Microwave assisted synthesis of zinc oxide (ZnO) nanoparticles in a noble approach: utilization for antibacterial and photocatalytic activity

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Abstract

In this study, zinc oxide (ZnO) nanoparticle was synthesized by a novel route of microwave assisted method using ascorbic acid and PVA as capping agent and stabilizing agent. A prominent peak at 378 nm by UV absorption ensured the synthesis of ZnO nanoparticles. The crystalline property revealed that synthesized ZnO nanoparticle is highly crystalline having wurtzite structure. A significant band at ~ 498 to 557 cm^{-1} in FTIR was assigned to the characteristic stretching mode of Zn–O bond. EDX spectrum showed that ZnO nano particles were composed only with Zinc and oxygen. SEM image disclosed that ZnO nanoparticles were spherical in shape with an average particle size of 70–90 nm for the combined effect. The antimicrobial and ant-biofilm application of synthesized ZnO nanoparticle was studied against *Salmonella typhi*, *Klebsiella* spp. SK4, *E. coli* RN89, *E. coli* (gram-negative) and *S. aureus*-8a (gram-positive) bacteria and exhibited excellent activity against the strains. Prepared nanoparticle also found to possess outstanding photocatalytic activity against methylene blue degradation under sunlight with a T_{50} and T_{80} value of 71.44 and 165.87 min respectively.

Keywords Zinc oxide · Microwave radiation · Photocatalytic activity · Antibacterial activity · Antibiofilm activity

Abbreviations

NPs	Nanoparticles
PVA	Polyvinyl alcohol
g	Gram
mg/mL	Milligram/milliliter
TSA	Tryptic soy agar
TSB	Tryptic soy broth
CFU	Colony forming unit
FWHM	Full-width at half-maximum
ROS	Reactive oxygen species
MB	Methylene blue
EDX	Energy-dispersive X-ray
SEM	Scanning electron microscope

1 Introduction

Recently metal and metal oxide nanoparticles have increased the interest to be used in many areas of technology [1–4]. Among various metal oxides nanoparticles, zinc oxide (ZnO) has received much attention due to its unique physical, chemical and effective biological properties [5]. Usually ZnO nanoparticle has higher durability, less toxicity and high heat resistance which make it attractive for transparent electronics, piezoelectric, catalytic reactions and more specially in biomedical application like delivery of biomolecules (drug, gene, etc.), cancer therapy, tissue engineering, bioimaging, biosensing etc.

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[6–8]. ZnO nanoparticle exhibits significant antimicrobial activity because nano-sized ZnO can easily interact with bacterial surface and destroy them through either toxification or structural aberration [9]. Again, due to the ability of imparting toxicity through generating reactive oxygen species (ROS) in aqueous media ZnO nanoparticles are now considering as potential for controlling phytopathogenic fungi like *Botrytis cinerea*, *Penicillium expansum* and *Fusarium* sp. [10, 11]. Beside this, zinc oxide is a distinctive electronic and photonic n-type semiconductor material with a wide band gap of 3.37 eV and high exciton binding energy of 60 meV at room temperature, which make it attractive for potential catalytic and photochemical activities [9, 12].

Profound studies have been done on various approaches for the preparation of ZnO nanoparticle namely, sol–gel, micro emulsion, spray pyrolysis, electro-depositing, ultrasonic, chemical vapor deposition, hydro-thermal and precipitation methods [13, 14]. Most of these techniques were not extensively used on a large scale, but chemical synthesis has been widely used because of its simplicity and low expressive cost [15]. Again, controlling the structural and morphological properties of nanoparticle can be done more perfectly by chemical synthesis method than physical process [16]. For the chemical synthesis of well-structured nanoparticle, both capping and reducing agents, reagent concentration, pH, solvent composition and other synthesis parameters play a vital role [17, 18]. By using the capping and reducing agents such as ethylenediamine, citrates, ascorbic acid, amino acid, poly-acrylamide, polyvinylpyrrolidone (PVP) etc., the size and morphology of ZnO can be controlled [19–21]. Among various capping agents, polymer based capping agent like Poly vinyl alcohol (PVA) is now considered for some metal nanoparticle formation [22–24]. Generally, PVA is a synthetic polymer which is most effective in film forming, emulsifying, and has an adhesive quality because of its hydrophilic and biocompatible properties [25]. Ascorbic acid (AA) or vitamin C, on the other hand, is a biomimetic organic material which can be extracted from plant roots, aquatic macrophytes and phytoplankton [26]. Ascorbic acid has the tendency to form complexes in the solution with various metal ions such as Au^{3+} , Al^{3+} , Ni^{2+} , Ti^{4+} , and Zn^{2+} and has been widely used as a reducing agent in the synthesis process of metal oxide and metal nanomaterials [27, 28]. Again, ascorbic acid acts as an inhibitor for the hydrolysis of Zn^{2+} , therefore it can be used as modifier material for modified the structural and morphology properties of ZnO nanoparticle [29, 30].

