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Nanoscale synthesis and characterization of zinc oxide nanoparticles and investigation of its properties with antibacterial and antifungal activity

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ABSTRACT
In the proposed work, a facile convenient and meagre method of sol-gel assisted hydrothermal method was used for the synthesis of pure zinc oxide nanoparticles (ZnO NPs). The structural and optical morphology are inspected using various techniques of the synthesized nanoparticles and at different molar concentrations of 1, 2 and 3 moles respectively. The X-Ray Diffraction (XRD) pattern revealed the hexagonal crystal structure for pure ZnO nanoparticles and the crystal size is noted to be increasing with the increase in concentration. The Scanning Electron Microscope (SEM) illustrated the crystalline growth being proportional to the concentration of nanoparticles. The Fourier Transform Infrared Spectroscopy (FTIR) confirmed the presence of various functional groups such as O-H, C-O, -C-O-C, C-H and C-N groups. The elemental composition studied using Energy- Dispersive X-Ray Spectroscopy (EDX) revealed the presence of Zn and O particles and the purity of ZnO NPs were noted. Optical characterization of the sample using UV-Vis-NIR Spectroscopy revealed the band gap (E_g) being decreased from 5.91eV to 5.11eV with the increase in molar concentration of ZnO NPs. Anti- bacterial studies were carried out at pre- defined concentration (25 m/l to 100 m/l) using gram positive and gram-negative bacteria to test the potential of synthesized ZnO NPs as inhibitory agents.

Key words: Zinc Oxide, Nanoparticles, Antimicrobial activity, sol-gel, chemical synthesis, NPs

I. INTRODUCTION

Clearly, nanoscience and nanotechnologies are not contemporary in the present years. Earlier decade Chemists were involved in making large molecule polymers made up of nanoscale subunits and nanotechnologies were employed in creating the tiny features on a computer chip for the past 30 years. Normally, nanotechnology deals with the particles which are having the size in the range of about 1-100nm. Recently many researchers have been actively working on various developments over the decades. This has led a way for nanotechnologies to find place in a wide variety of applications such as cosmetics, drug delivery, biosensors, textile and various other biomedical applications. Metal oxide nanostructures have caught the attention of various researchers and is being considered in many areas of technology. among all the metal oxides discovered so far, zinc oxide (ZnO) has received much scrutiny in recent times. zinc oxide nanoparticles are currently in the prominent positions of research owing to their peculiar properties and ample applications. Zinc oxide (ZnO) nanoparticles are the ones having a diameter of about 100nm. These nanoparticles are found to posses' high catalytic activity and their surface area is at maximum. They are present in the nature as both powders and dispersions. In the periodic table, zinc is at block D- period 4 element and oxygen are at block P- period 2 elements. These nanoparticles are found to exhibit various properties like optoelectronic properties which ensures great transparency, increased electron mobility and broad band gap. At room temperature,

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the ZnONPs accounts for a band gap of about 3.4eV approximately. Because of this, these micro crystals absorb the light in UV-A and UV-B region of spectrum in a coherent manner. Studies suggested that the greater impact of these particles on biological function depends solely on the concentration, pH, morphology, and biocompatibility. There are wide variety of ways to synthesize ZnO NPs- physical, chemical and biological methods. Usually, ZnO NPs that were synthesized by chemical routes depicts biological compatibility and exist sustainably. Here, we put forward a simple low-costsol-get assisted hydrothermal method to synthesize ZnO NPs of various molar concentration (1, 2 and 3 wt. %) using zinc acetate as precursor. The NPs were subjected to various characterization techniques such as XRD, SEM, FTIR, EDX and UV-Vis-NIR spectroscopy to analyse the structural, optical and morphological properties. These methods delivered the parameters like crystalline structure, size of the NPs, elemental composition, presence of various functional groups, absorbance and band-gap energy. The anti-bacterial and anti-fungal activities of ZnO NPs were tested using Kirby Bauer Agar Well Diffusion method against the gram-positive and gram-negative bacteria. The results showed the high inhibiting property of ZnO NPs as they were able to suppress the micro-organisms completely

II. EXPERIMENTAL SECTION

The main objective of this proposed work is to synthesize ZnO NPs using frugal way of sol-gel assisted hydrothermal method with the help of Zinc Acetate as precursor and make use of the particles to study the applications in healthcare.

PROPOSED METHOD

It is clear from various studies that nanotechnology will rule the world in upcoming generations as the researchers have put forth all their attentions in bringing forward the fresh pen. Here we propose a facile method of synthesis of ZnO NPs and understanding their structural, morphological and optical parameters to use them in investigating anti-bacterial properties. X-Ray Diffraction (XRD) analysis is done to determine the crystalline structure and crystalline size of the synthesized nanoparticles. Scanning Electron Microscope (SEM) were used to study the topography of the material. Fourier Transform Infrared (FTIR) spectroscopy reveals the presence of various functional groups in the material. Energy Dispersive X-Ray (EDX) spectroscopy can predict the composition of various elements present in the synthesized NPs. The optical properties like absorbance and band-gap energy are studied using UV-Vis-NIR Spectroscopy. We put forward Kirby Bauer Agar Well Diffusion Assay in determining the activity of synthesized ZnO NPs in inhibiting the growth of micro-organisms.

