

# Antimicrobial applications of green synthesized zinc oxide nanoparticles

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**Abstract:** Zinc oxide (ZnO) nanoparticles were fabricated by green process in presence of aqueous extract of Watermelon rind. High Performance Liquid Chromatography technique was used to assess the biomolecule content of the aqueous extract prepared from watermelon rind. Citrulline an essential amino acid present in watermelon rind was found to act as capping agent for fabrication of ZnO nanoparticles. The shapes of produced ZnO nanoparticles were found to be anisotropic with few nano rods which were confirmed by TEM analysis. The prepared ZnO nanoparticles were used as antimicrobial agent against pathogens. Higher activity was found against *S. paratyphi B* strains. Further, the ZnO nanoparticles were assessed for its heavy metal removal capacity from aqueous solution. The results suggest that watermelon rind can be a potential precursor for fabrication of ZnO nanoparticles with versatile applications.

**Keywords:** Watermelon rind; Nanoparticles; Biomimetic; Adsorption; Antimicrobial

## 1. Introduction

In recent years, application of nanoparticles within size range of 1-100nm has received significant attention due to their novel properties and has come up as an area of extensive research and widespread applications in various fields [1-3]. Several physical and chemical procedures have been used for synthesis of ZnO nanoparticles in relatively short period [4]. But the process of physical and chemical methods is limited because of cost and time-consuming process. To fulfill the growing need of environmentally friendly nanoparticles, researchers are using microorganisms for synthesis of various metal nanoparticles [5]. The maintenance of culture and media limits the application of biological routes of synthesis. Agro waste materials contain rich organic functional groups which exhibit diverse applications. Many agro waste products contain variety of macro molecules. The use of agrowaste for synthesis of noble metal nanoparticles are recently explored and found to be effective route [6]. The organic molecules present in agrowaste products acts as capping cum reducing agent. Though the use of agrowaste products are explored for synthesis of noble metal nanoparticles, the application in fabrication of metal oxides and sulfides are not fully explored. Hence, the present study reports the use of agricultural waste product for synthesis of ZnO nanoparticles. An

extensive literature survey revealed that there are no reports on the synthesis of ZnO nanoparticles using agricultural wastes such as watermelon rind.

Watermelon (*Citrullus lanatus*) rind, a common agricultural by-product, is a natural and rich source of the non-essential amino acid citrulline containing abundant carboxyl and amino groups, which has remarkable capability of binding heavy metals from aqueous solutions [7]. Watermelon rind (WR) consists of pectin, citrulline, cellulose, proteins and carotenoids [8,9]. Only half of the fruit is considered to be edible leaving the other half like rind and peel as waste products. Earlier watermelon rind has been used as novel biosorbent for removal of heavy metals and dyes from aqueous solution [10,11]. In order to reduce the agro-waste, a novel method has been proposed to make effective utilization of watermelon rind for biosynthesis of ZnO nanoparticles.

In the present study, ZnO nanoparticles have been developed in bioinspired method using watermelon rind aqueous extract. The ZnO nanoparticles have been characterized by UV-VIS, XRD, TEM techniques. Further the potential of ZnO nanoparticles have been evaluated as nano sorbent for removal of Pb<sup>2+</sup> and Cd<sup>2+</sup> ions from aqueous solution and as antimicrobial agent against pathogens.

## 2. Material and methods

### 2.1 Preparation of extract

The watermelon rind was processed to powder according the procedure reported in literature [10]. The aqueous extract was prepared by adding 1 g of dried watermelon rind powder to 50 ml of distilled water and boiled in a water bath at 85 °C for 20 min. Later, the cooled extract solution was filtered using whatman filter paper and fresh supernatant solution was used immediately for experimental purpose. The supernatant solution was named as Watermelon Rind Extract (WRE)

### 2.2 Synthesis of ZnO nanoparticles

For synthesis of ZnO nanoparticles, 0.25 M Zinc acetate solution was prepared in distilled water. To 50 ml of zinc acetate solution, ammonium hydroxide was added continuously drop by drop until precipitation got completed. The reaction mixture was stirred in a magnetic stirrer at room temperature and freshly prepared 10 ml of WRE was added and stirred overnight. The reaction mixture was centrifuged at 10000 rpm for 15 min and the supernatant solution was discarded and pale white solid obtained was dried at 75 °C for 3 h in hot air oven. The dried precursor obtained was powdered and characterized.

