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RESEARCH ARTICLE

Cytotoxicity of zinc oxide nanoparticles to lymphocytes using *Enterococcus faecium* bacteriocin and assessment of their antibacterial effects

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ABSTRACT

Objective(s): Multidrug-resistant *Enterococcus faecium* can grow in a variety of settings and cause infections that can be fatal, making it a serious threat. Partially purified and characterized bacteriocins with antimicrobial efficacy demonstrated antimicrobial activity against gram-negative bacteria.

Methods: Zinc oxide nanoparticle (ZnO-NPs)were synthesized by a biological method from suspensions of *E. faecium* bacteria isolated from the Gums of healthy people at different time points (24 and 48 hour), and temperatures ranging from (35-37)°C to pH (5- 5.30).

Results: The size of ZnO-NP particles has been determined. The biosynthesized ZnO-NPs' peak of absorption was visible in the UV-VIS spectrum at 267 nm. The mean dimension of the biosynthesized ZnO-NPs was determined by atomic force microscopy (AFM) to be within 259.2 nm. Three different peak shapes in the XRD spectra demonstrated the production of ZnO NPs.Analysis using X-ray (EDX) demonstrates the zinc content of the ZnO-NPs. SEM was utilized to evaluate dimensions and form. The vast majority of the particles were spherical and uniform in shape, based on SEM images. The minimum inhibitory concentration (MIC) was determined at concentrations ranging between 1000,500, 250,125,64 µg/ml. The minimum inhibitory concentration for ZnO-NPs prepared from E. faecium using the microtiter plate method was 250 µg/mL. The toxicity of zinc oxide nanoparticles was tested on human lymphocytes.

Conclusions: ZnO-NPs were synthesized successfully using an easy-to-use, low-cost, green, high-throughput, and environmentally friendly technology that showed remarkable antibacterial effectiveness against a variety of bacterial species.

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INTRODUCTION

Enterococci are a large genus of Lactic acid bacteria (LAB) and are gram-positive, catalase-negative, facultative anaerobes, and nonspore-forming bacteria. [1] The intestinal flora of the host is affected by homeostasis management and improvement by LAB, which are probiotics present in the mammalian intestine. [2] and [3]. Enterocin, a bacteriocin obtained from the genus Enterococcus that includes *E. faecium, has strong inhibitory effects on susceptible bacterial strains*. Bacteria have many positive properties that make them particularly interesting for various applications [4]. Nisin, a bacteriocin from the group of antibiotics, has been approved for use as a food preservative. The analysis was performed on viable plate counts of canned meat samples treated with volatile rosemary oil and nisin A at effective concentrations. [5] E. *faecium*, of the genus Lactobacillus, is a commensal microorganism in the intestine of mammals. LAB bacteria are naturally tolerant to high thermal

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stress and are known to be active over a wide range of pH values [6]. Furthermore tasteless, odorless, and colorless, these peptides are antimicrobial and have a higher potential stability. Because it is protein-based, proteolytic enzymes can readily hydrolyze it. Consequently, the duration of bacteria fragments' survival in the human body and environment is limited, thereby diminishing the likelihood of targeted germs interacting with destroyed antibiotic fragments. [7].In recent years, researchers have used nanotechnology evalution antimicrobial agents such Zinc oxide synthesis has gained great interest because it is a clean and environmentally friendly approach and has a wide range of applications in biotechnology and medical fields. ZnO-NPs are among the most frequency metal oxide nanoparticles and are unique, used in many antibacterial. ZnO NPs display different shapes and sizes with minimal surface areas that allow them to rupture microbial cells as applications antibacterial agents [8]. Quorum sensing systems have been discovered in a number of studies to be potential targets for antimicrobial drugs that prevent the growth of biofilms over time [9]. ZnO-NPs have distinctive features that are not present in other nanoparticles, including improved biofilm penetration, increased solubility, and effective drug delivery. [10].In the current research on an environmentally safe and green technique was utilized to synthesize ZnO-NPs from Bacteriocin-like inhibitory substance (BLIS) . Additionally, we investigated, for the first time, Cytotoxic effect of ZnO-NPs on lymphocytes. It has been determined Properties of green biosynthesized ZnO-NPs .

MATERIALS AND METHODS

Materials

Zinc acetate ($Zn(CH_3CO_2)_2$) was purchased from Sigma (USA). De Man, Rogosa and Sharpe (MRS) broth HiMedia was purchased from (India). MTT Kit was purchased from Intron Biotech (Korea)

Methods

Sample collection and bacterial identification

50 samples from patients who frequented a personal dental clinic were used in this investigation. With great care to avoid exposing the specimens to oral contaminants, sterile dental explorers and small-sized bonding brushes were used to collect swabs containing specimens, which are small fragments of profoundly infected dentin, from deep lesion sites. These disorders have been diagnosed using a variety of assays. [11]

Sample collection of Proteus mirabilis (P. mirabilis)

Patients were transported to the Al-Yarmouk Teaching Hospitals in Baghdad between September 2023 and November 2023, when urine samples were taken from a range of sources. A urinary tract infection was seen in 150 people in general. Urine collected via midstream catheterization was immediately sent in sterile containers to the laboratory for culture. To establish preliminary proof of identity, were cultured on a selective and differentiatial medium such blood agar and MacConkey agar, cultures were incubated at 37°C for a whole day. [12]

Isolation and identification of P. mirabilis

The characteristics of a colony, such as its size, color, texture, the company, form, and swarming phenomenon, were employed to make the first diagnoses. Each isolate of P. mirabilis had a number between 1 and 70. After incubating at 37°C for the entire night, swarming phenomena assay on blood plates made from agar [13].

Biochemical tests

Several biochemical tests have been to diagnose including oxidase, catalase, indole, methyl red, urease, and motility tests, according to [14].

Bacteriocin-like inhibitory substance (BLIS):

The antimicrobial spectrum of the isolated lactic acid bacteria obtained from MRS broth culture was analysed. After 24 hours of incubation at 37°C and under the appropriate conditions for each strain, the BLIS were recovered by centrifugation (60,000 rpm) for 15 min at 4°C [15]. The isolate with the highest bacteriocin production was chosen using the Agar Well Diffusion Assay (AWD) and Filter Paper Disc Method (FPD)

Screening for BLIS production by E. faecium

Filter Paper Disc Method (FPD)

Bacteria were applied with the BLIS mixture on the outermost layer of Mueller Hinton agar (MHA) plates in order to detect the presence of inhibitory substances. MHA plates were covered with five millimeter-diameter sterile filter paper discs that had been saturated with 100 µl of BLIS solution [16]. The plates were incubated aerobically at 37°C. We measured and recorded the inhibiting zones that formed around the paper discs.

