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# Green approach to synthesize zinc oxide nanoparticles using leaf extract of Aloe vera and its effect against *Bacillus cereus*

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***Abstract:*** *Nanoparticles of zinc oxide (ZnO) have been investigated in numerous studies to determine their potential therapeutic properties, including antibacterial activity. It has been discovered that nanoparticles of zinc oxide exhibit antibacterial activities against a variety of pathogens, including B. cereus. This study focuses on the biosynthesis of ZnO NPs using an extract from the Aloe vera plant. A total of 100 stool samples were obtained from children suffering from diarrhea, aged between 3 months and 9 years, from various hospitals between November 2022 and February 2023. Out of 100, 55 B. cereus bacteria were identified using selective medium (Mannitol egg yolk polymyxin agar), of which 21 were B. cereus bacteria confirmed using the VITEK 2 System. Genomic DNA was isolated from the isolates, with DNA concentrations ranging from 15-19 ng/µl. Aloe vera samples were collected from the local market in Baghdad in January 2023. Aqueous extracts of Aloe vera leaves were obtained using conventional methods. ZnO nanoparticles were prepared using the Aloe vera extract, then identified and characterized. The nanoparticles were characterized using: Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), field emission microscopy (FEM), and ultraviolet-visible spectroscopy (UV). The two samples were inoculated in culture media containing different concentrations of the nanocomposite nanoparticles (1, 2, 3 mg/ml) and the same concentrations of the Aloe vera plant extract, which showed different effects on the growth of B. cereus bacteria. The minimum inhibitory concentration (MIC) was determined to be 15.6 mg/ml.*
***Keywords:*** *Bacillus cereus, Aloe vera, zinc oxide nanoparticles*

## INTRODUCTION

Nanotechnology is concerned with the study and application of particles ranging in size from 1 to 1000 nm. Nanoparticles have unique qualities due to their small size in comparison to bulk equivalent, making them perfect for applications in fields such as electronics, energy, environment, and health(1).

Nanoparticles can be metallic, polymeric, or lipophilic. They may be created with desired characteristics like as size and form using physical, chemical, and biological processes. However, due to the high cost and toxicity of the chemicals required in synthesis, physical and chemical approaches have received little attention. As a result, substantial research into the biological production of metallic nanoparticles such as silver, gold, titanium dioxide, magnesium oxide, copper oxide, iron oxide, aluminum oxide, and zinc oxide has been conducted (2)(3).

Among these, zinc oxide nanoparticles (ZnO NPs) have piqued the interest of experts all over the world due to their therapeutic properties. Zinc oxide nanoparticles exhibit unusual UV filtration, semiconducting, and catalytic activity, which has piqued the interest of scientists throughout the world. Furthermore, these nanoparticles are non-toxic, physiologically safe, and biocompatible. Zinc oxide nanoparticles are also used in cosmetics and sunscreen creams because they may absorb damaging UV-A and UV-B rays (4).

Zinc oxide is safe (21 CFR 182.8991) and can be used as medication, according to the US Food and Drug Administration. Antimicrobial agents of zinc oxide nanoparticles can be employed to destroy harmful bacteria. Depending on particle size, shape, concentration, and period of contact to the bacterial cell, they first break the cell wall, then penetrate and collect in the cell membrane, eventually causing death by interfering with metabolic operations (5).

**Samples collection:** A total of 100 stool samples were collected from 100 paediatric patients suffering from diarrhea at Al-Alawi Children's Hospital, Central Children's Hospital and Almadaen Hospital - Baghdad during the period from November 2022 to February 2023. The median age of paediatric patients was 5.4 years with a range between 3 months to 9 years.

Out of 100 samples, *B. cereus* was isolated and identified only from 21 samples. Isolation and identification of *B. cereus* was performed by cultivating the stool samples in MYP agar plates. Fifty-five (55%) samples were showed positive growth in MYP plates, while 45 samples were failed to exhibit growth in MYP plate**s.**

Green Synthesis of ZnO nanoparticles using *Aloe vera* leaves extract

Green method synthesis of nanoparticles increases the rate of inhibition when compared with conventional delivery and also keeps the antimicrobial activity for the longer time.

Antibacterial activity of *Aloe vera* extract and ZnO NPs on *B. cereus*

The resulted *Aloe vera* extract and ZnO nanoparticles were tested for their antibacterial activity against the selected isolates through the use of well diffusion method and the result were examined after 24 hrs of incubation.The results showed a

remarkable inhibition zone of ZnO nanoparticle that prepared from *Aloe vera* had effective antibacterial activity than the effect of plant extract with an inhibition zone of different diameters as shown in table (1-1) and figure(1-1).

