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Biosynthesis of ZnO Nanoparticles using *G. nepalense* Leaf Extract, Characterization and their Antibacterial Activity

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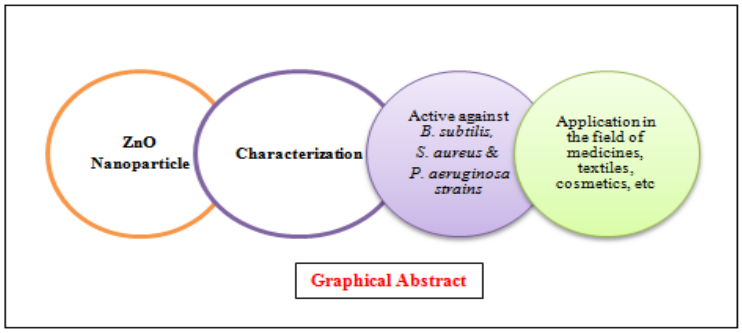
ABSTRACT

In this research work, crystalline nature and formation of ZnO nanoparticles using G. nepalense leaf extract was confirmed by XRD technique. UV-Vis spectrum showed the presence of characteristic absorption peak at 388nm of ZnO nanoparticles. Presence of water soluble phytochemicals which are responsible for the reduction of Zinc ions and stability of ZnO nanoparticles, were predicted by FTIR study. Antibacterial activity was investigated by determining the diameter of zone of inhibition against Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa strains using agar well diffusion method. Diameter of zone of inhibitions of ZnO nanoparticles at 35, 17 and 40 mm against Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa, respectively, were measured. In another study, MIC and MLC values were also determined.

Graphical Abstract:



Keywords: Crystalline, reduction, antibacterial activity.

INTRODUCTION

One of the unique behaviour of ZnO nanoparticle is antimicrobial behaviour and this behaviour proved to be very useful to control the rapid increase in the bacterial diseases/infections due to cytotoxic behavior of ZnO nanoparticles. Fiercely increase in the research on ZnO nanoparticleis due to its nano size because nano size particle has large surface area which results in the increase in number of active sites on the surface that enhance efficiency to act against the pathogens. But plant mediated synthesis of ZnO

nanoparticle glorifies this efficiency because of the presence of bioactive components in the plant extract [1,2]. These bioactive components having free hydroxyl, amino, carbonyl etc. groups play an important role in the reduction of Zinc ions and stability of ZnO nanoparticle. Plant extracts mediated i.e. biological synthesis of ZnO nanoparticles is also a green, cost effective and eco-friendly approach [3-12]. And in this research, work an attempt has been tried to synthesize ZnO nanoparticles using aqueous leaf extract of *G. nepalense*. *G. nepalense* (Geraniaceae) is commonly known as *Phori* or *Syunli*, perennial, hairy herb with creeping rhizomatous rootstocks. Leaves are sub orbicular, palmately 5-7 lobed, segments irregularly lobed, toothed, lower leaves petiolate and upper ones sessile. It is common in montane zones, above 1400m height, widely distributed in Himalaya, China, Japan and Myanmar. Plant infusion used in fever and renal disorders but roots paste is applied in itching [13]. A study on this plant proved that it contains polyphenolic compounds and of significant anti-inflammatory activity [14].Green Chemistry approach strives to achieve sustainability at the molecular level [15] and use of *G. Nepalese* leaf extract for the synthesis of ZnO nanoparticles is also a green approach.

Fig 1. *G. nepalense*

MATERIALS AND METHODS

Chemicals used in the preparation of ZnO nanoparticles were provided by the Department of Chemistry, HNB Garhwal University, Campus Pauri (Uttarakhand).

Preparation of aqueous leaf extract of *G. Nepalese:* Healthy leaves of *G. nepalense* were collected from the forests of Pauri Garhwal, identified from GUH, HNB Garhwal University, Uttarakhand and its accession no. **GUH 20756** was collected. Exactly weighed 05gm of dried finely powdered *G. nepalense* leaves was taken in a 250 ml Erlenmeyer conical flask containing 100ml deionized distilled water and heated for 25-30 min at 70^0C. Then leaf extract was filtered in a separate conical flask and stored for further research work.