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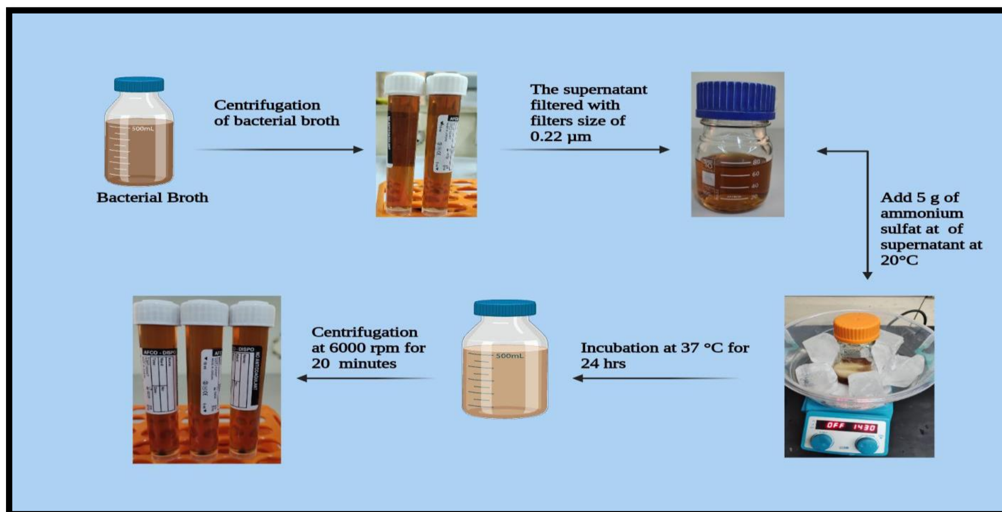

Fig. 1. Partial purification of Bacteriocin-like inhibitory substance (created by Biorender.com)

Agar well diffusion method (AWD)

Bacteria were spread on the surface of MHA plates using the AWD method. The wells were cut into pour plates with a sterile 5 mm diameter cork borer filled with 100 l of FLAB suspension. The plates were kept at room temperature for two hours before being incubated for 24 to 48 hours. After incubation, the zones of inhibition in millimeters were measured to determine the antimicrobial activity of the BLIS [17].

Determination of the optimal conditions for BLIS

1- Determination of the optimal production pH

The production and growth of inhibitory substances in the BLIS were tested and examined to determine the impact of pH. MRS broth was prepared in 10 ml tubes and adjusted with 0.5 Hydrochloric Acid (HCl) or 0.5 NaOH after autoclaving to achieve different pH values of 5, 6, and 7 [18]

2- Determination of optimum incubation:

The impact of the incubation period was investigated. Ten millilitres of a selected strain were incubated in modified MRS broth medium for different durations (24, 48 and 72 hours) at 37°C under anaerobic conditions. [19]

3- Effect of nitrogen source and carbon source (sugars):

Peptone water, yeast extract, lactose, and glucose were added to the Brain heart infusion broth (BHI) at concentrations of 1%. [20]

Partial purification of the bacteriocin-like inhibitory substance

Ammonium sulfate precipitated at varying concentrations to produce partially pure crude bacteriocin. Ammonium sulfate was precipitated by gradually adding it to the crude enzyme while swirling it continuously on ice to different saturation degrees.

Zn $(CH_3CO_2)_2$ stock solution was dissolved in 50 ml of deionized water to prepare the stock solution**.** [21]

ZnO -NP Biosynthesis

For the purpose of to generate the Zn NPs, 3 ml of the final concentration of 1 mM Zn (CH3CO2)2 at room temperature and pH 5 was mixed with 7 ml of BLIS bacteria. Every color change that occurred over the three days when the flasks were incubated at 37°C was noted [22]. After being left to incubate for 27 hours, the reaction mixture that was used for creating the ZnO NPs was centrifuged. The precipitate was then removed using deionized water. The resulting pellet-shaped were then placed in a hot air oven that was set at 120°C to completely evaporate the liquid to obtain powdered nanoparticles [23] Color shifts can be used to determine the production of NP. [24].

Characterization of ZnO NPs:

UV-Visible (UV-VIS) spectral analysis

The samples were scanned from 200 to 900 nm at a speed of 500 mm/min, with a blank reference used for spectrophotometer correction [25].

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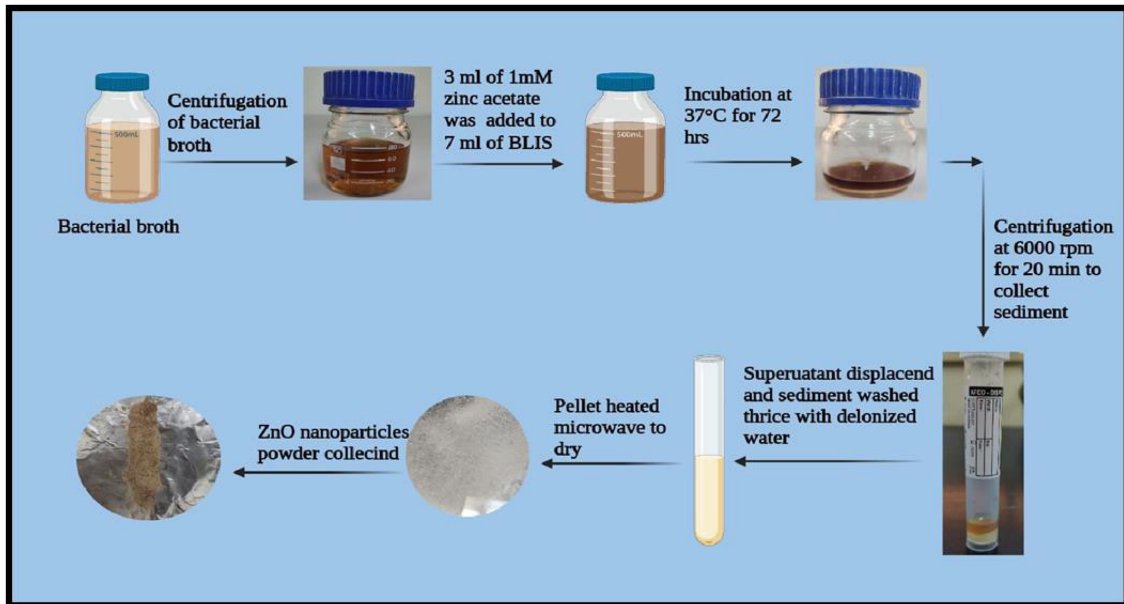

