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Biogenic synthesis of ZnO Nanoparticles using *Polygonum chinense* leaf extract and their Antibacterial activity

Farhana Sultana[1], Jayanta Barman[2] and M.C.Kalita[3]

[1]Biotech Hub, A.D.P. College, Nagaon, India.

[2]Department of Physics, A.D.P. College, Nagaon, India.

[3]Department of Biotechnology, Gauhati University, India.

Abstract

Now-a-days, biological reduction agents are being used in the fabrication of nanoparticle to minimize the effects of toxic chemicals. ZnO nanoparticles has been gaining much attention for its semi conducting properties, high catalytic and photochemical activity, unique antifungal, antibacterial, wound healing and UV filtering properties. The present study states a green approach for the synthesis of zinc oxide nanoparticles employing aqueous leaf extract of *Polygonum chinense* as a reducing/capping agent. The prepared ZnO nanoparticles were characterized using UV-visible spectroscopy (UV-vis), fourier transform infrared spectroscopy (FTIR) and X-Ray diffraction (XRD). FT-IR spectra revealed the functional groups and the presence of protein as the stabilizing agent for surrounding the ZnO nanoparticles. The average particle size was calculated as 16.64 nm by using Scherrer's formula. The antibacterial activity of the ZnO was tested against both gram positive and gram negative bacteria like *Escherichia coli* (MTCC 739), *Klebsiella pneumonia* (MTCC 432), *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 441)

by disc diffusion method. ZnO nanoparticles showed significant antimicrobial activity against the tested bacterial strains.

Keywords: Nanomaterial, capping agent, photochemical, Metal Oxide, Antibacterial activity

INTRODUCTION

Biosynthesis of nanoparticles is a bottom-up approach which employs biological sources or their components for the synthesis of nanoparticles. Among the biological entities employed, plant mediated synthesis is very common where the bioactive phytochemicals derived from plants are utilized for the production of nanoparticles. Recently, plant extract mediated synthesis of nanoparticles has become one of the popular alternatives over the conventional methods. Biosynthesis or green synthesis approaches has been gaining attention as they are cost effective, novel and usage of toxic chemicals and harsh conditions for reduction and stabilization are avoided (1). Various metal and metal oxide nanoparticles have been successfully synthesized using biological sources (2-5). ZnO nanoparticles (ZnO NP) has been gaining much attention for its semi conducting properties, high catalytic and photochemical activity, unique antifungal, antibacterial, wound healing and UV filtering properties (6). ZnO NPs are an ideal for biological applications as they are environment friendly, non-toxic, biosafe and biocompatible (7-8). As per the US Food and Drug Administration, ZnO with other four zinc compounds have been listed as generally recognized as safe (GRAS) material (9). Recently, ZnO NPs have been used in food packaging materials and various matrices. ZnO incorporated into the packaging matrix offers preservatory effects to the food materials (10). Now-a-days, ZnO NPs have been applied in sunscreens, paints and coatings as they are transparent to visible light and offer high UV absorption (11). ZnO NP are also used as an ingredient in antibacterial creams, ointments and lotions, self cleaning glass, ceramics and deodorants (12).

The genus Polygonum belongs to family Polygonaceae and comprises about 150 species. *Polygonum chinense* (Common name: Chinese knotweed) is a popular ethno medicinal plant of N.E. India. It is used in herbal remedies, such as for the treatment of dysentery, diarrhoea, dyspepsia etc and also used as food. The present study states a green approach for the synthesis of zinc oxide nanoparticles employing aqueous leaf extract of *Polygonum chinense* as a reducing/capping agent

2. MATERIAL AND METHODS

2.1. Materials

Zinc acetate dihydrate and all other chemicals were purchased from Merck Chemical Reagent Co. Ltd. India. All glassware was washed with sterile distilled water and dried in hot air oven before use. Leaves of *Polygonum chinense* were collected from Nagaon District,Assam.

2.2. Preparation of *Polygonum chinense* leaf extract

Fresh leaves of *Polygonum chinense* were collected and cleaned by washing several times with running water and subsequently with distilled water. Leaves were dried at room temperature in shade until all moisture was lost (10–12 days). Dried flowers were then ground to yield coarse powder, 10 gm of which was boiled in 100 mL of double distilled water for 15 min. The aqueous extract was then cooled, filtered using Whatman No.1 filter paper and stored at 4^0C for further use.

