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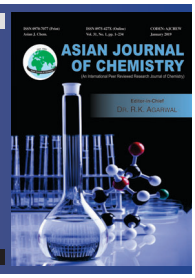

Green Synthesis, Characterization and Antibacterial Activity of Zinc Oxide Nanoparticles using Leaf Extract of *Rubia cordifolia*

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This work reports the synthesis of zinc oxide nanoparticles using leaf extract of *Rubia cordifolia*, characterization and the antibacterial activities against selected pathogenic bacteria. X-ray diffraction showed that the particle are hexagonal wurtzite in nature and average crystallite size is 23.61 nm. The SEM image revealed that the particles are sheets in shape. TEM image indicates that the size is found in the range of 23.45 nm. Further, as-formed zinc oxide nanoparticles revealed significant antibacterial activity against gram negative *Pseudomonas aeruginosa* and *Escherichia coli* and gram positive *Micrococcus luteus*, *Staphylococcus aureus* and *Streptococcus pneumonia* bacterial strain. The current investigation demonstrates convenient consumption of *Rubia cordifolia* leaf extract as a fuel for the synthesis of zinc oxide nanoparticles through green synthesis method to attain significantly antibacterial activity.

Keywords: Zinc oxide nanoparticles, *Rubia cordifolia*, Antimicrobial activity.

INTRODUCTION

Recent advances in the field of nanotechnology, particularly the ability to prepare highly ordered nanoparticulates of any size and shape, have led to the development of new biocidal agents. Nanomaterials are called "a wonder of modern medicine" and it is stated that antibiotics kill possibly a half dozen different disease-causing organisms but nano-materials can kill some 650 cells. Metal nanoparticles have various functions that are not observed in bulk phase and have been studied widely because of their exclusive catalytic, magnetic, optical, electronic and antimicrobial wound healing and anti-inflammatory properties [1]. Synthesis of nanomaterials has been done by many methods such as chemical, biological and physical. By the progress of chemical and physical methods environmental contaminations has produced and also a large amount of dangerous byproducts resulted by the chemical synthesis of nano-materials. Due to this reason there is a requirement of green synthesis which involve a clean, harmless, environmental friendly and non-toxic route for the synthesis of nanoparticles. Green method did not involve use of high temperature, pressure, energy and toxic chemicals [2].

In recent years, zinc oxide classified as an important semiconductor in group II-VI, having distinctive scientific and technological consequences, with a direct broad band gap and a great excition-binding energy, is a greatly preferential metal oxide with a massive list of attractive properties. Zinc oxide is useful material in many field due to its remarkable properties, includes high electrochemical coupling coefficient, wide range of radiation absorption and photostability. A potential platform having wide range of narrative uses has been represented by nanotechnology which controlled the synthesis of nano-materials as a dimension range 1-100 nm. Zinc oxide nanoparticles have been used in many industrial areas due to its high UV radiation absorption. They are used in making gas sensors, solar cells, photocatalyst and making UV protected cosmetics. Therefore, researchers have a great attention towards the synthesis of zinc oxide nanoparticles. It's a material of concern for pro-ecological systems and biomedicine because of its low toxicity, biocompatibility and biodegradability. Zinc oxide having range of nanometric ZnO means that it can be classified with new materials with likely applications in many fields of nanotechnology. Zinc oxide can arise in one- (1D), two- (2D), and three- (3D) dimensional structures. One dimensiona

structures include combs, nanorods, needles, rings,wires, helixes, belts, ribbons, springs and tubes. Nanoplate/ nanosheet and nanopellets structures are examples of 2D structures while ZnO obtained in 3D structures, such as dandelion, coniferous urchin-like, flower and snowflakes, *etc*. [3,4]. Zinc oxide nanoparticles have been used to purify water because ZnO nanoparticles can absorb arsenic and sulphur, since ZnO nanoparticles have much high surface area than bulk zinc oxide material [2].

Rubia belongs to Rubiaceae family comprises about 60 species. *R. cordifolia* has a perennial climber with very long, cylindrical roots with a thin red bark. Stems of this plant are ribbed, long, rough with woody base. Extensive amount of anthraquinone obtain from the plants belonging to Rubiaceae family [5]. Leaves are variable, arranged four in a whorl, cordate-ovate to ovate-lanceolate, base slightly cordate, petiols quadr-angular and shining [6]. In this study, we reported a rapid, eco-friendly, novel biosynthesis of zinc oxide nanosheets using *Rubia cordifolia* leaf extract as capping and stabilizing agent. These nanoparticles were further investigated as antibacterial activity against *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumonia* and *Pseudomonas aeruginosa.*

EXPERIMENTAL

All chemicals used without any purification. Zinc nitrate (99.9 % purity) and sodium hydroxide pellets (99 %) was used supplied by Sigma-Aldrich chemicals. Fresh and healthy leaves of *R. Cordifolia* were collected from park of forest department, Khirshu, Uttarakhand state of India. The aqueous extract of *R. cordifolia* was prepared using 10 g of dried and washed leaves, boiled with 100 mL of double distilled water at 60-70 ºC for 30 min. The extract was filtered through Whatman filter paper.

