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Synthesis and Characterization of Zinc Oxide Nanoparticles (ZnO NPs) and their Biological Activity

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Abstract

Zinc Oxide Nano particles promise an important role in various industries like pharma, fertilizer, textiles and garments due to their specific properties such as anti-fungal, antibacterial and anti-rust. Zinc oxide nanoparticles were prepared by eco-friendly wet chemical method. Formation of ZnO nanoparticles was confirmed by using spectroscopic and microscopic investigations. Samples were characterized using Ultra-Violet Spectroscopy, Fourier Transform Infrared, Scanning Electron Microscopy, Transmission Electron microscope, X-ray Diffraction and four probe Voltage-Current characterizations. Antifungal and antimicrobial activity

Keywords:

Zinc Oxide Nanoparticles (ZnO NPs), Antibacterial activity, Antifungal Activity, XRD, FTIR, SEM,TEM, UV, VI. Bacillus Subtilis, Pseudomonas aeruginosa, Candida,A.niger

INTRODUCTION-

Due to their special characters metal oxide nanoparticles are utilized in different capacity as pharma for making special targeting drugs, in Agriculture industry for fertilizer and environmentally friendly pesticides. In paint industries, these are useful in Nano-electronics component. Metal oxide nano particles are used for making biomedical Nano-devices. Metal oxides nanoparticles are reported to show very crucial features in many areas of physics, chemistry and material Nano science (1). Microwave imaging with zinc oxide nanoparticles as contrast-enhancing agents

Zinc oxide nanoparticles are very important class of metal oxide nanoparticles. It is environmentally friendly and has various set of applications. It has diverse properties depending upon its morphology (2). It is widely used in pharm industry, fabrication of Nano

electronics devise, paint industries ,garments and textiles industries and has various biomedical application .

We have synthesized and characterized zinc oxide nanoparticles (ZnO NP) and observed various biological effects on different type of microorganism. Zinc Oxide Nano particles have reported to have anti-fungal, anti-bacterial property which gives wide application in drugs and food processing industries. Zinc oxide Nano particles is used in area of antibacterial and photo catalysis (3).Zinc Oxide nanoparticles have shown antimicrobials agents against pathogenic and spoilage microorganism (4). Metal oxide nanoparticles provide an area this is certainly big in terms of their unique optical, magnetic, catalytic and electrical properties [5]. It is widely used being as a food products preservative because of the anti-microbial prospective. ZnO has also been utilized in the packaging of meals cans such meat, corn, fish and peas to prevent spoilage and preserve the colour [6].

Different techniques have been used to prepare zinc oxide nanoparticles such as hydrothermal [9–12], solvo thermal [13,14], micro emulsion [15], sol-gel [16,17] and thermal decomposition of precursors [18,19]. Using wet chemical method of synthesis, in accordance with Raghupathi et al. and Applerot et al., ZnO nanoparticles exhibit maximum degree of antibacterial activity with the reduce in average particle size[20,21]. Method of synthesis of nanoparticles strongly affects the design and size of nanoparticles, which determines the properties of nanoparticles [22,23].

Synthesis of Zinc Oxide Nano Particles

Zinc oxide nanoparticles were prepared using wet chemical method. ZnO NP were synthesized by using 0.2 M solution of $Zn(NO_3)_2$ and 0.4M NaOH. The nitrates of zinc metals were dissolved in water and sodium hydroxide was added drop wise at room temperature followed with continuously stirring. .

The stirring was done for 6.30 hours at 83 degree Celsius. The reaction mixture was filtered first and dried in oven at 65 degrees Celsius. Hydroxides were calcined in furnace at 610 degree Celsius to obtain zinc oxide nano particles.

These NPs were characterized using SEM, TEM, FTIR, UV and I-V.

Characterization of zinc oxide nano particles

Zinc oxide nano particles were characterized using X-ray diffraction, scanning electron microscope, transmission electron microscope, Fourier transform infrared, UV spectrum and four probe voltage-current method .

X-ray diffraction analysis

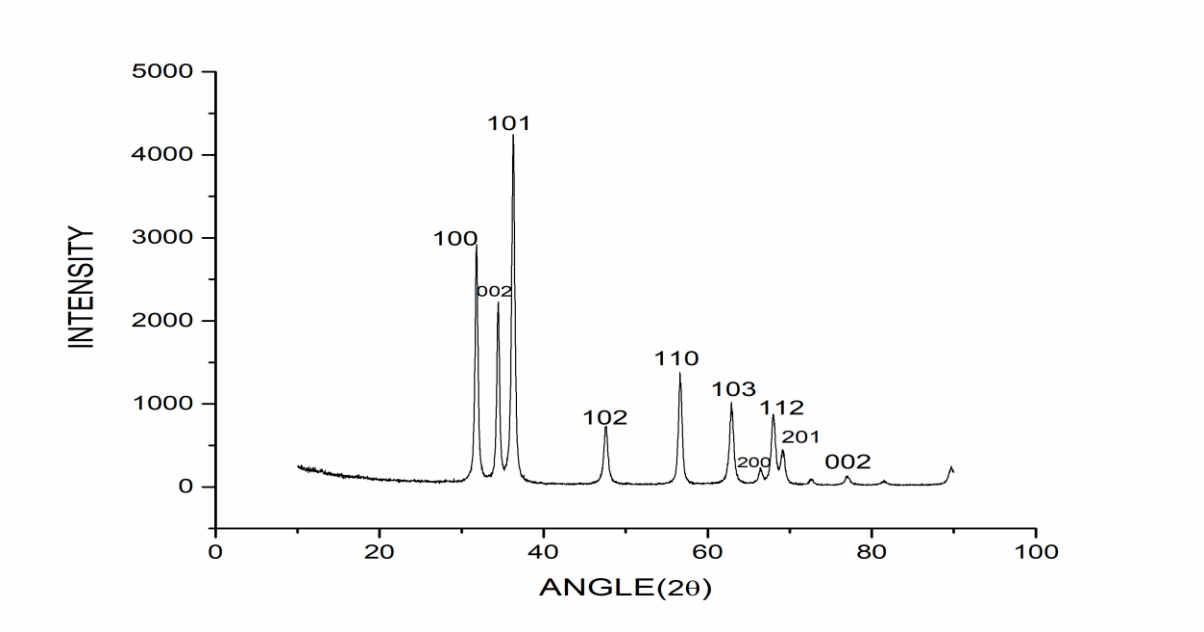
Synthesized particles were subjected to X-Ray diffraction studies to obtain the crystallinity and particle size. In XRD spectrograph of zinc oxide nanoparticles, peaks for ZnO-NPs synthesized using sheep fecal matter appears at $2\theta = 31.72°$ (100), 34.41° (002), 36.22° (101), 47.67° (102), 57.71° (110), 62.91° (103), 68.01° (112), 69.10° (201) and 77.047° (202) respectively.

Particle size was calculated by utilizing Debey-Scherrer equation

$$D = 0.9\lambda/\beta Cos\theta$$

Here D, k, λ, and β represent the average crystal size, shape factor (0.9), wavelength (0.15416) and Bragg angle θ of the X-ray (1.5406 Å) Cu Ka radiation, respectively. The ZnO NPs of typical size had been calculated as 25.57 nm.

XRD GRAPH ZnO NPs



Scanning electronics microscope (SEM) analysis

The morphology of NPs was analyzed by scanning electron microscopy. The average particles size obtained are 66.015nm,50.49nm, 0.106micro meter, 39.83nm, 38.611nm as shown in figure (a), (b), (c), (d) and (e) respectively.

