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Antibacterial Activity of ZnO Nanoparticles Prepared by Microwave Assisted Chemical Method.

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ABSTRACT

Zinc Oxide (ZnO) nanoparticles of three different sizes were prepared by using microwave assisted chemical method. Zinc acetate dihydrate, sodium hydroxide and deionized water were used as precursors. X-ray diffraction (XRD) studies of the prepared ZnO nanoparticles indicate polycrystalline nature. The preferential orientation and crystallite size depends on microwave power. Scanning electron micrograph results indicates the formation of nanoparticles. The antibacterial study of the prepared ZnO nanoparticles was carried out by disk diffusion method. It was found that antibacterial activity of ZnO nanoparticle depends on size and concentration.
Keywords: ZnO, nanoparticle, XRD, antibacterial activity, *E.coli, S.aureus*

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INTRODUCTION

Zinc Oxide (ZnO) is a well-known semiconductor material having wide applications such as bio sensor [1], solar cell electrode [2], varistor [3] and catalyst [4].Nano-sized ZnO particles are being used in sunscreens[5] and into fabrics[6] due to its ability to absorb UV-radiation which prevent the skin injury and fading of fabrics. Recently, it was shown that nanostructured ZnO were biocompatible, biodegradable and biosafe materials [7] and hence can have wide medicinal applications. Undoped and doped ZnO nanoparticles showed greater antibacterial activity on both Gram-positive and Gram-negative bacteria [8-10].The mechanism towards antibacterial activity of ZnO nanoparticles is not well understood. However, the generation of hydrogen peroxide [11]from the surface of ZnO nanoparticle is the main factor and binding of nanoparticle on the bacterial surface due to electrostatic forces[12]causing damage in the cell membrane and intercellular contents was the suggested mechanism. Also the mechanism strongly depends on size and concentration.

Vapour and liquid phase technique are the two broad classification method for preparing size and shape selective nanoparticles. Liquid phase techniques such as co-precipitation, sol-gel, microemulsion, hydrothermal, template and biomimetic were most widely used due to simple in operation and a promising one to obtain different size and shape.However, each technique has its own merits and demerits in terms of purity of particles, equipment cost and synthesizing time [13]. Of which, microwave based synthesis of nanoparticles is an important tool in the design of green synthetic approach and has several advantages such as rapid volumetric heating, higher reaction rate, reaction selectivity, purity, product yields and energy saving [14].Also microwave assisted methods was found to be a promising technique for preparing different nanostructured materials ranging from quantum dotto complex morphologies by using different solvent or surfactant such as water, ethylene glycol, benzyl alcohol, mixed solvent[14]. However the usage of different toxic solvent or surfactant requires repetitive washing and centrifugation of the product. Therefore, it is highly desirable to develop facile methods for the rapid fabrication of desired size ZnO nanoparticle in low-toxic solvents.

In this paper, we report a simple microwave assisted method to synthesize spherical shaped ZnO nanoparticles of different size by varying power and time of microwave irradiation using deionized water as solvent. The prepared ZnO nanoparticles were characterized for structural, morphological and optical property. The antibacterial activity of the prepared ZnO nanoparticles was studied as a function of size using disk diffusion method.

MATERIALS AND METHOD

All the reagents used in the work were of analytical grade and utilized as received without further purification. The synthesis was carried out in a domestic microwave oven (Onida-20l) which is slightly modified to hold the refluxing condenser.Aqueous zincacetate dihydrate ($Zn(CH_3COO)_2.2H_2O$; 99.99%, Sigma Aldrich) of 0.1M and 0.2M of sodium hydroxide (NaOH; 99.99%, Sigma Aldrich) were utilized as precursors. Sodium hydroxide solution was

added slowly to zinc acetate solution under constant stirring at room temperature. Then the resultant solution is transferred in the specially designed refluxing condenser flask kept inside the microwave oven. Microwave irradiation proceeded at three different power level (80W, 160W and 360 W) for 30 min. After microwave processing, the solution was allowed to cool in the oven to room temperature. The resultant precipitate was then separated by centrifugation (6000 rpm), and washed with deionized water and absolute ethanol for several times. The powder was dried in an oven at 80 °C for 24 h and annealed at 400 °C for 1 h in air. Structural studies of the powder were carried out using PANalytical X-ray diffractometer of X'per PRO model. Morphological and elemental studies were carried out using HITACHI Scanning Electron Microscope of S-3000H model. The antibacterial activity of ZnO nanoparticle on *E.coli* and *S.aureus* was studied through disk diffusion method.

