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Green Synthesis of Zinc oxide nanoparticles using stem extract of *Withania somnifera* and its antibacterial effect

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Abstract- In this study, we report the synthesis of Zinc oxide nanoparticles highly explored in the field of nanotechnology, biotechnology, medicine and others fields. The nanoparticles were successfully synthesized by the acetate derivatives of zinc oxide and plant stem extract of the indigenous ayurvedic plant *Withania somnifera.* 10 mM of Zn $(CH_3COO)_2$. $2H_2O$ was mixed with the plant stem extract at different concentration and the reaction temperature was maintained at 65°C. The white colour paste obtained was dried, collected and packed for characterization. In UV-visible Spectrophotometer analysis absorption peak was observed at 303 nm, which is specific for Zinc Oxide nanoparticles. The hydrodynamic diameter of the synthesized nanoparticles ZnO NPs was observed to be 232 nm with zeta potential 13 ± 11 mV. The Field Emission Scanning Electron Microscope (FESEM) characterization reveals the presence of zinc oxide nanoparticle. In its clustered form, the average size of nanoparticles was measured to be 100 nm. All nanoparticles showed different degree of antibacterial activity against Gram Positive *Micrococcus luteus* (ATCC4698), *Staphylococcus aureus* (BAA2686) and Gram-negative bacteria *Escherichia coli* (PTA8019) and *Salmonella typhi* (ATCC29630). The antibacterial activity was inversely proportional to the size of the synthesized zinc oxide nanoparticles.

Key words: Green synthesis, Characterization, Zinc oxide nanoparticles and Antibacterial activity

INTRODUCTION

Nanotechnology has seen significant growth in the past decade, especially in its applications in medicine and biotechnology.[1-3] Nanotechnology has opened new possibilities in drug delivery, gene delivery, Nano medicine and biosensors.[4-5] Nanoparticles are of interest due to their high surface - to - volume ratio, which makes them highly reactive compared to bulk materials.[6-8] Nanoparticles can be synthesized through physical, chemical and biological methods. Green synthesis methods, which use materials like plant extract and microorganism, are cost - effective, nontoxic and eco-friendly.[9-12] The eco-friendly synthesis method reduces the usage of harmful substances since it makes use of microorganisms like fungi, bacteria, and algae as well as renewable resources like leaf, root, and flower extracts.[13-15] The focus of the work is on synthesizing ZnO nanoparticles from the plant *Withania somnifera*, known as 'Ashwagandha'.[16] Zinc oxide nanoparticles exhibit desirable optical properties, such as high electron mobility, large exciton for sensor fabrication.[17-18] *Withania somnifera* is a traditional medicinal plant belonging to the Solanaceae family. It has been used for various purposes, including as an antibacterial, antioxidant, aphrodisiac, liver tonic and anti-inflammatory agent.[19] The plant contains phyto chemicals, which are naturally occurring compounds, found in plants, are known for their health benefits. *Withania*

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somnifera, also known as "Indian Ginseng" has been traditionally used for rejuvenation and is an important component of Ayurvedic formulations.[20-23] The plants stems are reputed to have antioxidant, immune - modulatory and hematopoietic properties.[24-25]

MATERIALS & METHODS

Sample Collection:

The plant material, *Withania somnifera*, stem, was collected from the campus of Vinoba Bhave University Hazaribagh. It is a medically important plant. Fresh green stem was harvested during the month of June to December.

Preparation of *Withania somnifera* stem extract:

10 gm of fresh leaves were washed with running normal tap water followed by distilled water and then cut into small pieces, in 250 ml Erlenmeyer flask containing 100 ml distilled water. The soaked pieces were boiled at 65°C for 15 minutes and then extract was allowed to cool down to room temperature, filtered through Whatman No. 1 filter paper and was stored for further use for one week.

Synthesis of Zinc oxide nanoparticles (ZnO NPs):

10mM Zinc acetate dihydrate Zn $(CH_3COO)^2.2H_2O$ was dissolved in 100ml distilled water. Then 2 ml of NaOH solution was added drop wise into the Zinc acetate solution and 10 ml of *Withania somnifera* plant stem extract was added to the same. The colour of the reaction mixture was changed to white colour after 48 hrs of incubation time, showing the synthesis of zinc oxide nanoparticles. The precipitate was separated from the reaction solution by centrifugation at 5000 rpm for 20 min and pellet was collected. Pellet was dried, using a hot air oven at 70°C and preserved for further experimental use.

