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GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING *RUBIA CORDIFOLIA* ROOT EXTRACT AGAINST DIFFERENT BACTERIAL PATHOGENS

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ABSTRACT

Wide application of nanoparticles stimulates the need for synthesizing them But, the conventional methods are usually hazardous and energy consuming. This lead to focus on “green synthesis”of nanoparticles which seems to be easy efficient and ecofriendly approach. In this study the green synthesis of zinc oxide nanoparticles was carried out using root extract of *Rubia Cordifolia* a reducing agent and their antimicrobial activity against various bacterial pathogens. Zinc oxide nanoparticles are known to be one of the multifunctional inorganic nanoparticles with effective antibacterial activity. Microbiological tests were performed using varying concentrations of green ZnO NPs with size 14.18 nm. Green synthesized NPs showed antibacterial activity against *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli*. Zinc Oxide nanoparticles were characterized by XRD, SEM, EDX and TEM. The green synthesis of Zinc Oxide nanoparticles appears to be cost effective, eco-friendly and easy as compare to other methods for the nanoparticles synthesis and its antibacterial activity.

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INTRODUCTION

Nanotechnology is the technological innovations in the 21st century. Research and development in this field is growing rapidly throughout the world. A major contribution of this field is the development of new materials in the nanometer scale. These are usually particulate materials with at least one dimension of less than 100 nanometers, even the particles could be zero dimension in the case of quantum dots. Metal nanoparticles have been of great interest due to their distinctive features such as catalytic, optical, magnetic and electrical properties. Nanoparticles present a higher surface area to volume ratio with decrease in the size, distribution and morphology like, nanoroads, nanoflowers, nanowires, nanodendrites and nanoparticles of the particles.[1,2]

In recent years, green synthesis of metal nanoparticles is an interesting issue of the nanoscience and nanobiotechnology. There is a growing attention to biosynthesis the metal nanoparticles using organism. Among these organism, plants seem to be the best candidate and they are suitable for large scale biosynthesis of nanoparticles. Nanoparticles produced by plants are more stable, and the rate of synthesis is faster than that in the case of other organisms. Moreover, the nanoparticles are more various in shape and size in comparison with those produced by other organisms.[3]

ZnO nanoparticles are believed to be nontoxic, biosafe, and biocompatible and have been also used as semiconductor, magnetic material, electroluminescent material, piezoelectric sensor and actuator, nanostructure varistor, field emission displaying material, thermoelectric material, gas censor, constituent of cosmetics etc.[4,5] The antibacterial activity of ZnO has been studied largely with different pathogenic and nonpathogenic bacteria such as *S. aureus and E. coli.*[5] ZnO have been reported to be activated by UV and visible light in order to generate highly reactive oxygen species such as OH^-, H_2O_2, O_2^{2-}, which is harmful to bacterial cells. It is suggested that the aldehyde groups are responsible for reduction of zinc oxide to zinc oxide nanoparticles and also stabilize the nanoparticles.[6]

The family Rubiaceae comprises of about 450 genera and 6500 species and includes trees, shrubs and herbs. Rubia comprises about 60 species. *Rubia cordifolia* (Rubiaceae) is a perennial climber with very long, cylindrical, flexuose roots with a thin red bark. In Sanskrit it is known as Manjistha. Stems often have a long, rough, grooved, woody base. Plants belonging to this family are known to contain substantial amounts of anthraquinone. Leaves variable, arranged four in a whorl, cordate-ovate to ovate-lanceolate, base slightly cordate,petiols are quadrangular and shining. Stipules are absent. *R. cordifolia* is used traditionally in the treatment of liver fluke, dysentery, maggots, wounds, skin disorders and has anticancer activity etc. [7,8] Therefore many research paper published on the greensynthesis of nanoparticles using many plants extract, but there are no published report on the green synthesis of zinc oxide nanoparticles of *Rubia Cordifolia* root extract. Therefore, the present study reported the synthesis of Zinc Oxide nanoparticles using aqueous root extract of *Rubia Cordifolia* and its activity against different bacteria. The structure, size and morphology of synthesized product were investigated by the standard characterization techniques.