RAW MATERIALS

Analytical grade reagents- zinc acetate -Zn $(CH_3(CO_2)_2)$, Iso-propyl-alcohol, and ammonium hydroxide were purchased from Sigma Aldrich in India Ltd. Antibacterial and antifungal investigations using Bacillus subtilis, pseudomonas and A. niger were studied from Eumic Analytical Lab and Research Institute, Tiruchirappalli.

PREPARATION OF ZnO NPs

A definite amount of Raw Zinc acetate (Zn $(CH_3CO_2)_2$) is weighed in weighing machine with different mole concentrations such as 1, 2 and 3 moles. The collected samples were stored in an air tight container for further synthesis. In a beaker of 100 ml, 50 ml of distilled water is measured and the sample was added to it and was stirred well. The solution mixture of (Distilled water + Zinc acetate (Zn $(CH_3CO_2)_2$)) was placed on the magnetic stirrer and then it was rotated at RPM of 650 to 750. After continuous stirring for 1hour the homogenous solution was obtained. Few amounts of ammonia were added to increase pH value up to 8. The influence of addition of ammonia in varying the pH of the solution were tabulated in Table as shown below. Then the solution kept in the hot plate to maintain 80°C and solution converted into gel form. Then, the gel formed nanoparticles is kept in hot temperature bath to maintain the temperature of 100°C for 6 hours.

Table 1 Influence of ammonia in varying the Ph

Sample	Ammonia hydroxide	Ph value approximately		Result
		Before adding ammonia	After adding ammonia	
1 Mole	30ml	10^{-5}	10^{-8}	Neutral
2 Mole	52ml	10^{-5}	10^{-8}	Neutral

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3 Mole	67ml	10^{-5}	10^{-8}	Neutral

CHARACTERIZATION OF ZnO NPs

The characterization techniques of synthesized ZnO NPs of different mole concentration (1,2 and 3 wt.%) is done using XRD, FTIR, UV spectra, EDX, and SEM. Study of optical properties were done with the help of a double beam UV Spectrometer representing a spectral range of 200 to 800nm. Presence of various functional groups were analysed using a FTIR spectrometer representing the regions from 4000cm^{-1} to 400cm^{-1}.XRD diffraction patterns was revealed using an x-ray diffractometer (SHIMADZU) in which the required sample are scanned in the range of 2q of about 10 to 70^{0}c and producing a rate of about 0.04m/s. the crystalline size of the particles is demonstrated using the same diffractometer by applying Debye- Scherrer equation. the prepared nanoparticle is subjected to JSM 6360 Scanning Electron Microscope in order to study the morphological properties which under vacuum condition operates on a voltage of about 10 to 20KV. The elemental composition of the prepared NPs was demonstrated by EDX Spectrometer.

PREPARATION OF CULTURE MEDIA

Nutrient Agar Medium

Nutrient agar medium is one of the most commonly used medium for several routine bacteriological purposes:

Ingredients: Grams/Litre
Peptone : 5gm
Beef extract: 3gm
Agar: 15gm
Sodium chloride: 5gm
Yeast extract: 1.5gm
p^H : 7.0

After adding all the ingredients into the distilled water, it is boiled to dissolve the medium completely and sterilized by autoclaving at 15 Ib psi pressure (121°C) for 15 minutes.

Nutrient Broth

The nutrient broth was assembled by the same composition lacking agar. At the adding all the ingredients into the distil water it is boiled to dissolve the medium completely and sterilized by autoclaving at 15 Ib psi pressure (121°C) for 15 minutes.

ANTIMICROBIAL ACTIVITY

Microbial Inoculum Preparation

The nutrient broth was prepared, then identified bacterial colonies inoculated into the broth culture were used for antimicrobial activity.

Kirby Bauer Agar Well Diffusion Assay

The nutrient agar medium was developed and sterilized by autoclaving at about 121˚C 15 lbs. pressure for 15 minutes and then aseptically the medium is poured into the sterile Petri plates and allowed to solidify the Bacterial broth culture that was swabbed on each Petri plates using a sterile bud. Then wells were made by well cutter. This procedure was repeated for each Petri plates then the Petri plates were incubated at 37˚C for 24 hrs. After incubation the plates were monitored for the tract of inhibition.

III. RESULT AND DISCUSSION

The required amount of Zinc Acetate aqueous solution is mixed with a few drops of ammonia solution by stirring it continuously in magnetic stirrer for about an hour at a constant temperature. Once the required pH is obtained, it is found that the solution yields the products as Zn $(OH)_2$ and ZnO. The presence of ZnO was confirmed using XRD technique as discussed below.

X-Ray Diffraction (XRD) Analysis

XRD analysis is used to determine the crystal structure and crystalline size of the nanoparticles. The XRD pattern of synthesized ZnO NPs for 1, 2 and 3 mole concentrations respectively are shown in figure 1 (a - c). The XRD peaks at 2q= 31.6723^0, 34.37^0, 36.18^0, 41.25^0, 45.27^0, 47.50^0, 54.38^0, 62.65^0, 63.45^0, and 67.84^0 corresponds to the (101), (002), (101), (210), (211), (102), (110), (103), (311), and (112) crystal planes and hexagonal crystal structure were obtained as per the Standard Joint Committee on Powder Diffraction Standard (JCPDS No, 89-1397). However, XRD patterns revealed sharper peaks, indicating its crystalline structure and along with the sharp peaks some additional peaks were also observed. IN order to calculate the average crystalline size of the synthesized NPs, Scherrer Equation is employed as follows.

$$\text{Crystalline size}\left(D_{avg}\right) = \frac{K\lambda}{\beta\cos\theta}$$