However, a newly derived physical route for the synthesis of nanoparticle is the utilization of microwave radiation which has the advantages of rapid reaction, controlling over reaction conditions and also ensure

the high yield of nanoparticles [31]. Beside, as microwave assisted reduction method has the advantages of homogeneous volumetric heating, and high reaction rate as compared with other physical and chemical methods and both PVA and ascorbic acid can be a good competent for capping agent and reducing, therefore, we focused on the synthesis of ZnO nanoparticle by a combination of microwave radiation along with poly vinyl alcohol (PVA) and ascorbic acid for utilizing it in antibacterial and photocatalytic purposes.

2 Materials and methods

2.1 Materials

Analytical grade Zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, ACS reagent grade, Merck-KgaA, Germany) was used. Poly (vinyl alcohol), PVA ($[\text{CH}_2\text{CH}(\text{OH})]_n$, ACS reagent grade, Merck-KgaA, Germany) and ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$, 99%, Sigma-Aldrich Chemie GmbH, Germany) was used for the synthesis of ZnO nanoparticle. All of the solutions were prepared by using di-mineralized water and Chemicals were utilized without further purification.

2.2 Synthesis of ZnO nanoparticle

In order to synthesize the ZnO nanoparticle, 0.7-gram (g) PVA was dissolved in 60 mL distilled water by stirring through magnetic stirrer. 7.04 g Ascorbic acid was dissolved in 100 mL distilled water. After then 7.04 g $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ is added to PVA solution and diluted. Ascorbic acid solution is added drop wise to Zn-salt solution with continuous stirring. The solution is heated in a microwave oven (Miyako, MD-80D20ATL-DJ, 800 W, 2.45 GHz, Japan) at 400 W for 3 min. The solution was kept for overnight for precipitation. Precipitation was then centrifuged three times at 6000 rpm for 25 min to ensure the complete separation of nanoparticles. Centrifuged nanoparticles were then air dried in an oven (DO-150, Han Yang Scientific Equipment Co. Ltd. Seoul, Korea) at 105 °C for three hours and calcined at 500 °C in a furnace (NX2-2.5-10G, Henan, China) for next four hours. The nanoparticles synthesized by this route was identified as sample A. In sample B only microwave heating and ascorbic acid was used while sample C was synthesized by using PVA, ascorbic acid and normal heating (at 120 °C until color change to white) instead of microwave radiation. Drying in oven at 105 °C for three hours and calcination at 500 °C for next four hours were also done for sample B and sample C.

2.3 Characterization of ZnO nanoparticle

The prepared ZnO nanoparticles were characterized for their structural, optical and nanostructure properties. The optical absorption spectra of ZnO were observed using UV-vis spectrometer (UV-1601, Shimadzu, Japan). The bonding between different atoms and chemical information of the particles was collected by FTIR spectra (FTIR 8400S spectrophotometer, Shimadzu Corporation, Japan) in the wavenumber range of 4000–400 cm^{-1} (resolution: 2 cm^{-1} ; scans: 30). The crystal structure and crystallite size of ZnO nanoparticles were obtained by using X-ray diffractometer (Ultima IV, Rigaku Corporation, Japan) with Cu-K α radiations ($\lambda = 1.5406 \text{ \AA}$) in 2θ range from 20° to 80° operated at a voltage of 40 kV and current 40 mA with scan speed of $3^\circ/\text{min}$. From the XRD analysis, full-width at half-maximum (FWHM) data were used to determine average crystallite size. The average crystallite size (D) of the ZnO particle was calculated using Debye–Scherrer's formula of Eq. (1) [32]:

$$D = \frac{k\lambda}{\beta \cos\theta} \quad (1)$$

where β is the line broadening at FWHM and k is the shape factor which is equal to 0.9 in this case. The structural parameters like (dislocation density strain, and stacking fault) were also calculated from the XRD data by using Eqs. (2)–(4) respectively [33].

