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Brassica oleracea Mediated Synthesis of Zinc Oxide Nanoparticles and its Antibacterial Activity against Pathogenic Bacteria

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Green synthetic methods of nanoparticles are simple, non-toxic and environmentally benign process which decline the demerits of conventional physical and chemical methods. The current study focusses on the synthesis of zinc oxide nanoparticles using fresh *Brassica oleracea* extract. Zinc oxide nanoparticles were characterized using UV-vis spectrophotometry, which showed exciton absorption peak at 380 nm. X-ray diffraction analysis, scanning electron microscopy, atomic force microscopy, energy dispersive X-ray analysis and thermogravimetric analysis were used to determine the shape, size, crystallinity and purity of nanoparticles. The antibacterial activity of zinc oxide nanoparticles was determined by agar well diffusion method against Gram-positive and Gram-negative bacteria.

Keywords: Zinc oxide nanoparticles, Green synthesis, *Brassica oleracea* extract, Antibacterial activity.

INTRODUCTION

Nano-sized materials are known to play important role in basic and applied sciences thus the metal and metal oxide nanoparticles have been intensively studied in the past decade [1,2]. The metal oxides particularly exhibit fascinating properties such as electronic, catalytic, magnetic and antimicrobial activity owing to their large surface area to volume ration due to a high fraction of atoms [3,4]. Zinc oxide nanoparticles have a wide band gap of 3.3 eV at room temperature and high excitation binding energy of 60 meV, which helps it to demonstrate outstanding catalytic, optical, photochemical and electronic properties [5,6]. Zinc oxide nanoparticles are known to possess unique UV filtering, antibacterial, antifungal properties also which leads to its extensive use in cosmetic and healthcare industries [7-9]. Previous studies suggest that the strong antibacterial activity of zinc oxide nanoparticles against a variety of pathogenic Gram-positive and Gram-negative bacteria attributed to its capacity of releasing Zn^{2+} ion from ZnO complex and interruption of lipid bilayer integrity, ROS generation and subsequent damage to DNA and protein of the host cell [10,11]. Chemical and physical methods of nanoparticle synthesis have been replaced by feasible eco-friendly alternatives like nanoparticle synthesis using microorganisms, organic sugar, starch, enzymes and plants [12,13]. Plants extracts act as a reservoir of several pharmacologically active metabolites and allow the synthesis of nanoparticles in well-defined size and shape controlled manner [14]. *Brassica oleracea* contains a wide range of biologically active compounds like glucosinolates, polyphenols, alkaloids, flavonoids and micronutrients such as vitamin C, carotene and folic acid-fibers [15]. The present work aims to investigate the role of *Brassica oleracea* extract in synthesis and stabilization of ZnO nanoparticles. The morphology and crystal structure were characterized using UV-visible spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), atomic force microscopy (AFM), energy dispersive X-ray analysis (EDAX) and thermogravimetric analysis (TGA). The antibacterial activity of zinc oxide nanoparticles was evaluated using agar well-diffusion method against *Escherichia coli* and *Streptococcus pyogenes* and *Rhizobacteria*.

EXPERIMENTAL

UV-visible spectrophotometer with a wavelength range of 300-700 nm was used for confirmation of nanoparticle synthesis. X-ray diffractometer operating at 30 mA and 40 kV was used to determine the purity and crystallinity of the nanoparticles. SEM analysis was conducted to analyze the surface morphology of the nanoparticles. Nanoparticles were sputtered on a carbon coated copper grid for EDAX analysis. AFM was used to determine the particle size and distribution of the nanoparticles. TGA analysis was done to analyze the change in a physical and chemical property of biosynthesized zinc oxide nanoparticles with temperature.

Preparation of *B. oleracea* extract: *Brassica oleracea* (10 g) was washed with running tap water and then rinsed with Milli-Q water. Leaves were dried, chopped and soaked in 100 mL Milli-Q water. The solution was boiled at 60 ºC for 30 min and then allowed to cool at room temperature. *Brassica oleracea* extract was filtered through Whatman filter paper No. 1 and the filtrate were stored at 4 ºC for analyses [16].