Where, D represents the crystalline size, K is the Scherrer constant (0.9), □ □ is the wavelength of light used for diffraction (0.154 nm), □ is the FWHM (full width at half maximum) at 2□ □ and □ is the angle of reflection. Thus, the calculate average size of the ZnO NPs are obtained from the above XRD pattern as 34.2nm for 1 Molar, 37.9nm for 2 molar and 42.6nm for 3 molar concentrations respectively. It is clear from the above analysis that

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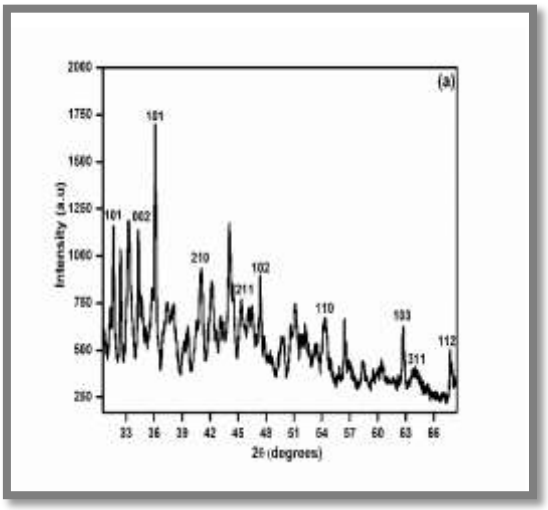
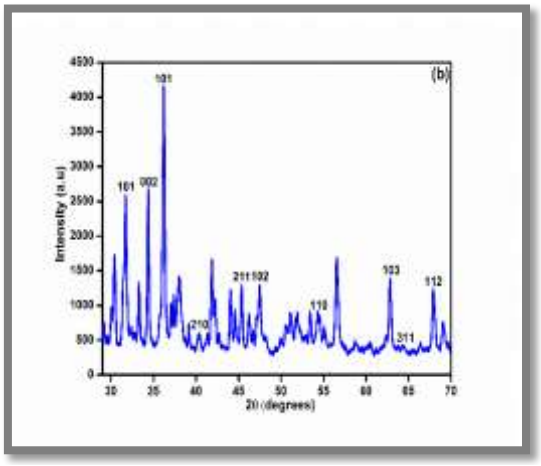
the average size increases with increase in the concentration. The different values of FWHM (full width at half maximum) at various peaks contributes to the variation of crystalline size at respective peaks. It is also clear that the peaks are narrower on increasing the mole concentration of nanoparticles which results in increase in the crystallite size. Also, with the calcination temperature, there are chances for the crystalline size to increase gradually. XRD spectra reveals that the different molar concentration used has strong influence on the structural parameters of the prepared ZnO NPs.





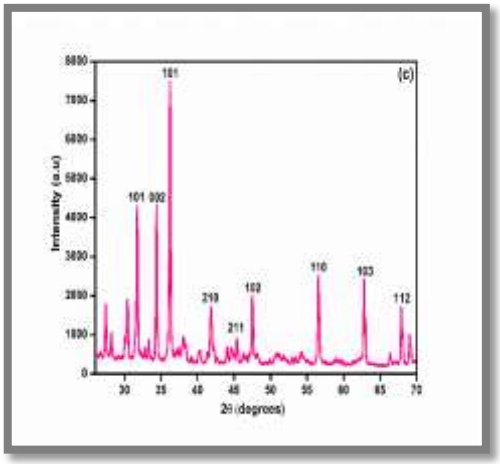


Figure 1 (a-c): XRD patterns of ZnO NPs for different molar concentration

Scanning Electron Microscopy (SEM) Analysis

The small amount of powdered Zinc Oxide is taken and SEM analysis is performed and various parameters of the particle is studied. Fig 2 (a) shows that, it has high magnification for 1 mole of Zinc Oxide NPs. With different mole concentrations it exhibits the crystalline growth by increasing the concentrations. The study revealed that the ZnO nanoparticles are in hexagonal structure and are agglomerates of nano crystallites. The prominent well-structured growth observed from the SEM micrographs of the fine particles confirms the development of hexagonal crystal structure of the ZnO nanoparticles. Fig 2 (b) shows the 2 moles of magnified image of Zinc Oxide NPs. Larger magnification images suggest that they are the clusters of various smaller sized grains in the present samples. The cluster of smaller sized grains gets triggered by greater energy due to the increase in substrate temperature that causes greater potency of nanoparticles and thereby resulting in larger grain sized structures. Clearly, the increase of surface area ratio with volume plays a vital role in the intensification of optical properties of the materials. Meanwhile, the reduction of grain size in nm ranges confirms the quantum confinement effect to the ZnO NPs, which confers as a blue shift due to increase of the bandgap. It not only increases the material property but also amplifies the multifaceted applicability. Thus the 2 moles of ZnO are stronger than the pure material. Furthermore, fig 2 (c) shows the 3 moles of ZnO magnified image done under the SEM analysis. With the increase in the substrate temperature, the band gap is also found to increase proportionately. The spectrum depicts sharp peeks that confirm the presence of Zn nano particles. The

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number of crystal particles present is reduced when compared to figure 2 (b).