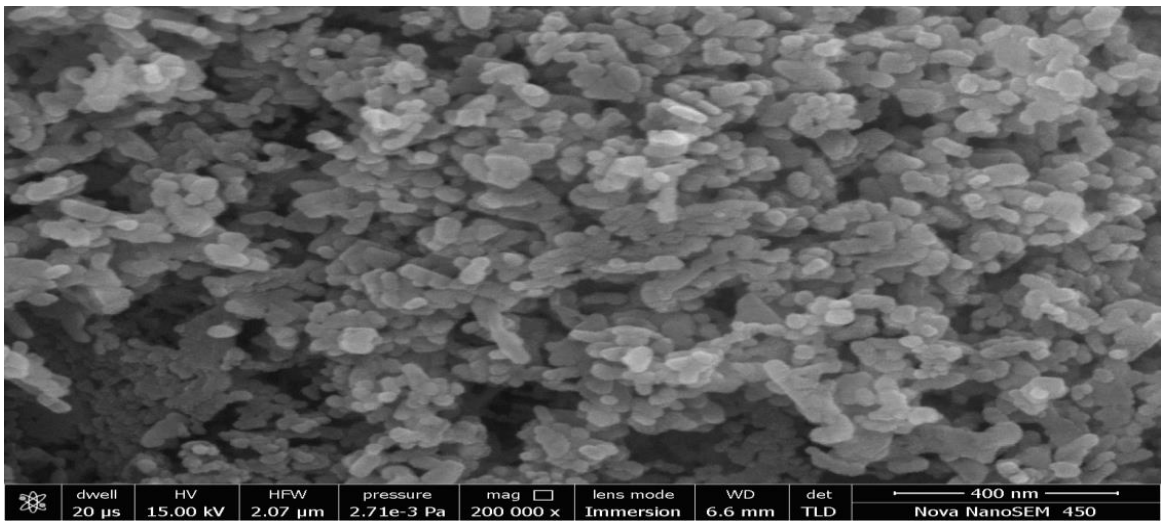


Figure-a

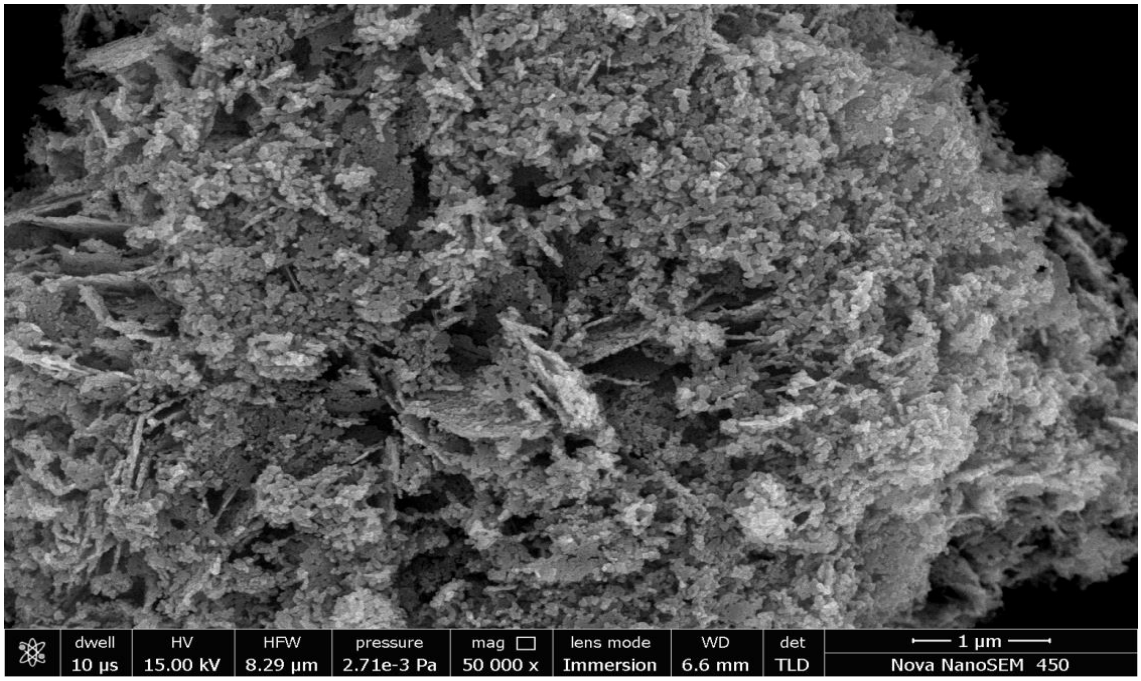


Figure -b

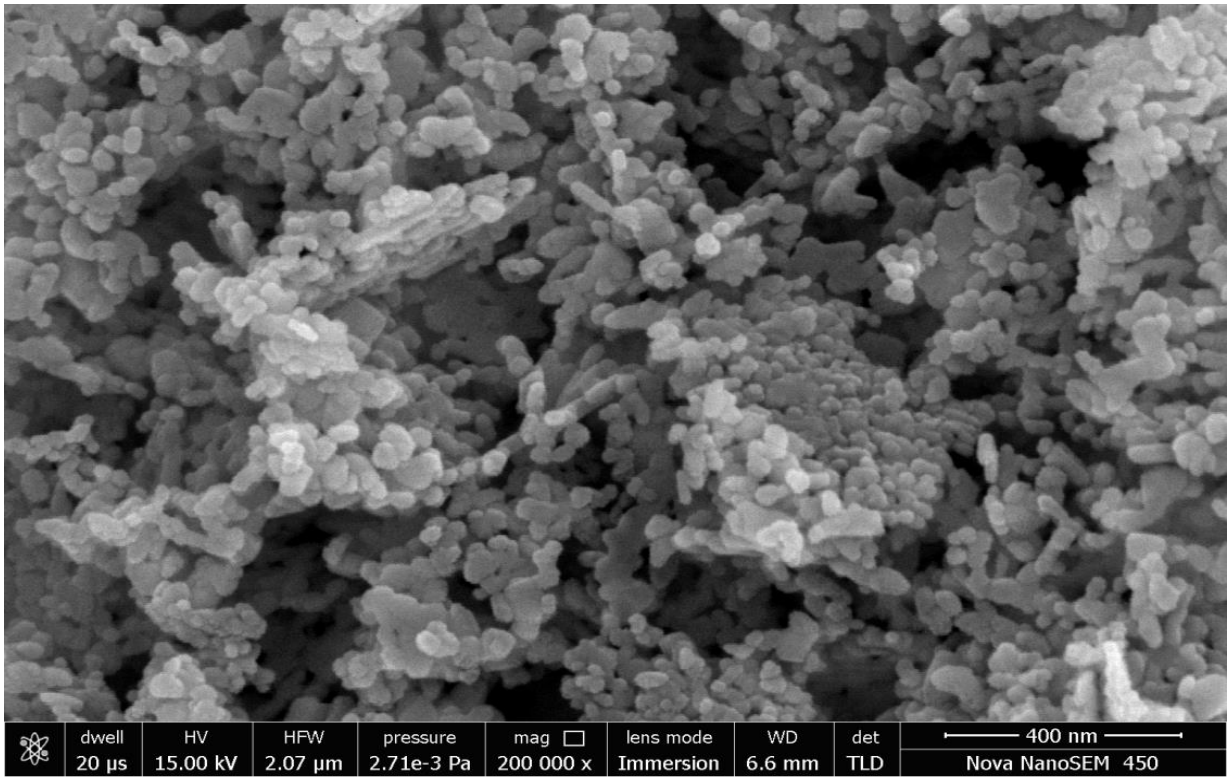


Figure -c

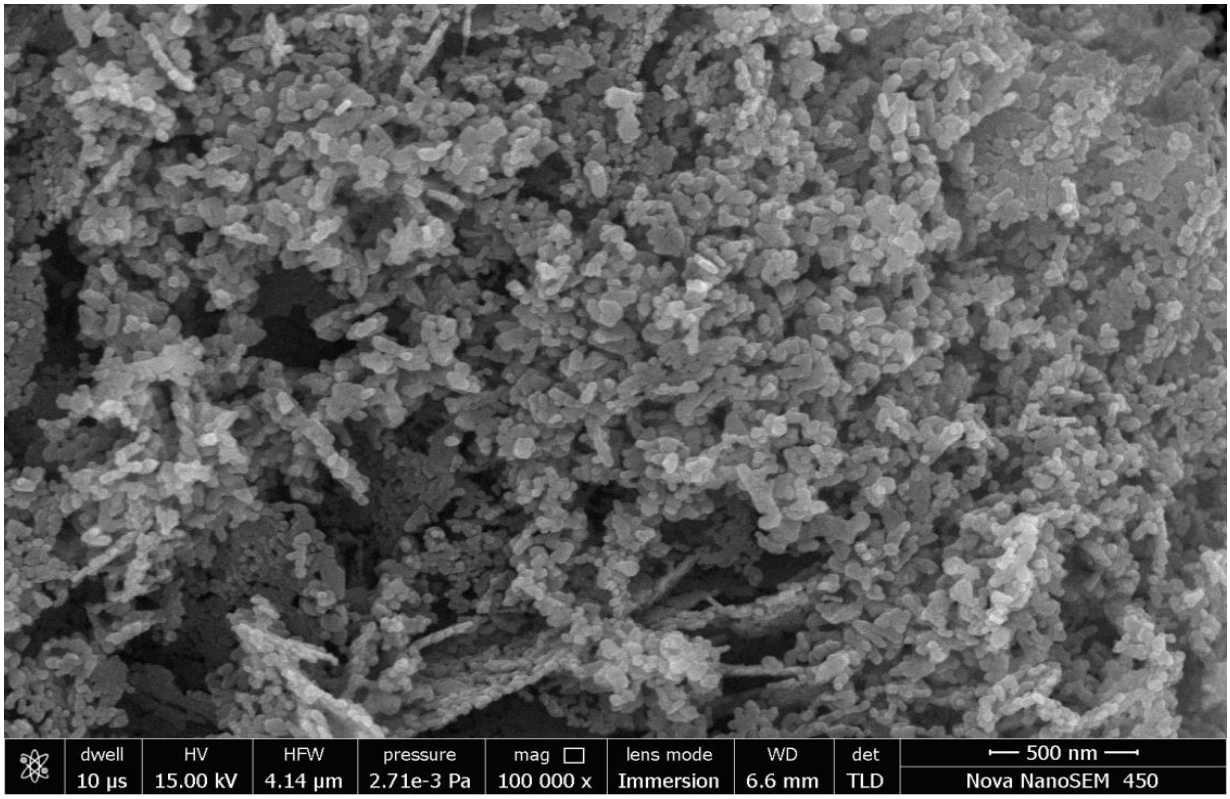


Figure -d

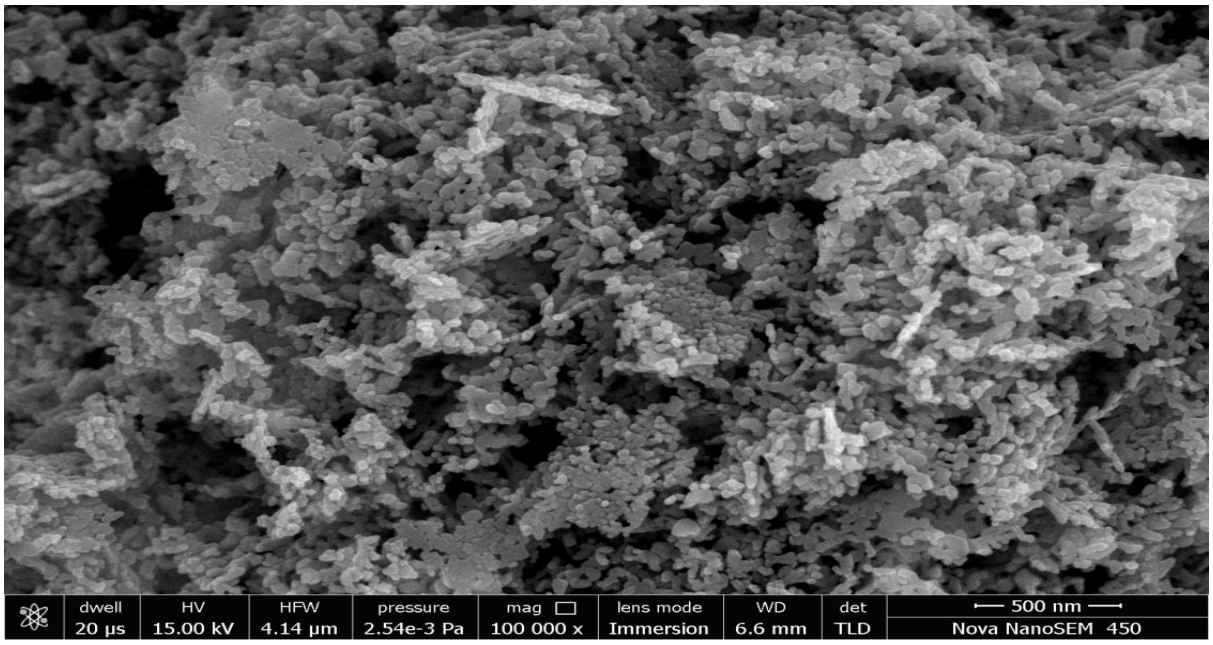


Figure -e

Transmission Electron Microscopy Analysis

TEM images proclaim about the dimensions of synthesized nanoparticles are with in nanometer scale. TEM imaging was carried out to observe the sizes and structural morphologies of the ZnO-NPs. As shown in Fig. (a), (b),(c),(d),(e) the shapes of the ZnO-