### 2.3 Detection of capping agents

In order to identify the biomolecule's/capping agents present, High Performance Liquid Chromatograph analysis (Waters 1525 Binary pump) equipped with UV detector (Waters 2487 dual wavelength) was carried out for WRE. Prior to sample injection the system was equilibrated for 15 min with 0.01 % Ortho phosphoric acid and methanol (75:25) as mobile phase at a flow rate of 1ml min<sup>-1</sup>. The detector was set at 207 nm and 20 µl of WRE was injected into C18 column (Waters symmetry) with a runtime of 30 min. Different standards were also run at the above optimised conditions for detection of major compounds.

### 2.4 Adsorption studies

The stock solution of Pb<sup>2+</sup> and Cd<sup>2+</sup> ions was prepared by dissolving appropriate nitrate salts and diluted to required concentration with double distilled water. Batch mode adsorption experiments were employed to access the capability of ZnO NPs to remove the toxic metals such as Pb<sup>2+</sup> and Cd<sup>2+</sup> ions from aqueous solution. To 20 ml of varying concentrations (50-200 mg L<sup>-1</sup>) of metal solutions 1 g L<sup>-1</sup> of ZnO NPs were added and agitated in a rotospin unit at 50 rpm for 30 min in room temperature. After agitation the solution was centrifuged at 10000 rpm for 10 min and the resulting supernatant solution was subjected to Atomic Absorption spectrometer (AA240, Varian, Australia) to know the residual metal concentration.

### *2.5 Antimicrobial studies*

The screening of antibacterial activity for the ZnO NPs suspension was conducted adapting the methodology of Mehmood et al., [12]. The bacterial test pathogens were maintained in Nutrient broth and incubated overnight in shaker to reach the bacterial population of 10<sup>8</sup> CFU/ml. Muller Hinton agar (per liter of distilled water: 300 g beef infusion, 17.5 g casein acid hydrolysate, 1.5 g starch, 20 g agar, pH 7.2) was freshly prepared and dispensed into sterile petri plates. Wells of 8 mm diameter were punched into the agar medium and filled with 50 µl of WRE ZnO NPs. Nutrient broth was loaded as negative control and antibacterial antibiotic disk streptomycin was used as positive control. The plates were incubated for 4 days at 28 ± 2 °C and monitored for every 12 hours. The antibacterial activity was evaluated by measuring the inhibition halo against test pathogens

## **3. Results and discussion**

Watermelon rind is known to be rich in phenols, cellulose and proteins. An attempt was made to identify the phyto constituents of WRE by HPLC analysis. HPLC chromatogram of WRE displayed six peaks at different retention times (RT) depicting presence of six different molecules. Similarly, the WRE after synthesis of ZnO NPs was subject to HPLC and found to have six signals. It was observed that the intensity of peak at RT 3.5 was found to diminish after fabrication of nanoparticles. This observation suggested that the peak at RT 3.5 might have bound to ZnO NPs as capping agent. In order to identify the molecule at RT 3.5 various standards (based on literature reports) were run at similar chromatographic conditions. The RT of standard Citrulline was found to match with the RT of peak identified as capping agent for ZnO NPs formation. Citrulline which is considered as mere metabolic intermediate especially in the synthesis of urea cycle is found abundantly in watermelon rind [7,13 and 14].

### *3.1 Characterization of ZnO NPs*

An UV-Vis spectrum is an important technique to ensure the formation of nanoparticles provided surface plasmon resonance (SPR) exists for the metal [15]. The SPR arises due to the collective oscillation of free conducting electrons induced by an electromagnetic field. The formation of ZnO NPs was initially confirmed by UV-Vis spectrometer (Hitachi Model: U-2800 double beam spectrometer). The UV-Vis spectrum of ZnO NPs exhibits a sharp peak around 370 nm due to its Surface Plasmon resonance (Fig not shown here). Similar observations have been earlier reported for ZnO NPs by Sangeetha et al [4] for aloe barbadensis miller leaf extract and Jayaseelan et al [16] for *Aeromonas hydrophila* bacteria.