Fig. 2. Biosynthesis process of ZnO NPs Nanoparticles (created by Biorender.com)

Atomic force microscopy (AFM) analysis

We measured the average diameter of the produced NPs using atomic force microscopy. A thin layer of the prepared NPs was created by applying a few drops to a silica glass plate, the deposited film glass plate was then scanned using AFM. [26].

Fourier Transform Infrared-red Spectroscopy (FTIR)

The functional groups involved in the formation of the ZnO NPs were determined using Fourier transform infrared (FTIR) spectroscopy, the dried NPs were embedded into a thin KBr disc, and all spectra were determined using a Perkin-Elmer model spectrum within the range of 3900-400 cm. [28]

Energy dispersive X-ray (EDX) spectroscopy

The elemental composition of materials, including nanoparticles, can be determined using energy dispersive X-ray spectroscopy (EDX). EDX detects the characteristic X-rays emitted by the elements within a sample when it is bombarded with high-energy X-rays [29].

X-ray diffraction (XRD)

The crystal structure of the NPs was ascertained using XRD. Various techniques exist for powdered sample grinding in XRD, contingent upon the sample matrix, sample dimensions, and/or quantity of produced material required [27].

Field emission scanning electron microscopy (FESEM)

Utilizing fields scanning electron microscopy (FESEM), the shape and structure of the generated nanoparticles was investigated. The sample surface was scanned with an electron beam in order to produce pictures. Interaction between the sample's atoms and electrons and its topography. [30]

Determination of the minimum inhibition concentration (MIC) of the ZnO-NPs

The lowest inhibitory concentrations (MICs) of ZnO-NPs against P. mirabilis and E. faecuim, which were cultivated in the appropriate conditions for an entire night, were determined by the microdilution method. The ZnO-NPs disappeared and diluted in the suitable culture media. ZnONPs were produced in different concertation (1000, 500, 250, 125, and 62.5 μg/mL). ZnO-NPs were identified using an agar well diffusion assay after E. faecuim and P. mirabilis were grown on MHA. [31].

Isolation of human lymphocytes

A healthy, nonsmoking female donor who was free of infectious diseases at the time of blood collection had her lymphocytes removed. After

dilution with a comparable amount of phosphate-buffered saline (PBS), blood was transferred onto 3 milliliters of Ficoll-Paque and subjected to a 20-minute centrifugation at 2000 × g. The lymphocyte-containing layer, also known as the buffy coat or white layer, was carefully removed from the plasma layer and placed into a fresh 15 ml tube. The transferred cells were diluted with 10 mL of PBS and centrifuged at 1000 × g for 10 minutes before being removed from the supernatant. Lymphocytes were washed and suspended for 5 minutes at 37°C in erythrocyte lysis buffer (150 mM NH4Cl, 10 mM NaHCO3, 1 mM EDTA, pH 7.4). Then, the cells were washed twice with PBS. [32] Cells were cultured in 96-well flat microtiter plates with a final volume of 2001 complete culture medium (RPMI-1640) per well. After incubation, the medium was removed, and twofold serial dilutions of SeNPs (25, 50, 100, 200, and 400 g/ml) were added to the wells containing PC3 and HdFn cells. To the lymphocyte-containing wells, different twofold serial dilutions of ZnO-NPs (1000, 500, 250, and 125 g/ml) were added. Each well received 10 µl of MTT solution after exposure. After that, the plates were incubated for 4 hours at 37°C and 5% CO2. Finally, at a wavelength of 575 nm, the absorbance was measured with an ELISA reader. [33]

RESULTS

Identification of Bacterial Isolates

Fifty *E. faecium* specimens were taken from tooth root canals for this research. After the samples were analysed, only nine isolates had been found, as shown in Table 1.

Proteus mirabilis isolates

Two hundred and fifty specimens from urine catheters have been collected in total between September and December of 2023. Each sample was administered directly onto blood agar and MacConkey culture plates. Only 35 specimens (46.6%) of all the specimens were found to be.Phyto mirabilis, as shown in Table 1.

Screening of the Antimicrobial Activity of E. Faecium

The (FPD) and the (AWD) was used to separate the isolates that had a capacity to create an inhibitory compound (BLIS) in order to study the microbial productivity of BLIS. Approximately five E. faecium isolates were identified to produce inhibitory compounds during the first screening, and inhibition zones measuring between 10 and 26 mm. For the entire duration of the secondary screening, isolate displayed the most inhibition diameter. The most successful E. faecium (E20), and the filter paper disk methodology produced the best results. The filter paper disk and agar well diffusion techniques were commonly used for evaluating five isolates for the formation of BLIS.

Optimum conditions for bacteriocin production:
A-Optimal pH:

The optimal pH for production, according to the results, was 5 when the diameter of the inhibitory zone reached 21 mm. The lower inhibition zone of 5.30 mm at pH 7 is depicted in Fig. (3). The antimicrobial activity of the BLIS was found to be stable at pH values ranging from 4 to 8. Low pH could significantly reduce the need for neutralizing agents.

Optimum incubation time:

BLIS production was observed at different incubation times, with the highest production occurring after 72 hours of incubation, resulting in the largest inhibition zone, measuring 21 mm. BLIS activity, on the other hand, decreased after 24 hours, with the lowest inhibition zone measuring 5 mm. as shown in Figure (4). Found that the highest activity from *E. faecuim* was observed after a three-day incubation period.

