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Therapeutic Role of Zinc Oxide Nanoparticles in Inhibiting Pathogenicity and Toxin Production of Bacillus cereus

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Abstract

Nanomaterial's represent powerful tools in disease detection and biomedical applications due to their unique structural and functional properties. Nanowires, in particular, offer remarkable sensitivity and nanoscale dimensions, especially when functionalized with antibodies capable of selectively binding to essential biomolecules such as DNA, proteins, or other cellular components.In this study, Aloe vera samples were collected from the local market in Baghdad, and aqueous leaf extracts were prepared using standard extraction methods. Zinc oxide (ZnO) nanoparticles were subsequently synthesized using the Aloe vera extract as both a reducing and stabilizing agent. The synthesized nanoparticles were characterized using Fourier-transform infrared spectroscopy (FTIR), field emission microscopy (FEM), confirming their structural integrity and optical features.The antibacterial activity of ZnO nanoparticles and Aloe vera extract was assessed against *Bacillus cereus*. Bacterial cultures were exposed to different concentrations of the nanocomposite nanoparticles (1, 2, and 3 mg/ml) as well as equivalent concentrations of the plant extract. Both treatments inhibited bacterial growth, with ZnO nanoparticles exhibiting a more pronounced antibacterial effect. The minimum inhibitory concentration (MIC) was determined to be 15.6 mg/ml.To further evaluate the molecular impact, quantitative real-time PCR (qRT-PCR) was performed to analyze the expression levels of the cytK1 and cytK2 genes in B. cereus isolates treated with ZnO nanoparticles and Aloe vera extract. RNA and cDNA concentrations were measured using the Quantus Fluorometer prior to analysis. The results demonstrated significant modulation of target gene expression by both treatments, with ZnO nanoparticles exerting a stronger regulatory effect, particularly in isolates harboring the cytK gene.The findings highlight that ZnO nanoparticles synthesized via Aloe vera extract possess enhanced antibacterial activity and gene regulatory effects compared to the crude extract alone. These results underscore their potential application as eco-friendly nanobiomaterials for antimicrobial and molecular therapeutic strategies.

Keywords: *Bacillus cereus*, zinc oxide nanoparticles, Aloe vera Extract.

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Introduction

Bacillus cereus, as it is commonly known as (sensu lato), is primarily associated with gastrointestinal disorders; however, it is an opportunistic human pathogen associated with a wide range of other infections, including severe eye infections, periodontitis, necrotizing fasciitis, endocarditis, nosocomial acquired bacteraemia, osteomyelitis, sepsis, liver abscess, pneumonia, and meningitis, particularly in postsurgical It creates various chemicals during the idiophase, including degradation enzymes, cytotoxic agents, and cell-surface proteins, which may contribute to pathogenicity. However, the importance of B. cereus in these devastating, and usually deadly, clinical infections in humans is largely underappreciated [1]

Bacillus cereus has been linked to a variety of health problems, including food poisoning and systemic infections. While most strains of B. cereus are harmless, some strains can produce toxins that can cause illness when ingested. Symptoms of B. cereus food poisoning can include nausea, vomiting, diarrhea, and abdominal cramps, while systemic infections can cause symptoms such as fever, chills, and muscle aches. Bacteria, for example, can create enzymes that tear down tissues, leading to skin infections and potentially necrotizing fasciitis (a dangerous bacterial infection that affects the skin and soft tissues). Bacillus cereus systemic infections are uncommon but can be severe. When germs enter the circulation and travel to other regions of the body, these illnesses can arise. Systemic infections can cause sepsis, a potentially fatal illness, in extreme situations [2]

Cytotoxin K (CytK) generated by Bacillus cereus is widely thought to be related with foodborne diarrhoea. CytK has two different variants: cytK-1 from Bacillus cytotoxicus and cytK-2 from Bacillus cereus. Surprisingly, the prevalence of cytK was lower in food poisoning and clinical isolates than in ambient samples