Biosynthesis of ZnO nanoparticles: *Brassica oleracea* extract (25 mL) was added to 75 mL of 1 mM of zinc sulfate solution in a 250 mL Erlenmeyer flask and the reaction mixture was incubated at 80 ºC for 24 h, 100 rpm. Reduction of zinc ions to zinc nanoparticles was confirmed using visual observation and UV-visible spectroscopy after 24 h. ZnO nanoparticles were collected by centrifuging the reaction mixture at 8000 rpm for 15 min. ZnO nanoparticles were double washed using Milli-Q water and then overnight dried in a hot air oven. Obtained ZnO nanoparticles was used for characterization.

Antibacterial activity of zinc oxide nanoparticles: The agar-well diffusion method was used to determine the antibacterial activity of ZnO nanoparticles. Different concentration of zinc oxide nanoparticles was tested against *Escherichia coli* (Gram -ve), *Streptococcus pyogenes* (Gram +ve) and *Rhizobacteria*. The fresh bacterial suspension was dispersed on the surface of nutrient agar plates. Different concentration of nanoparticles (20, 40 and 60 µL) was incorporated into the wells and the plates were incubated at 37 ºC for 24 h. Ampicillin was used as positive control. Zone of inhibition was recorded in each plate [17].

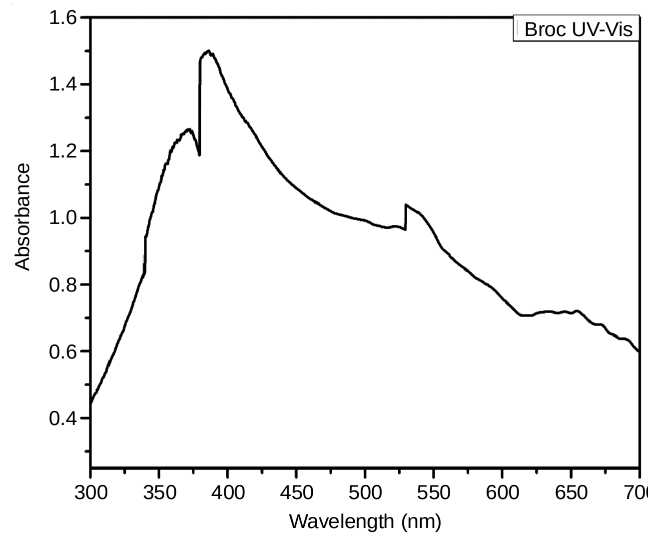

Fig. 1. UV-visible spectrum of zinc oxide nanoparticles

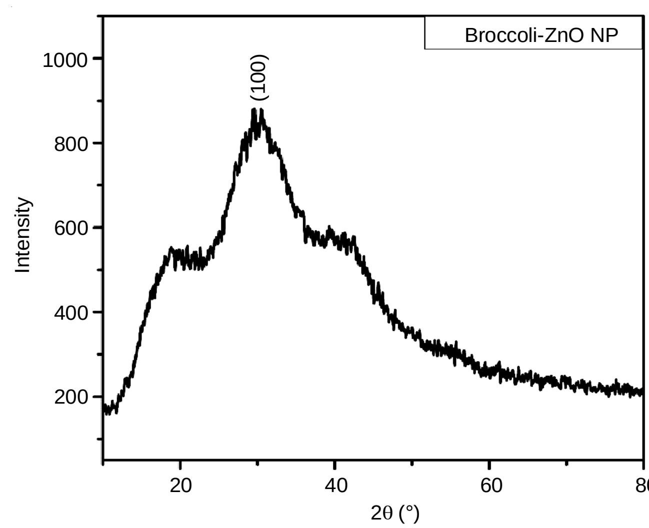

Fig. 2. XRD spectra of *B. oleracea* extract synthesized ZnO nanoparticles

RESULTS AND DISCUSSION

UV-visible analysis: UV-visible absorption spectroscopy is widely used for the confirmation of nano-sized particles. Fig. 1 shows strong exciton absorption peak at 380 nm wavelength which confirmed the synthesis of zinc oxide nanoparticles synthesized from *Brassica oleracea* extract, which is in good agreement with green synthesis of zinc oxide nanoparticles using *Pongamia pinnata* and *Cassia fistula* [18,19].