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NPs are spherical small agglomeration. A average particle size-18.26nm, 24.61nm, 26.66nm, 17.14nm in figure (a),(b),(c),(d) and figure (e) exhibit crystallite structure

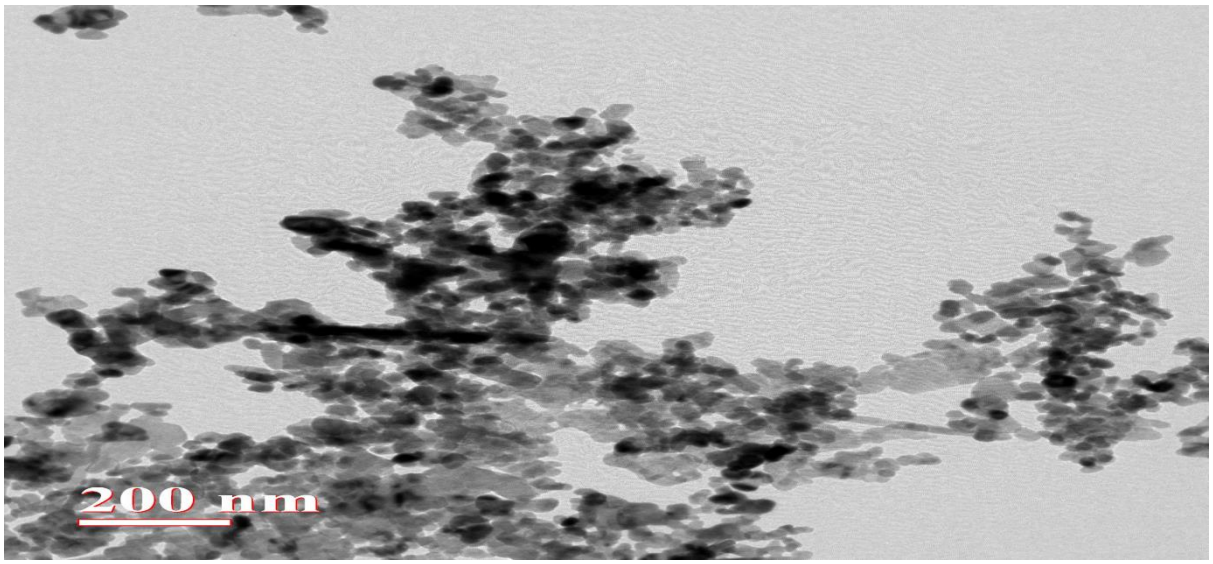

Figure -a

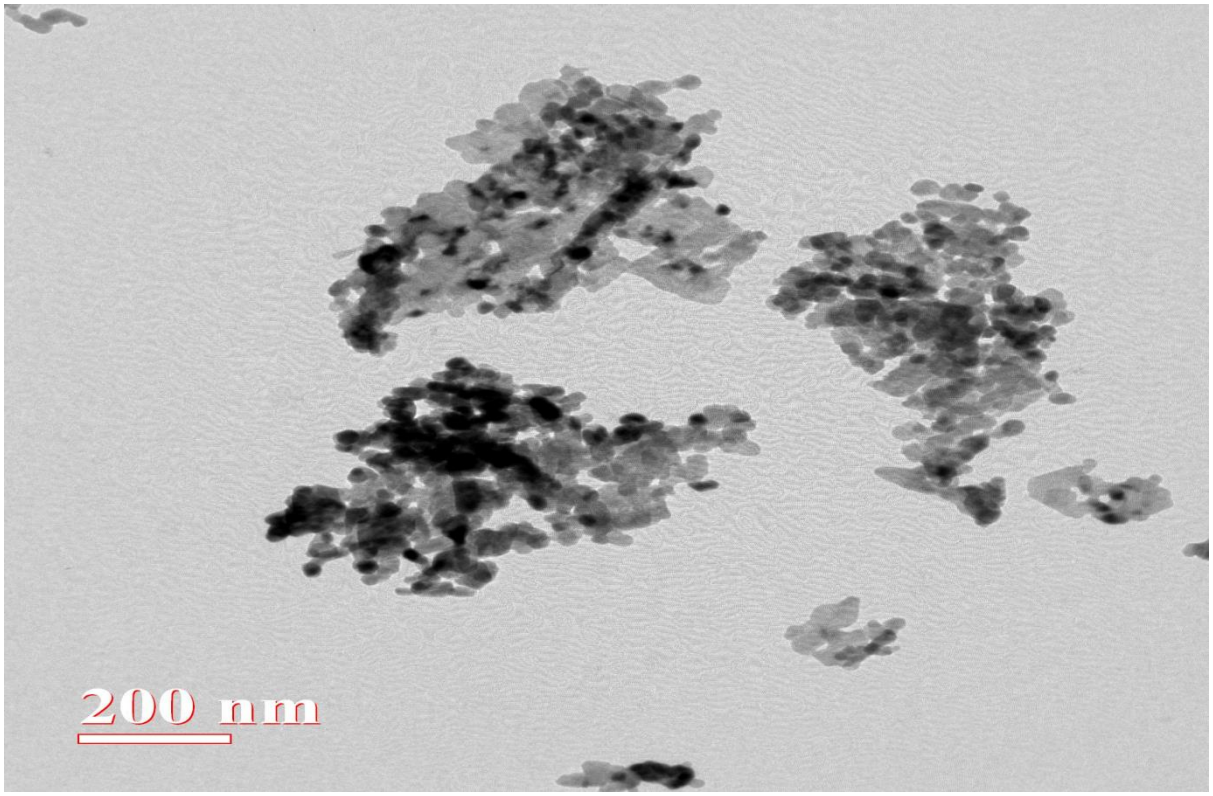

Figure -b

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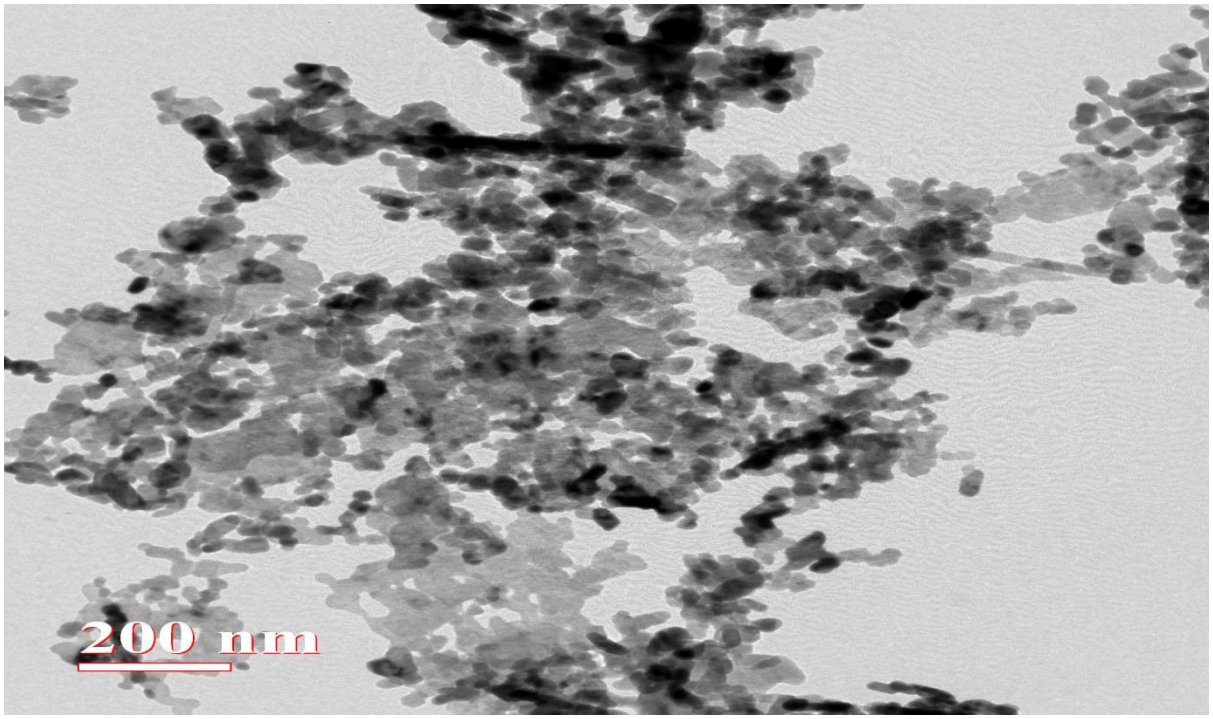