2.3. Synthesis and optimization of synthesis parameters for zinc oxide nanoparticles

Zinc oxide nanoparticles were synthesized using zinc acetate dihydrate Zn $(CH_3COO)_2$. $2H_2O$ (13). 50 mL of 0.01 M solution of zinc acetate was taken and leaf extract was added to zinc acetate solution in volumes ranging from 0.25 mL to 2 mL. The pH of the mixture was maintained at 12 and the solution was stirred continuously for 2 h. A white precipitate resulted which was then dried at 60^0C overnight. Prior to drying, the precipitate was centrifuged at 15,000 rpm for 5 min and washed twice with sterile de-ionized water.

2.4. Evaluation of antibacterial activity

The preparations and procedures for determination of antibacterial activity were done according to the manual on Antimicrobial Susceptibility Testing by Dr. M.K. Lalitha (2004). All the microbial cultures were produced from MTCC; Chandigarh, India was maintained on nutrient agar slants which were stored at -4^{o}C. The four bacterial species tested are *Staphylococcus aureus (MTCC 96), Bacillus subtilis(MTCC 441), Escherichia coli (MTCC 739), Klebsiella pneumonia (MTCC 432).* Müeller-Hinton agar (3.8%) media was used for inoculation. The dried surface of a Müeller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. 50mg of the ZnO NP were mixed with 1ml DMSO. Using a sterile puncher, wells of 8mm diameter were made on the inoculated agar plates. Three wells were made in each agar plates. Wells are then saturated with 10, 30 and 50µl of each extracts. The wells were allowed to dry and the plates are then inverted and placed in an incubator set to 37°C for 18 hrs. Wells with the solvent used for dissolution were used as

negative control and 1mg/ml amoxicillin were used as positive controls. After incubation time, each plate is examined. The diameters of the zones of complete inhibition are measured, including the diameter of the well. The experiment was performed in triplicate.

3. RESULTS

3.1 UV-Visible spectra analysis

UV–VIS spectrum of ZnO nanoparticles was recorded, by taking 0.1 ml of the sample and diluting it with 2 ml deionized water using spectrophotometer (Systronics), in the wavelength region 200 to 800 nm. Figure 1 shows the UV-Vis spectra of ZnO nanoparticles prepared from the extracts of *Polygonum chinense*.

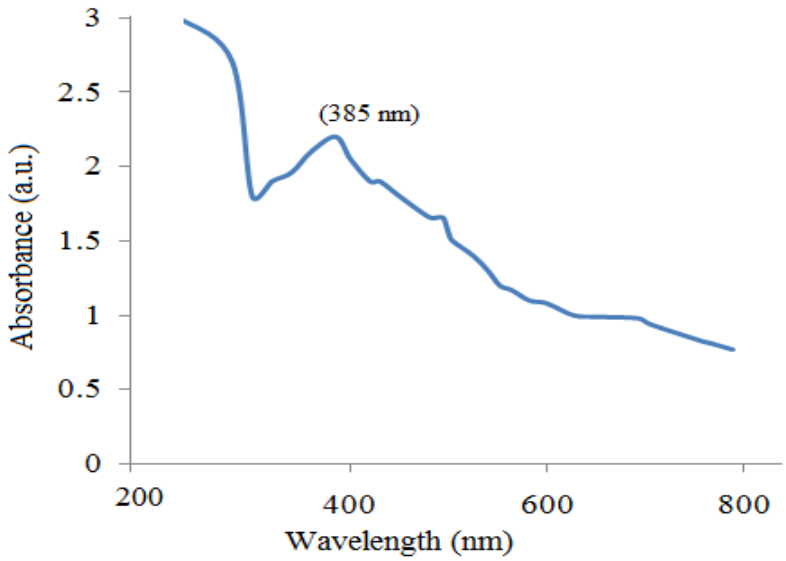

Figure 1: UV-vis spectra showing absorption of Zinc nanoparticles (ZnO NPs) synthesized using *Polygonum chinense* aqueous leaf extract.

The nature and value of the band gap of ZnO particle can be determined using the fundamental absorption that corresponds to electron excitation from the valence band to conduction band.

The relation between the absorption coefficient (α) and the incident photon energy (hv) can be written as

$$(\alpha\, hv)^{1/n} = A(hv - E_g) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where, A is a constant and E_g is the band gap of the material and exponent

which depends on the type of transition. For direct allowed transition n=1/2 and for indirect allowed transition n=2.

To determine the possible transition, $(\alpha\ h\nu)^2$ versus hv were plotted and corresponding band gaps were obtained by extrapolating the straight line portion of the curves to $(\alpha\ h\nu)^2=0$. The calculated band gap energy found to be in between 1 to 1.09 eV.