$$\text{Dislocation density } (\delta) = \frac{1}{D^2} \quad (2)$$

$$\text{Strain } (\epsilon) = \frac{\beta}{4\tan\theta} \quad (3)$$

$$\text{Stacking fault } (\alpha) = \frac{2\pi^2}{45(3\tan\theta)^{0.5}} \times \beta \quad (4)$$

Morphology and elemental compositions were investigated using scanning electron microscopy (JSM-7600F, JEOL, Tokyo, Japan) embedded with energy-dispersive X-ray (EDX) spectroscopy.

2.4 Antibacterial activity

For the calculation of zone of inhibition, a well diffusion method by agar plates was used [34]. Clinical pathogenic bacteria *Klebsiella* spp. KH15, *Salmonella typhi* SH7, *Escherichia coli* EH19 and *Staphylococcus aureus* 8a were developed on nutrient agar plate and maintained at 37°C for the whole night. This overnight culture of bacteria in nutrient broth (Oxoid Limited, United Kingdom) was then utilized in the experiment. In this technique, sterile nutrient agar

plate was equipped for each bacterium. All bacterial culture was adjusted in optical density (OD) 0.1 using UV-visible Spectrophotometer (Spectrumlab 1200RS, Japan). The spectrophotometer was first made auto zero using blank to eliminate the effect of assay reagents. These three bacterial pathogens were then coated over the agar plate with the help of sterile swab of cotton. Then these plates were permitted to dry. After that, one wells were bored by a sterile well cutter (7.0 mm diameter) in each agar plate. Subsequently, the suspension of NPs (20 mg/mL, 30 mg/mL) was poured into individual wells for each strain. The plates were allowed to place for 1 h for complete diffusion and then incubated at 37°C for 24 h (h) and measured the diameter of inhibitory zones in mm.

2.5 Anti-biofilm activity

Glass slides used for the study were washed with 70% alcohol and treated by UV for 15 min. Three selected isolates *E. coli* EH19, *Salmonella typhi* SH7, *Klebsiella* spp. KH15 and one-gram positive strain *Staphylococcus aureus* were used for the study and was grown on tryptic soy agar (TSA) (Oxoid Limited, United Kingdom) overnight at 37°C and stored at 4°C for further experiment. After 24 hr single isolated colonies for each were inoculated into 5 mL tryptic soy broth (TSB) to get over night culture which allow the formation of biofilm. Biofilm treatment by ZnO nanomaterials was done on glass cover slips incubated in 12-well plate. Biofilm cover slips were washed with sterile water for 3 times. Washed cover slips were then placed on 50 mL Falcon tube. 15 mL of sanitizing agents were added on falcon tube. Falcon tubes were placed in thermo centrifuge machine and were treated at 300 rpm for 5 min. Slips were then taken out from falcon tube, and washed with sterile water 3 times to remove loosely attached cells. To count viable cells, bacteria were enumerated by agar plating after ten-fold dilutions and incubated on tryptic soy agar (TSA) at 37°C for 24 h. Developed colonies were counted and converted into colony forming unit/mL (CFU/mL) [35]. The efficacy synthesized nanoparticle antibiofilm activities were evaluated by the Eqs. (5)–(7) respectively.

$$\text{Survival Fractions (SF)} = \frac{\text{Untreated Viable Cell}}{\text{Treated Viable Cell}} \times 100\% \quad (5)$$

$$\text{Percentage Kill (PK)} = (1 - \text{SF}) \times 100\% \quad (6)$$

$$\text{Log Reduction (LR)} = \log_{10}\left(\frac{1}{\text{SF}}\right) \quad (7)$$

2.6 Photocatalytic study

To evaluate photocatalytic activity of ZnO nanoparticle under sun light, methylene blue (MB) was chosen as a model pollutant for photocatalytic degradation. Stock solution of 50 ppm (parts per million) MB was prepared in distilled water by dissolving 20 mg of methylene blue in 400 mL distilled water. Initial concentration of methylene blue, in term of light absorbance, was measured by ultra-violet visible spectrometer with a maximum absorption at 666 nm. After then 100 mL of stock solution is taken in a 100 mL beaker and then 100 mg of ZnO nanoparticles are added in the beaker. The solution was then exposed to sunlight (from 11 to 3 pm of a particular day), after then 3 mL solution was withdrawn and suspended ZnO powder was separated by centrifugation at 6000 rpm for 10 min. This degraded solution was then used for absorbance measurement.