Effects of Nitrogen and Carbon Sources

In isolate E20, the yeast extract was found to be a nitrogen source for the production of inhibitory substances, resulting in a 28 mm inhibition zone. This was a better nitrogen source than tryptone. These findings support the hypothesis that yeast

Table 1. Prevalence of *Proteus mirabilis* and *Enterococcus faecium*

Type of bacteria	Source of samples	No. of samples	Gram stain No. (%)	*Proteus spp* and *Enterococcus spp* No. (%)	*p.mirabilis E.faecium* No. (%)
p.mirabilis	Catheter urine	150	100(66.6%)(GV-)	75(75%)	35(46.6%)
E.faecium	Tooth root canals	50	23 (46 %)(Gv+)	18(78.26%)	9(50%)

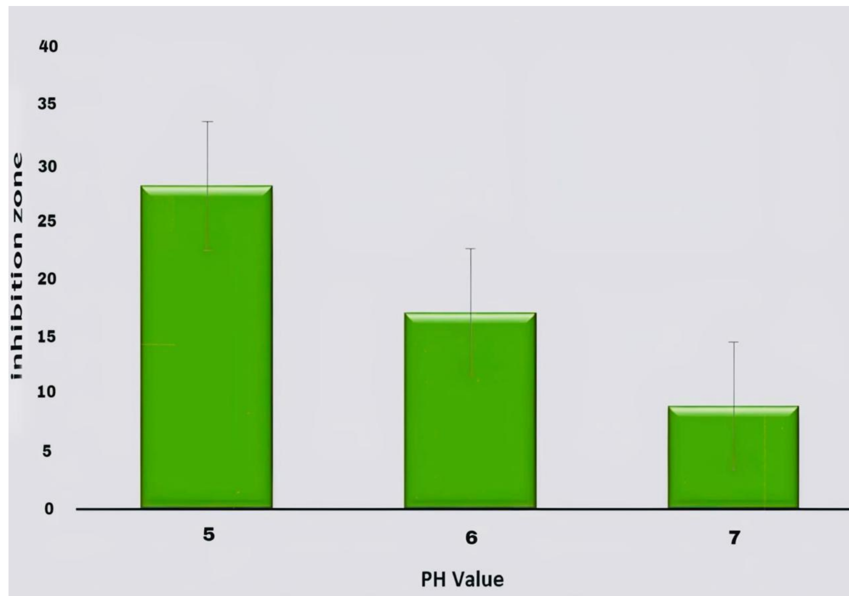

Fig. 3. Effect of pH on BLIS production.

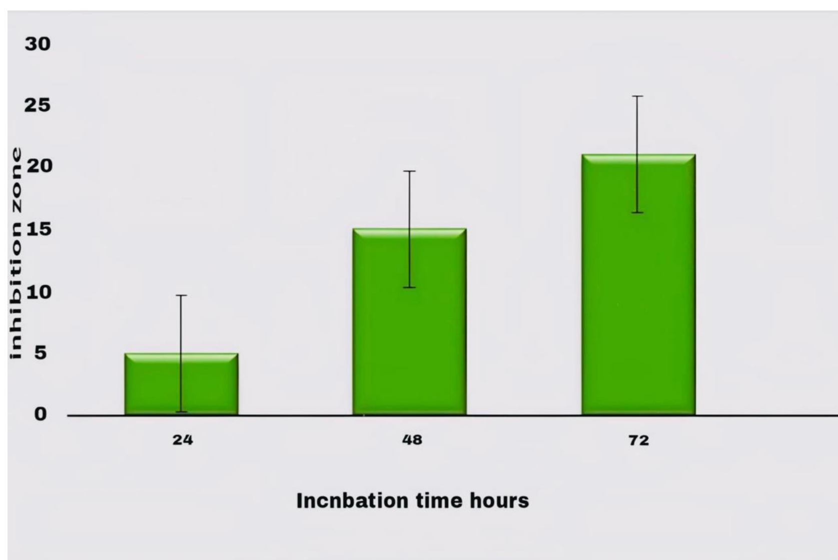

Fig. 4. Effect of incubation time on BLIS production.**: $p \leq 0.01$, NS: not significant, SD: standard deviation, n = 3.

extract is the best organic nitrogen source for bacteriocin production. proposed the effect of different carbon sources on bacteriocin-producing lactic acid bacteria and found that glucose and sucrose were the best carbon sources. Figure (5)

Partial purification of the bacteriocin-like inhibitory substance

Using a step-gradient extraction technique with ammonium sulfate in 60% isopropanol in 100 mM phosphate buffer (pH 5), bacteriocins produced by E. facuim were partially isolated. Bacteriocins utilized as unprocessed, partially purified preparations have to be applied accordingly to the experimental model.

ZnO- NPs Biosynthesis:

Using zinc oxide, nanoparticles were created. The free extract from the BLIS. The generation of a light yellow to white precipitate served as an indicator of nanoparticle production. Once the material was microwave-dried, a glossy white powder was obtained. Fig (6)

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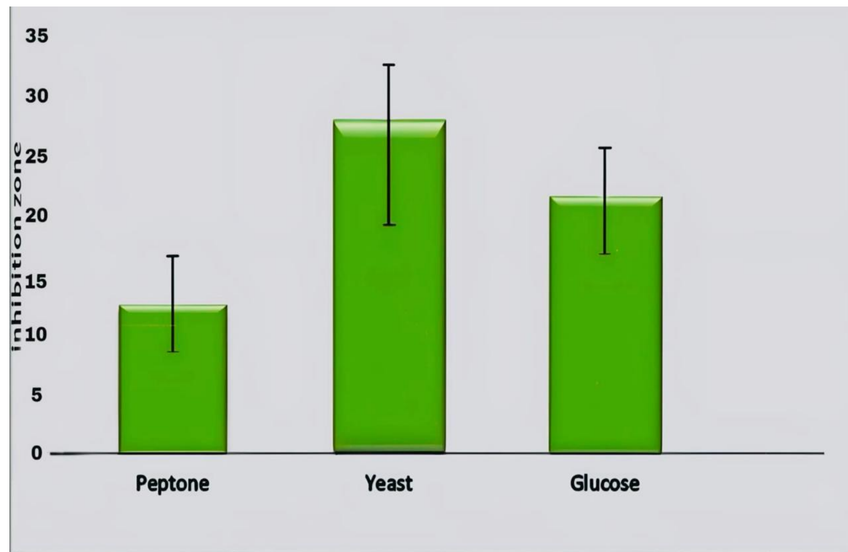
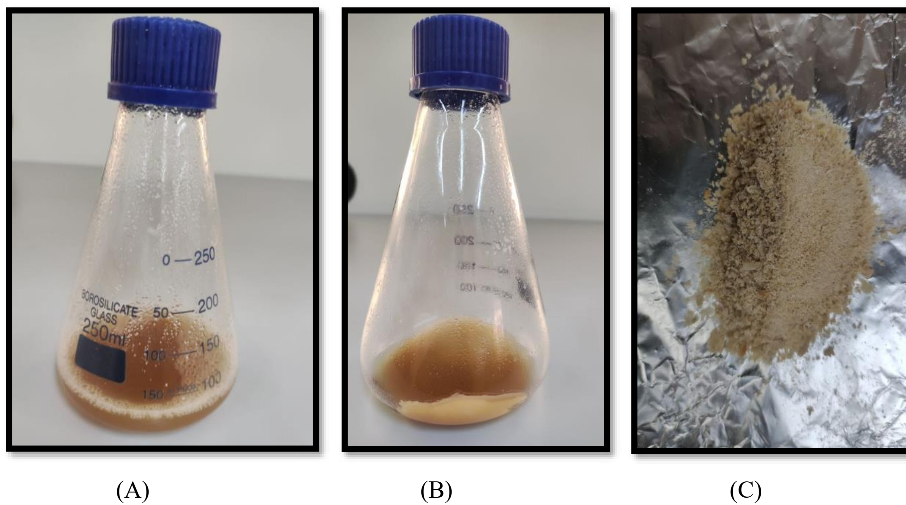