Powder XRD (Bruker D8 Advance model) analysis of WRE fabricated ZnO NPs was carried out to confirm the structure (Fig.1). The XRD pattern shows various distinct diffraction peaks at 31.74, 34.41, 36.23, 47.52, 56.57, 62.85, 66.37, 67.90, 69.06, 72.59 and 76.91 2θ values, which could be assigned to (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202) planes of Wurtzite structure. The strong and narrow

diffraction peaks of ZnO NPs indicate that the product has well crystalline structure. The Scherrer formula was applied to the major intense peaks and average particle size was found to be around 33nm.

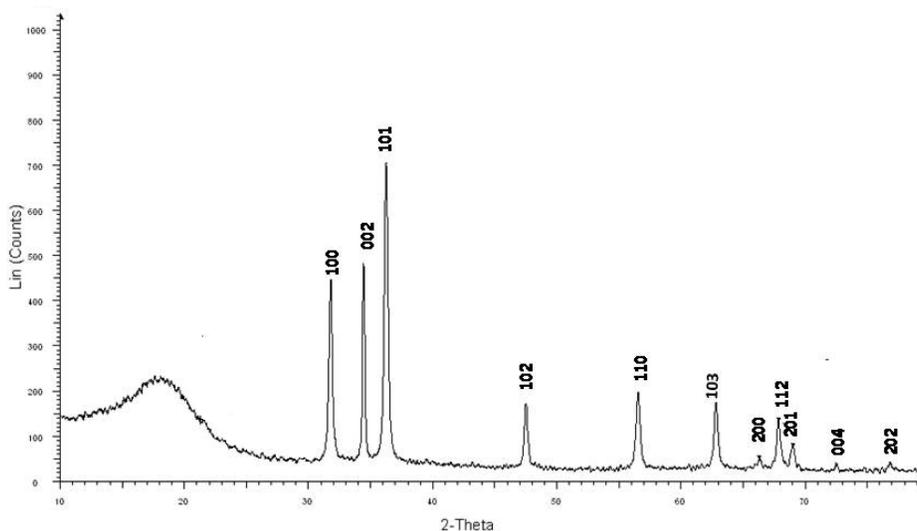


Fig.1 XRD pattern of ZnO NPs mediated by WRE

The morphology and size of the particles is determined by TEM (Philips, CM200 model) images and are shown in Fig. 2. The TEM observations of the samples indicate the shape anisotropy and the particles display rich variety of shapes in varying sizes. In addition to nanospheres, some pronounced anisotropic nanostructures and nano rods were observed. These nanoparticles size are found roughly in the range of 20-55nm for anisotropic structures and 12nm for nano rods. The selected area electron diffraction (SAED) depicted in Fig. 2 exhibit concentric rings with intermittent bright rods, indicating that these nanoparticles are highly crystalline in nature. These finds are corroborated with the XRD pattern (Fig. 1) of the synthesized ZnO nanoparticles.

### 3.2 Application of ZnO NPs as nano adsorbent

The ZnO NPs were used as nanosorbent for the removal of  $Pb^{2+}$  and  $Cd^{2+}$  ions from aqueous solution. The results of the experiments are represented in Fig.3. It can be seen from Fig.3 that the percentage removal of both the metal ions are very high at different initial metal ion concentrations (50-200 mg L<sup>-1</sup>). The maximum sorption capacity of ZnO NPs was found to be 176.2 and 153.4 mg g<sup>-1</sup> for  $Pb^{2+}$  and  $Cd^{2+}$  ions respectively. The sorption was found to be rapid for both the metals which depict the practical utility of the ZnO NPs in real time process for the treatment of industrial effluents loaded with metal ions. The high sorption capacity can be attributed to high specific surface area and the capping agent present on the surface of the ZnO NPs. In the present study the capping agent citrulline which are believed to be bound to ZnO NPs are well known binding agents for metal ions. Further study on the optimization of removal process, kinetics and isotherms are in progress.