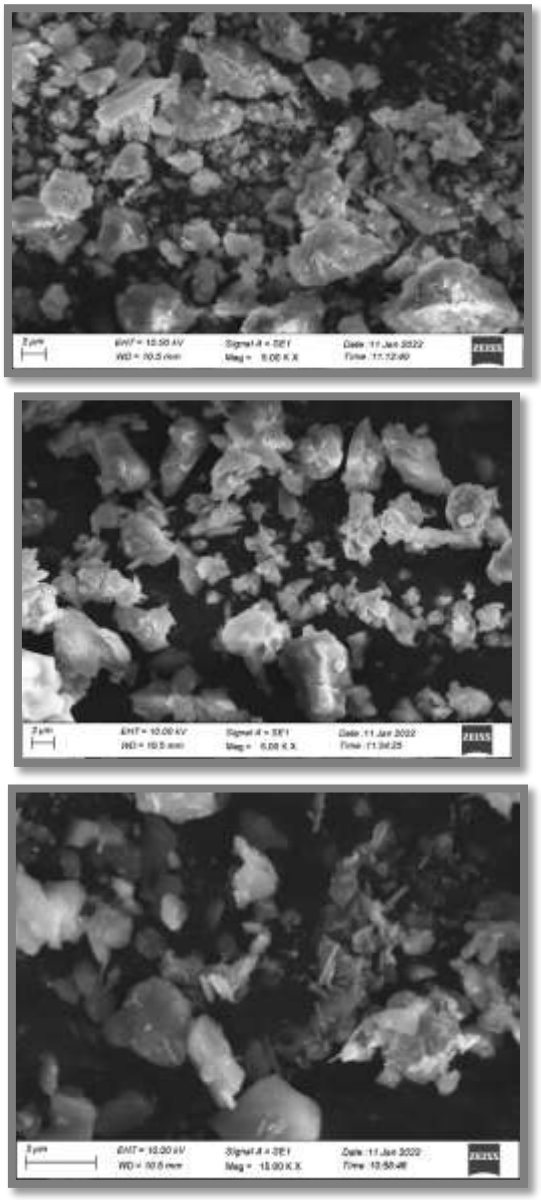

Fig 2 SEM images of the ZnO NPsof differentmole concentrations. (a) 1 mole (b) 2 mole (c) 3 mole

EnergyDispersive X-Ray spectroscopy (EDX) Analysis

The purity of ZnO NPs was determined via the EDX analysis. The figure 3 shows the EDX spectrum of ZnO NPs. EDX was used in order to determine the element composition that present in the samples. Some weight errors have occurred during the result reveal, because the unwanted particles in ZN and O were ± 4.92 and ± 1.03. Results revealed that the EDX data was composed of two elements which are Zn (67.85%) and O (32.15%). This Result has confirmed that the ZnO NPs has high Purity. In the previous studies the mass percentage of Zn and O were 73.9% and 26.1% respectively and values are shown in table 2. Finally, we got the theoretical expected mass percentage of Zn and O as 34.06% and 65.94%. Thus, the EDX result revealed that the synthesized ZnO NPs were of high purity, which contain high Zn and O element composition.

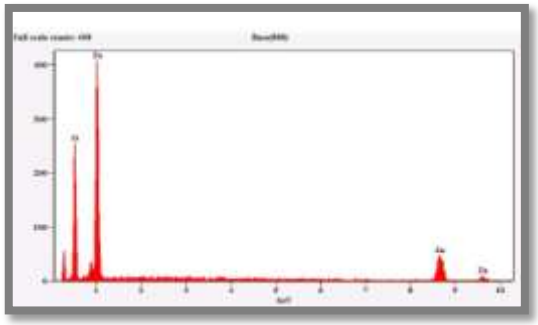

Figure 3: Elemental analysis of ZnO NPs with 3 mole concentration

Element Line	Weight %	Weight % Error	Atom %
O K	32.15	± 1.03	65.94
Zn K	67.85	± 4.92	34.06
Total	100.00		100.00

Table 2: Elemental analysis values od prepared ZnO NPs

Fourier Transform Infrared (FTIR) spectroscopy Analysis

FTIR is one of the constructive methods to reveal the composition of the products. Figure 4 (a-c) is a typical FTIR spectrum of 1,2 and 3 moles of ZnO nanoparticles respectively in the range of 400 – 4000cm^{-1}. According to figures, the bands are observed at 3342.59cm^{-1}, 3174.22cm^{-1}, 3160.65cm^{-1}, 3147.77cm^{-1}, 2828.23cm^{-1}, 2501.54cm^{-1}, 2351.09cm^{-1}, 1587.24cm^{-1}, 1577.70cm^{-1}, 1567.56cm^{-1}, 1402.64cm^{-1}, 1401.48cm^{-1}, 1401.17cm^{-1}, 1342.06cm^{-1}, 1341.95cm^{-1}, 1340.16cm^{-1}, 1289.69cm^{-1}, 1050.08cm^{-1}, 1049.31cm^{-1}, 1020.35cm^{-1}, 1020.15cm^{-1}, 963.25cm^{-1}, 936.05cm^{-1}, 935.20cm^{-1}, 691.37cm^{-1}, 690.30cm^{-1}, 680.56cm^{-1}, 621.76cm^{-1}, 621.56cm^{-1}, 619.46cm^{-1}, 520.81cm^{-1} and 520.60cm^{-1}.The peak in the region between 400cm^{-1} – 600cm^{-1} is attributed to ZnO stretching mode frequency. The bands at 3342.59cm^{-1}, 1587.24cm^{-1}, 1577.70cm^{-1} and 1567.56cm^{-1} are characteristic of O-H stretching vibrations of hydroxyl groups. The bands at 3174.22cm^{-1}, 3160.65cm^{-1} and 3147.77cm^{-1} may be

