic, magnetic, optoelectronic and information storage. ZnO is a nontoxic, safe inorganic antibacterial agent that is capable of killing various disease causing micro-organisms. Zinc oxide is the active ingredient in many diaper rash crèmes, which is a pretty clear indication that it's gentle and safe for even the most sensitive skin [2-7].

Ajwain-*Trachyspermum ammi*, also known as Ajowan caraway or bishop's weed or carom, is an annual herb in the family of Apiaceae. In Tamilnadu region it is kown as Omam. It is widely grown in arid and semi-arid regions where soils contain the high level of salts. Ajwain is profusely branched annual herb, 60-90 cm tall. Stem is striated, inflorescence compound umbel with 16 umbellets, each containing up to 16 flowers. The seeds contain 2–4.4% brown colored oil known as ajwain oil. The main component of this oil is thymol, which is used in the treatment of gastro-intestinal ailments, lack of appetite and bronchial problems. Ajwain is a traditional herb and is widely used for curing various diseases in humans and animals.

Many herbals in India are the chief and best source of medicinal plants and plant products. From many centuries till date especially in Tamilnadu, these medicinal plants have been extensively utilized in Siddha medicine. Recently, many such plants have been gaining importance due to their unique phytoconstituents and their versatile applicability in various developing fields of research and development. Nano-biotechnology is one of the most dynamic division of research in novel material science whereby plants and different plant products are finding an essential use in the synthesis of nanoparticles (Nanoparticles).Genrally the plant sources containing the phytoconstituents viz., Tannins, Alkaloids, Polyphenols, Flavonoids, Citric acid are good reducing agents. Many numbers of reducing agents have been associated with environmental toxicity or biological hazards. But they must be eco, health friendly and safe to produce metal oxide nanoparticles in an aqueous environment [8-11]. The green synthesis of metal oxide nanoparticles using plants has received more attention as a suitable alternative to chemical procedure and physical methods [12-15]. Extracts from plants may act both as reducing and capping agents in nano particle synthesis [16]. This is cost effective and eco friendly for the large scale production of metal oxide nanoparticles. The synthesis of ZnO nanoparticles using herbal seed extracts in antibacterial discipline is very interesting in the scientific and medical communities, largely due to the physical and chemical properties and is the center of attention of this article.

MATERIALS AND METHODS

In the extract process, *Trachyspermum ammi* seeds were collected, washed thoroughly with double distilled water and dried in air and powdered. About 15 g of *Trachyspermum ammi* seeds powder was mixed with 50 mL of double distilled water and boiled for 10 minutes. This extract was filtered through nylon mesh, followed by Millipore hydrophilic filter (0.22 um) and used for further experiments. In the second step, about 2 ml of the extract was slowly added drop wise to the 25 ml solution of 0.05 M $ZnNo_3$ through a dropping funnel under constant magnetic stirring for 2 hours at 50°C. In the third step the precipitate obtained was washed with ethanol, separated by centrifuge and dried at room temperature at 35°C to get ZnO nanoparticles in solid form as known as sample a. The same procedure was carried out in a similar way for the preparation of sample b and c where the 4 ml and 6 ml of the *Trachyspermum ammi* seed extract solutions were added respectively. In the present work, attempt has been made to understand the structural, morphological and optical properties of nano crystalline ZnO nanoparticles prepared through co-precipitation method using *Trachyspermum ammi* seed extract where the extract acts as the zinc salt reducing agent as well as capping agent. The prepared ZnO nanoparticles have been characterized by powder X-ray diffraction (PXRD), ultraviolet (UV) visible spectrophotometer, photoluminescence (PL), field emission scanning electron microscopy (FE-SEM) and energy dispersive X-ray analysis (EDAX). The highlight of this work is the antibacterial activity of green synthesized ZnO Nanoparticles using the popular herbal *Trachyspermum ammi*–carom seed extracts.