Figure -c

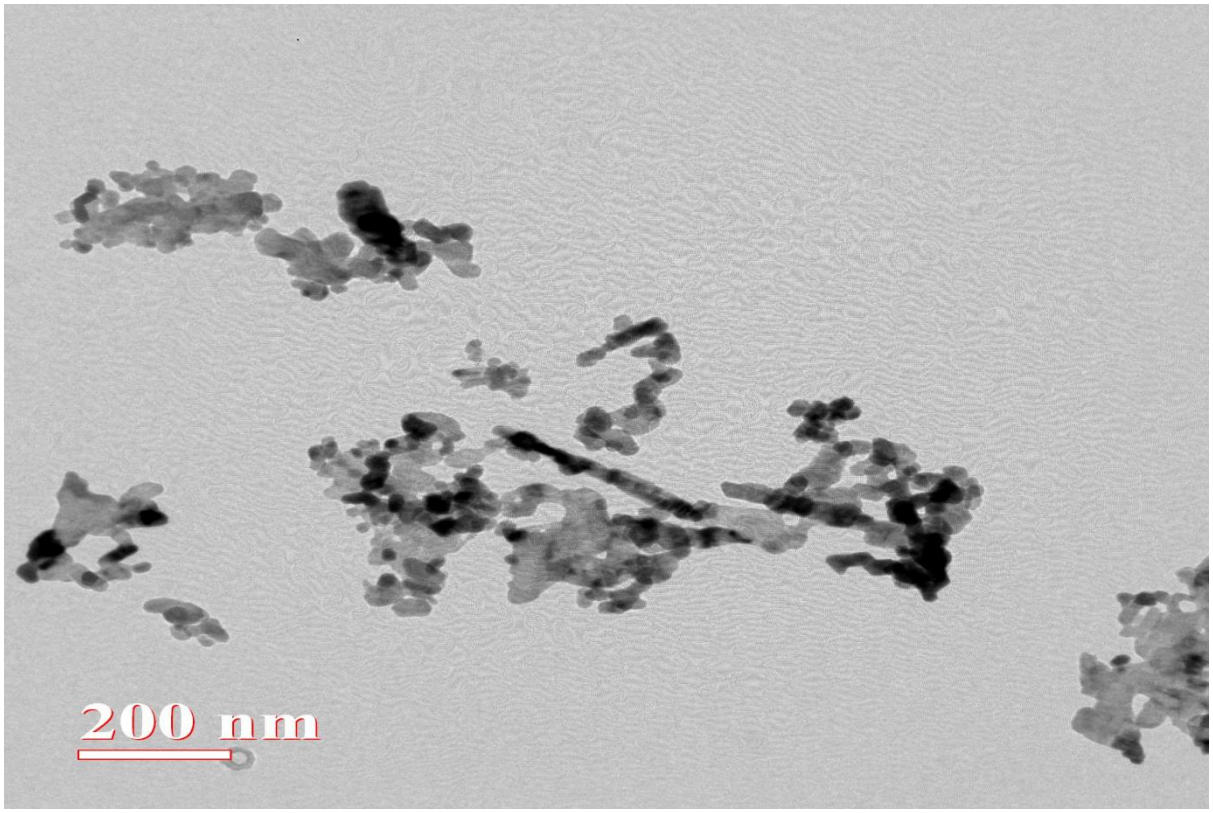


Figure -d

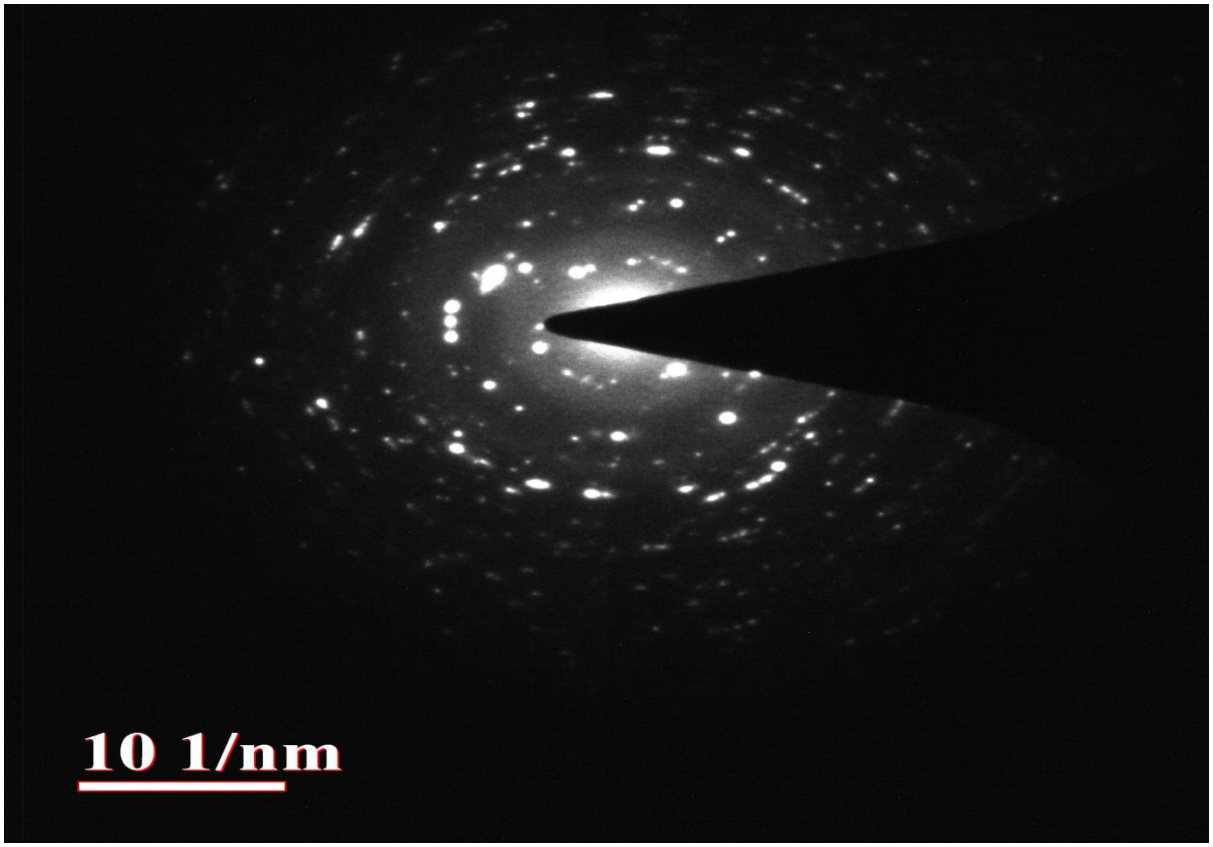
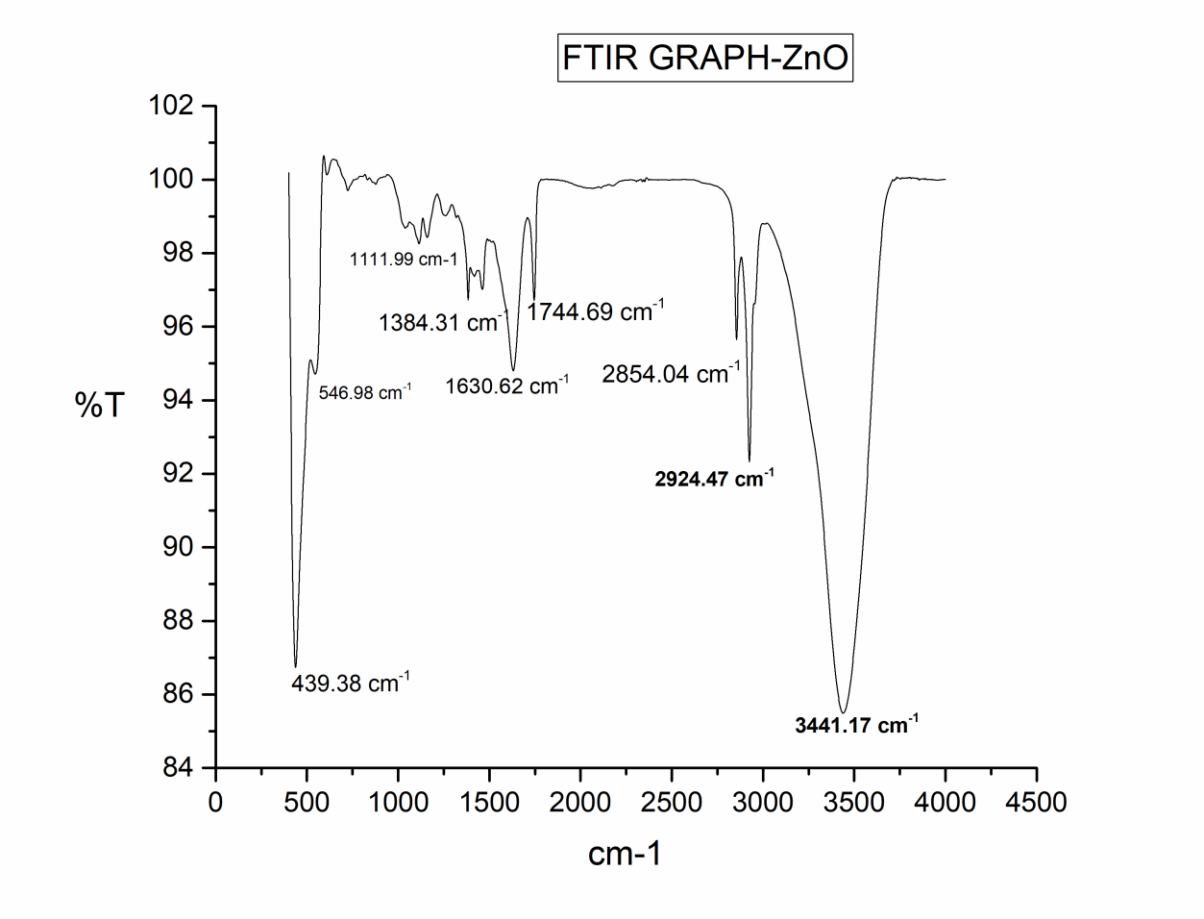