Preparation ZnO nanoparticles: 50mL of *G. nepalense* leaf extract was taken in a 250mL of Erlenmeyer conical flask. It was heated on magnetic stirrer at 70^0 C for 10 min. Freshly prepared 100mL of 100mM Zinc acetate dihydrate solution was added drop by drop to it, maintaining temperature at 70^0C and 08-10 pH range of the solution adding few drops of 1M NaOH solution. Colour of the solution changed from light brown to yellowish brown and after few minutes, reduction of zinc ions began. Reaction conditions were maintained for 30 min, cooled at room temperature and solution containing reduced zinc ions was centrifuged for 10 min at 5000 rpm, then three times with water and ethanol, to remove undesirable/uncoordinated material. Yellowish material was dried in the oven at 100^0C, to collect the Zinc oxide nanoparticles.

Fig. 2 Images of plant extract, solution of reduced Zinc ions and ZnO nano-powder.

Determination of Antibacterial Activity

Culture Media and Inoculums: For antibacterial test, Soyabean casein digest agar of Hi Media Pvt. Bombay, India was used. The bacteria were inoculated into Soyabean casein digest agar and incubated at 37^0C for 18h and suspension was checked to provide approximately, 10^8 CFUmL^{-1}.

Microorganisms used: Pure cultures of test bacterial organisms viz. *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were used for the research work.

Determination of diameter of zone of inhibition by well diffusion method: The agar well diffusion method [16] was modified. Soyabean casein digest agar medium (SCDM) was used for bacterial cultures and culture medium was inoculated with the bacteria separately suspended in nutrient broth. A total of 8mm diameter wells were punched into the agar and filled with nanoparticles solution prepared in DMSO and solvent blanks. DMSO was used as negative control. Standard antibiotic (Erythromycin, 1mgmL^{-1}) was simultaneously used as the positive control. The plates were then incubated at 37^0C for 18h. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition. The procedure for assaying antibacterial and antifungal activity was performed in triplicates to confirm the readings.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC): MIC and MLC values of potent samples were determined by the method [17,18] with some modifications. Nanoparticles solution was prepared in highest concentration (100µL) in sterile DMSO and is serially diluted with N-saline (0.85% NaCl) and similar quantity of bacterial suspension was added to different test tubes and incubated for 48h.

RESULTS AND DISCUSSION

UV-Visible Spectral Analysis: UV-Vis spectrum of ZnO nanoparticles is shown in Fig 3a. The spectroscopic study was carried out at room temperature and absorption was measured at different wavelengths ranging from 250nm–700nm. Characteristic absorption peak at 388nm can be assumed to be of ZnO nanoparticles.

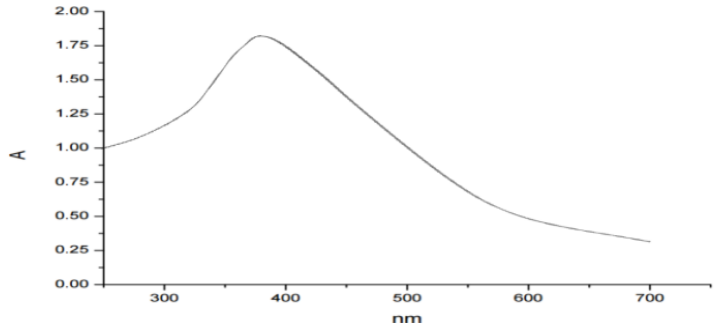


Fig 3a. UV-Vis spectrum of biosynthesized ZnO nanoparticles

XRD Analysis: Sharp diffraction peaks revealed that biosynthesized ZnO nanoparticles were made up of high quality crystals. All the diffraction peaks in the XRD pattern can be indexed to ZnO. The diffraction peaks at 2θ: 31.91°, 34.56°, 36.38°, 47.67°, 56.70° and 63.01° are respectively indexed to (100), (002), (101), (102), (110) and (103) planes of ZnO. Peaks confirmed that ZnO nanoparticles has been taken place. Slight dislocation of ZnO peaks may be due to the use plant extract.