Zinc oxide (ZnO) crystallizes have two main forms, one quartzite in the form of pyramidal crystals, a hexagonal structure of zinc sulphide, and the other is a cubic zinc alloy (Gu et al. 2013). ZnO has different forms in the form of nanostructures that exhibit a variety of properties such as piezoelectricity, optical transparency, conductivity, solar cell, optical luminance, optical nonlinearity and others. Access to different forms of nanotechnology depends only on different processing techniques [3]

Aloe vera contains a variety of active compounds, including polysaccharides, anthraquinones, and phytosterols, that are believed to be responsible for its many health benefits. One of the most well-known use of aloe vera is for wound healing properties. Polysaccharides found in the plant's leaves have been demonstrated to encourage the development and repair of skin cells. This may aid in the healing of cuts, burns, and other forms of wounds (Kumar and Yadav 2014). Aloe vera is also thought to contain anti-inflammatory qualities, which may aid in the relief of pain and swelling associated with arthritis and other inflammatory disorders. The plant's gel includes chemicals including acemannan, which have been proven to have immune-modulating properties and may aid in the reduction of inflammation in the body [4]

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Isolation and Identification of *B. cereus* from stool

Stool specimens were plated directly onto mannitol-egg yolk polymyxin (MYP) agar and spread by using a sterile wipe to make a uniform dispersion of the solution on the media's surface then incubated aerobically at 37◦C for 24 h. Following the 24-h incubation period, the MYP agar plates for the specimens were observed. Individual *B.cereus*-like colonies (pink colored will appear as smooth, with a slight irregular margin). The suspected colonies were subjected for their identification by VITEK 2 System. The colonies were transferred from the first screening step (mixed culture) into separate agar plates and incubated at 37◦C for 24 h to obtain a pure growth (MYP). The pure culture was kept at 4°C for a further study [5]

Table 1-1: The number (%) of stool samples with positive growth result in MYP agar plates.

No. of Samples	+ve Growth (%)	-ve No Growth (%)
100	55 (55%)	45 (45%)

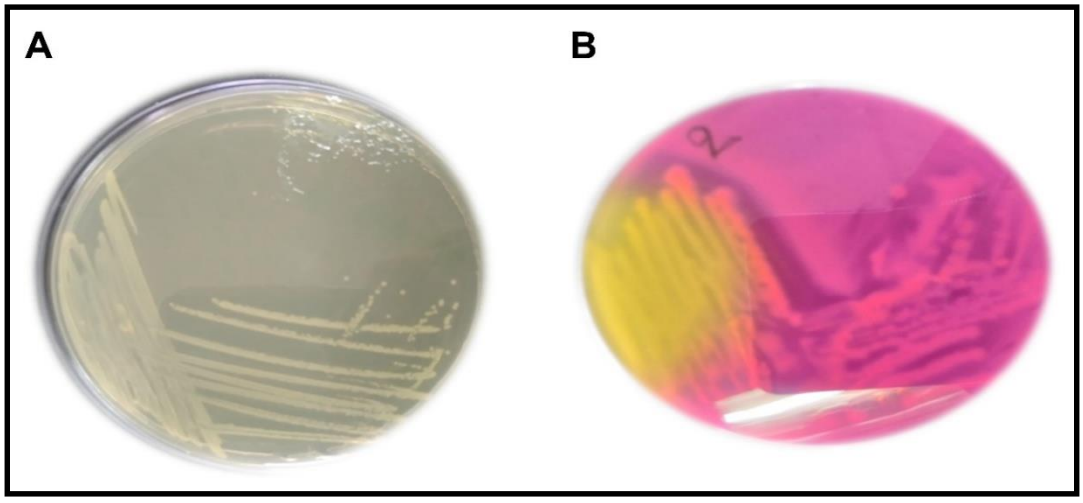

Figure 1-1: Growth of colonies of *Bacillus cereus* on (A) Nutrient agar, (B) MYP agar plate after incubation at 37°C for 24 hours.