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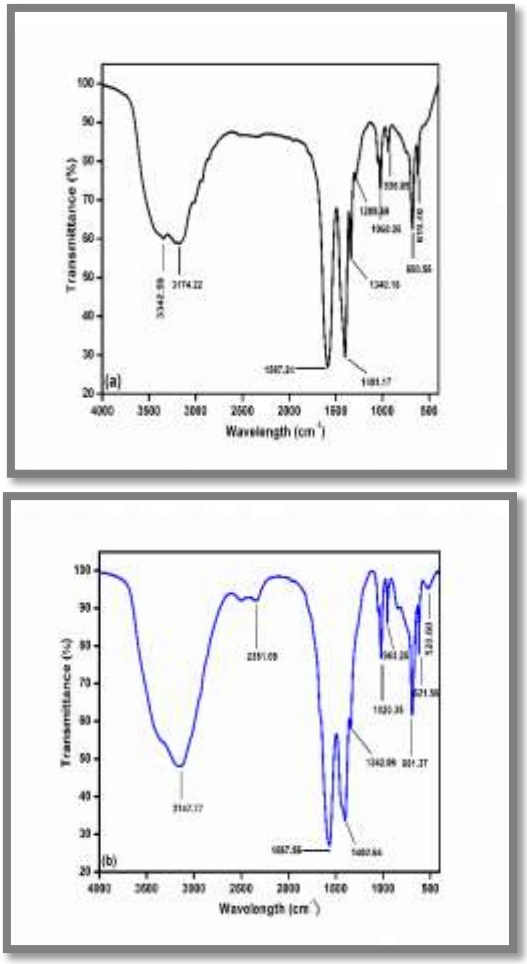
due to the C-H stretching. The peaks at 1342.06cm^{-1}, 1341.95cm^{-1}, 1340.16cm^{-1} and 1050cm^{-1} may attribute to C-0 and -C-O-C stretching modes. The bands resulting at 1049.31cm^{-1}, 1020.35cm^{-1} and 1020.15cm^{-1} correlates to C-N stretching of amines. The bands at 936.05cm^{-1} and 935.20cm^{-1} represents the O-H bending of carboxyl groups. The bands at 680.59cm^{-1}, 619.46cm^{-1}, 520.81cm^{-1} and 520.60cm^{-1} denotes the C-Br stretching of alkyl halides.



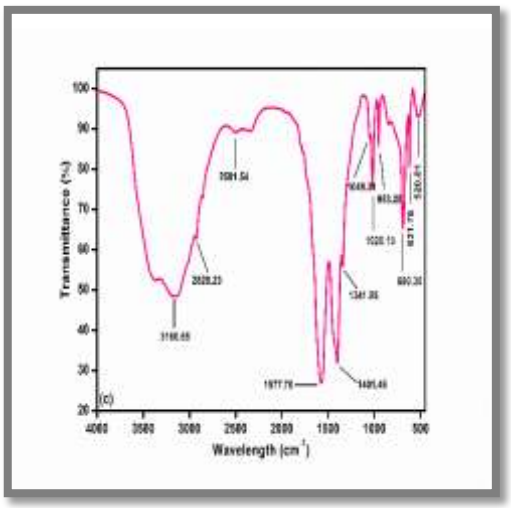


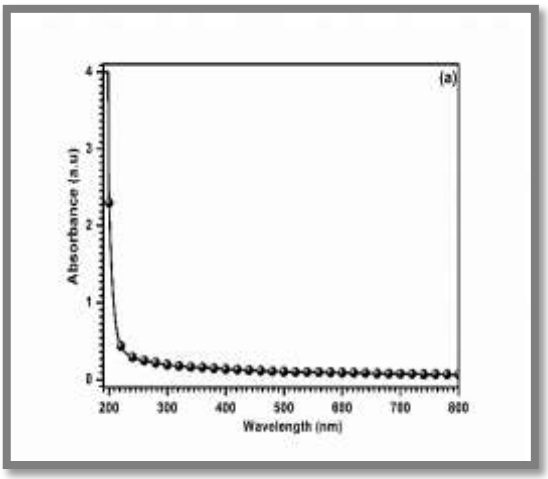
Figure 4: FTIR spectrum of Prepared ZnO NPs of 1, 2 and 3 mole concentrations

UV-Vis-NIR Spectroscopy Analysis

UV-Vis absorption spectroscopy was used to study the optical properties of ZnO NPs. Figure 5 (a-c) reveals the absorption spectra of ZnO nano particles at different molar concentration such as 1, 2 and 3 moles respectively. From the figures, a strong optical absorption peak is observed below 370 nm. It is understood from that the rise is molar concentration cause the wavelength to shift to slightly bigger values. The maximum absorbance is observed for 3 mole ZnO NPs. The absorption which is presents near the visible range confirms the existence of some kind of defect energy levels in the synthesized Zinc Oxide Nanoparticles. From the UV-Vis spectra, the optical energy-gap (E) values are obtained from the following equation,

$$\text{Band gap}\,(\alpha h\nu) = A(h\nu - E)^n$$

Where, α is the absorption coefficient, hν is the energy of the photon, A is the proportionality constant that varies with the material and n represents the refractive index.