Table (1-1): Zone of Inhibition produced by *Aloe vera* extract and ZnO NPs against two *B. cereus* isolates

| ***Antibacterial Name*** | ***Concentration (mg /ml)*** | ***Diameters of Inhibition Zone (mm)*** | |
|---|---|---|---|
| | | **Isolate1** | **Isolate2** |
| ***ZnO NPs*** | 1 | 52 | 31 |
| | 2 | 55 | 33 |
| | 3 | 58 | 34 |
| ***Aloe vera Extract*** | 1 | 46 | 32 |
| | 2 | 50 | 33 |
| | 3 | 51 | 35 |

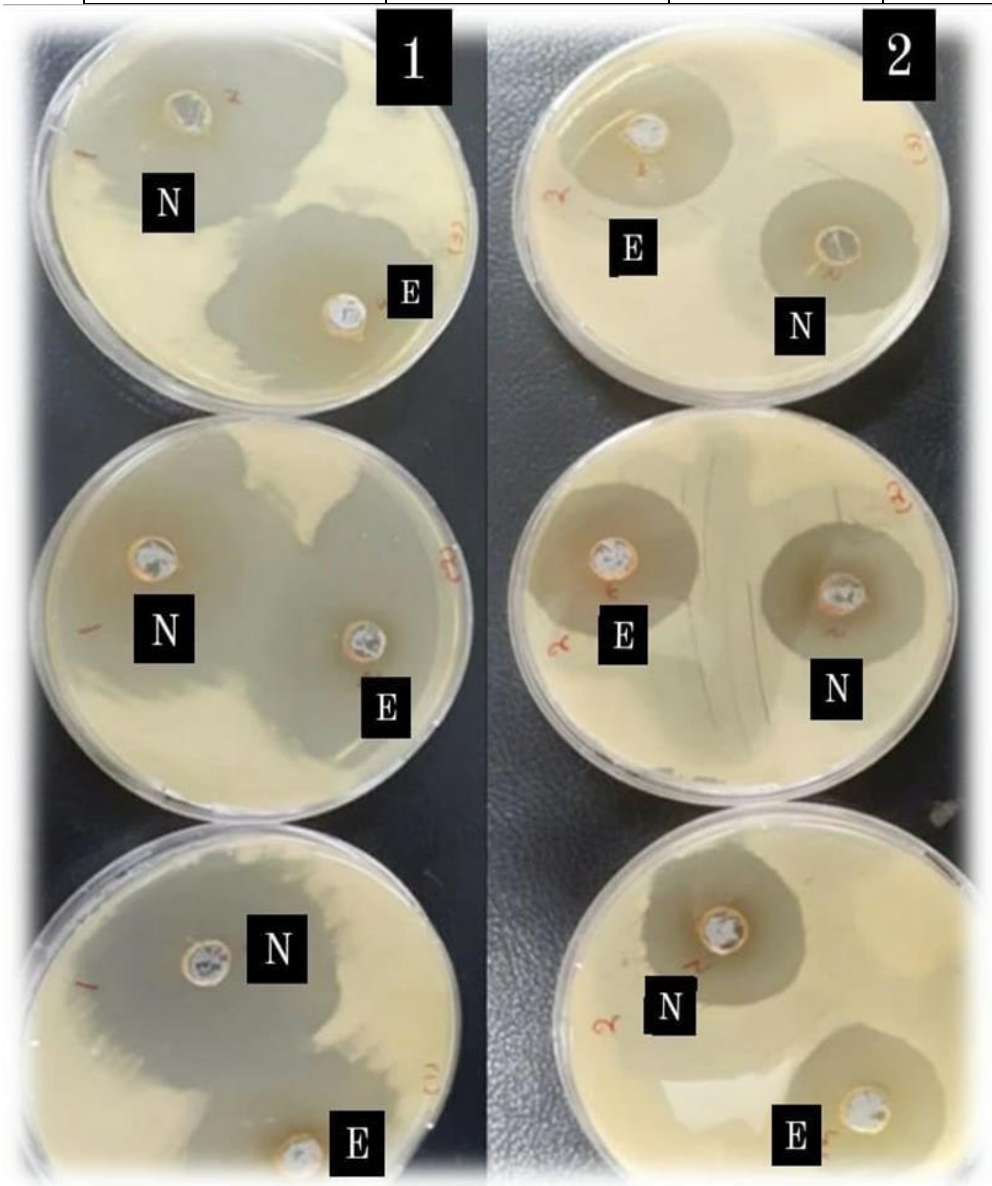


**Figure (1-1): Effect of different concentrations (1, 2, 3 mg/ml) of *Aloe vera* extract(E) and synthesized ZnO nanoparticles(N) on the growth of *B. cereus*.**