The photocatalytic degradation and rate of degradation of MB aqueous solution was calculated by the Eqs. (8) and (9) respectively [36].

$$\% \text{ Degrdaton} = \frac{A_0 - A}{A_0} \times 100\% \quad (8)$$

$$\ln\left(\frac{A}{A_0}\right) = -kt \quad (9)$$

where A_0 and A are the initial and post-irradiation absorbance of MB solution at 666 nm as measured by the UV-Vis spectrophotometer, respectively, and k is the rate constant of the reduction reaction.

Time required for 50% and 80% degradation of dyes (T_{50} and T_{80}) were calculated by the following Eqs. (10) and (11).

$$T_{50} = \frac{0.693}{k} \quad (10)$$

$$T_{80} = \frac{1.609}{k} \quad (11)$$

2.7 Statistical analysis

Test were done three times for each experiment and data are represented as mean $\pm$ standard deviation (SD). Significant study ($p \leq 0.05$) was done by analysis of variance (ANOVA) using IBM SPSS Version 25.0.

3 Results and discussion

3.1 Synthesis of ZnO nanoparticles in presence of ascorbic acid

When zinc oxide precursor solution after dissolving in water get mixed with ascorbic acid it immediately forms a complex with ascorbic acid as shown in Fig. 1. When the as formed metal ion chelate complex is dried and calcined at 500 °C in a furnace for four hours it gets converted into ZnO nanoparticle [37]. The PVA helps to prevent the agglomeration of ZnO nanoparticles by acting as a stabilizing agent. The microwave radiation used in the synthesis reaction to provide the energy required for the reaction, enabling particle size controlling features and also to accelerate the reaction.

3.2 Optical property

Figure 2 shows the UV-visible absorption spectra of the synthesized ZnO nanoparticles. It can be seen from the figure that there is a prominent absorption peak at 378 nm, 380 nm and 380 nm was observed for sample A, sample B and sample C respectively which is due to π - π^* electronic excitation of ZnO [38]. The red shift in UV absorption in case of sample B and sample C is due to the higher particle sizes of ZnO NPs as compared to the sample A. It also exhibits that significantly sharp absorption of ZnO represents the monodispersed nature of the nanoparticle's distribution which are beneficial for both photocatalytic and antibacterial activity. From the Tauc's plot, the bandgap of the sample A was calculated to be 3.35 eV as shown in onset of Fig. 2.

3.3 X ray diffraction characterization

Figure 3 shows the XRD pattern of synthesized ZnO nano-powder. The peak broadening in the XRD pattern clearly represents that smaller crystallite size of the materials present in the samples. The sharp diffraction peaks confirm the good crystallinity of the synthesized particles. All the XRD peaks were indexed as hexagonal wurtzite phase of ZnO nanoparticles match with JCPDS Card No. 36-1451 as shown in figure. The prominent diffraction peaks labeled on the figure which were located at 31.63°, 34.3°, 36.20°, 47.24°, 56.35°, 62.51°, 67.52° and 68.7° correspond to the (100), (002), (101), (102), (110), (103), (112), (201) planes were for the synthesized ZnO nanoparticles which is similar to other finding [39]. Further it also indicates that the synthesized particles were free of impurities as it does not contain any

Fig. 1 Possible synthesis mechanism of ZnO nanoparticles in the presence of ascorbic acid

Fig. 2 UV spectrum of synthesized ZnO nanoparticles. The Tauc's plot of band gap calculation for sample A is given in inset

Fig. 3 XRD spectrum of synthesized ZnO nanoparticles

characteristics XRD peaks other than ZnO peaks. The various structural parameters (crystal size, dislocation density, strain and stacking fault) of the synthesized ZnO nanoparticle are shown in Table 1. The average crystallite size of the sample-A, B and C was calculated from Debye's Scherrer equation using FWHM data to be about 22 nm, 20 and 20 nm respectively. PVA and microwave radiation or normal heating did not make effect significantly on the crystalline size as the main reaction for the