RESULTS AND DISCUSSION

3.1 PXRD ANALYSIS

The crystallinity was determined by XRD powder diffraction. Analysis was performed by using an XRD SHIMADZU X-ray diffractometer equipped with a Cukα (λ=1.54Å) source. The XRD patterns of all samples a, b and c of ZnO nanoparticles were recorded from 30^0 to 80^0 for 2Ө angles with diffracted intensities as shown in Fig.3.1. The diffraction planes with indices 100, 002, 101, 102, 110, 103, 200, 112, 201, 004, 202 could be indexed to the ZnO wurtzite structure and good they show agreement with JCPDS Data Card No: 36-1451.

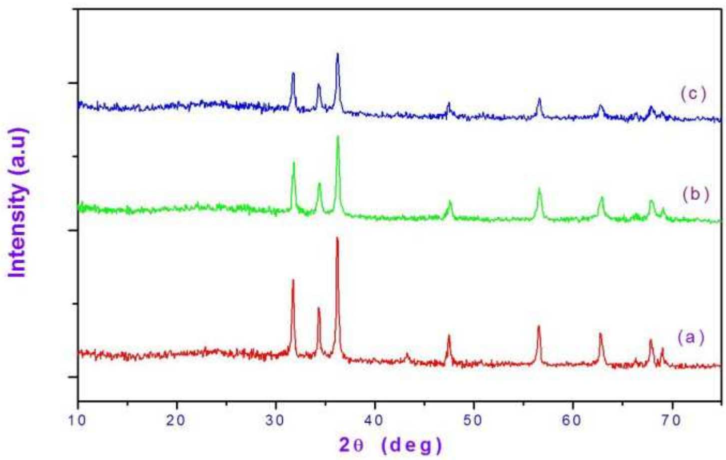

Fig.3.1 XRD patterns of green synthesized ZnO Nanoparticles samples a, b, c

Wurtzite lattice parameters such as the values of d, the distance between adjacent planes in the Miller indices (h k l) (calculated from the Bragg Equation, λ=2dsinθ), lattice constants a, b, and c, inter-planar angle and unit cell volumes are calculated from the Lattice Geometry equation [17-19]. The (101) peak with relativey high intensity indicates the preferred orientation of all the samples. Table.1 shows that the Crystallite size, d - spacing, Crystallites volume V, Lattice parameters, *c*/*a* ratio, cell volume v, number of unit cell NU, bond length ι, dislocation density δ of ZnO Nanoparticles.

Table.1 Structural parameters of green synthesized ZnO Nanoparticles for samples a, b, c

sample	2Θ	D nm	d Å	V $(nm)^3$	Lattice Constant a (Å)	c (Å)	c/a	v $(Å)^3$	NU $x10^6$	ι (Å)	δ X 10^{-4} nm^{-2}
a	36.271	39.510	2.474	67.340	3.2498	5.206	1.6019	57.452	3.594	1.873	6.4839
b	36.236	35.212	2.515	62.253	3.2430	5.194	1.6017	54.214	3.701	1.836	8.0652
c	36.245	28.112	2.765	60.258	3.2421	5.190	1.6008	50.155	3.956	1.821	12.653

3.2 FE-SEM & EDAX ANALYSIS

The synthesized ZnO nanostructures are morphologically characterized by the FE-SEM images, as shown in Fig.3.2. The ZnO nanostructure shows the uniform hexagonal plates, irregular and highly aggregated nanoparticles with rough surface.