XRD analysis: X-ray diffraction analysis was conducted to confirm zinc oxide phase of the nanoparticles. The XRD peak identified at (100) reflection at 31.74º can be indexed to the spherical zinc oxide phase (Fig. 2). Result obtained was in agreement with the data from JCPDS file and lemon leaf mediated zinc oxide nanoparticles [20]. The crystalline structure of the particles was confirmed by the strong diffraction peak obtained.

SEM and EDX analysis: SEM analysis was conducted to study the morphology of nanostructures. Some agglomerates particles were also found. SEM image suggests the synthesis of ZnO nanoparticles with an average size of about 80 nm (Fig. 3A). The signal characteristic peak of only zinc and oxygen was further analyzed using EDAX analysis. Presence of Zn and O alone without any unknown signals suggests the purity of ZnO nanoparticles (Fig 3B). The results obtained are in accordance with root extract of *Zingiber officinale* assisted green synthesized metal oxide nanoparticles [21].

AFM analysis: The topography of *Brassica oleracea* synthesized zinc oxide nanoparticles were recorded using AFM analysis (Fig. 4). AFM analysis revealed the monodispersed nature and spherical shape of ZnO nanoparticles. The zinc oxide nanoparticles synthesized using *Passiflora indica* also in agreement with the present results [22].

TGA analysis: Thermogravimetric analysis (TGA) analysis revealed the changes in physical and chemical property of the biosynthesized zinc oxide nanoparticles at room temperature to 800°C at 20 ºC/min. Thermogram of synthesized ZnO nanoparticles has been shown in Fig 5. The curve represents mass loss between 30-120 ºC by 9.453 % due to evaporation of surface water. The mass loss continues up to 500 ºC by the rate of 50.65%.

Antibacterial activity of zinc oxide nanoparticles: Antibacterial activity ZnO nanoparticles were tested against the bacterial pathogens *Escherichia coli*, *Streptococcus pyogenes* and *Rhizobacteria* by agar well diffusion method and the zone of inhibition