Figure -e

Fourier Transform Infra-Red Analysis

Fourier transform infrared spectroscopy was used to detect the functional groups in the compound. The FTIR spectrum of ZnO-NPs in the wave number was ranged between 400 to 4000 cm1 is shown in below figure. The broad peak at 3441.17 cm1 corresponds to the O–H group.

Stretching vibration of the alcoholic group, the medium peaks are observed at 2924.47 cm1 and 2854.04cm1 for the C-H stretching vibration of alkanes. The strong peak at 1744.69cm1 for the C=H Stretching vibration of carbonyls group. Peaks at 1630.62 cm-1 is due to C=C Alkene functional group.



The medium peak at 1384.31cm1 corresponds to O-H bending in Phenol. The peak at 1111.99 cm1 is observed for the C-H Stretching of aliphatic ether. The previously observed bands were shifted to 3441.17, 2924.47, 2854.04, 1744.69, 1630.62, 1384.31, 1111.99, 546.98 and 430.38 (Zn-O band) cm−1 in the ZnO-NPs.

These observed peaks were due to different functional group.At 3441.17 cm1 a broad peak is obtained due to hydrogen bond in functional alcohol group.

UV-Visible Spectroscopy Analysis

The preliminary verification of the existence of zinc oxide was carried out by UV Visible analysis that is spectral. The suspension system concentration was 0.19 mg/mL. The sample exhibited peak absorption at 380nm as shown in Figure A. The UV-visible wavelength range was between 200 and 800 nm. The sharp peak obtained at 380 nm confirmed the presence of ZnO NPs in the mixture. The band gap Energy was found to be 3.1ev. This was calculated using Tauc's plot obtained from the values of the UV spectrum. The intercept of Tauc's plot directly gave the value of the energy gap.

UV-Visible is spectral range of Zinc oxide nanoparticles with Tauc's land energy that is showing space intercept at 3.1 ev

The wavelength range UV-visible was between 200 and 800 nm. The top is certainly razor-sharp at 380 nm confirmed the presence of ZnO NPs into the blend (Fig A). The broad

absorption band ranges that varies towards longer wavelength could be because of the activity regarding the electronic cloud regarding the total skeleton associated with ZnO NPs. The Energy gap (band gap) had been determined from the UV-visible spectroscopy. The Energy gap (band gap) was determined from the Ultraviolet spectroscopy that is noticeable. Optical band gap of ZnO NPs was found to be 3.1 ev, calculated using the Tauc's Equation:

$(\alpha h\upsilon)^2 = A\ (h\upsilon\text{-}Eg)$

Where α may be the absorption coefficient, hυ represents the vitality regarding the photon, A is the proportionality constant and differs aided by the material, and n signifies the index. The graph was plotted between hυ(Show by A) and $(\alpha h\upsilon)^2$ (Show by B) on X and Y axis respectively. The intercept on X axis show the band gap here is 3.1 ev.