Physicochemical Characterization

The physicochemical characterization of zinc oxide nanoparticles was done using standard techniques. UV - Visible spectroscopy was done to determine the optical properties of the nanoparticles. The spectrum scan was performed at a range of 200 - 800 nm using a UV-visible spectrophotometer (Carry 5000, Agilent, Santa Clara, CA, USA) to perform the scanning. Further the hydrodynamic size and zeta potential of the ZnONPs was determined in the disc diffusion medium through dynamic light scattering (DLS) using zetasizer (Malvern, UK). The size of the zinc oxide nanoparticles was further determined by electron microscopy by using FE-SEM (Carl Zeiss, Jena, Germany).

Antibacterial activity of synthesized zinc oxide nanoparticles

The antibacterial activity of synthesized zinc oxide nanoparticles was performed by well diffusion medium, *Escherichia coli, Micrococcus luteus, Staphylococcus aureus* and *Salmonella typhi*. Fresh overnight culture of each strain was washed regularly onto the individual plate. The 25µl, 50 µl, 75 µl and 100 µl of Zinc oxide nanoparticles solution were placed onto the plates and incubated for 24 hours at 37°C. Commercial distilled water was placed as control. After incubation period got over, different levels of zone of inhibition were observed and measured.

RESULT & DISCUSSION

Green synthesis and characterization of zinc oxide nanoparticles

The green synthesis of zinc oxide nanoparticles (ZnO NPs) was carried out by using *Withania somnifera* stem extract as shown (figure 1). The synthesized nanoparticles were dried and suspended in an aqueous medium for characterization. The physical and optical characteristics of the nanoparticles were identified. The UV-Visible spectrum analysis exhibited a sharp peak at a 303 nm (figure 2A) with a small broadness of the curve presenting the presence of some intermediates. The size and morphology of zinc oxide nanoparticles determined by FESEM showed multiertities shape with an average size of 100 nm (figure 2B). On determining of stability and size, using dynamic

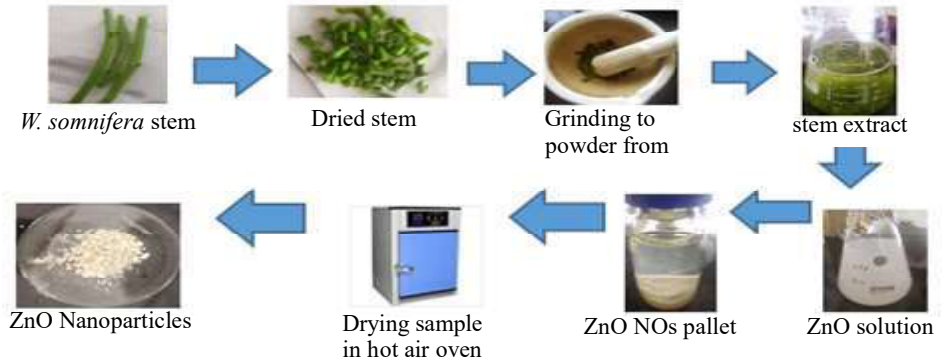

Figure 1. Preparation of ZnO NPs using *Withania somnifera* Stem extract.

light scattering (DLS), showed the hydrodynamic diameter of ZnO NP as 126±10 nm in aqueous medium (figure 2C). Zeta potential was found to be 19±08 mV in aqueous medium (figure 2D).