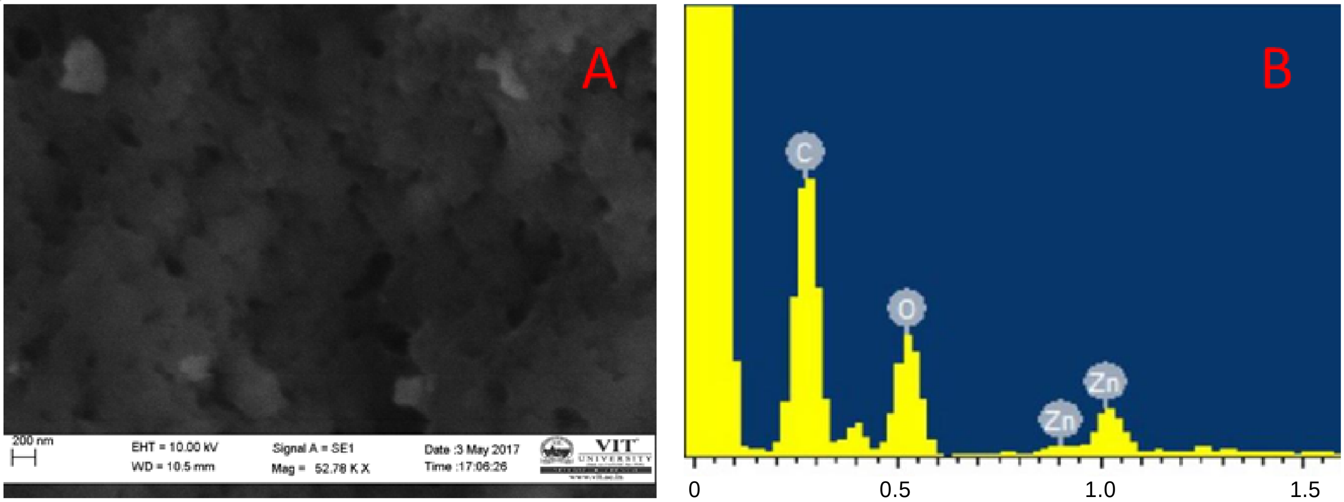

Fig. 3. (A) SEM micrograph of ZnO nanoparticle (B) EDAX of ZnO nanoparticle

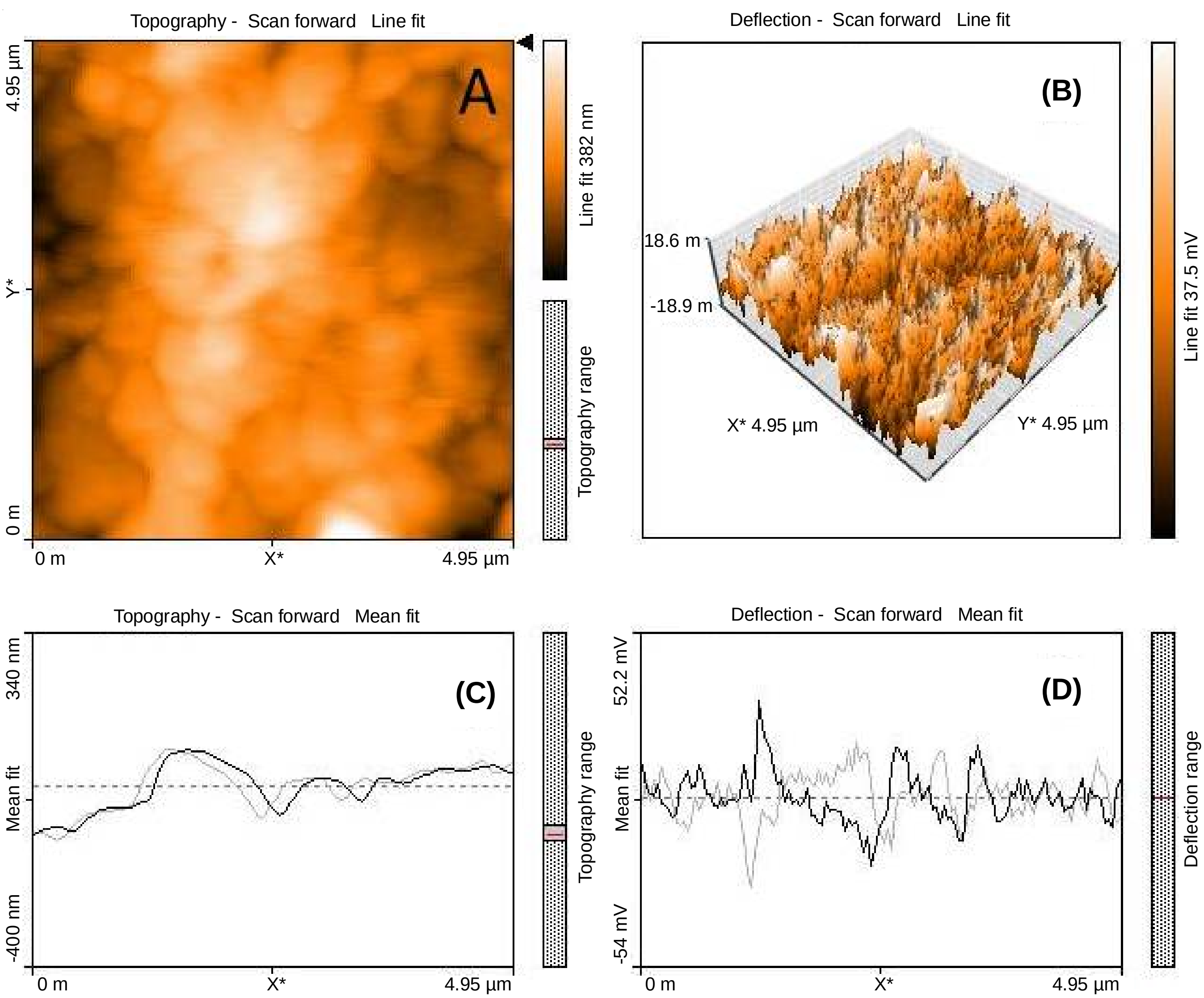

Fig 4. AFM results of zinc oxide nanoparticles (A) Topographical 2D image of zinc oxide nanoparticles (B) 3D image of the zinc oxide nanoparticles. (C) & (D) particle size distribution

values were determined. The highest zone of inhibition was found in *Rhizobacteria* with a zone diameter of 28 mm at 60 µL concentration and the lowest zone of inhibition was found to occur for *Escherichia coli* at a concentration of 20 µL. Moderate zones of inhibition were observed for *Streptococcus* pyogenes at all the concentrations. The plants are the major sources used