RESULTS AND DISCUSSION

Structural studies

Figure 1 (a–c) show the XRD pattern of ZnO powders obtained from different microwave power. All the XRD patterns aggress with Joint Committee on Powder Diffraction Standards (JCPDS) card [36-1451] indicating polycrystalline nature of the prepared powder with hexagonal (wurtzite) crystal structure. The planes were indexed as (1 0 0), (0 0 2), (1 0 1), (110), (103) and (112) with respect to standard card. The obtained XRD peaksof all the powders shows broadened in their shape when compared with standard JCPDS peak. Also the broadening decreases as microwave power increased. These effects can be classified into instrument and specimen broadening. Instrument broadening originates from the non-ideal optical effects of the diffractometer and from the wavelength distribution of the radiation. In the present work instrumental broadening was corrected by using a standard defect free silicon sample.Specimen broadening arises due to small crystallite (grain) size and strain (lattice distortion). Grain size causes the radiation to be diffracted individually. The prepared ZnO nano-powder shows polycrystalline in nature, and hence large number of grains with various relative positions and orientations cause variations in the phase difference between the wave scattered by one grain and the others. The total intensity scattered by all grains is the sum of individual intensities scattered by each grain. On the other hand, lattice strain broadening is caused by varying displacement of the atoms with respect to their reference-lattice positions. A uniform compressive or tensile strain (macrostrain) results in peak shift of X-ray diffraction lines, whereas a non-uniform of both tensile and compressive strain results in broadening of diffraction lines (microstrain). The following well known Scherrer's formula was utilized to determine grain size and microstrain

$$D = \frac{0.9\lambda}{\beta \cos\theta} \text{ and } \varepsilon_{hkl} = \frac{\beta}{4\tan\theta} \text{ ----------(1)}$$

Where D is the size of the grain in the direction perpendicular to the reflecting planes, θ is the diffraction angle, K is the shape factor and is equal to 0.9, λ is the wavelength of x-ray and β the full width at half maximum of prominent peaks in radian ε_{hkl} is microstrain. The calculated

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grains was found to be in the ranges of 10 to 30nm, 40 to 80nm and 90 to110nm for the powders obtained from the microwave power of 80W, 160W and 360W respectively. This indicates that the prepared ZnO powder consists of nanoparticles and size strongly depends on microwave power. The microstrain found to decreases from 2.18 x 10^{-3} to 2.40 x 10^{-4} as microwave power increased and may be due to better crystallinity.