Synthesis of zinc oxide nanoparticles: An aqueous zinc nitrate (90 Mm) was added to aqueous leaf extract of *Rubia cordifolia* and adjusted pH to 12 by 1 M NaOH. The resulted solution was pale white in colour. Then solution centrifuged at 3000 rpm.The precipite was washed with distilled water followed by ethanol to remove the impurities. The solution was dried at room temperature and used for the characterization of zinc oxide nanoparticles [2].

X-ray diffraction analysis of ZnO nanoparticles: The particle size and nature of ZnO nanoparticles were determined using X-ray diffraction. This was carried out using Shimadzu XRD-6000/6100 models with CuK_{α} radians at 2θ angle. XRD is rapid analytical technique mainly used for phase identification of a crystalline material and provides information of unit cell dimensions. The average size of ZnO nanoparticles was determined by Debye Scherrer's equation:

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

where D is a average crystallite size, K is the Scherrer's constant, λ is the X-ray wavelength, β is the full width at half maxima (FWHM) and θ is the Bragg's diffraction angle.

EDX followed by SEM analysis of ZnO nanoparticles: Energy dispersive X-ray spectrometer (EDX) was carried out for determining the composition of nanoparticles followed by scanning electron microscopy (SEM) by using ZEISS EVO 18 scanning electron microscope machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of sample on the grid.

TEM analysis of ZnO nanoparticles: Transmission electron microscopy (TEM) was used to visualize the shape as well as the diameter of the synthesized nanoparticles. The images were obtained with a Tecnai, u-Twin 50-300 KV.

Antimicrobial activity: The zinc oxide nanoparticles synthesized from *R. cordifolia* leaf extract were tested for antimicrobial activity by agar well diffusion method [7] against organism like *Micrococcus luteus*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia* (clinically isolates). Strain was spread uniformaly on the plates using sterile cotton swab. Wells of size 8 mm in diameter have been made on Muller- Hinton agar plates using sterile cork-borer. Using micropipette 75 and100 µL of ZnO nanoparticles suspension poured into wells. After incubation at 37 ºC for 24 h the different levels of zone of inhibition measured for determining the antimicrobial activity of synthesized nanoparticles.

RESULTS AND DISCUSSION

The study on green synthesis of zinc oxide nanoparticles by natural leaf extract of *R. cordifolia* was employed and reported in this work. The aqueous zinc ions reduced to zinc oxide nanoparticles when added to the extract of *R. cordifolia* leaf extract. It was observed that the colour of solution turned into pale white colour, indicated the formation of zinc oxide nanoparticles. The formation of nanoparticles was determined by X-ray diffraction. Fig. 1 shows the XRD pattern for zinc oxide nanoparticles synthesized using leaves extract of *R. cordifolia*. The average crystallite size of ZnO nanoparticles was calculated from XRD pattern using Debye-Scherrer's equation. The calculated crystallite size is 23.61 nm and the crystal nature of ZnO nanoparticles is wurtzite hexagonal which is similar as reported by Darroudi *et al*. [8].

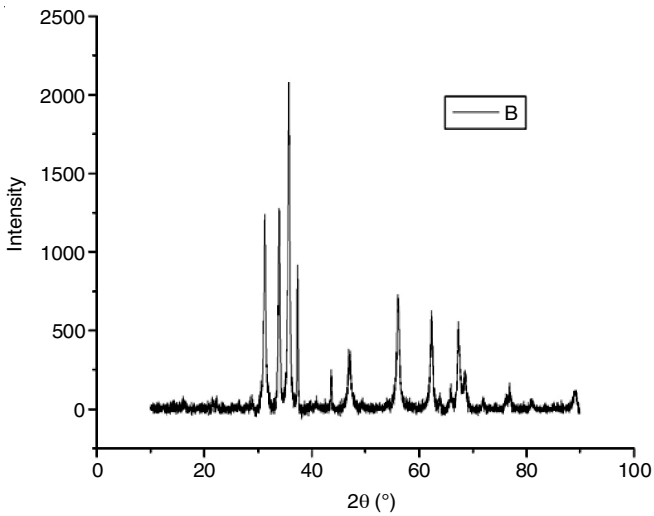

Fig. 1. X-ray diffraction pattern of ZnO nanoparticles of leaf extract of *R. cordifolia*

TEM analysis: In order to verify the results of X-ray diffraction analysis, zinc oxide nanoparticles were examined by TEM analysis. From TEM analysis (Fig. 2), the calculated mean diameter is 23.45, which is almost similar to the calculated particle size from X-ray diffraction patterns.