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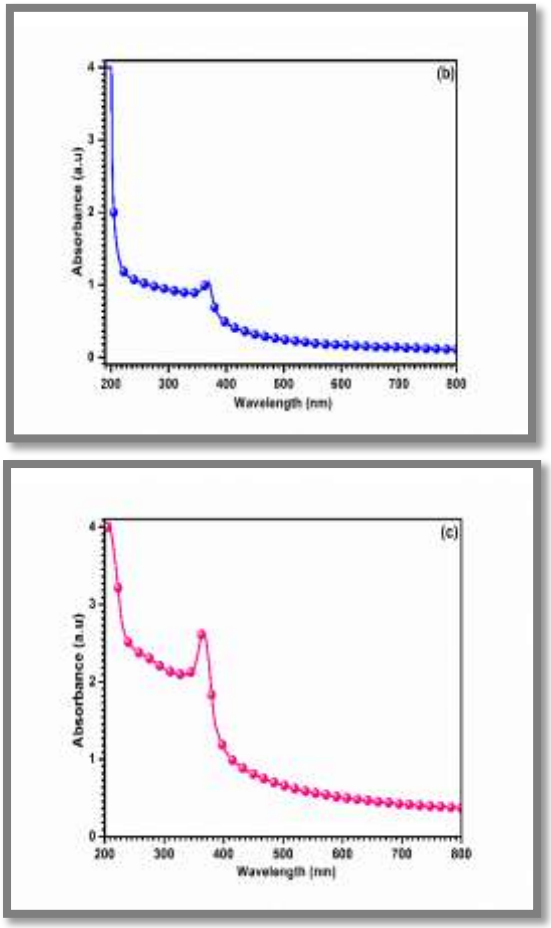

Figure 5(a-c): Absorbance Spectrum of ZnO NPs obtained at different mole Concentrations

The optical band gap values were discovered using Tauc's plot, among $(\alpha h\nu)^{1/2}$ vs. (hν) for ZnO NPs as shown in Fig. 6 (a-c). When n is 2, it specifies directly permitted transitions and intercept on E-axis gives the values of E_g. The energy gap values of 5.91, 5.75, and 5.11 eV respectively for ZnO NPs are observed from the extrapolation of the vertical strait line to E-axis. The band gap of ZnO NPs gradually decreased because of increases in Fermi energy level and DOS of E shrinking and another possible reaction is V_O that will initiate gradual effects close to Fermi level moving towards CB. The highest band gap observed in lower mole concentrations.

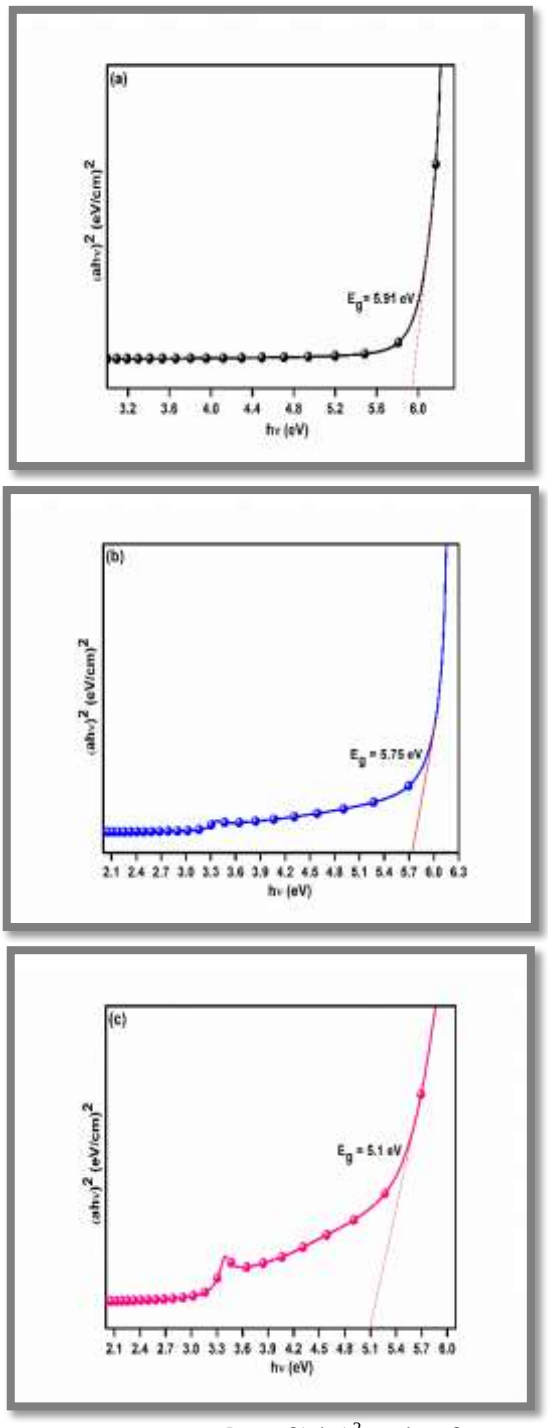

Figure 6: Plots of$(\alpha h\nu)^2$ Vs hν **of ZnOnanoparticles obtained at different mole concentration**

Antimicrobial activity

The antibacterial activity of Zn-1, Zn-2 and Zn-3 NPs was investigated by the well-known Kirby Bauer disc diffusion method. The technique was used against clinically isolated bacterial cultures such as, Bacillus subtilis (Gram positive),