Table 1 Average structural parameters of ZnO nanoparticle

Sample	FWHM (β) ($\times 10^{-3}$ rad)	Crystal size (D) (nm)	Dislocation density (δ) ($\times 10^{15}$ lines/m ²)	Strain (ϵ) ($\times 10^{-3}$)	Stacking Fault (α) ($\text{\AA}^\circ$)
A	7.01	22	2.1	3.88	0.11
B	8.05	20	2.70	4.33	0.10
C	8.24	20	2.85	4.38	0.10

Fig. 4 FTIR data of synthesized ZnO nanoparticles

synthesis of ZnO crystal was same for all samples (formation of metal ion chelates complex and then calcination to form ZnO NPs). From the Table 1 it can be concluded that, the ZnO NPs synthesized by combined effect

(sample A) possessed better crystallinity as compared to the other two samples ensuring by the inverse relation between FWHM and strain with crystal size. Again, the lower value of δ for sample A (2.1×10^{15} lines/m²) also implied that it had very few lattice defects and higher crystalline qualities as related to other samples.

3.4 Vibrational properties analysis

The FTIR spectrum of ZnO nanostructures were recorded in the range 400–4000 cm⁻¹, is given in Fig. 4. From the FT-IR spectrum, various functional groups and metal-oxide (MO) bond present in the compound were analyzed. In the FTIR spectrum, three sharp peaks were observed at 557.43 cm⁻¹, 489.92 cm⁻¹ and 524.64 cm⁻¹, which could be attributed to the Zn–O stretching vibration mode which is consistent with other report [40]. In these cases, variation of ZnO stretching vibrational mode was observed because the number of atoms that constitute the surface might be changed at different conditions. The peaks close to 890 cm⁻¹ and 3340 cm⁻¹

Fig. 5 **a–c** SEM images of sample A, sample B, sample C respectively; **d–f** Particle size distribution of sample A, sample B, sample C respectively; **g** EDX of sample A and **h** Elemental composition of sample A

Fig. 5 (continued)

are due to the -OH bending and stretching from PVA and ascorbic acid [41]. The other bands observed at $800\text{--}900\text{ cm}^{-1}$ is assigned to C-H bend and the bands found at $1600\text{--}1300\text{ cm}^{-1}$ correspond to the C=O bonds and O-C-O bonds [42]. The symmetric and asymmetric bending modes of CO bonds were observed at $1525\text{--}1580\text{ cm}^{-1}$ [38]. The bending at $\sim 2327\text{ cm}^{-1}$ is due to absorption of CO_2 molecules present in the air.

3.5 Morphology analysis

Figure 5 shows the SEM images for pure ZnO nanoparticles. It is clearly observed that the particles are spherical in shape for all of the samples (Sample A, B and C). However, the variation of particles size was observed for microwave radiation and PVA concentration used in sample preparation. SEM images shows that the nanoparticles have good powder dispersion when prepared using PVA (sample A & C), but in absence of PVA (sample B) the synthesized particles get aggregated as indicated in Fig. 5a–c. This is because PVA prevents agglomeration by acting as a

stabilizing agent of nanoparticles. From particle size distribution it has been found that average particle size of sample A is $70\text{--}90\text{ nm}$, sample B is $230\text{--}280\text{ nm}$ and sample C is $580\text{--}630\text{ nm}$. There was an observation that use of microwave radiation might reduce particle size which is ensured from the particle size distribution graph (Fig. 5d–f). This might be happened because microwave radiation accelerates the reaction rate and causes reduction in reaction time which reduces the particle size as compared to the sample C [37].

Figure 5g shows the EDX pattern of as prepared ZnO sample indicating that ZnO nanoparticles were composed of only Zn and O and no evidence of possible contamination was found during its synthesis which is also ensued by the XRD study (Fig. 2). Furthermore, from the elemental composition (Fig. 5h) it also indicates that the synthesized nanoparticle consists of Zn (85.85%) and O (14.14%). From all characterization we observed that the combined effect (utilizing microwave radiation, PVA and ascorbic acid) gives the most nanosized ZnO particles and therefore we did all the application study by using the sample A only.