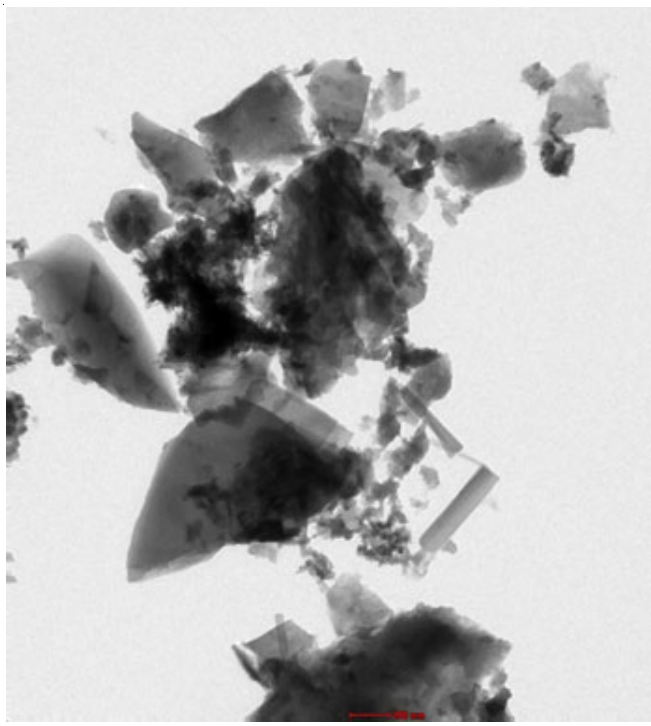

Fig. 2. TEM analysis of ZnO nanoparticles of leaf extract of *R. cordifolia*

EDX and SEM analysis: Fig. 3 confirmed the presence of ZnO with a sharp signal of Zn at around 1 keV and for O at around 0.5 keV. Peak of carbon is may be due to the presence of biomolecules of the plant extract. SEM morphology (Fig. 4) showed the well dispersed zinc oxide nanoparticles and sheet morphology of ZnO nanoparticles of leaf extract of *R. cordifolia*. The results are in agreement with the sheet morphology of zinc oxide nanoparticles of leaf extract as reported earlier [9].

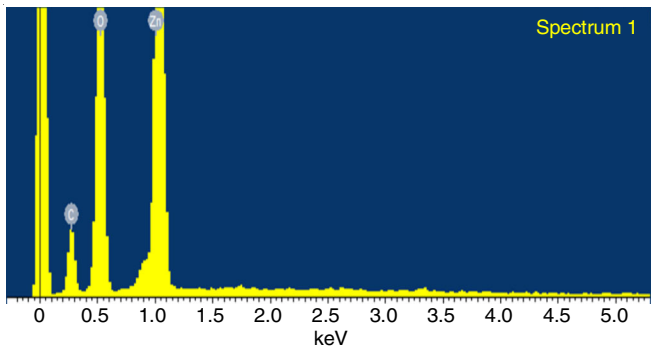

Fig. 3. EDX pattern of synthesized ZnO nanoparticles of leaf extract of *R. cordifolia*

Antimicrobial activity: The antimicrobial activity of zinc oxide nanoparticles synthesized by leaf extract was investigated against bacterial pathogens such as *Micrococcus luteus, Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* using well diffusion method. The diameter of zone of inhibition (mm) with synthesized ZnO nanoparticles using leaf extract of *R. cordifolia* suspension is represented in Table-1. The zone of inhibition of zinc oxide nanoparticles have been found against *Micrococcus luteus* (25 mm), *Escherichia coli* (17 mm) and *Staphylococcus aureus* (14 mm) and no activity has been found against *Streptococcus pneumonia* and *Pseudomonas aeruginosa*. The zone of inhibition is more for synthesized zinc oxide nanoparticles against *M. luteus* and *E. coli* as compared to erythromycin. Erythromycin does not exhibited any zone of inhibition since *E. coli* is not susceptible to synthesized ZnO nanoparticles.