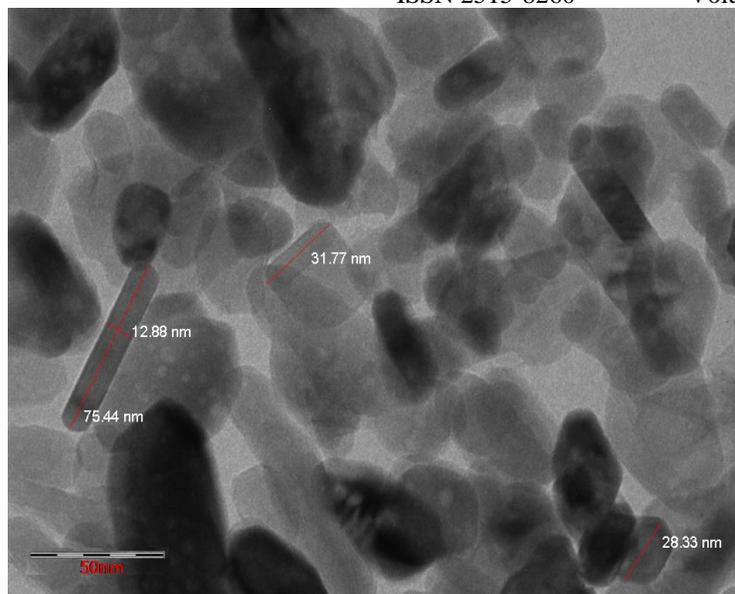


Fig.2 TEM image of synthesized ZnO NPs mediated by WRE

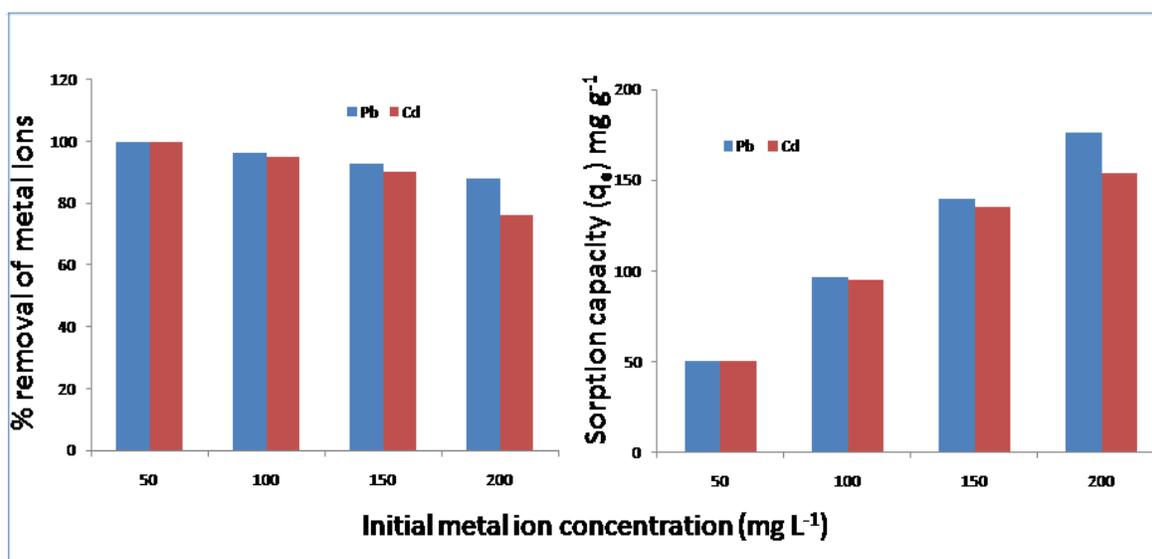


Fig.3 Plots of percentage removal and loading capacity of synthesized ZnO NPs towards Pb<sup>2+</sup> and Cd<sup>2+</sup> ions from aqueous solution

### 3.3 Antimicrobial activity

Recently several research groups have suggested that the ZnO nanoparticles have excellent antimicrobial activity against various pathogens. In view of this, the as synthesized ZnO nanoparticles were evaluated as antimicrobial agent against gram positive and gram-negative strains. The pathogens selected and the zone of inhibition values is summarized in Table.1. Interestingly it was observed that the zones of inhibition against the test pathogens exhibited by ZnO nanoparticles were very high compared to standard streptomycin disc. Highest zone of inhibition was observed for the *S paratyphi B* and least was the *E.Coli* (Fig.4). The ZnO nanoparticles may attach to the surface of the cell membrane disturbing permeability respiration functions of

the cell resulted in high activity. These results suggest that the ZnO nanoparticles synthesized by WRE are potential antimicrobial agents.