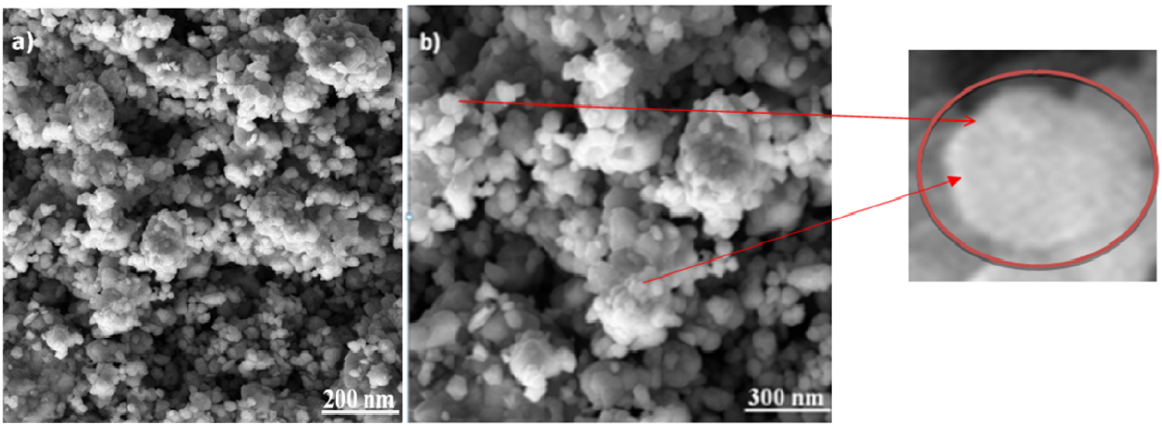

Fig.3.2 FE-SEM images of green synthesized ZnO Nanoparticles for sample c

The average diameter of the ZnO cluster is ~ 41nm has been observed. EDAX spectrum shows the high values of zinc (51%) and oxygen (29%) respectively as shown in the Fig.3.3. These results confirmed the presence of zinc as a majority label compared to oxygen in precursor material. The EDAX analysis shows that the optical absorption peaks of ZnO Nanoparticles and these peaks which are due to the surface plasmon resonance effect of zinc oxide Nanoparticles. The origin of these elements lies in the phyto components which are existed along with ZnO Nanoparticles [20-23].

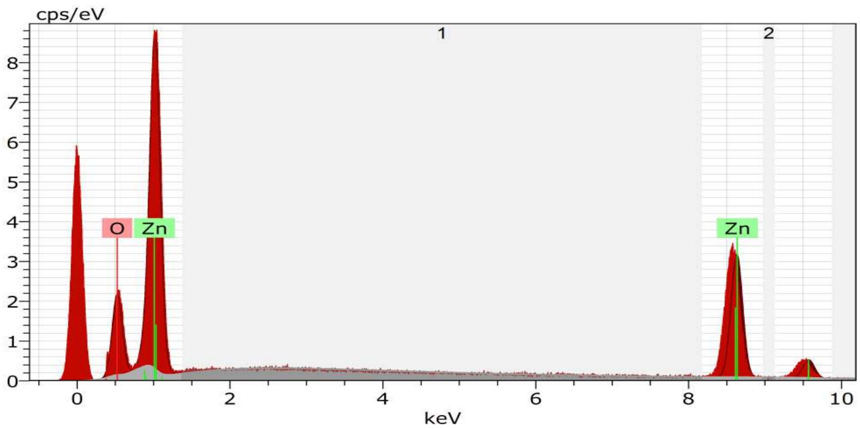

Fig.3.3 EDAX spectrum of green synthesized ZnO Nanoparticles for sample C

3.3 FT-IR ANALYSIS

Phytoconstituents present in the extract play a vital role for the reduction of ZnO and act as capping agent on ZnO Nanoparticles. The FT-IR spectra of synthesized ZnO are shown in Fig. 3.4. The absorption peaks at 445 cm^{-1}, 449 cm^{-1}, 665 cm^{-1} and 672 cm^{-1} for the samples a, b and c indicate the presence of ZnO nanoparticles and they are related to the stretching vibrations of Zn-O bonds [24, 25]. The sharp band around 1015 cm^{-1} for the three samples, indicates the symmetric stretching of C-O group present in the extract [26]. The FT-IR spectra also show the presence of bonds due to CH stretching around 2939 cm^{-1}.