TABLE-1
GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES DIFFERENT PLANT EXTRACT

S. No.	Name of the plant	Size (nm)	Shape	Ref
1	*Azadirachta indica*	20-45	Hexagonal	[25]
2	*Cassia fistula*	5-15	Hexagonal and sponge like irregular shape	[18]
3	*Solanum nigrum*	20-30	Hexagonal quasi-spherical	[26]
4	*Moringa oleifera*	16-20	Hexagonal	[27]
5	*Nephelium lappaceum*	50	Needle-shaped and hexagon	[28]
6	*Couroupita guianensis Aubl.*	Not mentioned	Nanoflakes	[29]
7	*Pongamia pinnata*	30.4-40.8	Spherical	[30]
8	*Caltropis procera*	90-100	Not mentioned	[31]
9	*Carica papaya*	11	Nano-flower	[32]
10	*Plectranthus barbatus*	30-60	Spherical	[33]
11	*Mangifera indica*	45-60	Spherical and hexagonal quartzite	[34]
12	*Brassica oleracea*	80	Spherical	This work

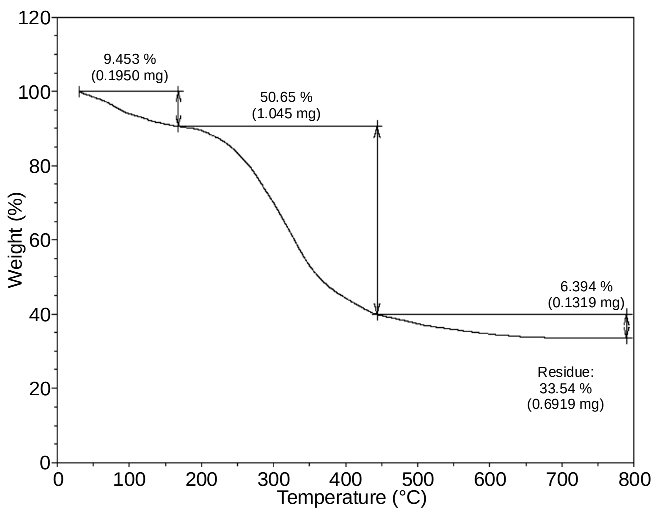

Fig. 5. Thermogravimetric analysis of synthesized ZnO NPs

for the synthesis of zinc oxide nanoparticles with different shape and size is shown in Table-1. Therefore, the present finding clearly suggests the potential application of *Brassica oleracea* extract prepared ZnO nanocrystals as an excellent antibacterial agent. The zinc oxide nanoparticles are actively involved in the antibacterial activity and may used for different types of biomedical applications based on this property [10,22-24].

Conclusion

In the present study, zinc oxide nanoparticles were synthesized using *Brassica oleracea* extract and characterized using UV-visible spectroscopy, XRD, SEM, EDAX, AFM and TGA. SEM analysis revealed the synthesis of spherical nanoparticle with individual particle size range of 80 nm and formation of aggregates. The ZnO nanoparticles were found to have spherical structure with an absorption maximum at 380 nm assigned to the intrinsic band-gap absorption. XRD and EDAX confirmed the synthesis of pure crystalline ZnO nanoparticles. AFM suggested the monodispersed nature of ZnO nanoparticles. Effect of ZnO nanoparticles for their antibacterial potential was tested against *Escherichia coli*, *Streptococcus pyogenes* and *Rhizobacteria* by agar-well diffusion method, which showed good activity against all the three tested bacterial pathogens. This study conclusively reports an eco-friendly approach for the synthesis of zinc oxide nanoparticles and highlights its potential to be used as an effective antibacterial agent.

CONFLICT OF INTEREST

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

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