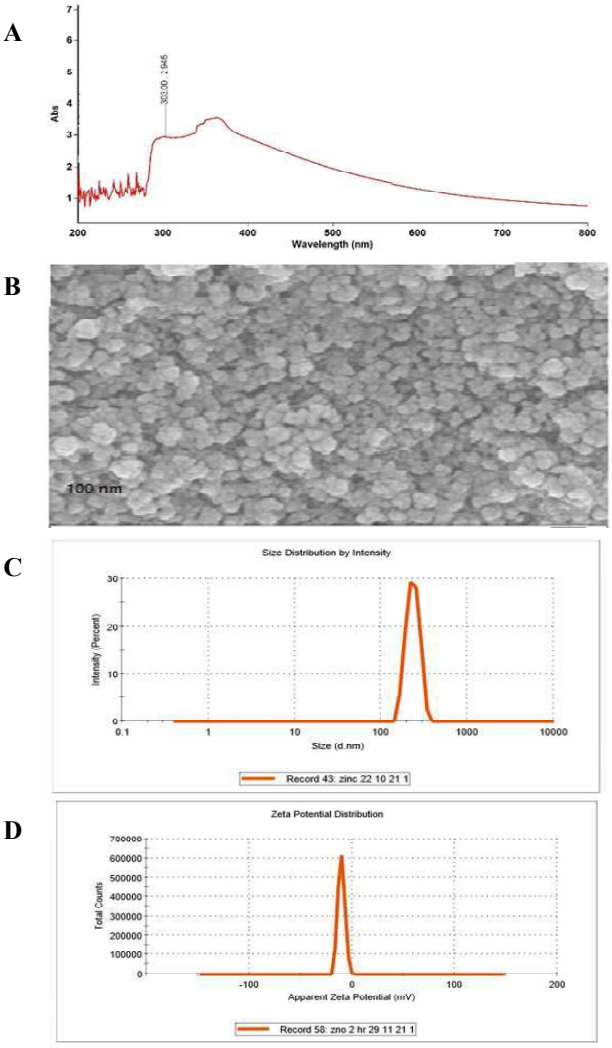

Figure 2:- Physicochemical characterization of green synthesised ZnO nanoparticles (A). UV - Visible spectroscopy of Zinc Oxide nanoparticles (ZnO NPs) (B). Optical image of ZnO NPs determined by FESEM, (C). Hydrodynamic diameter of ZnO NPs as determined by dynamic light scattering, and (D). Zeta potential of ZnO NPs determined by dynamic light scattering

Antibacterial efficiency of ZnO NPs

Antibacterial efficiency of zinc oxide nanoparticles was checked against four bacterial strains, gram - positive (*Micrococcus luteus* and *Staphylococcus aureus*) and gram - negative (*Escherichia coli* and *Salmonella typhi*), and their zone of inhibition was measured through well diffusion analysis as shown in figure 3 and table 1, the zone of inhibition against both bacterial strains was found to increase with an increasing in the concentration of Zinc oxide nanoparticles.

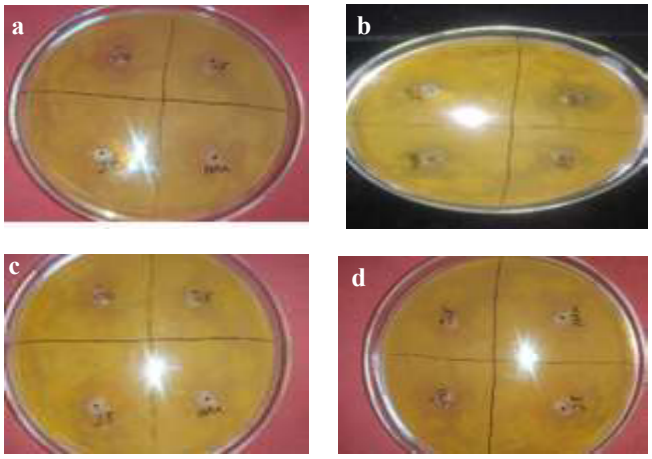

Figure 3: Antibacterial activity of ZnO NPs against (a) *E.coli* (b) *M.lureus* (c) *S. typhi* (d) *S.aureus*

Table 1: - Antibacterial properties of synthesized zinc oxide nanoparticles

Concentration of ZnO NPs	Zone of inhibition (mm)			
	E. coli	*Micrococcus luteus*	*Salmonella typhi*	*S. aureus*
25 µg/ml	04±1	07±1	05±1	06±1
50 µg/ml	06±2	09±2	06±1	08±1
75 µg/ml	07±2	11±2	09±2	09±2
100 µg/ml	9±1	12±1	11±2	11±2

CONCLUSION

In brief the present study describes a successful green synthesis of zinc oxide nanoparticles (ZnO NPs) using stem extract of *Withania somnifera* and characterization of their physicochemical properties. Zinc oxide nanoparticles were stable with a size of 100 nm and have the standard physicochemical properties with high antibacterial efficiency against both Gram - positive and Gram - negative bacterial strains.

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