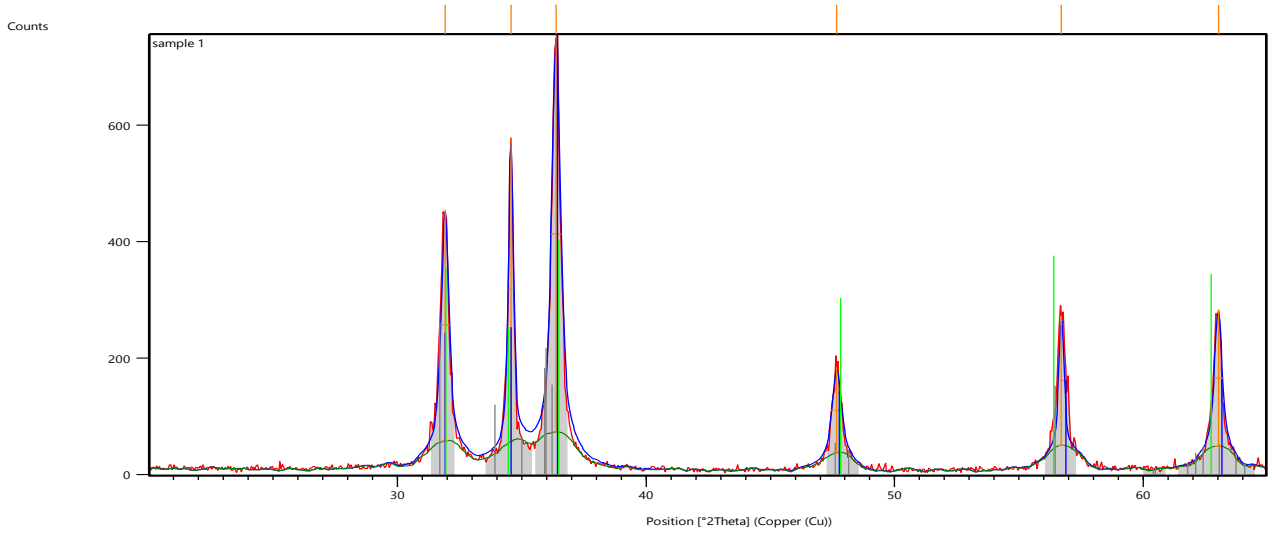


Fig 3b. XRD pattern of ZnO nanoparticles

TEM Micrograph: TEM micrograph (Fig. 3c) at 200 nanometer scale confirmed the size and shape of ZnO nanoparticles. From TEM micrograph, it can be seen that nanoparticles were distributed in the size ranging from 20-40 nm and spherical in shape. TEM micrograph is observed in acetone solution of the sample.

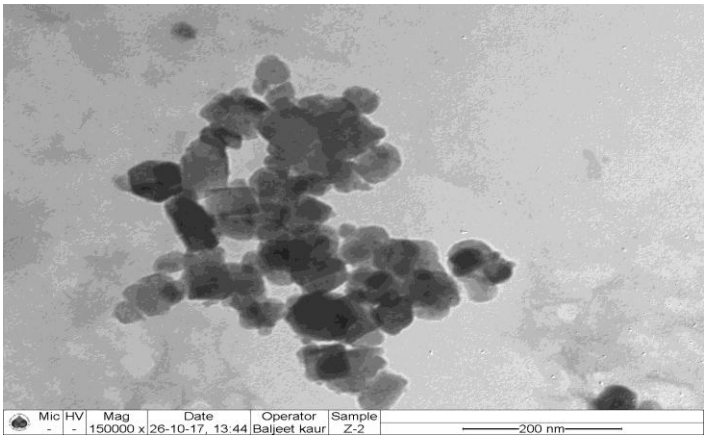


Fig 3c. TEM micrograph of ZnO nanoparticles at 200 nm scale

FT-IR Analysis: Absorption bands at 3410.10, 1585.83 and 1786.43 cm^{-1}corresponds to O-H, C=O and C=C stretching vibrations respectively, of polyols which involved in the synthesis and stability of ZnO nanoparticles. A study on this plant confirmed the presence of polyphenolic compounds [14].