MATERIALS AND METHODS

Zinc nitrate ($ZnNO_3$) of sigma Aldrich was used. All glasswares was washed with sterile distilled water and dried in an oven before use. The procedure for the synthesis was referenced from literature.[9,10]In this method of green synthesis there is no requirement for high pressure, energy, temperature or toxic chemicals¨

PREPARATION OF PLANT EXTRACTS

Plant materials were collected from Khirshu forest park ($78^0$52’2’’ and $30^0$10’15’’), Garhwal (Uttarakhand) India. The plant material were washed several times with water to remove the impurities and then dried at room temperature. The extract used for the reduction of zinc ions to zinc nanoparticles (ZnO) was prepared by placing 10 gm of washed dried fine cut material in 250 ml glass beaker along with 100 ml of double distilled water. The mixture was then boiled for 30 min at 60-70^0 C. The extract was cooled to room temperature and filtered using whatman filter paper. The extract was stored in a refrigerator for further experiments.

Preparation of zinc oxide nanoparticles

For the synthesis of nanoparticles 20 ml of root extract was taken heat for 10-15 min at 60-70^0C using a magnetic-stirrer. Then 50 ml of 91 Mm zinc nitrate was added drop wise to it under stirring. The color became yellowish. 50 ml of 1 M of NaOH added drop wise till the cream color precipitate of zinc hydroxide was formed. The reaction mixture was left for 5-10 min under stirring for complete reduction to zinc hydroxide. Now reaction mixture was left for 1 hour. Then the precipitate was collected by centrifugation at 3000 rpm for 10 min. The precipitate was washed 3 times with double distilled water. Then dried at room temperature and the sample were stored for further studies.

CHARACTERIZATION

XRD (X-Ray Diffraction) analysis:

The particle size and nature of the ZnO-NPs were determined using XRD. This was carried out with Shimadzu XRD-6000/6100 models with CuK radians at 2 θ angle. X-ray powder diffraction is rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The size of the ZnO-NPs 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.

SEM (Scanning electron microscopy) analysis of zinc oxide nanoparticles:

Size and structure of the nanoparticles analysis was done 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 the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

TEM (Transmission electron microscopy) analysis of zinc oxide nanoparticles:

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 assay:

Zinc nanoparticles of the root extract were tested for their antimicrobial activity against three gram positive and two gram negative pathogens *viz*, *Micrococcus luteus*, *Staphylococcus aureus* , *Streptococcus pneumoniae* , *Escherichia coli* and *Pseudomonas aeruginosa* (clinical isolates and got from the department of botany and microbiology, H.N.B. Garhwal university, Srinagar (uttarakhand) India) respectively by using agar well diffusion method.[11] A suspension of zinc nanoparticles of varying concentrations of 70ug/ml, 100ug/ml and 150ug/ml in 0.5% DMSO was used for screening the antimicrobial activity of nanoparticles against the test microorganisms. DMSO was used as a control. A 100ul of overnight culture of all the test microorganisms was spread on Muller Hinton agar plates aseptically and allowed to dry for 15minutes. Subsequently four adequately spaced wells of 8mm in diameter were punched into the plates using a sterile cork-borer. A 70ul zinc nanoparticles solution of each concentration was poured into each well under aseptic conditions. The plates were kept at room temperature to allow the extract to diffuse into agar medium. The plates were then incubated at 37°C for a period of 24h and observed for the zone of inhibition. The antimicrobial activity of the nanoparticles was determined by measuring the zone of inhibition surrounding the wells. The assay was performed in triplicates for the accuracy of the results.

RESULTS AND DISCUSSION

Biological synthesis of ZnO-NPs by using *R. Cordifolia* revealed that the pale white precipitate was appeared. *R. Cordifolia root* has been used for the reducing material as well as surface stabilizing agent for the synthesis of spherical shaped ZnO-NPs. The SEM analysis was used to determine the structure of the reaction products that were formed. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min . The SEM image showed relatively spherical structure.(Fig-1) Further, To obtain elemental composition on the nanoparticles, it is subjected to EDX analysis. EDX (Energy dispersive X-Ray spectrometer) identities the elemental composition of materials imaged in a SEM for all elements with an atomic number greater than boron.(Fig-2 and Fig-3)

XRD spectra showed strong diffraction peaks at 31.24, 33.9, 35.78, 47, 56.08, 62.36, and 67.56 degrees of 2 θ. Crystal lattice parameters a= 2.84 and c= 5.27 , indexed as the hexagonal wurtezite structure of ZnO (Fig- 4). High purity and crystallinity of the prepared ZnO-NPs confirmed the sturdy and clear peak. For other impurities no characteristic peaks were accessible. The average crystallite size of ZnO-NPs has been calculated is 14.18 nm.