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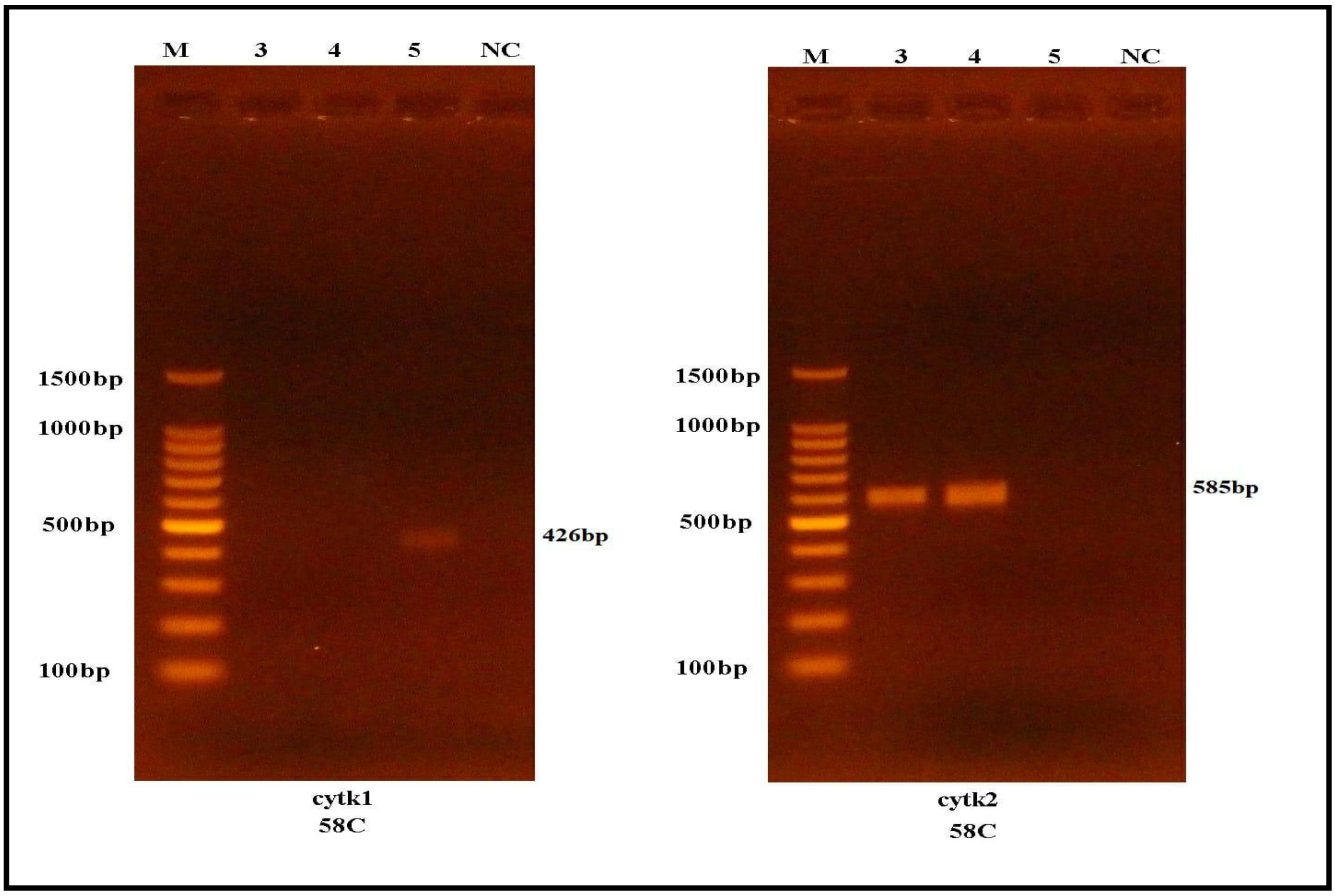

Figure 1-2: Polymerase chain reaction amplification of *cytK1* and *cytK2* genes of DNA isolated from suspected *Bacillus cereus* species. DNA fractionated on 1.5% agarose gel electrophoresis stained with ethidium bromide. M: 1500bp ladder marker. Lanes 3-5 resemble PCR products. NC: negative control.

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.

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The presence of yellowish white precipitates in the solution combination proved the presence of the plant extract shown in figure (1-3).

Figure (1-3): Formation of ZnO NPs (Left: before the reaction, right: after the reaction).