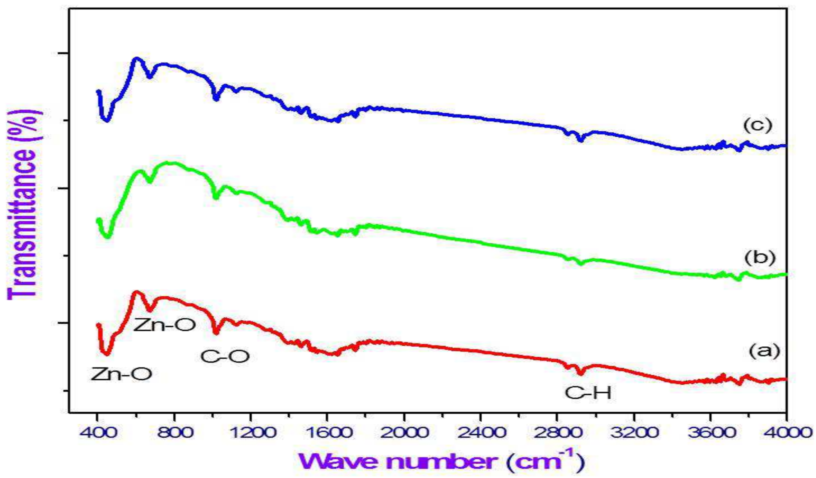

Fig.3.4 Typical FTIR spectra of green synthesized ZnO Nanoparticles for samples a, b and c

3.4 UV-VIS ANALYSIS

Optical absorption and optical energy bandgap measurements were carried out on ZnO nanoparticles for all the three samples a, b, c. Fig.3.5 shows the variation of the optical absorbance with the wavelength. The optical absorption coefficient has been calculated in the wavelength range of 300 - 800 nm. The absorption edge has been obtained at a shorter wavelength. The broadening of the absorption spectrum could be due to the quantum confinement of the nanoparticles. The ZnO Nanoparticles have good crystallinity and show strong blue emission, promising for applications in optical devices. UV absorption spectrum of ZnO nanoparticles show absorbance edge near 350 nm, which agrees with the reported value of 312 nm [27-28]. An attractive result is that the bandgap energy Eg decreases from 3.592 to 3.383 eV as the quantity of extract increases from 2ml to 6ml namely for samples a, b, c respectively. It is depicted in the fig.3.6

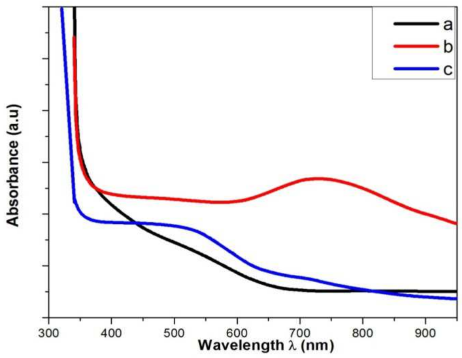

Fig.3.5 Uv-Vis Absorption curve of of green synthesized ZnO Nanoparticles for samples a, b and c

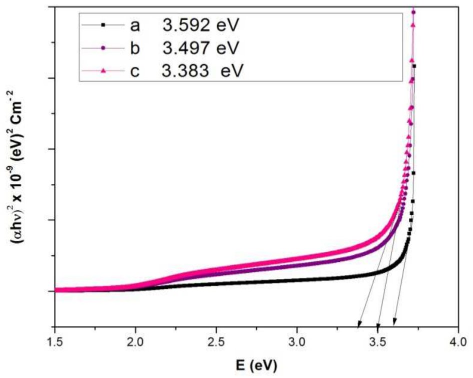

Fig 3.6 Band gap energy curve of green synthesized ZnO Nanoparticles for samples a, b and c

3.5 PL SPECTRA ANALYSIS

Fig. 3.7 shows the photoluminescence spectra of ZnO Nanoparticles for samples a, b, c over the wavelength range 350-450 nm on irradiating at excitation wavelength λ_{ex} = 300 nm. In the photoluminescence (PL) spectra of ZnO, typically there are emission bands in the UV and visible regions. In general, visible emission in ZnO is attributed to different intrinsic defects such as oxygen vacancies Vo, zinc vacancies V_{Zn}, oxygen interstitials [30]. The UV peak is usually considered as the characteristic emission of ZnO and attributed to the band edge emission or the exciton transition. Several emission bands, including band edge emission at 396 nm and defect related ultraviolet emission at 362 and 382 nm, violet emission at 420 nm and blue emission at 445 nm were observed [31,32].