The TEM analysis was used to determine the average diameter of the ZnO-NPs were formed. The average diameter of the NPs has been calculated by TEM analysis is 14.22 nm, which is approximately same as the size calculated by XRD analysis.(Fig-5)

Zinc oxide nanoparticles are generally known to produce antibacterial activity to a wide range of bacteria by operating various mechanisms. Arresting of microbial growth in the form of zone of inhibition was measured using various concentration of zinc oxide nanoparticle solution (70, 100 and 150 µL / well). The synthesized nanoparticles exhibited different degrees of antimicrobial activity against microorganism tested probably due to different mechanisms involved in killing of microorganism (Fig-6). Inhibition of microbial growth was observed as the zone of inhibition whose diameters are estimated (Table-1)

Table-1: Antimicrobial activity as zone of inhibition for various microorganisms against generated ZnO-NPs.

Different inhibition zone-mm cork borer diameter-8mm				
S.no	**organism**	**70 □L**	**100 □L**	**150 □L**
1.	*Micrococcus luteus*	22 mm	27 mm	27 mm
2.	*Staphylococcus* aureus	12 mm	13 mm	15 mm
3.	*Escherichia* coli	12 mm	14 mm	15 mm
4.	*Pseudomonas* aeruginosa	-	-	-
5.	Streptococcus pneumoniae	-	-	-

Interestingly, it was observed that synthesized nanoparticles showed potential antimicrobial activity with both gram positive and gram negative bacteria. Synthesized nanoparticles showed more activity toward *Micrococcus luteus.* NPs showed no inhibition toward *P. aeruginosa* and *S. pneumoniae.* Thus biological synthesis of zinc oxide nanparticle is able to generate biologically active zinc oxide nanoparticle with antimicrobial activity.