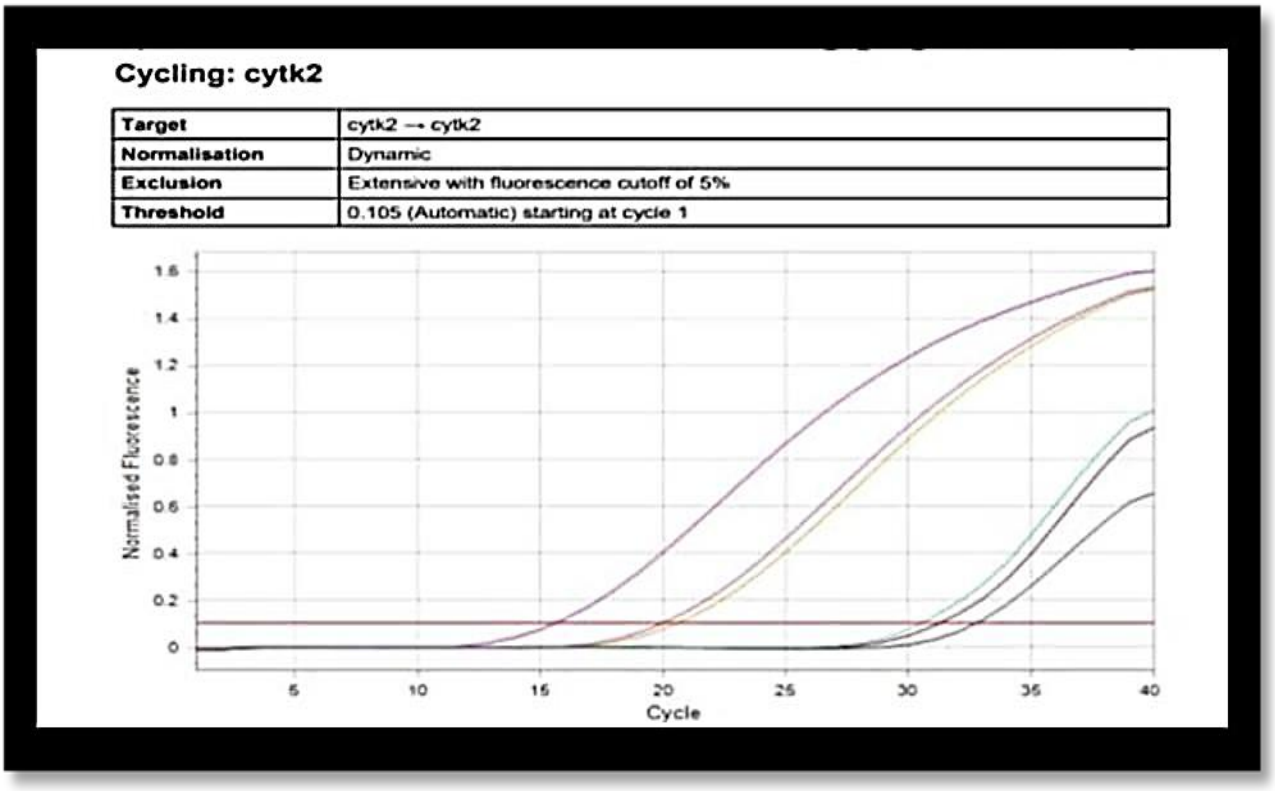


Figure (1-2): Amplification plot using SYBR green Real Time PCR Chemistry targeting region in the *cytK2* region as a reference gene to calibrate the target amplification

The capability of *B. cereus* to produce virulence factors plays a crucial role in its chronic colonization. *B. cereus* exhibits reduced growth rates, significant resistance to antibiotics, the secretion of various surface molecules, and virulence factors, thereby enhancing its pathogenicity (6).

Zinc oxide NPs is one of the common nanoparticles; it is one of metal oxide and inorganic compound nanoparticles (7). ZnO produce ROS, then ROS leading into destroy the bacteria membranes (8). $H_2O_2$and $Zn^{+2}$ formation have role in the antibacterial activity (9).

Many studies found that ZnO nanoparticles have been found to inhibit biofilm development and virulence factor production in (10).

Research has demonstrated that, zinc oxide nanoparticles suppress the expression levels of all virulence genes and biofilm in clinical isolates of *B. cereus* (11) (12) Therefore, it can be concluded that Zinc oxide nanoparticles have an inhibitory effect on virulence gene expression in *B. cereus*.

Strong efficiency against clinical isolates is demonstrated by the produced ZnO NPs; therefore, ZnO-NPs can be utilized as an alternate therapeutic agent for infectious diseases (13). ZnO NPs are highly effective in treating S. aureus infections as an anti-biofilm agent. It is suggested that ZnO NPs could be used as an adjuvant with other antibiotics that target S. aureus in light of these promising findings (14). ZnO NPs treatment decreases the preformed virulence factors. ZnO NPs can suppress the expression of oxidative stress- resistance genes in bacteria, damage the integrity of bacterial cell membranes, and decrease the hydrophobicity of bacterial cell surfaces (15).

Conclusions

ZnO nanoparticles synthesized using Aloe vera leaves extracted and characterized using the following techniques: Fouriesr Transform Infrared Spectrum (FTIR)، Scanning Electron Microscopic (SEM)، Atomic Force Microscope (AFM).

The current study demonstrates the possibility of biosynthesis of zinc oxide nanoparticles from Aloe vera. It has been shown that these nanoparticles exhibit antimicrobial activity against B. cereus

Biosynthesized zinc oxide nanoparticles appeared with highly effective antimicrobial activity against B. cereus.

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