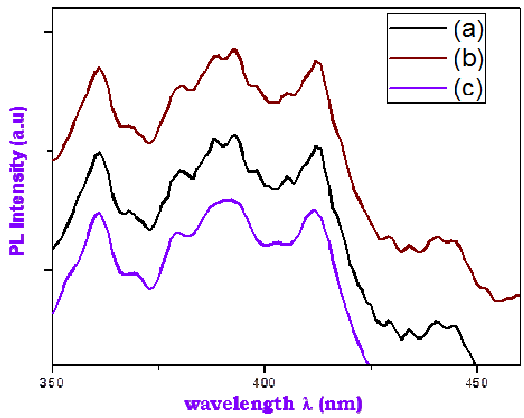

Fig 3.7 PL spectra green synthesized ZnO Nanoparticles for samples a, b and c

3.6 ANTIBACTERIAL ACTIVITY

Antibacterial activity of the ethanolic extract of the compound for samples a, b, c were determined using well diffusion method. It is performed by sterilizing Mueller Hinton agar media. After solidification, wells were cut on the Mueller Hinton agar using cork borer. The test bacterial pathogens such as Staphylococcus aureus and Pseudomonas aeruginosa were swabbed onto the surface of Mueller Hinton agar plates. Wells were impregnated with 25 µl of the test samples (ethanolic extract of the compounds). The plates were incubated for 30 min to allow the extract to diffuse into the medium. The plates were then incubated at 37°C for 24 hours, and then the diameters of the zone of inhibition were measured in millimeters as tabulated in Table.2.

Also effect of varying the quantity of Carom seed extract solution for samples a, b and c are represented by bar diagram in the Fig. 3.7. Each antibacterial assay was performed in triplicate and mean values were reported.

Solvent used: Ethanol, Standard used : Ampicillin 10 µg
Staphylococcus aureus : Zone of inhibition for Amp 10 µg - 17 mm
Pseudomonas aeruginosa : Zone of inhibition for Amp 10 µg - 12 mm

Table.2 The diameters of the zone of inhibition of *green synthesized* ZnO Nanoparticles for samples a, b, c

Test bacterial pathogens	Zone of inhibition in mm (in diameter)		
	S-a	S-b	S-c
Staphylococcus aureus	6	10	22
Pseudomonas aeruginosa	11	13	27

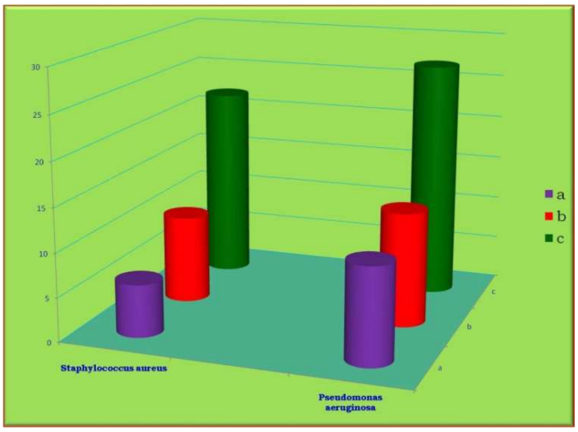

Fig. 3.7 Effect of varying concentrations of Carom seed solution for samples a, b and c on the Zone of Inhibition

The antibacterial activity of green synthesized ZnO nanoparticles was investigated on both Gram positive (Staphylococcus aureus) and Gram negative (*Pseudomonas aeruginosa*) bacteria by zone inhibition methods. The results of zone inhibition method as depicted in Fig. 3.8. The diameter of inhibition zones in mm around each well with ZnO Nanoparticles by carrom seed extract solution are found the highest antimicrobial activity against *Pseudomonas aeruginosa* (27 mm) than compared to standard tablet, as shown in the Fig. 3.8. The ZnO Nanoparticles formed in this experiment is found to have good antibacterial activities. From this study the antibacterial activity for both gram positive and negative bacteria were found to increases when quantity of seed extract increases as 2ml, 4ml and 6 ml namely for samples a, b and c. Also the inhibition efficiency of these samples is greater for *Pseudomonas aeruginosa than* Staphylococcus aureus.