Atomic Force Microscopy (AFM)

Atomic Force Microscopy (AFM) is a powerful technique for imaging and analyzing surfaces at the nanoscale. Figure A (3-8) shows granularity distribution of *Aloe vera* plant extract and ZnO NPs, while Figure B (3-8) depicts three-dimensional atomic force microscopy images *Aloe vera* plant extract and ZnO NPs. The size distribution of the *Aloe vera* plant extract averages around 50 nm to 55 nm across various percentages, and the surface morphology appears rough as in the studies mentioned[6]. In contrast, the ZnO nanoparticles exhibit an average size distribution ranging[7] from 18 nm to 30 nm across various percentages, and their surface morphology also appears rough The average diameter and height distribution of the *Aloe vera* plant nanoparticles and ZnO nanoparticles are presented in Table (1-2).

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Table (1-2): average diameter and height distribution of the Aloe Vera plant nanoparticles and ZnO nanoparticles

	Average Diameter	height distribution
Aloe Vera	**80.42 nm**	**44.26**
ZnO NPs	**96.59 nm**	**76.97**

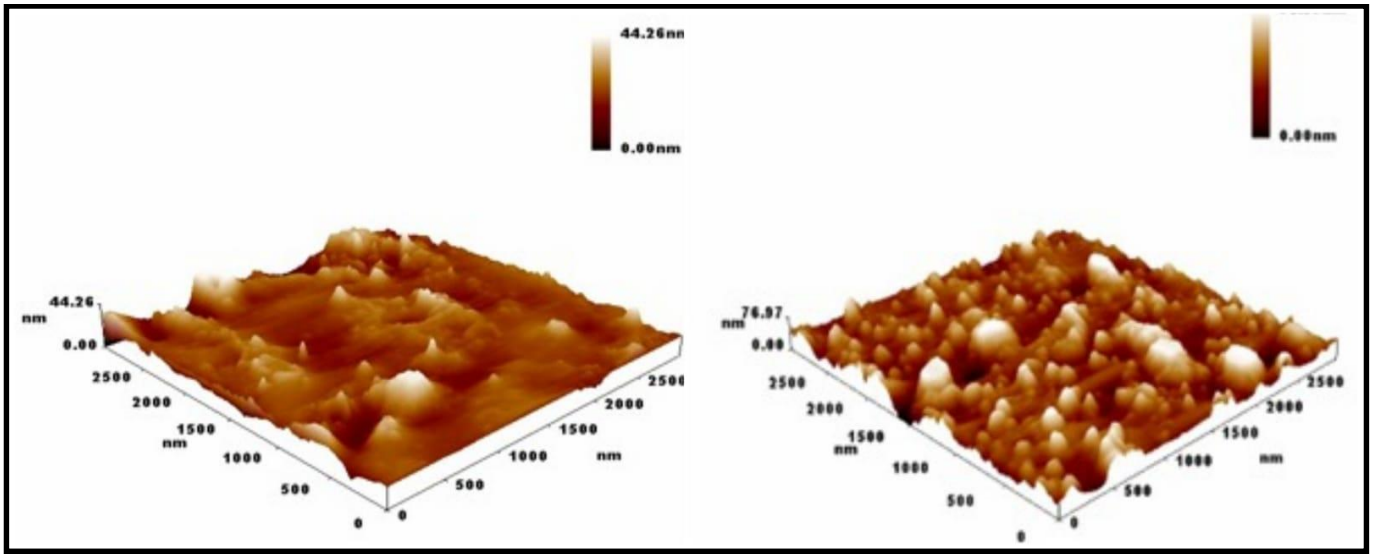

Figure (1-4): AFM 3-dimensional image of *Aloe vera* plant extract (Lift) and ZnO NPs (right).