Fig. 5. Effect of nitrogen and carbon sources. **: p ≤ 0.01, NS: not significant, SD: standard deviation, n = 3.



(A) (B) (C)

Fig. 6. Zinc nanoparticle biosynthesis
A: After incubation, cell-free extract with zinc acetate
B: Nanoparticle sedimentation
C: Drying of ZnO- NPS

Characterization of ZnO-NPs

UV–Vis spectral analysis

UV–visible spectroscopy is a widely used technique for determining the optical properties of nanoparticles. This test was carried out in the range of 200-400 nm for the ZnO NP solution. Figure (7) shows the presence of an absorption peak at 267 nm, indicating the successful biosynthesis of the ZnO- NPs from *E. faecium*.

Atomic force microscopy (AFM) analysis

AFM was used as a confirmatory technique to characterize the biosynthesis of ZnO-NPS by detecting its average diameter and morphology in both and three dimensions. The results of this study revealed that *E. faecium* biosynthesized ZnO-NPs with a diameter of 259.2 nm. Figure (8)

Fourier Transform Infrared Spectroscopy

The particular signals that infrared spectroscopy detects are a result of the substance-specific vibration of the molecules. FTIR spectroscopy, which uses absorbance and transmittance values between 400 and 4000 cm1, is a useful method

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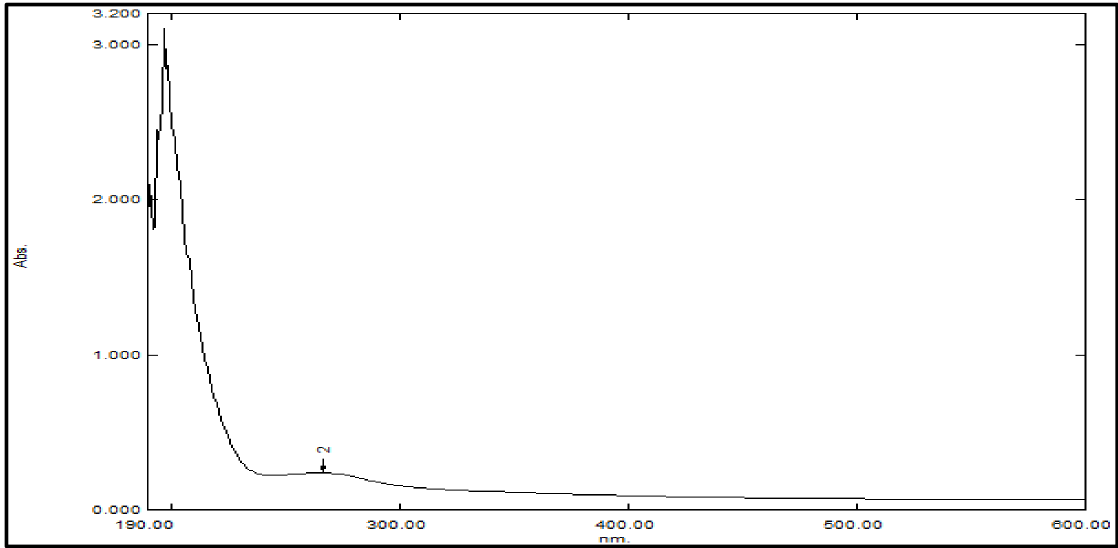