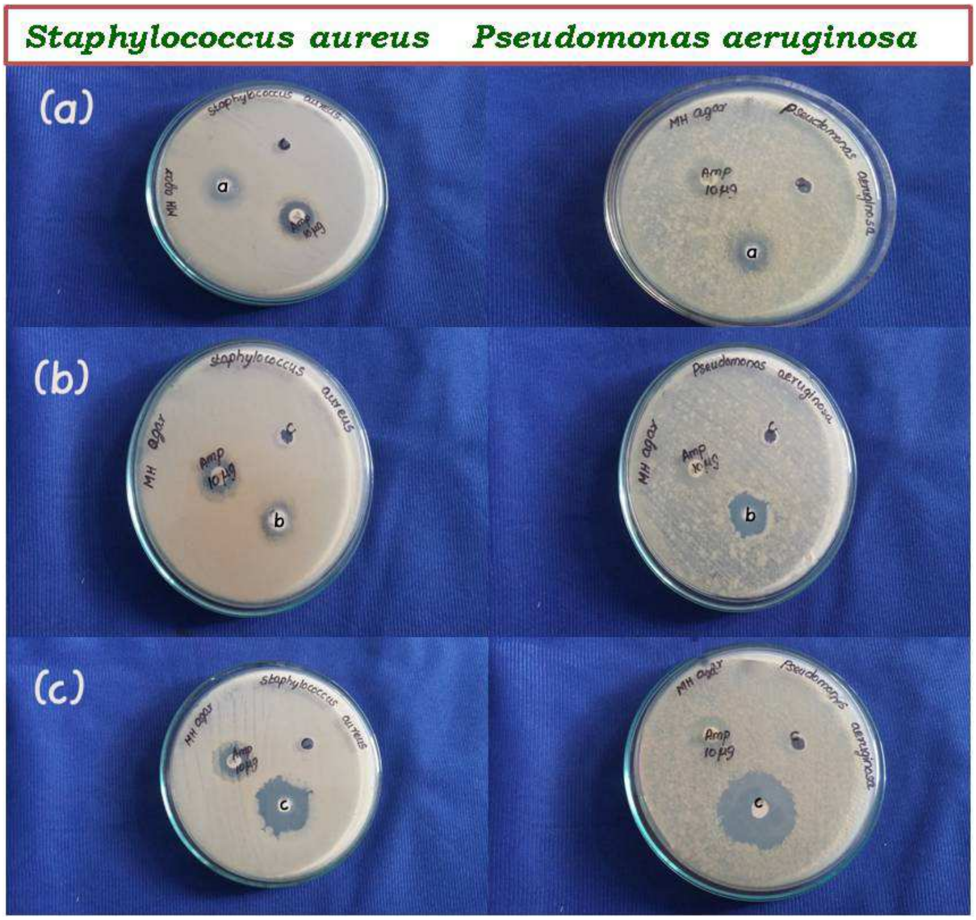

Fig.3.8 Zone of inhibition in mm for the green synthesized ZnO Nanoparticles against micro-organisms

CONCLUSION

ZnO nanoparticles were successfully synthesized by using Ajwain (Carom – *Trachyspermum ammi*) extract mediated reduction process. The structure, morphology and size (dimension) of prepared ZnO nanoparticles were examined by FT-IR, XRD, and FE-SEM. FT-IR spectra revealed the functional groups through stretching bands for ZnO nanoparticles which were found around 600–400 cm^{-1}. The average diameter of the nanoparticles is observed around ~41 nm. From the XRD study the average grain size is found to lie between 30–40 nm. As prepared ZnO nanoparticles show a direct transition in the range from 3.529 to 3.383 eV. It has the strong emission band at 382 nm and also a broad emission peak centered at 445 nm in visible region. The antibacterial activities of ZnO nanoparticles for both gram positive and negative bacteria were found to increases when quantity of seed extracts increases. In conclusion, the present work is so ready to lend a hand to the scientific society for using the ZnO nanoparticle as a potential agent to the biomedicine field. In addition, it is cost effective, nontoxic, safe and eco-friendly for the human society.

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