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Pseudomonas, (Gram negative) and Fungi A. niger, obtained from the Eumic Analytical Lab and Research Institute, Tiruchirappalli. Bacterial strains were maintained on nutrient agar slants (Hi media) at 4˚C. About 100 µL of these organisms were introduced into molten nutrient agar plates which spread uniformly, then the wells were made on the agar plates with sterile borer and the different concentrations (25, 50, 75, and 100 µL) of the Zinc (1, 2 and 3 mole) NPs and a positive control (reference standardGentamicin antibiotic) were added by using a micropipette, followed by incubation at 37°C for 24 hours. The diameter of the zone of inhibition (mm) and antibacterial activity were measured for Zinc (1, 2 and 3 mole) NPs**.**

Table – 3

Zone of inhibition values of Zinc (1, 2 and 3 mole) NPs against various microorganisms of Bacillus subtilis, Pseudomonas aeruginosa

Zinc 1 Mole	DMSO Extract 100 µl added and Zone of inhibition (mm/ml)				
	25 µl	**50 µl**	**75 µl**	**100 µl**	**Control (Gentamicin antibiotic)**
Bacillus subtilis	20	23	25	28	20
Pseudomonas aeruginosa	22	26	30	34	20
Zinc 2 Mole	DMSO Extract 100 µl added and Zone of inhibition (mm/ml)				
	25 µl	**50 µl**	**75 µl**	**100 µl**	**Control (Gentamicin antibiotic)**
Bacillus subtilis	18	20	23	26	20
Pseudomonas aeruginosa	20	23	28	32	20
Zinc 3 Mole	DMSO Extract 100 µl added and Zone of inhibition (mm/ml)				
	25 µl	**50 µl**	**75 µl**	**100 µl**	**Control (Gentamicin antibiotic)**
Bacillus subtilis	20	23	26	30	20
Pseudomonas aeruginosa	20	24	29	33	20

The antibacterial activity of synthesized Zinc (1, 2 and 3 mole) NPs was investigated by the Kirby Bauer disc diffusion method and shown in Figure-7. The zone of inhibition values of Zinc (1, 2 and 3 mole) NPs against Gram positive bacteria (Bacillus subtilis) and Gram-negative bacteria (Pseudomonas aeruginosa) are shown in Table-3. The synthesized compounds Zinc (1, 2 and 3 mole) NPs represented excellent inhibition activity against Gram positive bacteria (Bacillus subtilis) and Gram-negative bacteria (Pseudomonas aeruginosa). With an increase in concentration, the activity also proportionately increases.

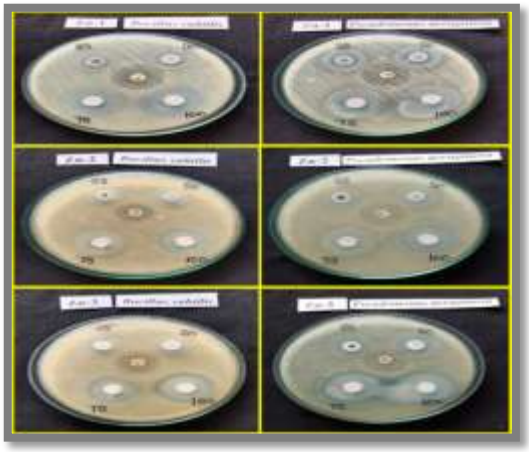

Figure 7: Antibacterial activity of zinc (1,2 and 3 mole) NPs.

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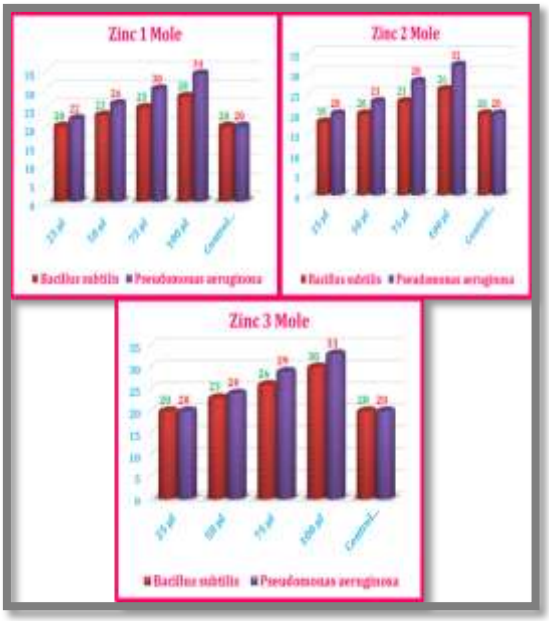

Figure 8: **Zone of Inhibition formed in the antibacterial activity comparison againstvariousmicroorganisms of Bacillus subtilis,Pseudomonas aeruginosaforZinc (1, 2 and 3 mole) NPs**.

The antifungal activity of Zn-1, Zn-2 and Zn-3NPs was investigated by a similar method and is shown in **Figure-3.** The zone of inhibition values of Zinc (1, 2 and 3 mole) NPs against A. niger are shown in **Table-2.** The synthesised compounds Zinc (1, 2 and 3 mole) NPs are highly effective againstA. niger. With an increase in concentration, the activity also increases proportionately.