Fig. 6 Antibacterial study for determination of inhibition zone by ZnO nanoparticle against **a** *S. typhi*, **b** *S. aureus*, **c** *Klebsiella* spp. and **d** *E. coli* bacterial strains

Table 2 Diameter of inhibition zone of ZnO nanoparticle at different concentration against gram-positive and gram-negative bacteria

Nanoparticle (ZnO) concentration (mg/mL)	Diameter of inhibition zone (mm)			
	<i>Salmonella typhi</i> 57	<i>Klebsiella</i> spp.-SK4	<i>E. coli</i> RN93	<i>S. aureus</i> -8a
5	R	R	R	R
20	12.5 ± 0.02 ^a	11 ± 0.03 ^c	10 ± 0.02 ^e	13 ± 0.01 ^g
30	14 ± 0.03 ^b	11.5 ± 0.01 ^d	10.5 ± 0.04 ^f	14.5 ± 0.04 ^h

Date are presented as mean ± SD and analyzed with one-way analysis of variance. Different letters represent statistical significance among different treatments ($p < 0.05$)

R-Resistant (no zone)

3.6 Antibacterial activity

The antibacterial activity of ZnO nanoparticle suspensions of different concentrations against gram positive (*S. aureus*) and gram negative (*Salmonella typhi*, *Klebsiella* spp. and *E. coli* RN89) bacteria were studied in aqueous lysogeny broth (LB). The well diffusion method was used to test the ability of the antibacterial agent to rupture the bacterial cells. The antibacterial activity studied against gram-positive and gram-negative bacteria at different concentrations of samples are shown in Fig. 6.

From the Fig. 6 and Table 2, it is lucid that prepared ZnO nanoparticle showed the highest antibacterial activity against *S. aureus*. The inhibition zone has been found to increase with increasing ZnO concentration. The highest inhibition zone of ZnO having concentration 20 mg/mL and 30 mg/mL were 13 ± 0.01 mm and 14.5 ± 0.04 mm respectively for *S. aureus*. The lowest inhibition was observed for *E. coli* RN93 for all concentration of ZnO nanoparticle solution ($p < 0.05$). The observed antibacterial activity is attributed to large surface area of ZnO nanoparticles and light absorption behavior of ZnO nanoparticles. ZnO nanoparticles are accumulated or deposited on

Fig. 7 Mechanism of antibacterial activity by ZnO NPs **Table 3** Comparison of antibacterial activity of ZnO NPs with previously reported works

Synthesis method	ZnO precursor	Concentration mg/mL	Bacterial strains	Level of activity	References
Green synthesis	Zinc nitrate	1	Gram positive	Weak activity	[37]
			Gram negative	Weak activity	
Sol-gel	Zinc acetate	0.50	Gram positive	Moderate activity	[48]
			Gram negative	Moderate activity	
Chemical	Zinc nitrate	200	Gram positive	Moderate activity	[49]
Microwave assisted	Zinc nitrate	8.65	Gram positive	Moderate activity	[50]
			Gram negative	Moderate activity	
Microbial route	ZnO	0.025	Gram positive	Moderate activity	[51]
			Gram negative	Strong activity	
Microwave assisted	Zinc nitrate	30	Gram positive	Moderate activity	This study
			Gram negative	Weak activity	

Here, weak activity (inhibition zone ≤ 12 mm), moderate activity ($12 \text{ mm} < \text{inhibition zone} < 20 \text{ mm}$) and strong activity (inhibition zone $\geq 20 \text{ mm}$) [47]

the surface of bacterial cells due to its nanometer scale. At the experimental condition, ZnO nanoparticle generates reactive species that actively inhibits the growth of bacterial cells. Theoretically, nanoparticle usually interrupt the permeability of both the cell wall, thereby hamper the DNA replication and protein synthesis process of microorganism by altering the structure of lipids, peptidoglycan, proteins and their DNA as shown in Fig. 7 [43].

In general, nanoparticle causes the microorganism resistance through toxic metal ion generation and by releasing of reactive oxygen species (ROS) on the surface of nanoparticles [44]. The results also suggested that ZnO

nanoparticle exhibits significant growth inhibitory effect with gram positive than gram negative bacterial culture. The results were consistent with previous report that the antimicrobial activity of ZnO is more effective for gram-positive than gram-negative bacteria as the former have simpler membrane [45]. A comparison study of antibacterial activity of ZnO nanoparticles synthesized by various routes and precursors is also given in Table 3 indicating that the as synthesized ZnO NPs also exhibited moderate antibacterial activity as reported by others. However, the cell wall of gram-negative bacteria has an extra outer membrane consisting of lipopolysaccharide and peptidoglycan layer which helps the gram-negative bacteria to reduce the impairment by ZnO nanoparticles [46].