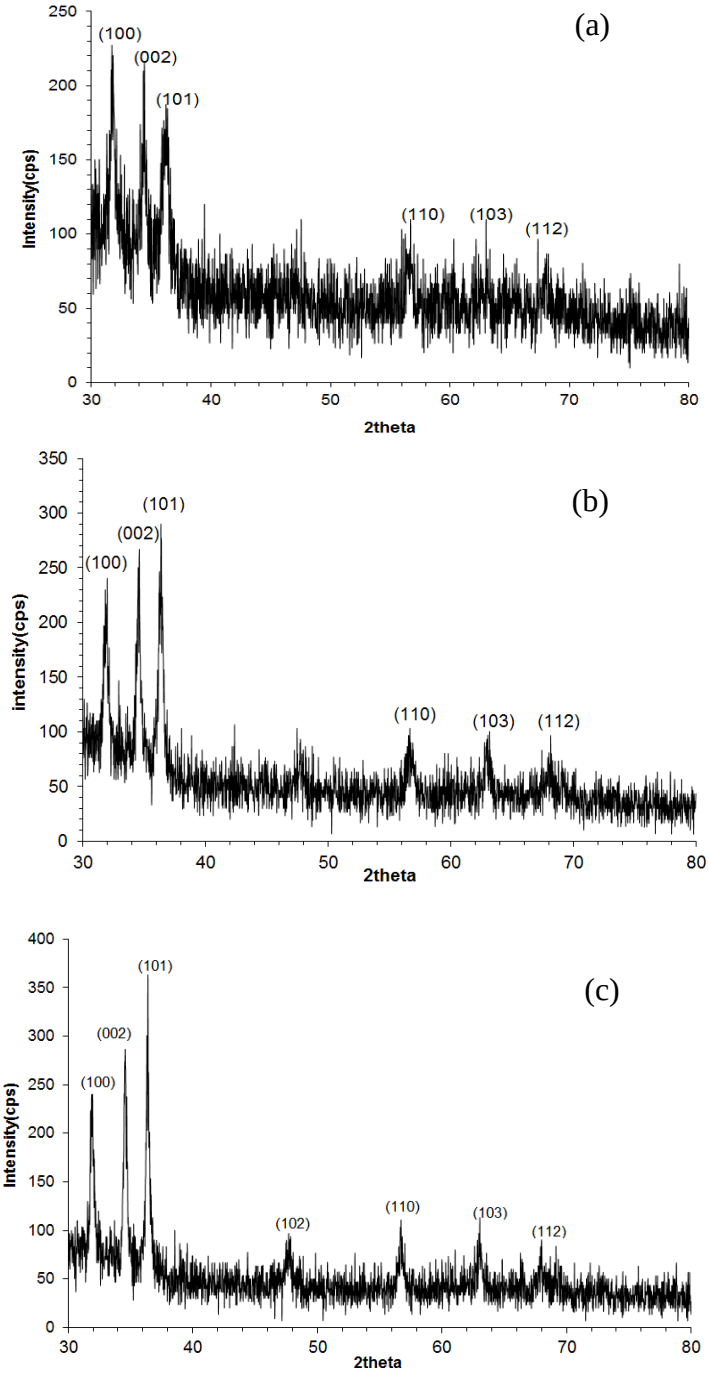

Figure 1: XRD pattern of ZnO particles obtained from microwave power of (a) 80W, (b) 160W and (c) 360W

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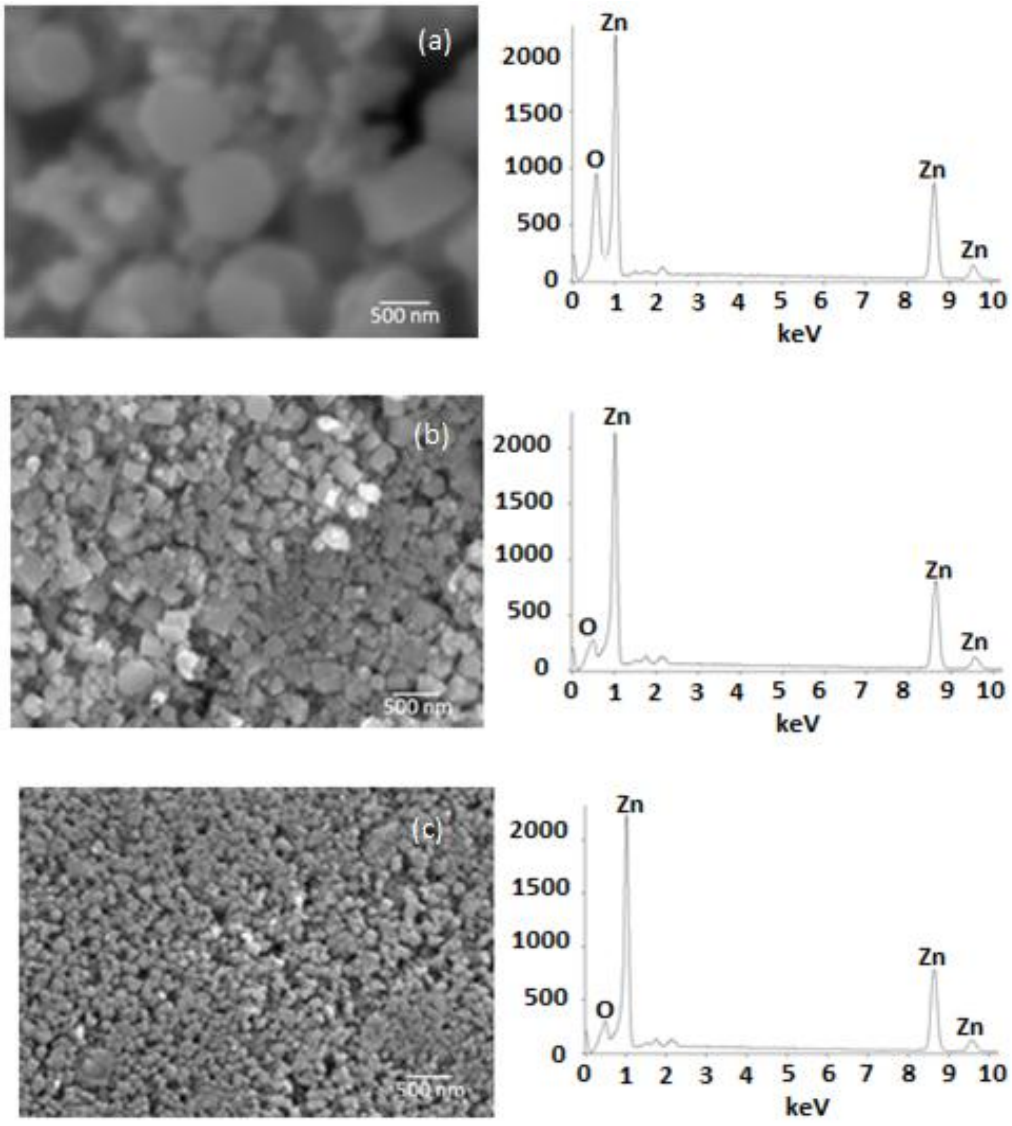