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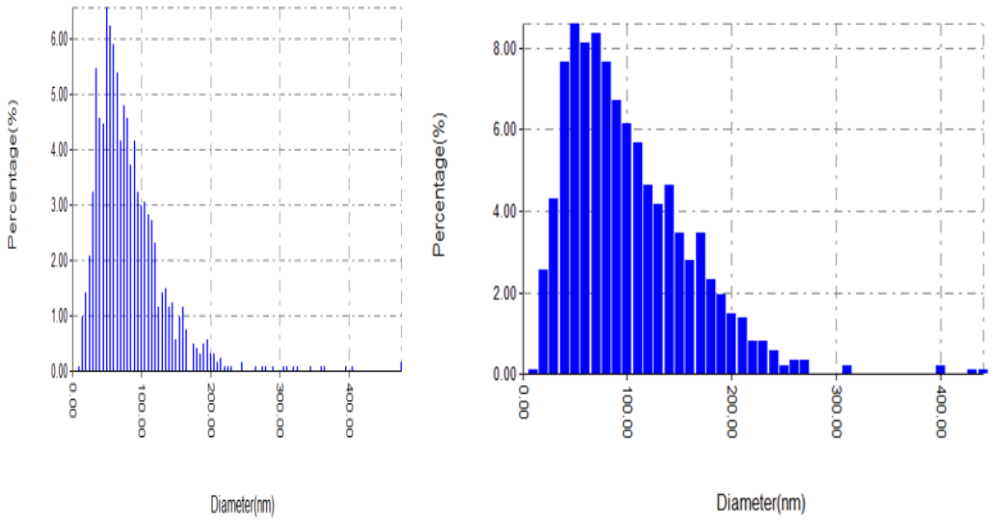

Figure (1-5):Result of AFM: (a) Histogram showing granularity Distribution of *Aloe vera* plant, (b)Histogram showing granularity Distribution of ZnO NPs.

Fourier Transform Infra-Red Spectroscopy (FTIR) Test

Fourier Transform Infrared (FT-IR) spectroscopy serves as a robust analytical tool for the examination of both synthesized zinc oxide (ZnO) nanoparticles and *Aloe vera* extract. This technique enables the identification of functional groups present in compounds and offers valuable insights into their molecular structures. By analyzing the FT-IR spectrum, absorption peaks corresponding to the vibrational modes of these functional groups become evident, allowing for the detection of distinct functional signatures, including OH, C=O, NH, CH, and more. Consequently, this confirms the presence of specific chemical bonds within the studied samples[8]

For the synthesized ZnO NPs, FT-IR analysis was conducted in the wavelength range of 4000–500 cm^{-1}, revealing significant findings. The broad peak observed at 3238 cm^{-1} signifies the presence of hydrogen-bonded groups, particularly hydroxyl groups (O-H) originating from phenolic compounds in the plant extract. Additionally, peaks spanning from 3966 to 3610 cm^{-1} represent the stretching vibration of O-H groups, while the peak at 2182 cm^{-1} corresponds to the C-C bond. Peaks at 1553 cm^{-1} and 1402 cm^{-1} indicate C=C stretching in aromatic rings and C=O stretching in polyphenols, respectively. Notably, strong absorption peaks in the range of 500-600 cm^{-1} unequivocally indicate the presence of ZnO nanoparticles, specifically highlighting the stretching frequency of Zn–O bonds Figure (3-10a) [9].

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In contrast, the FT-IR spectra of the *Aloe vera* extract, examined within the same wavelength range of 4000 to 500 cm^{-1}, exhibit distinct peaks. Peaks in the vicinity of 3920 cm^{-1} to 3671 cm^{-1} point to the presence of hydroxyl groups, while those at 2980 cm^{-1} are associated with the stretching vibration of C-H bonds. A weaker peak at 2666 cm^{-1} suggests C–H symmetric stretching in CH_2. The sharp peak at 1693 the peak appeared at 1637 cm^{-1} is attributed to the amino groups present in alcohol, phenol, and amines in the *Aloe vera* extract and the peak at1552 cm^{-1} indicates the presence of C=O stretching and, by extension, the existence of carboxyl components and polar phenolic compounds. The appearance of a peak at 1070 cm^{-1} suggests C-O vibrations, potentially originating from polyols like hydroxy flavonoids. In the lower frequency range, peaks at 898, 639, and 531 cm^{-1} indicate aromatic CH out-of-plane deformation Fig. (3-10b) [10]

It is noteworthy that the functional groups present in the *Aloe vera* extract can potentially facilitate the reduction of zinc ions (Zn^{2+} to Zn^{+1}), ultimately leading to the formation of zinc nanoparticles, as indicated in previous studies as in the studies mentioned .[11]

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[12]. Zinc oxide NPs is one of the common nanoparticles; it is one of metal oxide and inorganic compound nanoparticles[13]. ZnO produce ROS, then ROS leading into destroy the bacteria membranes [14]. H_2O_2 formation have role in the antibacterial activity [15]. Many studies found that ZnO nanoparticles have been found to inhibit biofilm development and virulence factor production in [16]. Research has demonstrated that, zinc oxide nanoparticles suppress the expression levels of all virulence genes and biofilm in clinical isolates of *B. cereus*[17, 18] 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 [19]. 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 [20]. 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 [21].