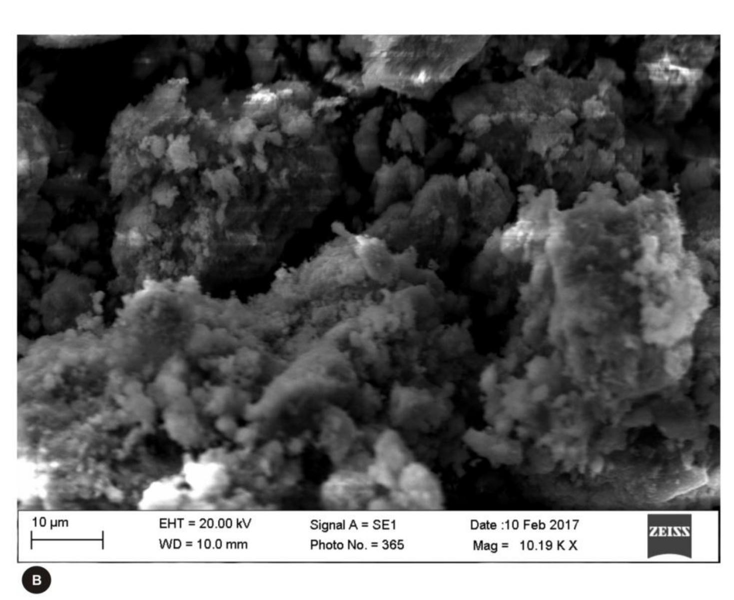

Fig-1: SEM analysis of ZnO-NPs of root extract of *R. Cordifolia.*

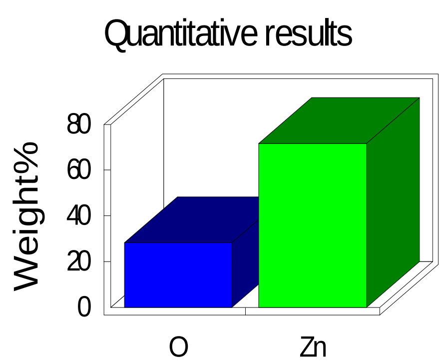

Fig-2: Quantitative analysis of ZnO-NPs of root extract of *R. Cordifolia.*

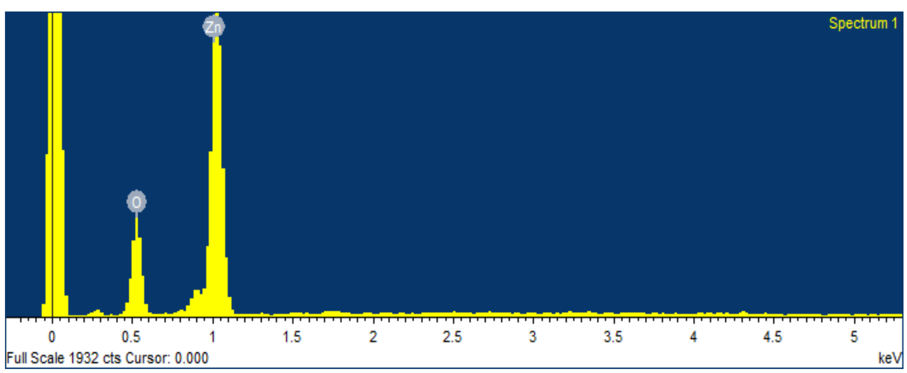

Fig-3: EDX measurement of ZnO-NPs of root extract of R *Cordifolia.*

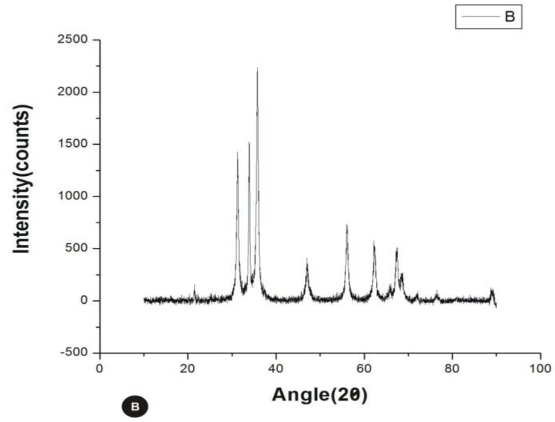

Fig-4: XRD analysis of ZnO-NPs of root extract of *R. Cordifolia.*

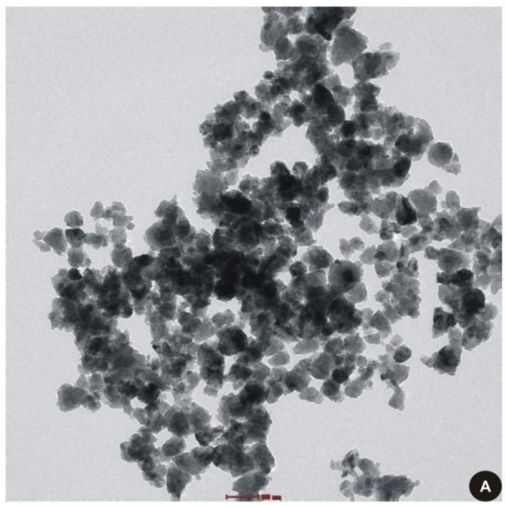

Fig-5: TEM analysis of ZnO-NPs of root extract of *R. Cordifolia.*

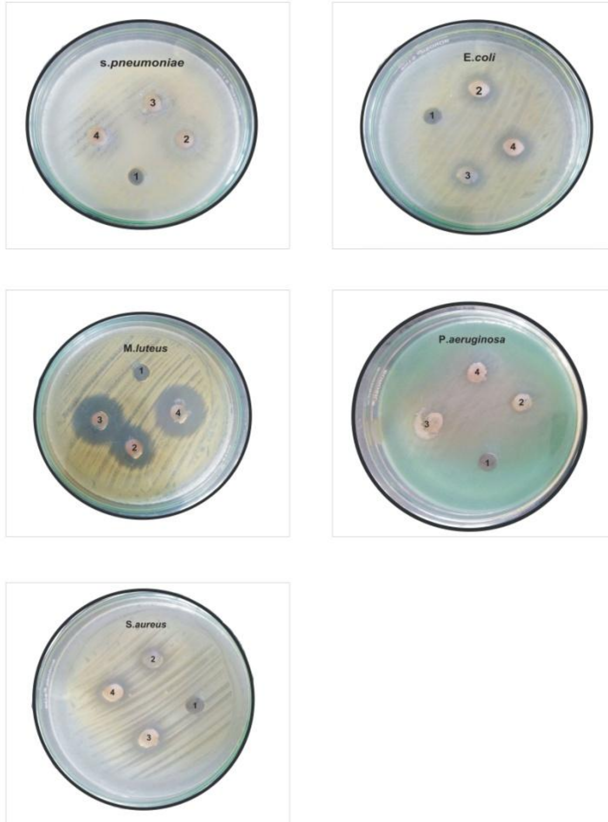

Fig-6: Antibacterial activity of ZnO-NPs of root extract of *R.Cordifolia* against different bacterial pathogens 1) control; 2)70 μL; 3) 100 μL; 4) 150 μL.

CONCLUSION

In the present work, we first report an eco-friendly and simple method for the synthesis of zinc oxide nanoparticles using *R. Cordifolia* root extract. XRD analysis reveals that the average size of the nanoparticles was found to be 14.12 nm which was calculated by Debye-scherrer equation. XRD and EDX results corroborated the purity of the synthesized ZnO-NPs. SEM analysis reveals the sphere structure of the nanoparticles. With the increasing drug resistance being reported among pathogens, searching for alternatives of effective antimicrobial therapy has become paramount. Synthesized ZnO-NPs using root extract of *R. cordifolia* show antibacterial activity against *M. luteus, E.coli* and *S.aureus*. The method of the present study offers several important advantageous features. The synthesis route is economical and eco-friendly, because it involves inexpensive and non-toxic materials for large scale synthesis.

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REFERENCES

1. Vidya C et. al., Green synthesis of ZnO nanoparticles by Calotropis Gigantea, *International Journal of Current Engineering and Technology*, 2013: special issue1:118-120.