Table – 4
Zone of inhibition values of Zinc (1, 2 and 3 mole) NPs againstAntifungal microorganisms of A. niger

Sample Name	DMSO Extract 100 µl added and Zone of inhibition (mm/ml) Antifungal activity (A.niger)				
	25 µl	50 µl	75 µl	100 µl	Control (Gentamicin antibiotic)
Zn 1 Mole	16	22	25	28	20
Zn 2 Mole	18	23	26	32	20
Zn 3 Mole	19	24	28	34	20

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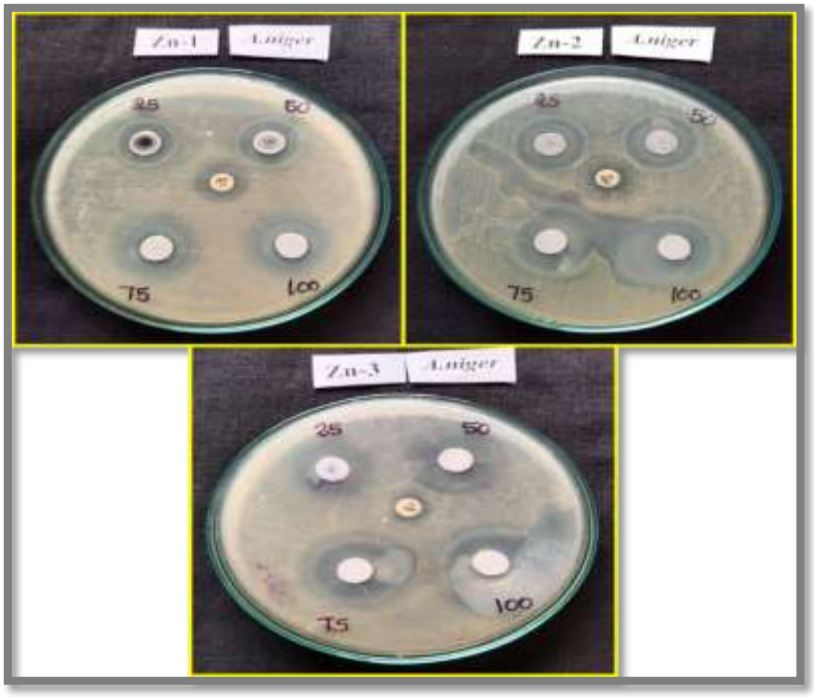

Figure 9: Antifungal activity of ZnO (1,2 and 3 mole) NPs

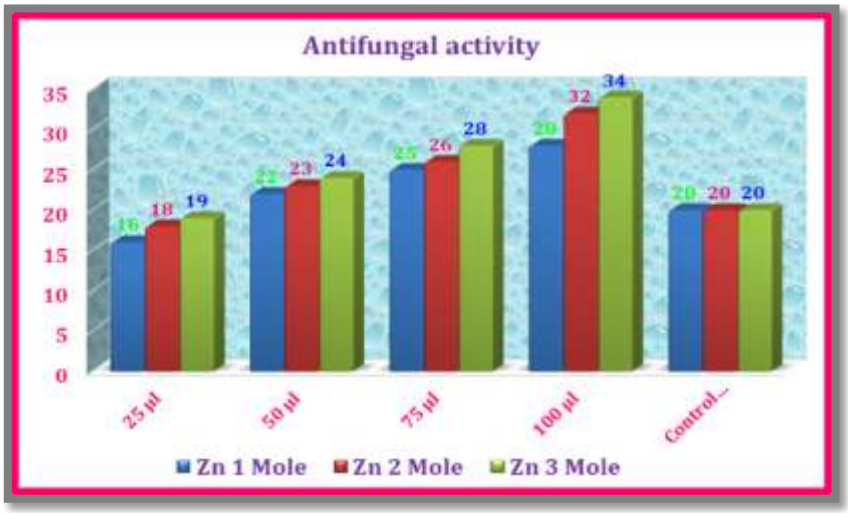

Figure 10: Zone of Inhibition formed in the antifungal activity comparison against Zinc (1, 2 and 3 mole) NPs for A. niger microorganism.

From this study, it was revealed that the Zinc (1, 2 and 3 mole) NPs showed excellent activity against Gram positive bacteria and Gram-negative bacteria. Zinc (1, 2 and 3 mole) NPs suppress the growth of gram-positive bacteria and Gram-negative bacteria to a maximum extent compared to that of the standard drug used. Zinc (1, 2 and 3 mole) NPs showed high activity against fungi, A. niger. Thus, Zinc (1, 2 and 3 mole) NPs associated with antibacterial agents can be a therapeutic option for the treatment of bacterial infections.

IV. CONCLUSION

We have successfully fabricated the ZnO NPs of different mole concentrations (1, 2 and wt. %) from zinc acetate using simple chemical process of sol-gel assisted hydrothermal synthesis and are stored cautiously to make sure that no impurities get added. The crystallite size and nature are studied using XRD technique revealing the average size in the range of 30-45 nm. The purity of the synthesized ZnO NPs were clear from EDX spectrum analysis which showed the theoretical mass percentage of Zn as 34.06% and O as

65.94%. The presence of various stretching and bending of functional groups were analysed from FTIR spectra by referring the band value to the MERCKS IR SPECTRUM TABLE & CHART. The crystal-clear structure of prepared ZnO NPs and the reasons to get such a predefined structure was studied using SEM analysis. The optical properties analysis using UV-Vis-NIR spectrum analysis revealed the parameters such as absorbance edge value and band gap energy and is found to be decreasing with the increase in mole concentration. The antibacterial and antifungal studies demonstrated the higher stability of ZnO NPs in suppressing the growth of microorganisms. In the coming generations, it is anticipated that the ZnO NPs will find a constant place in clinical as well as on- clinical research as therapeutic agents.

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