The absorption spectra also reveal that the excitonic absorption peaks for all these samples are blue shifted compared to the bulk indicating strong confinement. Size quantization of carriers in a small volume crystallite is well known to cause the blue shift. The shift of band gap can be utilized in determining the crystal radius (r) using the effective mass approximation relation [14-15].

$$\Delta E_g = E_g(\text{Film}) - E_g(\text{bulk})$$

$$=[h^2/8\mu r^2]-1.8e^2/\epsilon_0\epsilon_r r \quad \ldots\ldots(2)$$

Where $1/\mu = 1/m^*_e + 1/m^*_h$, μ is the reduced mass of electron and hole effective masses, $m^*_e=0.34m_0$ and $m^*_h=0.23m_0$ and $\epsilon_r=8.76$ is the permittivity of the sample. The values of band gap and particle size calculated from EMA model for different condition. The average particle size obtained 14.3 to 15.6 nm.

3.2 FTIR spectra analysis

FTIR- spectra is recorded to identify the functional groups involved in the ZnO particles prepared from the leaf extract of *Polygonum chinense*. The Figure 2 shows FTIR analysis for ZnO nanoparticles. The FT-IR spectrum showed strong absorption bands at 3340 cm^{-1} (O-H Stretch H-bonded), 2227 cm^{-1} (–C ≡ C– stretch; alkynes), the shoulder peak at 1638 cm^{-1} assigned for C=O group of carboxylic acids, the weak band at 1026 cm^{-1} can be assigned to the C-N stretching vibrations of aliphatic amines and 666 cm^{-1} correspond to C-H bend in alkynes.