Fig. 7. UV-visible spectrum ZnO NPs

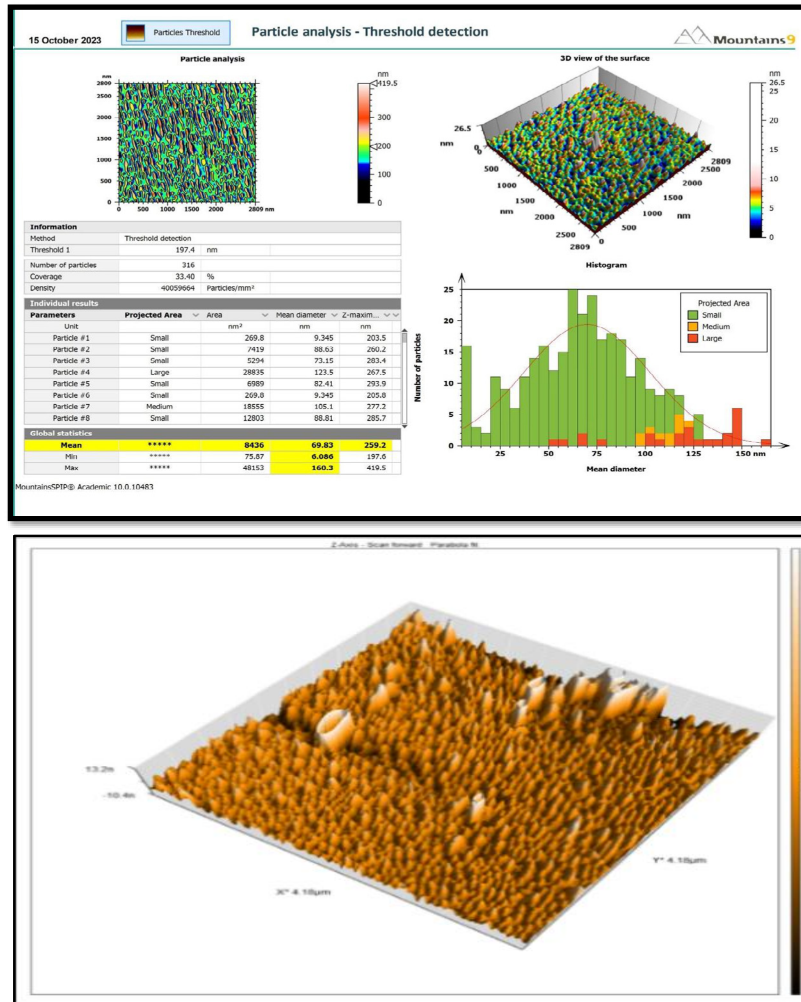

Fig. 8. 2D AFM image of ZnO-NPs biosynthesized

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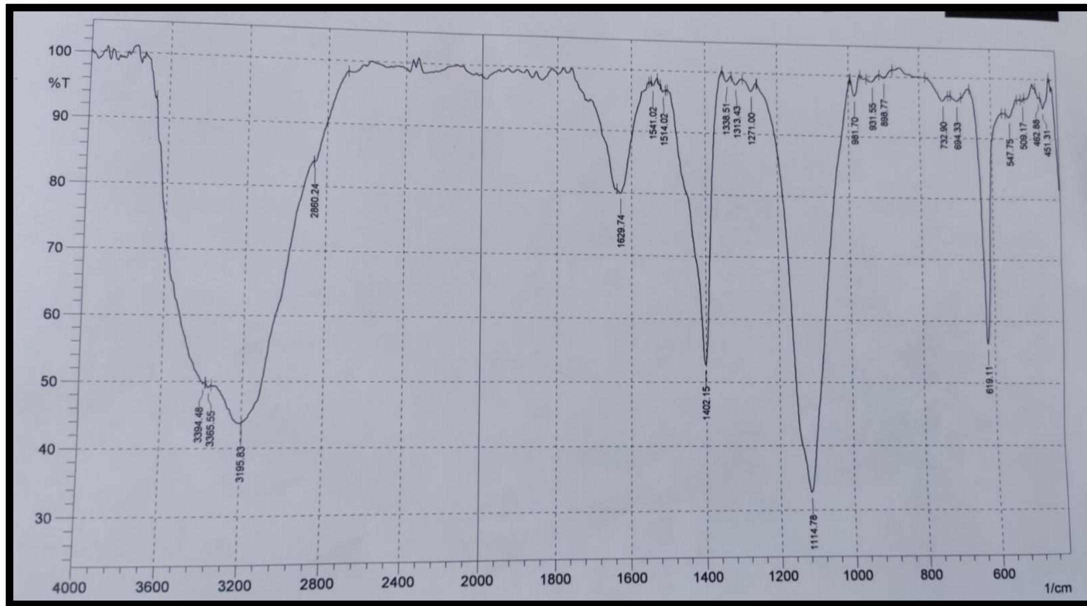

Fig. 9. FTIR results of the synthesized ZnO NPs

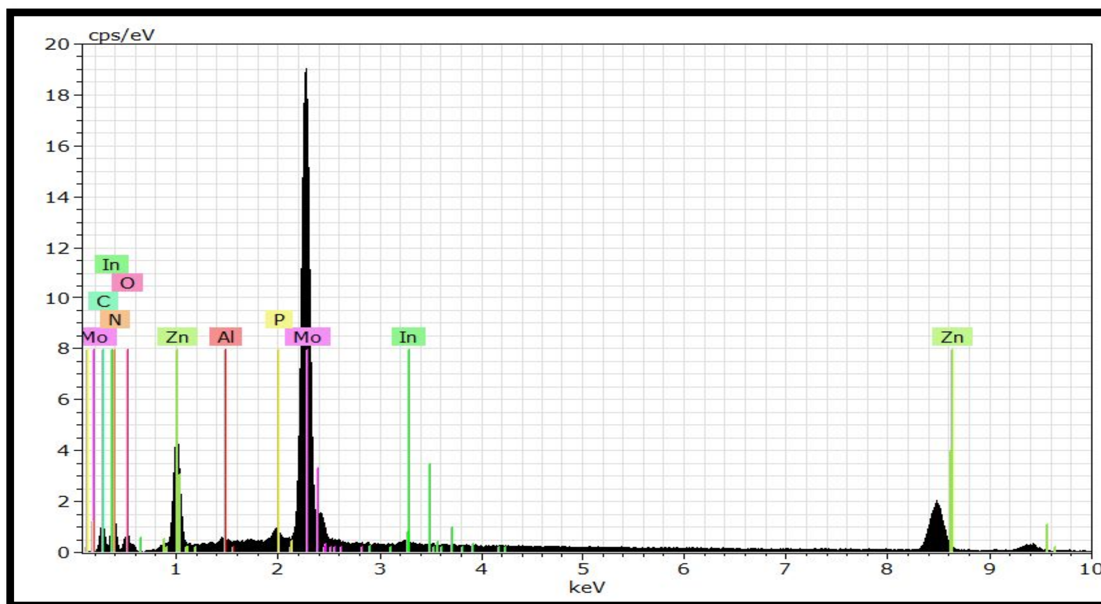

Fig. 10. EDX analysis of ZnO-NPs

for analysing the chemical bonding of NPs. The FTIR spectra of the biosynthesized ZnO-NPs are displayed in Figure (9).

Energy dispersive X-ray (EDX) spectroscopy

EDX analysis was used to determine the elemental composition of the biosynthesized ZnO-NPs, and the results are shown in Fig. (10). The presence of zinc in the EDX spectrum was determined in our study. showed that the atomic weight percentage of Zinc was 35.47%.