2. Vijaylaxmee Mishra, Richa Sharma, Green Synthesis of Zinc Oxide Nanoparticles Using Fresh Peels Extract of *Punica granatum* and its Antimicrobial Activities, *International Journal of Pharma Research and Health Sciences*, 2015; 3 (3): 694-699.
3. P.Ramesh, A.Rajendran, M.Meenakshisundaram, Green Synthesis of Zinc Oxide Nanoparticles Using Flower Extract Cassia Auriculata, *Journal of Nanoscience and Nanotechnology*,2014; 2 (1): 41-45.
4. R.Sharmila Devi and R. Gayathri, Green Synthesis of Zinc Oxide Nanoparticles by using Hibiscus rosa-sinensis , *International Journal of Current Engineering and Technology*, 2014; 4 (4): 2444-2446.
5. Krishna r raghupathi, Ranjit t koodali, and Adhar c manna, Size –Dependent Bacterial Growth Inhibition and Mechanism of Antibacterial Activity of Zinc Oxide Nanoparticles , *Langmuir*, 2015; 27(7): 4020-4028.
6. G. Parthasarathy, Dr. M. Saroja , Dr. M. Venkatachalam , Dr. S. Shankar , V.K. Evanjelene , Green Synthesis of Zinc Oxide Nanoparticles- Review Paper, *World Journal of Pharmaceutical Sciences* , 2016; 5 (4) : 922-931.
7. Rupali Patil, Mahalaxmi Mohan, Veena Kasture and Sanjay Kasture, Rubia Cordifolia : A Review, *Oriental Pharmacy and Experimental Medicine*, 2009; 9 (1): 1-13.
8. Devi Priya M, E.A. Siril , Traditional and Moder n Use of Indian Madder (Rubia Cordifolia L.) : An Overview, *Int. J. Pharm. Sci.* , 2014;25 (1): 154-164.
9. Niranjan Bala, S. Saha, M. Chakraborty, M. Maiti, S. Das, R. Basub and P. Nandy , Green synthesis of zinc oxide nanoparticles using Hibiscus subdariffa leaf extract: effect oftemperature on synthesis, anti-bacterial activity and anti-diabetic activity,*The royal society of chemistry* , 2015; 5 : 4993-5003.
10. Ghorbani, Synthesis of ZnO by precipitation method, *Orient. J. Chem.*, 2015; 31(2): 1219-1221.
11. Andrews JM., Determination of minimum inhibitory concentrations. *J. Antimicrob Chemother*; 2001;48 Suppl 1: S5-6.

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