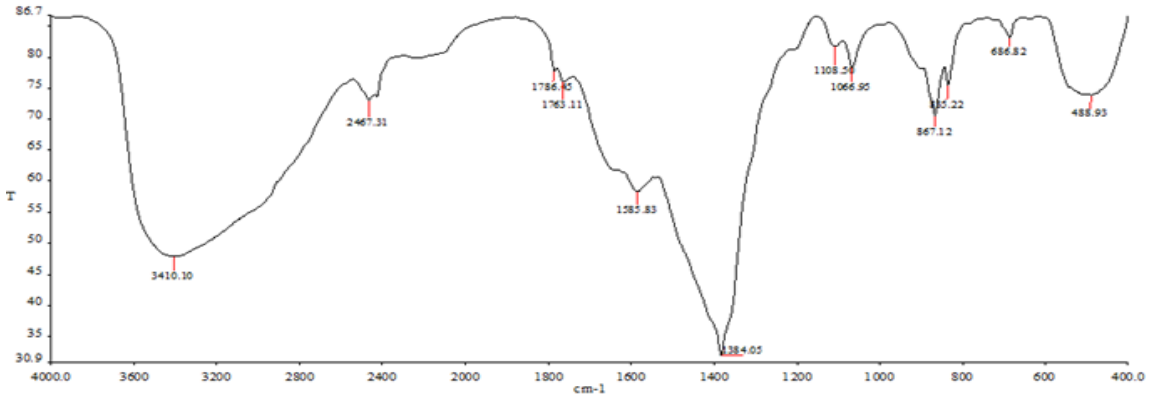


Fig 3d. FT-IR spectra of ZnO nanoparticles

Antibacterial Activity: Agar well diffusion method was used to determine antibacterial activity of biosynthesized ZnO nanoparticles against *B. subtilis*, *S. aureus* and *P. aeruginosa* strains. It was observed that these nanoparticles showed highest value of diameter of zone of inhibition against *P. aeruginosa* strain and lowest against *S. aureus* as given in the Table 1. MIC and MLC values of potent nanoparticles were found to be same for *Bacillus subtilis* and *Pseudomonas aeruginosa* but higher than *Staphylococcus aureus* (Table 2). And, it can be assumed that biosynthesized ZnO nanoparticles have potential antibacterial behaviour.

Table 1. Values of diameter of zone of inhibition

Sample/Controls (100 µL)	**Diameter of zone of inhibition (mm)**		
Nanoparticles	***Bacillus subtilis*** **(NCFT.583.08)**	***Staphylococcus aureus*** **(NCFT.576.08)**	***Pseudomonas aeruginosa*** **(NCFT.645.11)**
ZnO nanoparticles	35	17	40
Erythromyc in (1 mg/ml)	45	34	38

Table 2. MIC and MLC of the potent nanoparticles against the specific bacterial strains

Sample/Controls (100 µL)	**MIC and MLC (µL)**					
	Bacillus subtilis **(NCFT.583.08)**		***Staphylococcus aureus*****(NCFT.576.08)**		***Pseudomonas Aeruginosa*** **(NCFT.645.11)**	
	MIC	**MLC**	**MIC**	**MLC**	**MIC**	**MLC**
ZnO nanoparticles	20	30	6	12	20	30

APPLICATIONS

Biosynthesis of ZnO nanoparticles is a low cost, nontoxic and Eco friendly method. Resulted nanoparticles can be applied in the field of medicines, textiles, cosmetics, etc.

CONCLUSIONS

From the above investigation, it can be concluded that ZnO nanoparticles synthesized using *G. Nepalese* were highly crystalline, spherical and size in the range of 20-40 nm. Absorption band at 388nm showed the formation ZnO nanoparticles. FTIR data confirmed the presence of polyphenolic bioactive components in the ZnO samples. And biological study proved that biosynthesized ZnO nanoparticles had potential antibacterial activity.

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Conflict of Interest: The authors declare no conflict of interest.

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