Table 4 Effect of ZnO nanoparticle treatments against bacterial biofilm developed at 37 °C on glass coverslips

Bacterial strain	No. of surviving cells before treatment $\times 10^8$ (CFU/mL)	No. of surviving cells after treatment $\times 10^8$ (CFU/mL)	SF (%)	PK (%)	LR
ZnO (20 mg/mL)					
<i>E. coli</i> RN89	6.33 ± 0.02^a	5.05 ± 0.03^b	79.77	20.23	0.1
<i>Klebsiella</i> spp. KH15	6.43 ± 0.01^c	5.41 ± 0.02^d	84.13	15.87	0.08
<i>Salmonella typhi</i> SH7	5.21 ± 0.02^e	4.90 ± 0.01^f	94.04	5.96	0.09
<i>Staphylococcus aureus</i> -8a	6.48 ± 0.03^g	5.19 ± 0.04^h	80.09	19.90	0.1
ZnO (30 mg/mL)					
<i>E. coli</i> RN89	6.33 ± 0.03^a	4.75 ± 0.01^b	75.04	24.96	0.12
<i>Klebsiella</i> spp. KH15	6.43 ± 0.04^c	4.33 ± 0.03^d	67.31	32.69	0.17
<i>Salmonella typhi</i> SH7	5.21 ± 0.02^e	4.10 ± 0.02^f	78.69	21.31	0.1
<i>Staphylococcus aureus</i> -8a	6.48 ± 0.01^g	4.09 ± 0.05^f	63.12	36.88	0.2

Date are presented as mean $\pm$ standard deviation (SD) and analyzed with one-way analysis of variance. Different letters represent statistical significance among different treatments ($p < 0.05$)

3.7 Anti-biofilm activity

In this study, the anti-biofilm activity of ZnO against *Klebsiella* spp. SK4, *S.aureus*-8a, *E.coli* RN89 and *Salmonella typhi* SH7 was examined. Plate dilution and plate counting methods were used to measure the effectiveness of treatment. The results are shown in Table 4. From the table, it is observed that ZnO nanoparticle shows anti-biofilm activity against *E. coli* RN89, *Klebsiella* spp. KH15, *Salmonella typhi* SH7 and *Staphylococcus aureus* and the effect was concentration dependent. For instance, before treatment with ZnO NPs, highest amount of bacterial count was observed for *Staphylococcus aureus*-8a (6.48×10^8 CFU/mL) and it was reduced to 4.09×10^8 CFU/mL after the treatment for 30 mg/mL of ZnO whereas the count was reduced to 5.05×10^8 CFU/mL for 20 mg/mL of nanoparticles. The table shows that, 30 mg/mL of ZnO NPs killed 24.96%, 32.69%, 21.31% and 36.88% of *E. coli* RN89, *Klebsiella* spp. KH15, *Salmonella typhi* SH7 and *Staphylococcus aureus*-8a bacterial strains with an LR value of 0.12, 0.17, 0.1 and 0.2 respectively. It is also observed that, the highest amount of strains was survived for all the gram-negative bacteria for both treatment and the lowest survival fractions (63.12% and 80.09%) were observed for gram positive bacteria (*Staphylococcus aureus*) after the treatment with 30 mg/mL and 20 mg/mL respectively. This is because the gram-negative bacterium strains secrete more component for biofilm formation, which makes their biofilm more tolerant. Earlier report incontestable that ZnO nanoparticle interfere with biofilm integrity by interacting with Extracellular Polymeric Substances (EPSs). Different studies

shown that ZnO nanoparticle will act on the particle channels in microorganism biofilms as well as the metabolic activity of bacterium [52].

3.8 Photocatalytic activity

To evaluate photocatalytic activity of ZnO nanoparticle under sun light, 50 ppm methylene blue (MB) solution was chosen as a model pollutant for photocatalytic degradation. The degradation rate of MB solution at different time interval under sunlight irradiation is presented in the Fig. 8a, b.