X-ray diffraction (XRD)

spectra of ZnO NP powder revealed ten prominent peaks at 20 cm-1: 11.948, 20.241, 24.792, 28.271, 31.458, 45.19, 48.423, 56.242,

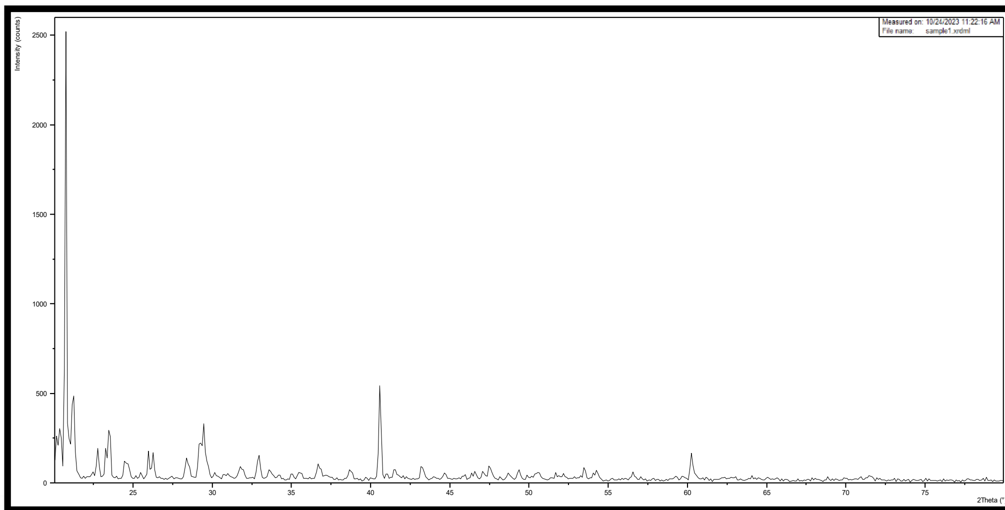

Fig. 11. XRD analysis of synergistic ZnO- NPs

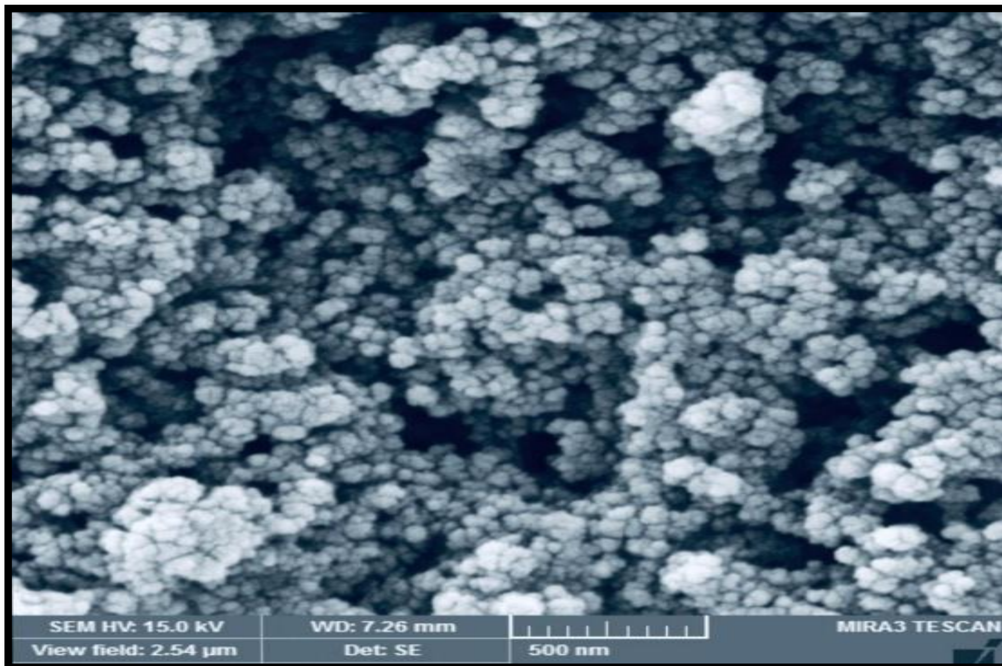

Fig. 12. SEM images of ZnO- NPs

63.191, 65.986, and 75.068 cm-1. The XRD pattern unequivocally supports the formation of zinc oxide NPs by biological materials, which is also supported by the resulting ICDD NOs 008, 79-2205, and 05-0664. Furthermore, these ZnO-NPs diffraction peaks revealed a hexagonal wurtzite structure, with a narrow diffraction peak confirming the formation of crystalline ZnO- NPs. As shown in Figure (11),

Field emission scanning electron microscopy (FESEM)

SEM was used to examine the size, shape, . SEM images showed that the particles were mainly spherical and consistent in shape. Fig. (12).

Antibacterial activity of the ZnO-NPs of E. facium

E. facium bacteria isolated from dental care

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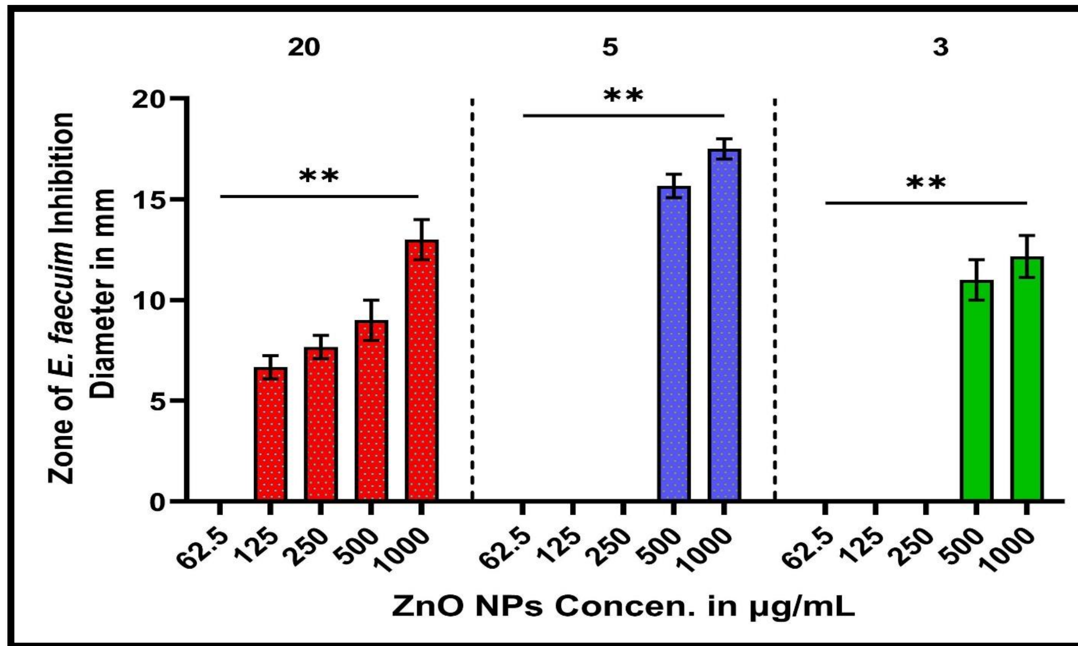