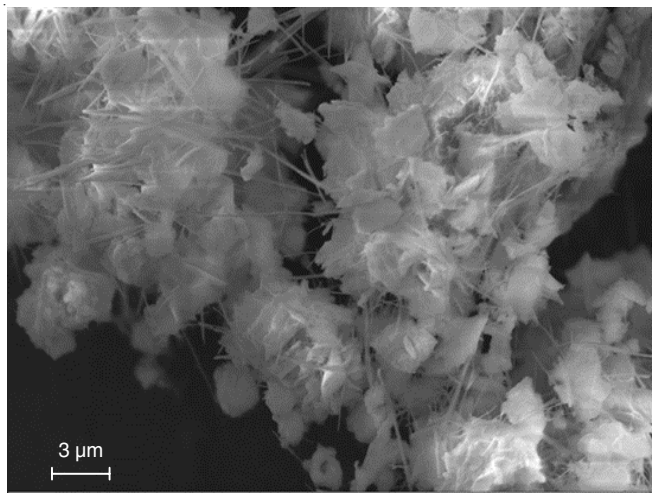

Fig. 4. SEM analysis of ZnO nanoparticles of leaf extract of *R. cordifolia*

TABLE-1
DIFFERENT ZONE OF INHIBITION OF SYNTHESIZED ZnO-NPs OF LEAF EXTRACT OF *R. cordifolia* AGAINST DIFFERENT BACTERIAL PATHOGENS (Inhibition zone - mm cork borer diameter - 8 mm), ERYTHROMYCIN AS A STANDARD DRUG

Organism	75 µL ZnO-NPs	100 µL ZnO-NPs	Erythromycin
Micrococcus luteus	22	25	23
Staphylococcus aureus	12	14	26
Escherichia coli	16	17	–
Pseudomonas aeruginosa	–	–	10
Streptococcus pneumonia	–	–	20

In present study, synthesized ZnO nanoparticles having average crystallite size of 23.61 nm and nanosheets in shape. In literature, several ZnO nanoparticles synthesized from different plants are reported to exhibit the antibacterial activity. (Table-2). However, the results of present study has been found to be comparable to the literature reports (Table-2). Hence, the green synthesized ZnO nanoparticles of leaf extract of *R. cordifolia* can also be used as good antibacterial agent.

Conclusion

The zinc oxide nanoparticles have been synthesized and characterized using *Rubia cordifolia* leaf extract. The characterization techniques *viz.*, XRD, SEM, EDX and TEM confirmed the formation of zinc oxide nanoparticles. The zone of inhibition in the antimicrobial screening indicated that synthesized ZnO nanoparticles has efficient antimicrobial activity against pathogenic microorganism. Antimicrobial activity of synthesized ZnO nanoparticles are concentration dependent.

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TABLE-2
GREEN SYNTHESIS OF ZnO-NPs USING LEAF EXTRACT OF VARIOUS PLANTS AGAINST DIFFERENT BACTERIAL PATHOGENS

Name of plant	Plant part	Shape	Size (nm)	Antibacterial activity (inhibition zone in mm)		Ref.
Aloe vera	Leaf	Rod shaped	500 nm in length	*E. coli*	7.0	[10]
				S. aureus	4.5	
Catharanthus roseus	Leaf	Spherical	23-57 nm	*P. aeruginosa*	13.0	[2]
				S. aureus	12.0	
				E. coli	10.0	
				Bacilus	12.0	
Solanum nigram	Leaf	Spherical	23.79 nm	*S. aureus*	18.0	[11]
				E. coli	7.0	
				V. cholera	11.0	
Hibiscus subdariffa	Leaf	Spherical, dumbbell	16-60 nm	*S. aureus* and *E. coli*		[12]
Brassica oleraceae	Leaf	Spherical and sheet	1-100 nm	*E. coli*	13.0	[13]
				B. subtilis	14.5	
				C. botulium	10.0	
				S. aureus	15.0	
				V. chlorae	9.5	
Camellia sinensis	Leaf		16 nm	*K. pneumonia*	10.3	[14]
				P. aeruginosa	3.3	
				E. coli	No inhibition	
				S. aureus	5.3	
Camillia sinensis	Leaf	Spherical (agglomerated)	Diameter-853 nm	*S. aureus*	25.0	[15]
				E. coli	29.0	
				P. aeruginosa	32.0	
Azadirachta indica	Leaf	Spherical	18 nm	*S. aureus*	24.3	[16]
				P. aeruginosa	21.3	
				E. coli	20.6	

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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