The degradation data (Fig. 8b) indicates that, in the presence of ZnO Nps under sunlight radiation, the degradation reaches almost 98% within 210 min. On the other hand, in the absence of ZnO Nps, only 3.001% of dye was degraded within 20 min and it reaches to almost 22% in 160 min (Fig. 8a). Thus it can be said that, under sunlight radiation ZnO Nps act as a catalyst for the degradation of MB. The kinetic study showed that the degradation of MB by ZnO NPs followed the first order reaction. The rate of reaction in presence and absence of ZnO Nps was 0.0097 and 0.0008 min^{-1} respectively and the reaction was more than twelve times faster in presence of ZnO NPs (Table 5). From the table, it is also revealed that 50% of the MB dye was degraded in 71.44 min and it took only 165.87 min for reaching 80% of its degradation. A comparison of degradation study along with the kinetic parameters of MB by ZnO NPs from various sources and synthesis routes are summarized in Table 6. From Table 6 it is very clear that, the ZnO NPs showed excellent activity against a higher initial dye concentration of MB as

Fig. 8 UV absorption spectra for degradation of MB by ZnO NPs in the **a** absence sunlight radiation and **b** presence of sunlight radiation. **c** Plot of $\ln(A_t/A_0)$ vs. time for both absence and presence of sunlight

Table 5 Comparison of degradation kinetics of MB dye solution by sunlight radiation in presence and in absence of ZnO NPs

Parameters	Degradation parameters in absence of ZnO NPs	Degradation parameters in presence of ZnO NPs
Equation	$Y = -0.0008x - 0.011$	$Y = -0.097x + 0.21$
Rate constant (k)	0.0008	0.0097
Degradation (%) at 20 min	3.001	12.62
T_{50} (min)	–	71.44
T_{80} (min)	–	165.87

compared to the other reported studies [55–57]. ZnO nanoparticle causes the photocatalytic degradation of MB dye under sunlight radiation due to the formation of various ROS like superoxide radical ($\cdot O_2$), hydroxyl radical ($\cdot OH$) and hydroperoxyl radical ($\cdot HO_2$) (Fig. 9) [53]. The radicals originate from the oxidation of absorbed water or absorbed OH^- and the presence of oxygen prevents the re-combination of electron-hole pairs which are generated by light of suitable energy absorbed by the ZnO nanoparticles [39]. The plenty of radicals produced by the absorbing more electron by surface oxygen enhances the discoloration and degradation of methylene blue by mineralization of the various intermediates

Table 6 Comparison study of methylene blue (MB) degradation by ZnO NPs obtained by various synthesis routes

Synthesis method	ZnO precursor	Doses mg/L	Dye conc. ppm	Degradation (%)	Time (min)	Reaction rate (k)	References
Green synthesis	Zinc nitrate	15	15	99	70	0.054 min^{-1}	[55]
Sol-gel method	Zinc Acetate	10	10	86	180	0.01 min^{-1}	[39]
Precipitation	Zinc Acetate	250	20	81	180	$8.4 \times 10^{-3} \text{ min}^{-1}$	[56]
Microwave-assisted green synthesis	Zinc nitrate	300	5	99.99	40	–	[50]
Biogenic route	Zinc acetate	250	0.1	83	60	$4.4 \times 10^{-3} \text{ s}^{-1}$	[57]
Microwave-assisted	Zinc nitrate	1000	50	98	210	0.01 min^{-1}	This study

Fig. 9 Possible Degradation mechanism of MB by ZnO NPs in presence of sunlight

formed during initial degradation and resulting in the CO_2 and H_2O generation in the final stage [54].

4 Conclusion

ZnO nanoparticle obtained from combined action of microwave heating, PVA and ascorbic acid is better in morphology and smaller in size. The average particle of combined effect ZnO nanoparticle was 70–90 nm and the average crystal was about 22 nm. Antimicrobial and anti-biofilm activity of synthesized nanoparticles confirmed that these ZnO nanoparticles can be a great candidate for the inhibition *Salmonella typhi*, *Klebsiella spp.* SK4, *E. coli* RN89 and *S. aureus-8a* microorganism and also against biofilm formation of microorganism. The prepared ZnO nanoparticle also exhibited good photocatalytic activity against methylene blue under sunlight radiation with a maximum degradation of 98% within 210 min. Thus, the above study reveals that synthesized nanoparticle with the effect of microwave heating and capping agent may find applications in biomedical and photocatalytic field efficiently.

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Author contributions KRA conduct the research work, SCP design, supervise, conduct and draft the manuscript, MA design and supervise the work, OS and MMR conduct specific experiments, MRN initially draft the manuscript, SNB initially draft the manuscript, SB and TDA helps to design the specific part of the manuscript. All the authors review the paper before the submission.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Availability of data and materials Research data have been provided in the manuscript.

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