Table 1 Antimicrobial activity of synthesized ZnO nanoparticles by WRE at 50 $\mu$ l dose

Pathogens	Zone of inhibition (mm)	
	ZnO nanoparticles	Streptomycin disk
<i>S. aureus</i>	33 $\pm$ 0.89	4 $\pm$ 0.12
<i>B. lichenformis</i>	37 $\pm$ 0.32	9 $\pm$ 0.38
<i>E. coli</i>	24 $\pm$ 0.67	10 $\pm$ 0.46
<i>S. paratyphi B</i>	52 $\pm$ 0.48	15 $\pm$ 0.92
<i>Pseudomonas</i>	43 $\pm$ 0.45	10 $\pm$ 0.64

The values are given in mean  $\pm$  SD of three replicates. The measurements are represented as mm for each pathogen

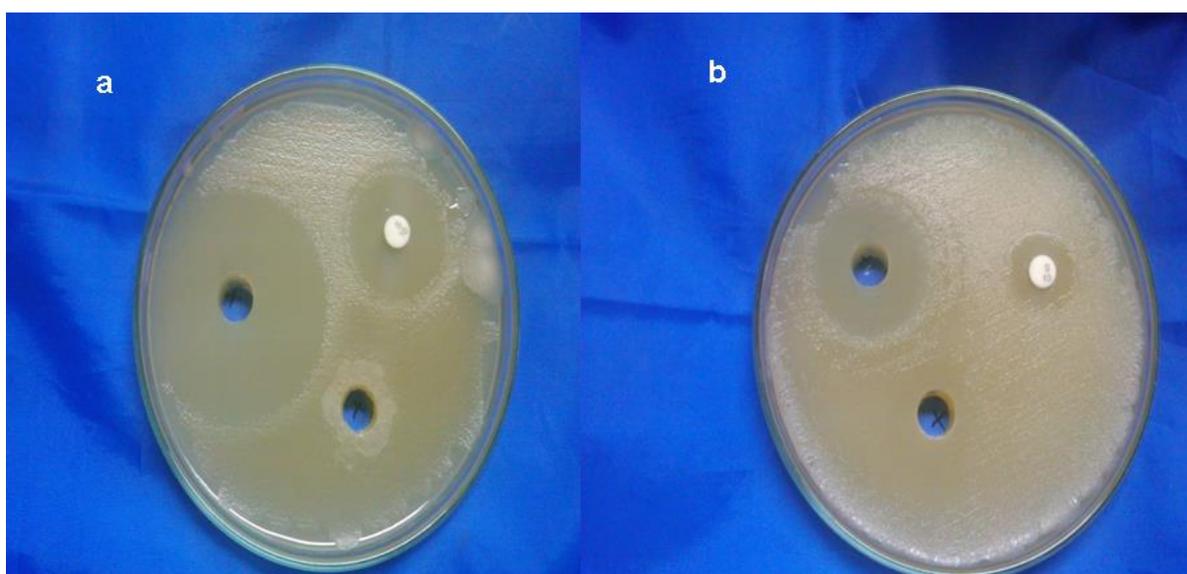


Fig 4 Antimicrobial activity of ZnO nanoparticles against pathogenic strains a) *S. paratyphi B* and b) *E. coli* by well diffusion method

#### 4. Conclusions

The present study reported the feasibility of watermelon rind to synthesis ZnO NPs in bioinspired way. The synthesized ZnO NPs was found to be anisotropic shapes with few nano rods. Citrulline an essential amino acid present abundantly in watermelon rind was identified to be the capping agent responsible for stabilization of ZnO NPs. As an application, the ZnO NPs was used in adsorption experiments as nano-sorbent and found to have great potential in removal of Pb<sup>2+</sup> and Cd<sup>2+</sup> ions from aqueous solution. Further the application was extended to biological sciences and evaluated as antimicrobial agent and found to be potential antimicrobial agent against gram positive and gram-negative strains. The results on heavy metal removal and anti-microbial activity suggest that the synthesized ZnO nanoparticles can be applied in real time for simultaneous removal

of heavy metal ions and killing of pathogens from waste water. These results suggest that watermelon rind an agro waste is capable of fabricating ZnO nanoparticles with potential applications in waste water treatment.

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