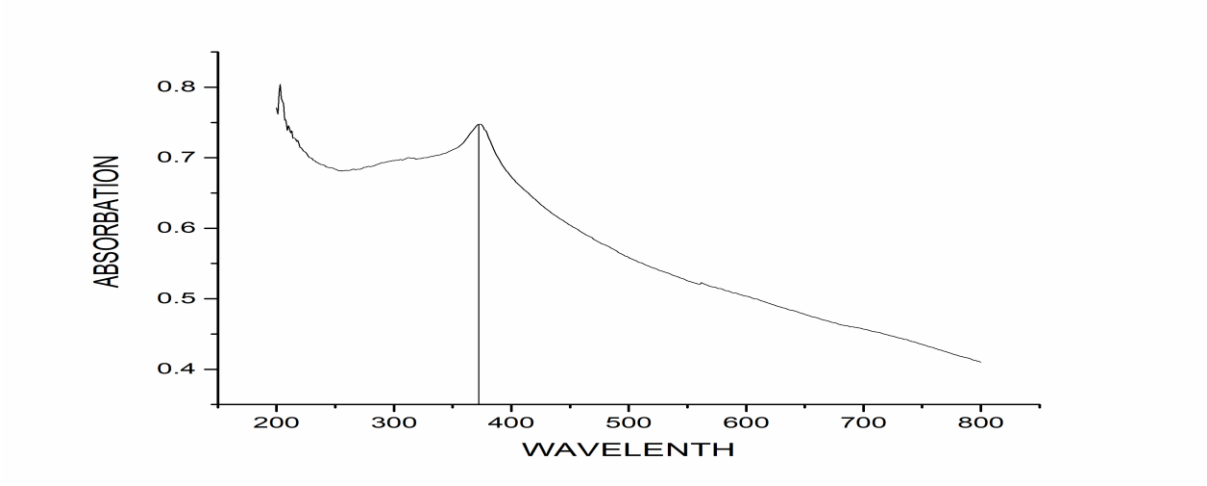


Figure-A

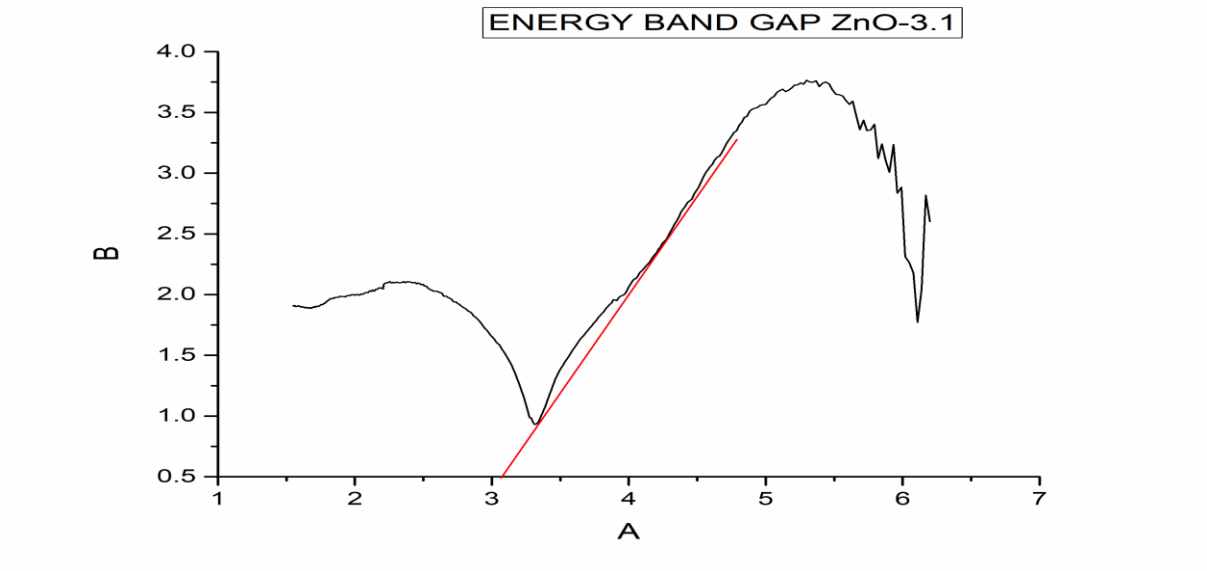


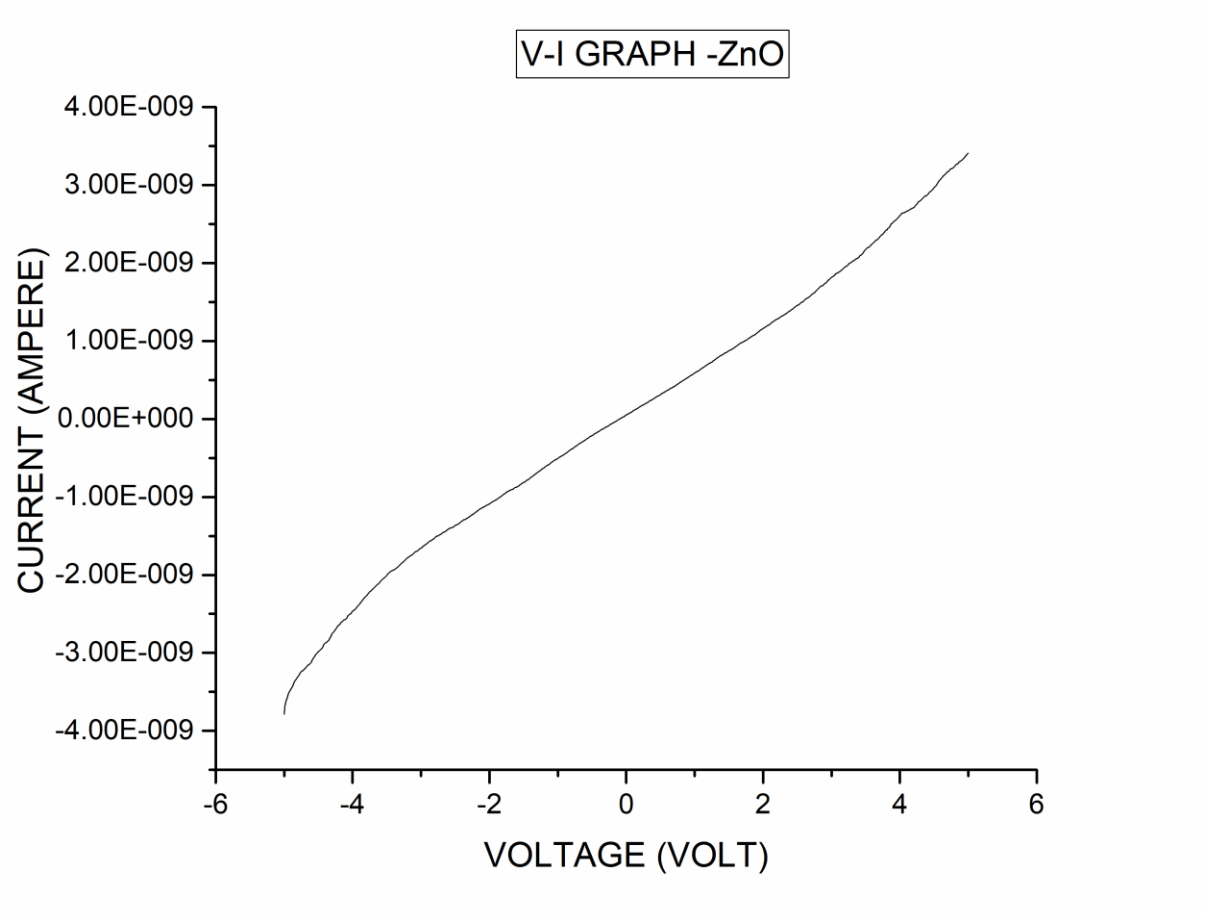
Figure-B

Voltage Current Characterization

Keeping track of electric characteristic of the product IV characterization was done using four probes. This study measured voltage and current. The end result is employed to find out resistivity of a product. Zinc oxide nanoparticles samples of thickness 1µ were utilized. The distance between probe (s) was kept 1mm. Thickness (t) of sample was less than distance between probe(s) or (t<S). To find the average resistivity, formula utilized was:

RESISTIVITY (ρ) =π/log (2) (V/I)

The average resistivity was found 17.88× 10^9 ohm.meter



Antibacterial Activity Analysis

In vitro activity that is anti-bacterial test was examined against gram-positive and gram-negative bacterial strains by the agar well diffusion method (Perez et al., 1990). Mueller Hinton agar number 2 (Hi-Media, India) had been used since the medium that is bacteriological. The extracts were diluted in 100% Dimethylsulphoxide (DMSO) at the concentrations of 10 mg/mL. The Mueller Hinton agar had been melted and cooled to 48 – 50ºC and a standard inoculum (1.5×108 CFU/mL, 0.5 McFarland) ended up being added aseptically to your molten agar and poured into sterile Petri dishes to offer a plate that is solid. Wells were prepared in the agar that is seeded. The test mixture (20, 40, 60 and 80 µl) ended up being introduced within the fine (6 mm). The dishes had been incubated for one

night at 37ºC. The spectrum this is certainly antimicrobial of extract was determined when it comes to microbial types in terms of zone sizes around each fine. The diameters associated with the zone of inhibition produced by the representative were compared to those generated by the control this is certainly commercial antibiotic (Ciprofloxacin). The research had been carried out 3 times for reduce the error plus the values that are mean provided.

Antibacterial Activity Against Bacillus subtilis

TEST SAMPLE	STANDARD(Ciprofloxacin)	20µl	40µl	60µl	80µl
ZnO	46mm	10mm	11mm	15mm	16mm

Antibacterial Activity against Pseudomonas aeruginosa

TEST SAMPLE	STANDARD(Ciprofloxacin)	20µl	40µl	60µl	80µl
ZnO	39mm	7mm	10mm	11mm	13mm

Determination of Antifungal Assay

Anti-fungal activity regarding the plant was experimentally investigated by agar well diffusion technique (Bonjar et al, 2005). Sabouraud's dextrose agar, SDA (Merck, Germany) had been employed for fungal medium. Suspensions of fungal spores were ready in sterile PBS (phosphate buffer saline) and adjusted to a focus of 106 cells/ml. Dipping a swab that is sterile the fungal suspension system and rolled on the surface of the agar medium. Wells were prepared in the agar this is certainly seeded. The test mixture (20, 40, 60 and 80 µl) had been introduced in the fine (6 mm). The dishes were incubated at 37°C. After incubation of 24 h bioactivities had been dependent on measuring the diameter of inhibition area (in mm). The diameters of the zone of inhibition produced by the agent were compared to those made by the control that is commercial (ketoconazole).

Within a study, Azam et al. [7] have reported that the game this is certainly antimicrobial both gram-negative (E. coli and P. aeruginosa) and gram-positive (S. and Bacillus subtilis) germs increased with increase in surface-to-volume ratio because of decrease in particle size of zinc oxide nanoparticles. It's been reported that the smaller size of zinc oxide nanoparticles exhibits better task this is certainly anti-bacterial micro scale particles [8]. The intensity of anti-bacterial activity is size centered. Intensity of anti-bacterial task is inversely proportional to the size of nanoparticles, So Nano-sized ZnO show good task this is certainly anti-bacterial volume ZnO [24, 25].

Antifungal Activity Against Candida

TEST SAMPLE	STANDARD (KETOCONAZOLE)	20µl	40µl	60µl	80µl
ZnO	31mm	25mm	26mm	28mm	30mm

ANTI FUNGAL ACTIVITY AGAINST A.NIGER

TEST SAMPLE	STANDARD (KETOCONAZOLE)	20µl	40µl	60µl	80µl
ZnO	23mm	11mm	12mm	13mm	14mm