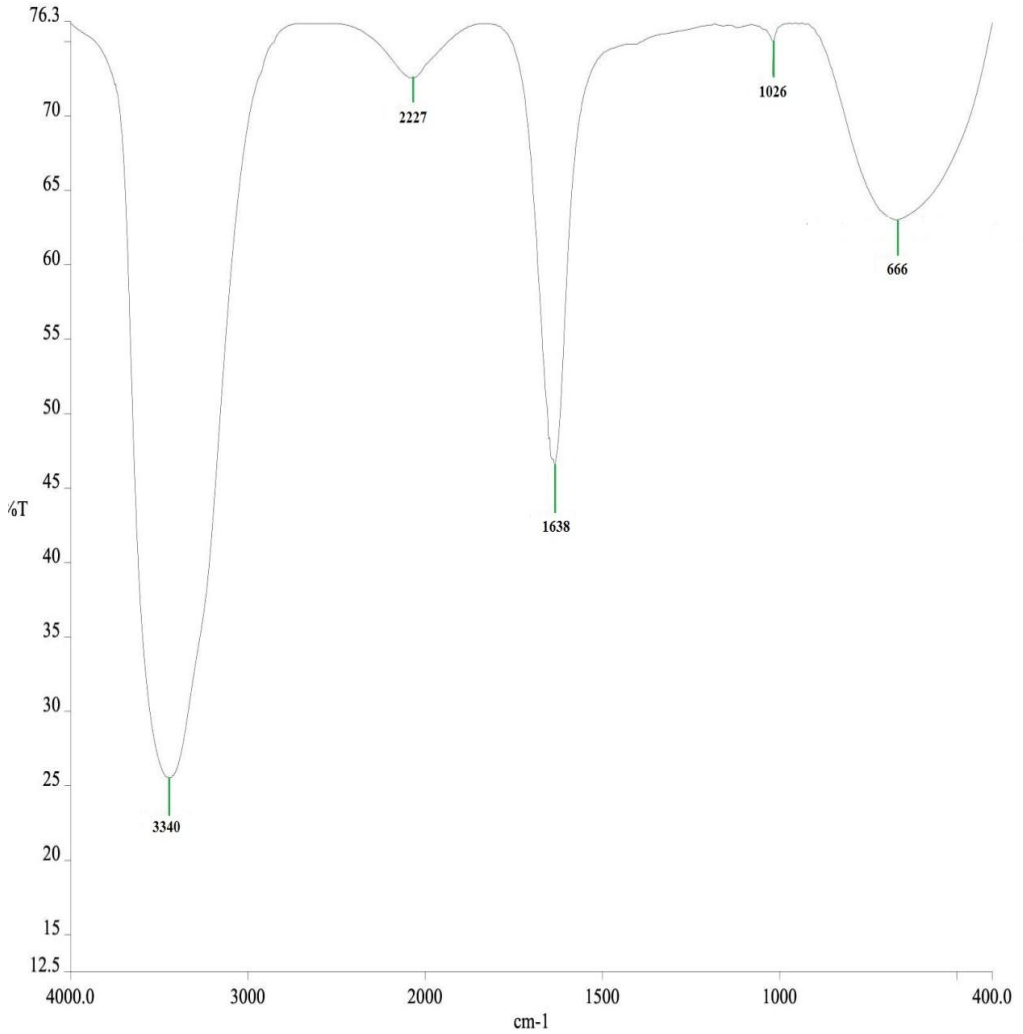

Figure 2: FTIR spectrum of Zinc nanoparticles (ZnO NPs) synthesized using *Polygonum chinense* aqueous leaf extract.

3.3 XRD:

The crystalline phase formation and size of metal oxide nanoparticles were analysed by X-Ray Diffraction (XRD) measurements**.** The powered sample was used for determining the formation of ZnO NPs by an X-ray diffractometer operated at a voltage of 40kV and a current of 30mA with Cu Kα radiation (λ =1.5416 A˚) . Crystal lattice indices and particle size were calculated using the X-ray diffraction pattern of ZnO NPs. XRD patterns of ZnO-NP are shown in Figure 3.

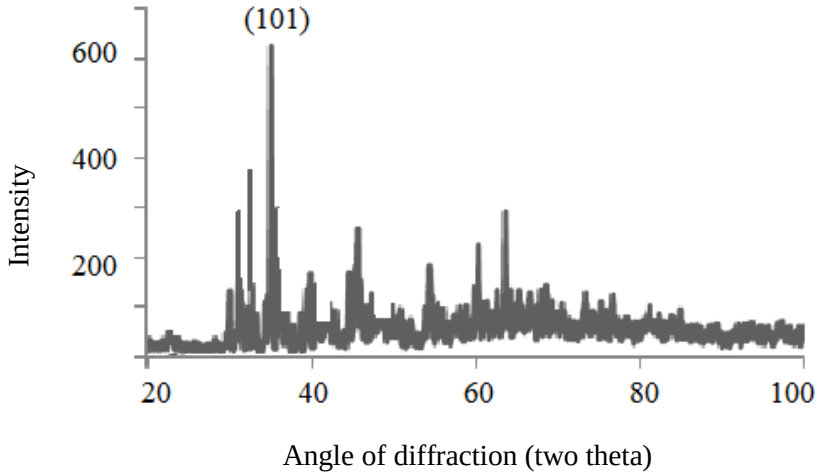

Figure 3: XRD pattern of Zinc nanoparticles (ZnO NPs) synthesized using *Polygonum chinense* aqueous leaf extract.

Diffraction peaks were observed at 2θ values of 31.85^0, 34.34^0, 36.96^0, 47.52^0, 56.39^0, 62.76^0, 68.0^0 and corresponding to lattice planes (100), (002), (101), (102), (110), (103), (112) respectively which are in good agreement with those of powder ZnO obtained from the International Center of Diffraction Data card (JCPDS-36-1451) attributed to hexagonal phase of ZnO (16-18).

Interplanar d-spacing was calculated using Bragg's Law equation:

$$2d \sin \theta = n\lambda$$

where, h is Bragg's angle of diffraction, ƛ is X-ray wavelength, i.e. 1.5406 A° and n= 1.

Further, particle size was calculated from the intense peak corresponding to (101) plane using Debye–Scherrer formula (17),

$$D = 0.89\lambda / \beta \cos \theta$$

Where, 0.89= Scherrer's constant {(shape factor), ranges from 0.9 to 1.0}, ƛ = X-ray wavelength (1.5406 A °), β =FWHM (Full Width at Half Maximum of the peak located at $2\theta = 36.96^0$ and θ=Bragg's angle of diffraction. The value of particle size was found to be 16.64 nm.

3.4 SEM Studies

The morphology of the ZnO NPs was studied by SEM observations, carried out on a JSM-6360 (JEOL) Electron microscope. As shown in fig. 4, the SEM analysis confirmed the size range of 20 - 30nm, a clear indication of the formation of ZnO nanoparticles.. Fig. 4 shows the formation of NPs that are self-aggregated in a close packed periodic array of hexagonal-like shape.