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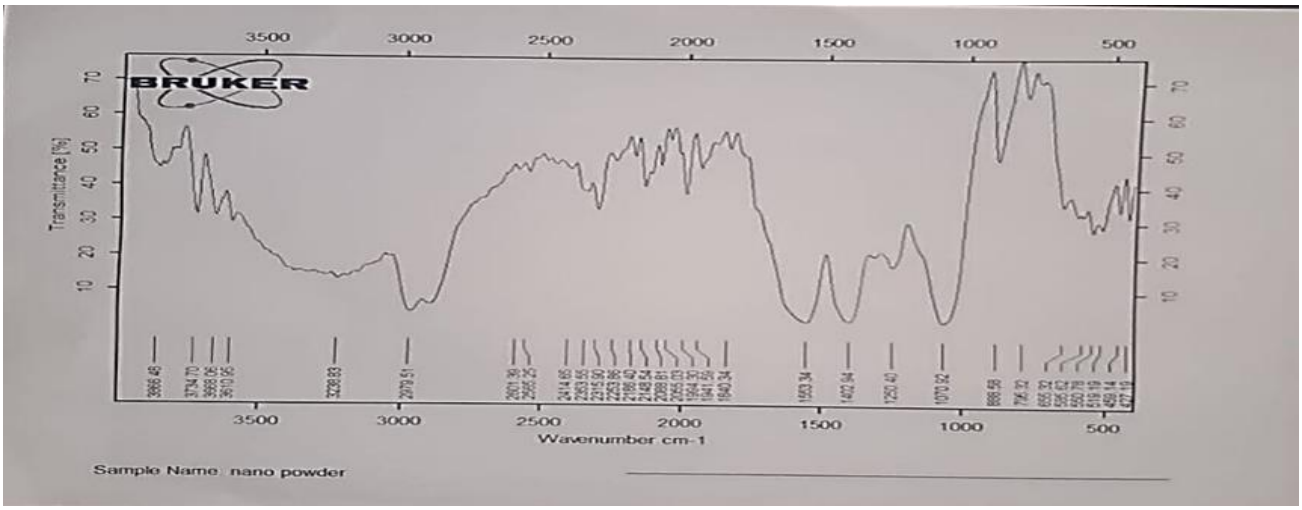
A)



B)