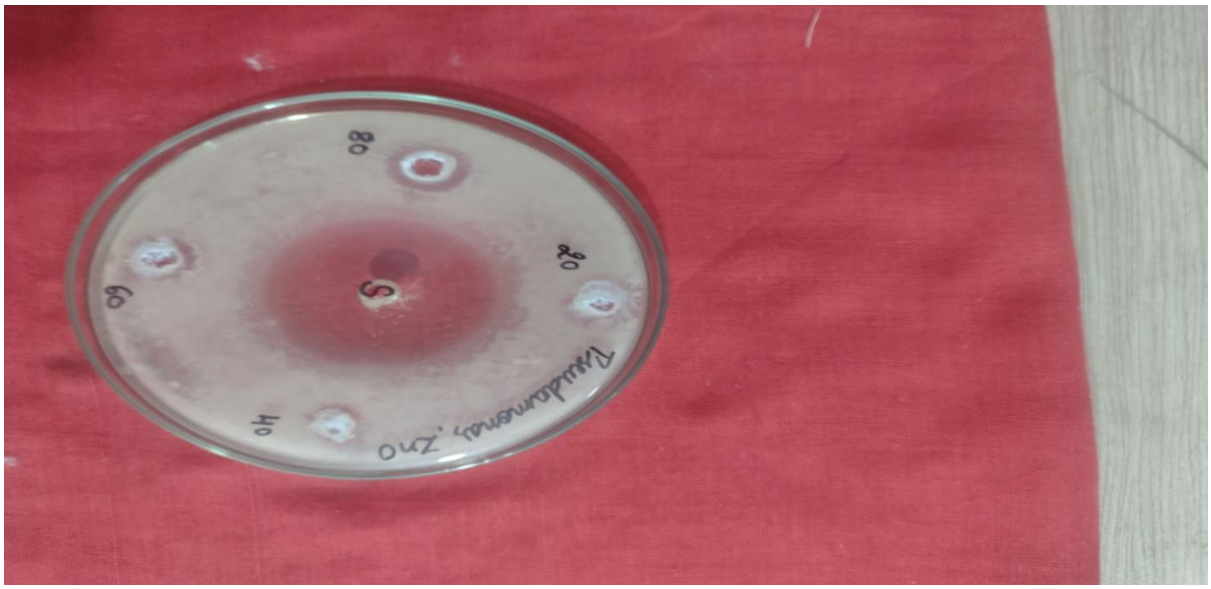


Figure-1

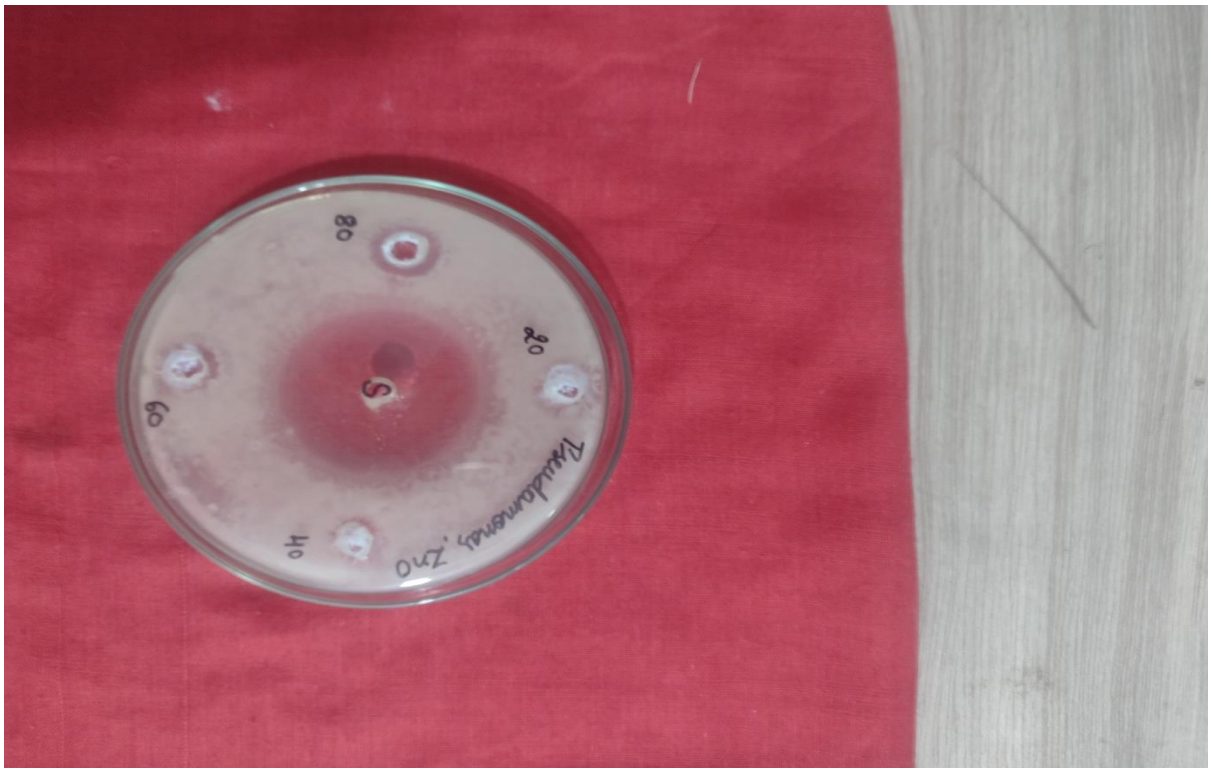


Figure-2

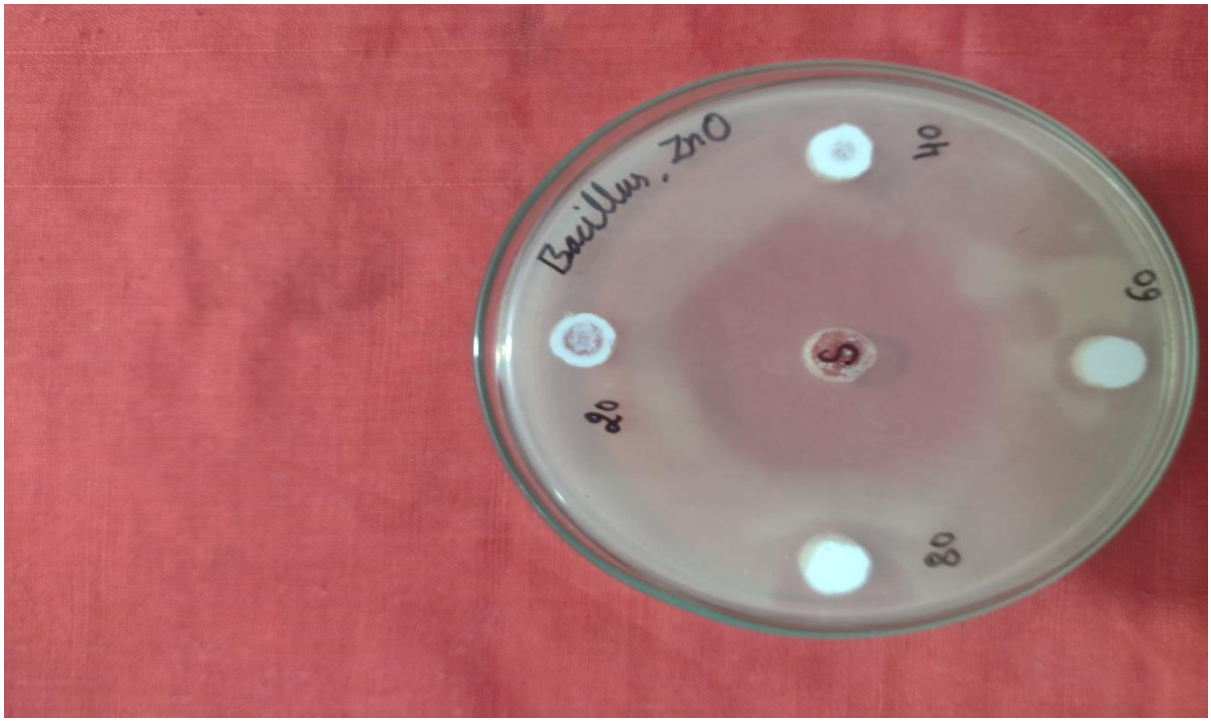


Figure-3

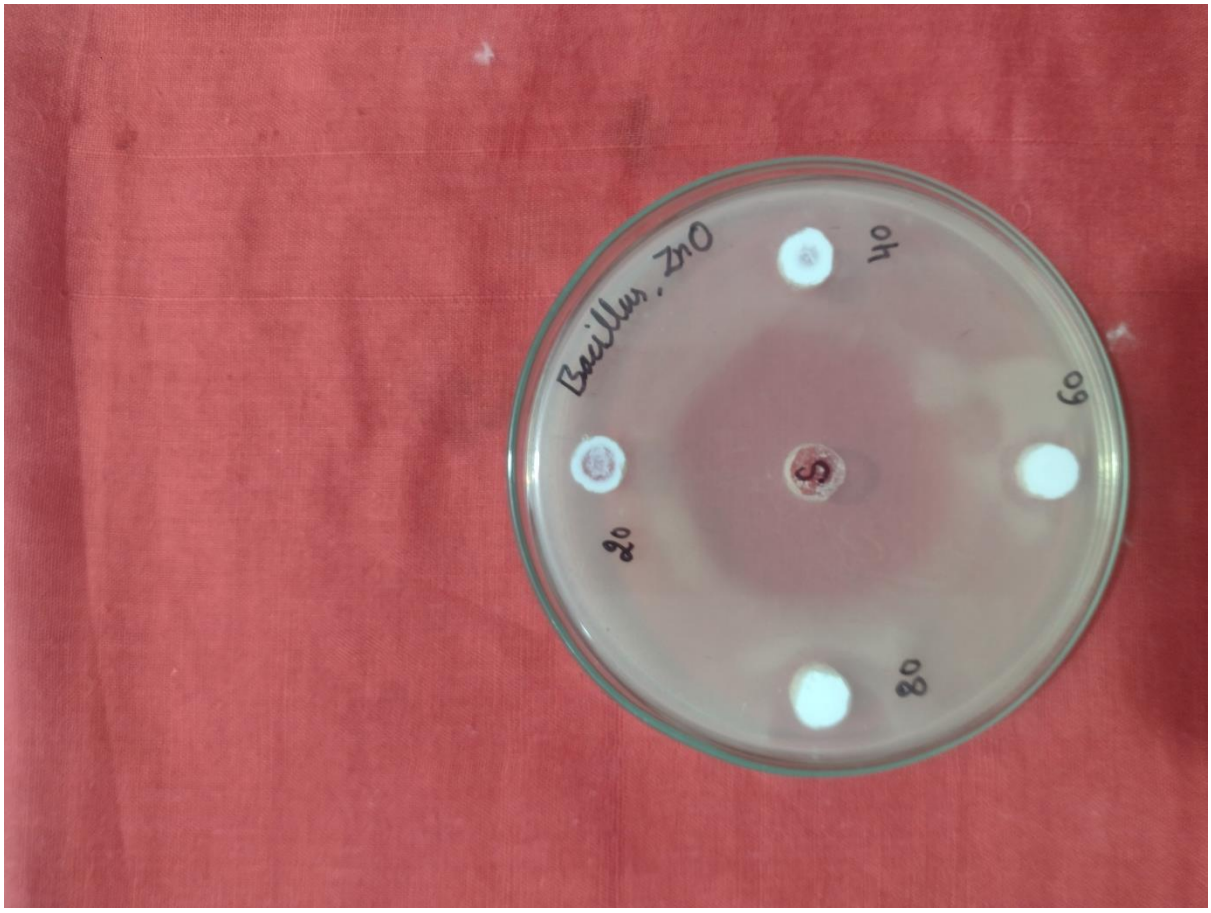


Figure-4

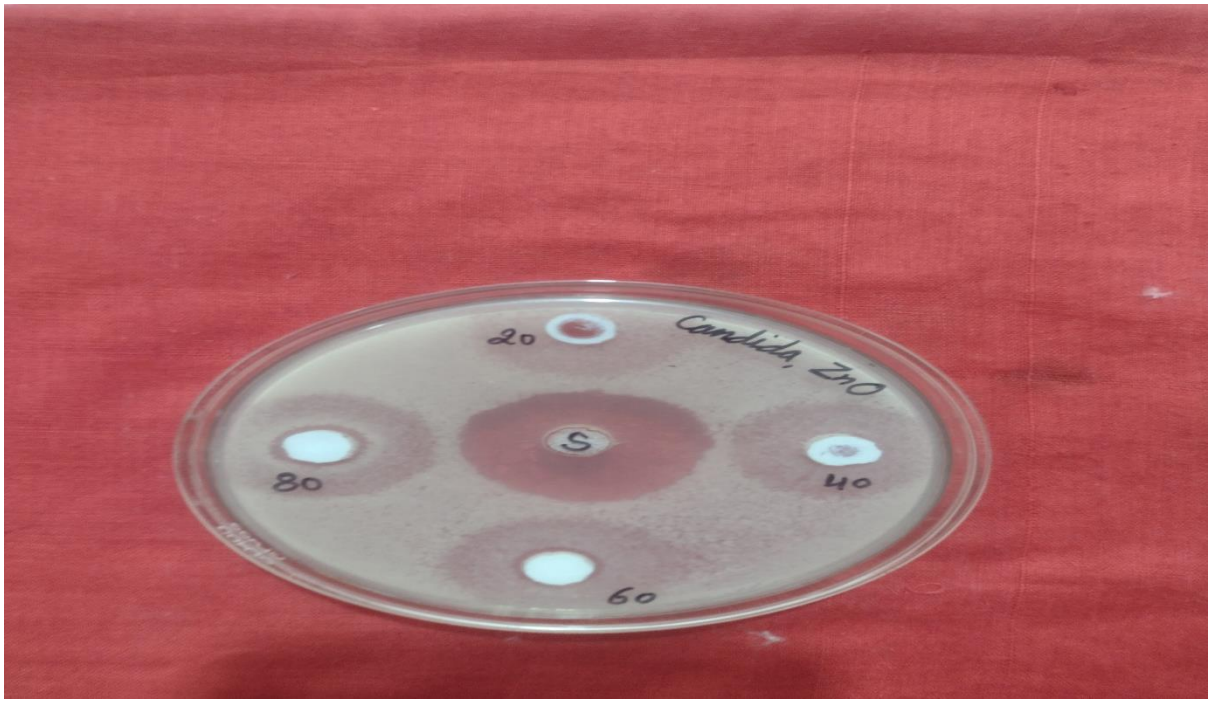


Figure-5

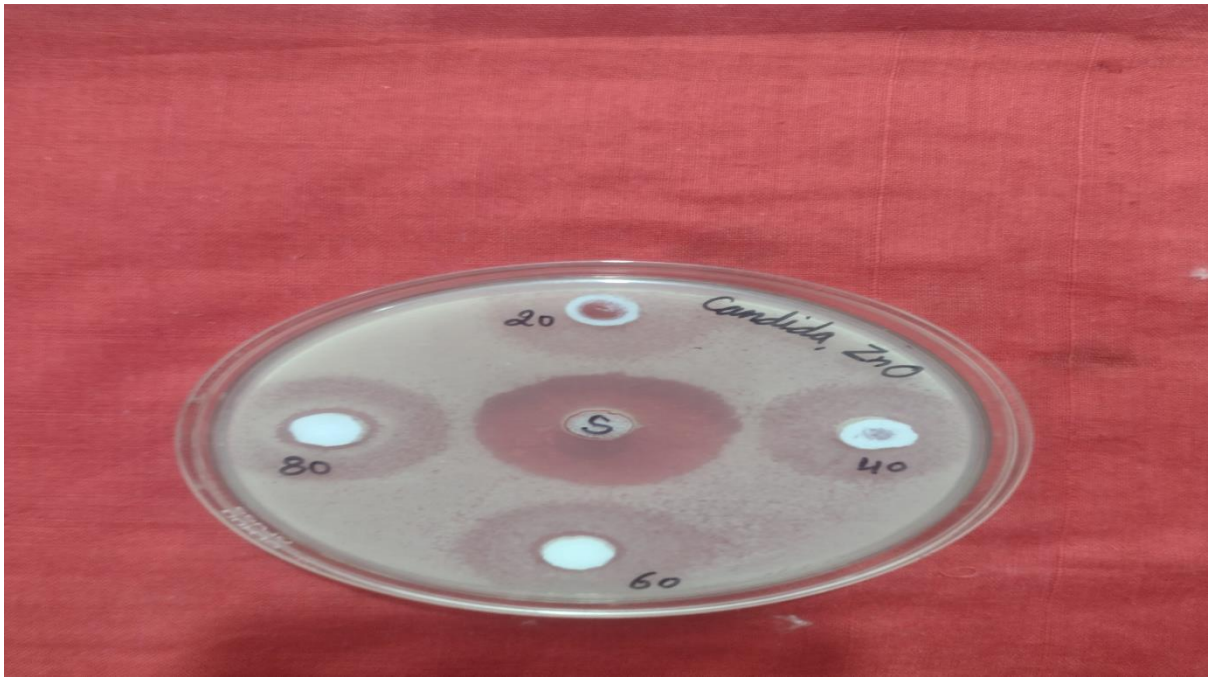


Figure-6

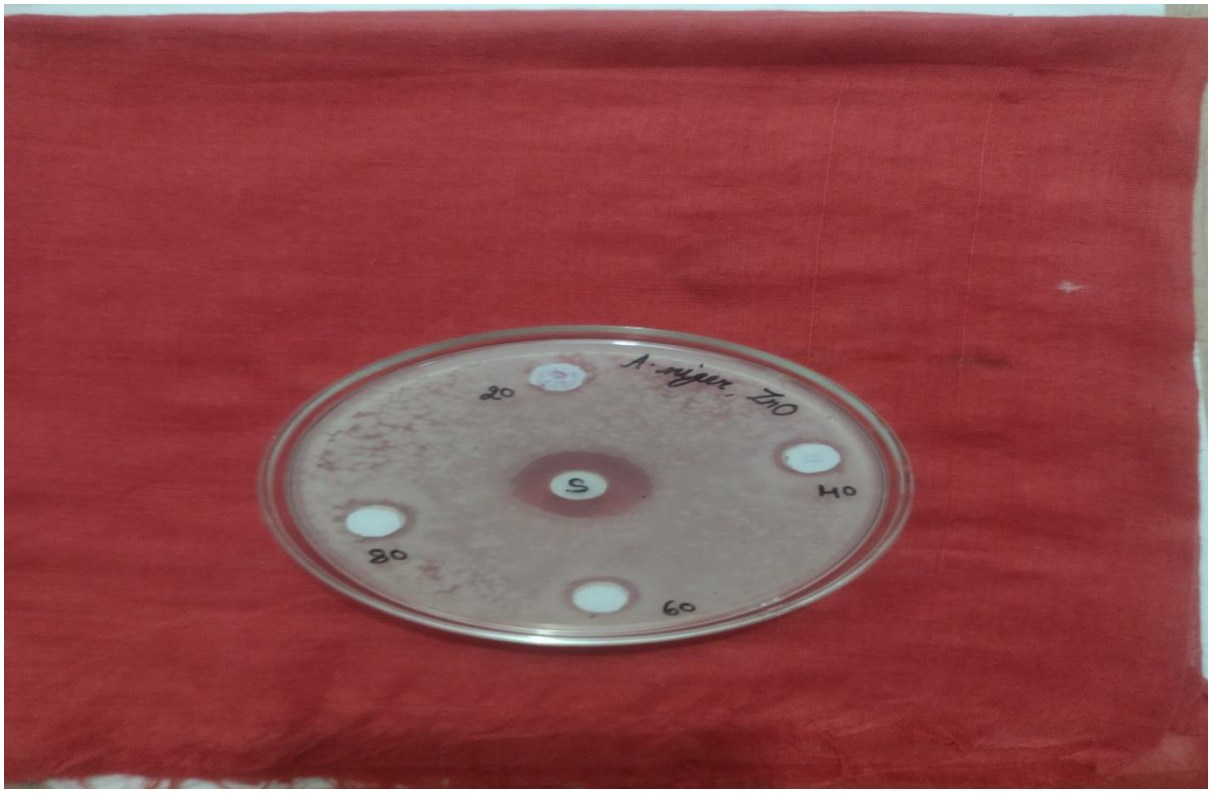


Figure-7

Summary

ZnO nanoparticles were synthesized utilizing by wet chemical method by the use of Zinc nitrate. The nanoparticles were analyzed. Anti-bacterial activity has been discovered to work on Fungus and bacteria. The nanoparticles were analyzed UV, TEM, FTIR, SEM and XRD and four probes. The utmost consumption is at 380 nm in Ultraviolet evaluation. FTIR of Zinc Oxide Nanoparticles showed different peaks of useful group as Alcohol / Phenol O-H Stretch, Alkyl C-H Stretch, Aliphatic Esters, Alkenes. The SEM and TEM analysis revealed particles morphology and measurements of zinc oxide nanoparticles. The XRD analyses showed crystalline structure and particle size of ZnO NPs. The resistivity of synthesis sample of ZnO-NPs ended up being come $17.88x10^{9}$. The biological activity of ZnO NPs revealed its Anti-bacterial and resistive property for the fungus.

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