Figure 2: SEM and EDS of ZnO particles obtained from microwave power of (a) 360W, (b) 160W and (c) 80W

Figure 3: Photograph of antibacterial results of ZnO nanoparticles with different size range (A) – 10 to 30 nm, (B) 30 to 50 nm and (C) 50 to 80 nm

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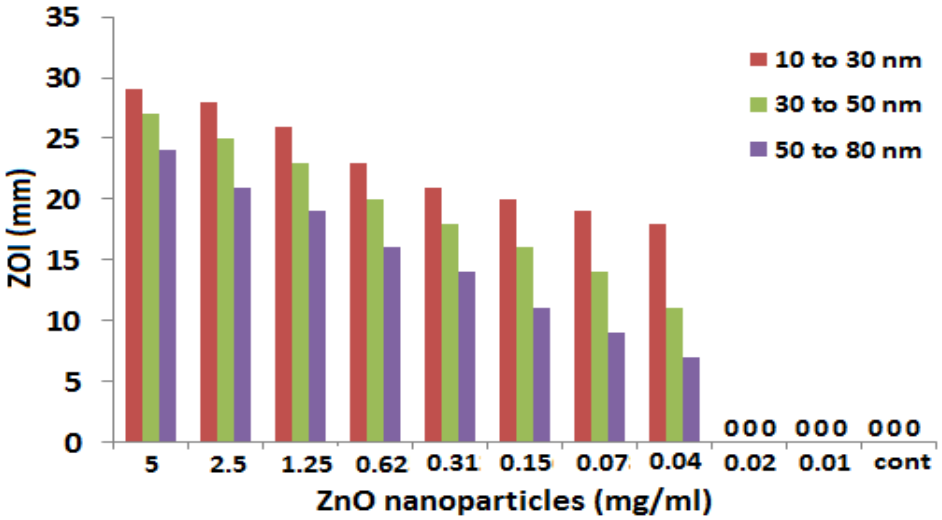