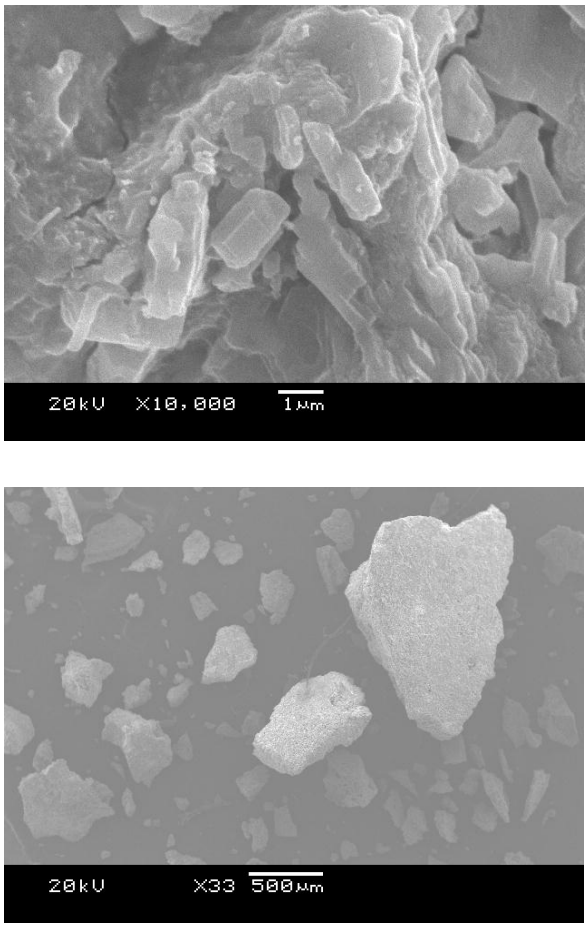

Figure 4: SEM image of Zinc nanoparticles (ZnO NPs) synthesized using *Polygonum chinense* aqueous leaf extract.

Antimicrobial activity:

The ZnO nanoparticles synthesized showed inhibition zone against the studied bacterial species.

The results are shown in the table below. The results depict that the nanoparticles are efficient in giving a zone of inhibition and significantly inhibit the pathogens.

Table 1: Zone of inhibition

(A- *E. coli,*B-*K. pneumonia,* C- *S. aureus ,* D- *B. subtilis*

1- Leaf Extract, 2-ZnO NP(Chemical synthesis) , 3-ZnO NP (green synthesis), 4-Tetracyclin(1mgml$^{-1)}$)

Bacterial Strains	**Zone of inhibition (mm)**			
	ZnO NP (Chemical synthesis)	**Leaf Extract**	**ZnO NP (Green synthesis)**	**Tetracyclin (1mgml^{-1})**
E. coli	9±0.14	10±0.11	14±0.13	35±0.08
K. pneumonia	13±0.11	11±0.16	16±0.15	32±0.09
S. aureus	12±0.15	11±0.14	13±0.11	31±0.09
B. subtilis	13±0.11	12±0.08	17±0.11	34±0.11

4. CONCLUSIONS

Green synthesis provides an environmental friendly, simple and efficient route for synthesis of nanoparticles. The reduction of metals by plant extracts avoids the usage of harmful and toxic reducing and stabilizing agents. The bio-reduction of aqueous zinc ions by the leaf extract of *Polygonum chinense* plant has been demonstrated. The reduction of metal ions through leaf extracts leads to the formation of zinc oxide nanoparticles of well defined dimensions. The synthesized ZnO nanoparticles were initially confirmed by UV-Vis spectroscopy at 385 nm. FT-IR analysis indicates the presence of phytoconstituents like amine, aldehyde, alcohols and phenol. XRD analysis reveals that the average size of the nanoparticles was found to be 16.64 nm. Toxicity studies of zinc oxide nanoparticles on pathogenic bacteria species have opened a new array of antimicrobial agent. The antimicrobial activity test performed by well diffusion method showed that at 50µg/ml concentration, zinc oxide nanoparticles have better antimicrobial properties. Nanomaterials reveal good result than other techniques used in water treatment because of its high surface area (surface/volume ratio). Earlier studies have also shows that zinc oxide nanoparticles have been used to remove arsenic from water, even though bulk zinc oxide cannot absorb arsenic. Biologically synthesized ZnO nanoparticles could be of immense use in water filtration for their efficient antimicrobial properties. It is suggested that these may be used in future at large scale water purification. The synthesis of ZnO nanoparticles is still in its developing stage and more research needs to be focused to improve the synthesis process which can control the size and shape parameters.

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