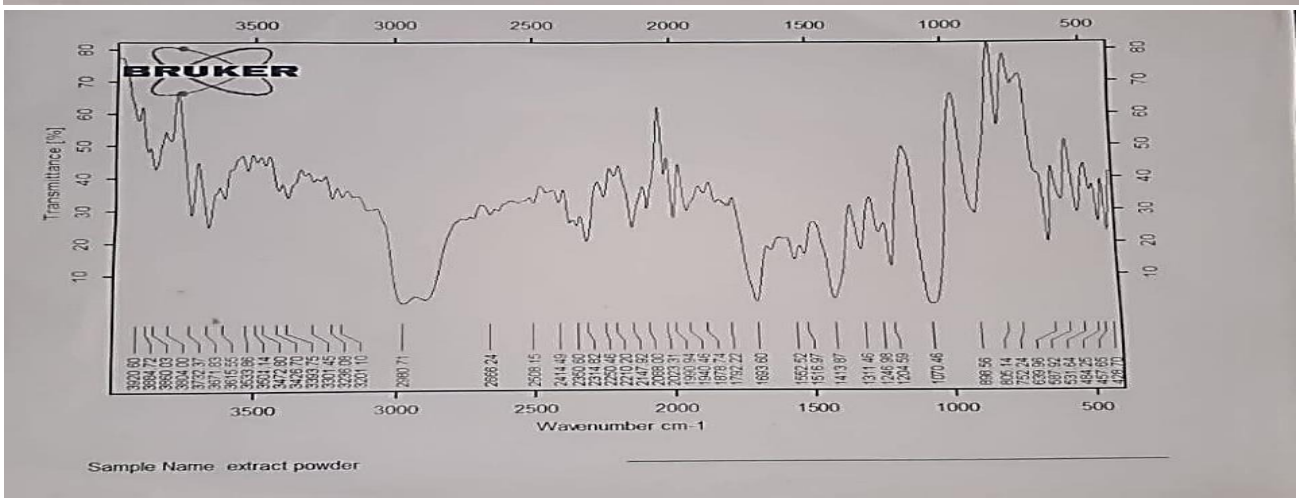


Figure (1-6): The FT-IR spectrum of ZnO NPs and *A. vera* extract.

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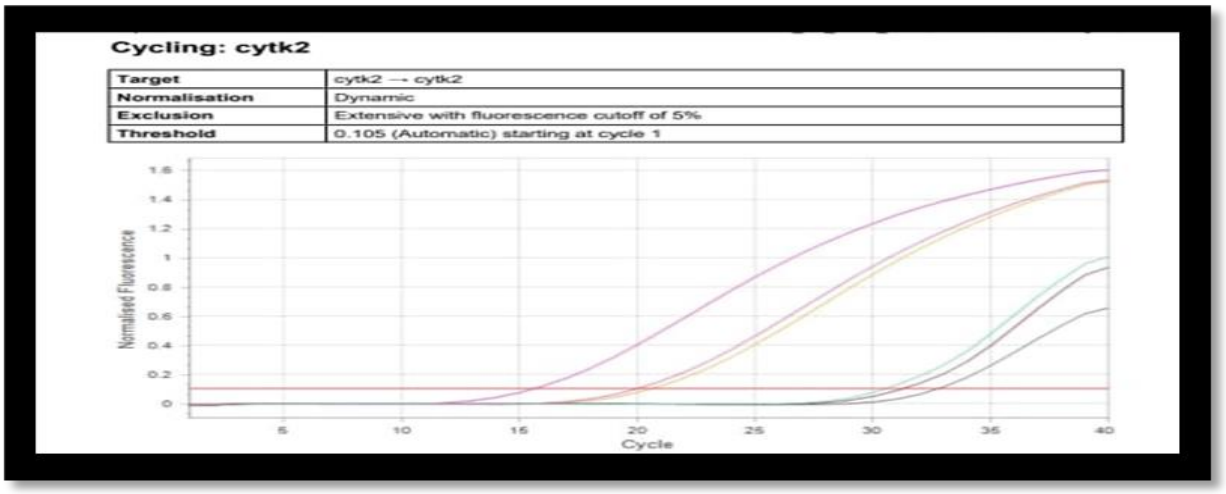

Figure(1-7) Amplification plot using SYBR green Real Time PCR Chemistry targeting region in the cytK2 region as a reference gene to calibrate the target amplification.

Conclusions

1. 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).
2. The growth of characterized *Bacillus cereus* isolates with cytK1, cytK2 gene were positively affected with different of ZnO NPs and aloe vera extract concentration (1,2,3) mg/ml.
3. The transcription levels in the *bacillus cereus* isolated increased in the treatment of 1 mg/ml ZnO nanoparticles more than the aloe vera extract.

Acknowledgments

1. Investigating other genes associated with antibiotic resistance and unravelling their mechanism of overcoming using ZnO NPs from aloe vera or other extract.
2. Utilizing manufactured zinc nanoparticles and comparing their effects with zinc nanoparticles from plant extract on *Bacillus cereus.*

Author's Declaration

- We hereby confirm that all the Tables in the manuscript are original and have been created by us.
- The study protocol was approved by the Medical Ethics Committee of Al-Nahrain University/College of Biotechnology. All members gave composed informed assent after checking on the review depiction. at [Al-Nahrain University/College of Biotechnology]. This approval underscores our commitment to ethical research practices and the well-being of our participants.

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