Figure 4: Size dependent zone of inhibition of ZnO nanoparticles for *S.aureus*

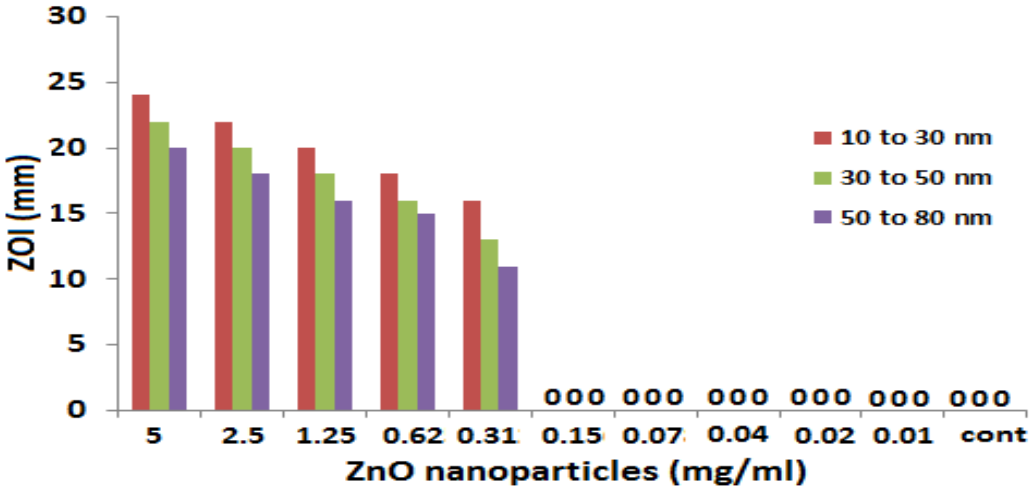

Figure 5: Size dependent zone of inhibition of ZnO nanoparticles for *E.coli*

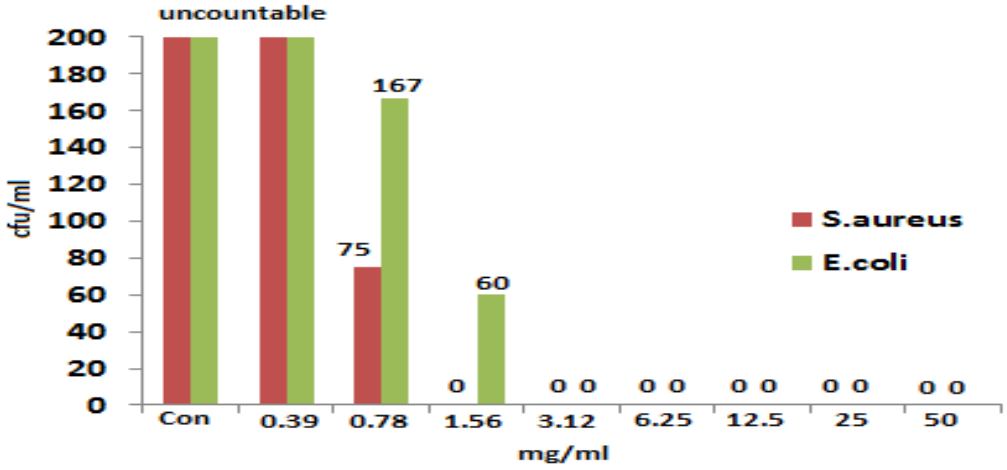

Figure 6: Number of colony forming units of *E. coli* and *S. aureus* of ZnO nanoparticle having size in the range of 10 to 30 nm

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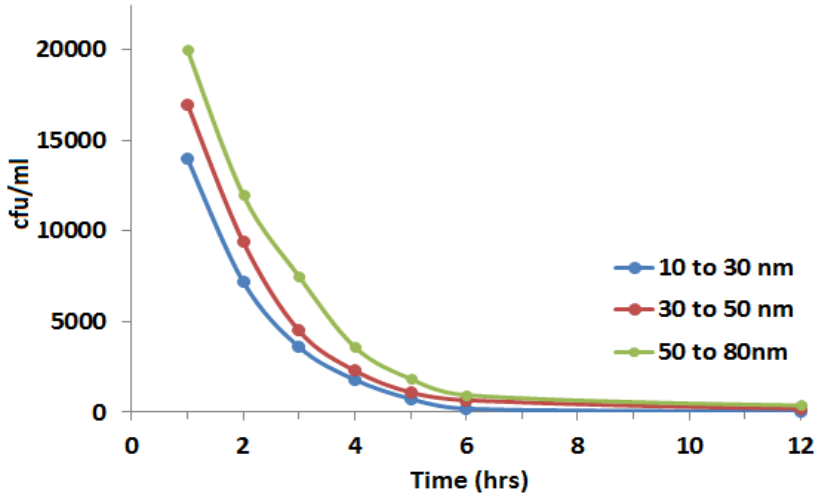

Figure 7: Number of colony forming units (cfu) of *S. aureus* with respect of time for different size of ZnO nanoparticle

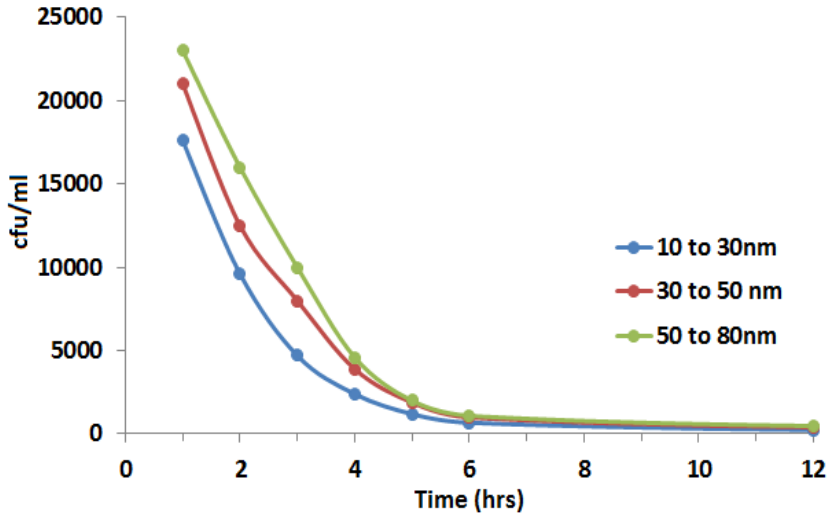

Figure 8: Number of colony forming units (cfu) of *E.coli* with respect of time for different size of ZnO nanoparticle

Morphology and compositional studies

Figure 2(a-c) shows the scanning electron micrograph (SEM) and Energy dispersive X-ray spectroscopy (EDS) pattern of prepared ZnO powders obtained at different microwave power. Scanning electron micrograph shows well defined grains randomly oriented and is agreement with the observed polycrystalline XRD pattern. Each grain is crystalline and indexed to wurtzite crystal structure. The observed grains size is comparable with that calculated value from XRD studies. The EDS spectrum of ZnO nanoparticle obtained at different microwave power reveals the presence of Zinc and Oxygen elements. The composition between Zn and O was found to be stoichiometric for the nanoparticles obtained at 80W and 160W ieZn : O (51.67 : 49.21 at%)

and (52.12 : 47.79 at%) respectively. However the ZnO nanoparticles obtained from 360W has oxygen deficient and non-stiochiometric in nature. This deficiency arises due to evaporation of precursor solvent and also the presence of excess Zn indicate n-type semiconductor due to electron donor nature of zinc metal.

Antibacterial activity studies

To study the antibacterial activity of the prepared ZnO nanoparticles on *E. coli* and *S. aureus*, the ZnO nanoparticles of 100 mg/ml wasdispersed in deionized water under ultrasonication at room temperature. To determine the antibacterial range of ZnO nanoparticles, desired volume of test bacteria is inoculated in nutrient broth medium with serially diluted three different sizes of ZnO nanoparticles from 100 to 0.78 mg/ml. After incubation for 24hrs at 37^{o}C, the colony forming unit (cfu) was estimated. The observation was found to be good in the range of 0.5 to 16 mg/ml for three different nanoparticle sizes.

To study the zone of inhibition, Muller-Hinton agar was prepared for 20 ml and poured into sterile petriplates. The microbial culture was then swabbed on the top of the solidified media and allowed to dry at room temperature for 20 minutes and disks were placed.Then 0.1 ml from each serially diluted ZnO nanoparticle solution was added on disks and zone of inhibition (ZOI) was measured after 24 hrs incubation kept at 37^{o}C. Figure 3show the photograph results of ZOI formation in three different sizes of ZnO nanoparticles on *E.coli* and *S.aureus* plates and indicate that the antibacterial efficacy increased with decreasing particle size. This may be attributed to increase in the surface area [15] as particle size reduced. Also, figure 4 and 5indicates that by increasing the ZnO nanoparticle concentration, the ZOI also increases and is different according to bacteria and, similar increasing trend was reported [16]. Figure 6 shows the number of colony forming unit of *E. coli* and *S. aureus* for different concentration of ZnO nanoparticles having size in the range of 10 to 30 nm. It was observed that a minimum of 3.0 mg/ml of ZnO nanoparticles is required for *E. coli* growth and 1.2 mg/ml for *S. aureus* and is termed as minimum inhibitory concentration. Thus the results suggest that the growth of Gram-negative bacteria is inhibited at high concentration of ZnO nanoparticle for all size range. Figure 7 and 8 shows the time-dependent antibacterial activity of ZnO nanoparticles with three different sizes. It shows that cfu of the tested bacteria decreased gradually and the rate of decrease is higher for low particle size.

CONCLUSION

ZnO of two different sizes were prepared by microwave assisted chemical method. X-ray diffraction studies indicate the polycrystalline nature with crystallite size in the range of nanometers obtained from Scherrer relation. The results indicate that ZnO nanoparticles inhibit higher for gram-positive than gram-negative bacteria. However the mechanism involved in the activity of ZnO nanoparticles is not clear and requires further study. Also in order to use ZnO nanoparticles in vivo condition, further studies should be performed investigating the toxic effect of ZnO nanoparticles on eukaryotic cells.

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