****: p < 0.05, NS: not significant, SD: standard deviation**

Fig. 13. zone of bacterial inhibition in mm with different concentrations of ZnO- against *E. Faecium* standard deviation (n = 3). Mean (± SD)

root canals was significantly reduced by ZnO nanoparticles, as shown by the MIC results and the widths of the areas of inhibition shown in Figure 7. More evidence of a statistically significant ($p < 0.05$) variation in the dispersed mean zone of bacterial inhibition after ZnO-NPs at different concentrations (1000, 500, 250, 125, and 62.5 µg/mL) treatment is presented in Fig. (13). Following treatment with 1000 µg/mL ZnNPs, the spread of the mean zone of bacterial inhibition showed a significant change ($p < 0.05$) accordance to the statistical analysis. The isolates of E. faecium varied substantially from each other.

Antibacterial activity of ZnO-NPs from P. mirabilis

P. mirabilis collected from urine and catheter urine were significantly reduced by ZnO nanoparticles, as demonstrated by the MIC results and the widths of the zones of inhibition shown in Figure 12. Fig. 14 offers additional proof that the dispersed mean zone of bacterial inhibition changed statistically significantly ($p < 0.05$) after ZnONPs at various concentrations (1000, 500, 250, 125, and 62.5 µg/mL) were added. Following treatment with the MIC of 1000 µg/mL ZnNPs, the statistical analysis revealed a significant change ($p < 0.05$) in the mean zone of bacterial inhibition. The P. mmirabilis isolates differed substantially from each other.

Cytotoxic effect of ZnO-NPs on lymphocytes

The cytotoxicity assay results showed that there was no significant reduction in the viability of human lymphocytes treated with biologically synthesized ZnO nanoparticles. These findings suggested that these methods could be used as a viable alternative to other physical methods currently associated with environmental toxicity. After 24 hours of interaction with ZnO-NPs, MTT assays revealed cytotoxicity in human lymphocytes. MTT assays have been widely used in cell culture experiments to determine the in vitro cytotoxicity of NPs. The results of the MTT assay revealed no significant ($p \leq 0.05$) increase in cell viability with increasing ZnO-NP. Figure 15 shows the results of the MTT assay for determining the viability of human lymphocytes treated with ZnO-NPs. In contrast, the toxic effect of chemically prepared ZnO-NPs on the MTT cell line

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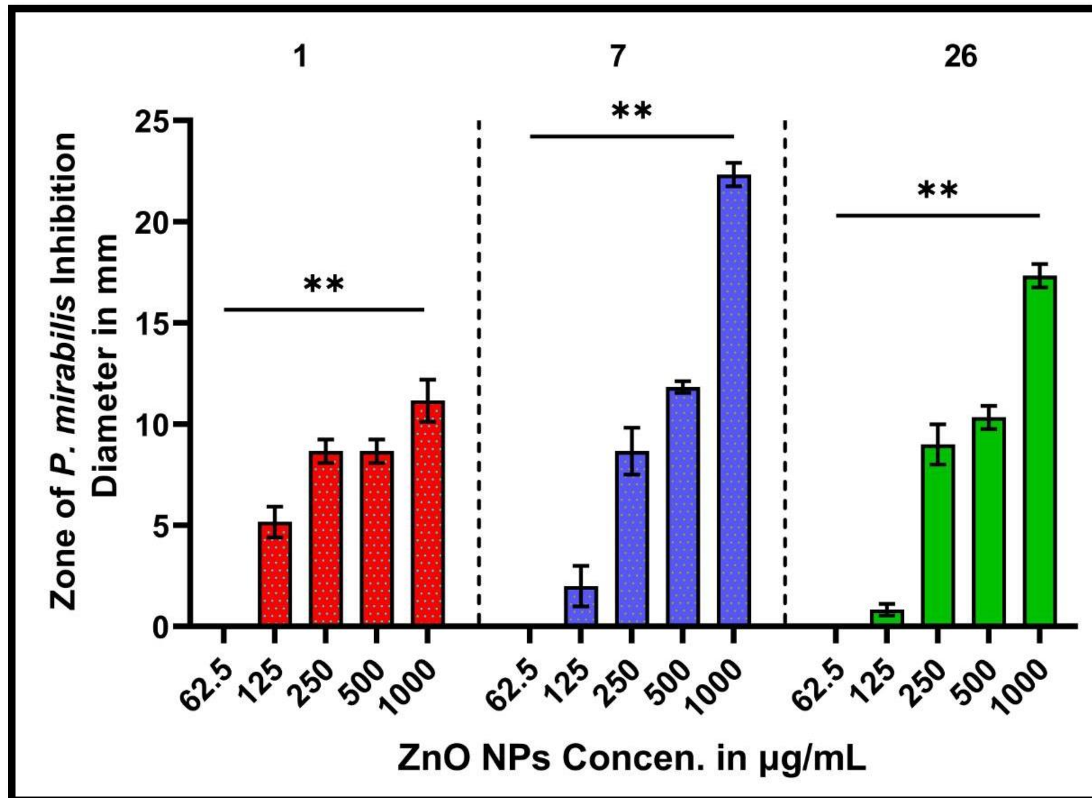

**: $p < 0.05$, NS: not significant, SD: standard deviation

Fig. 14. Mean (± SD) zone of bacterial inhibition in mm after treatment with different concentrations of ZnO-NPs (1000, 500, 250, 125, and 62.5 µg/mL) against *P. mmirabilis* standard deviation (n = 3).

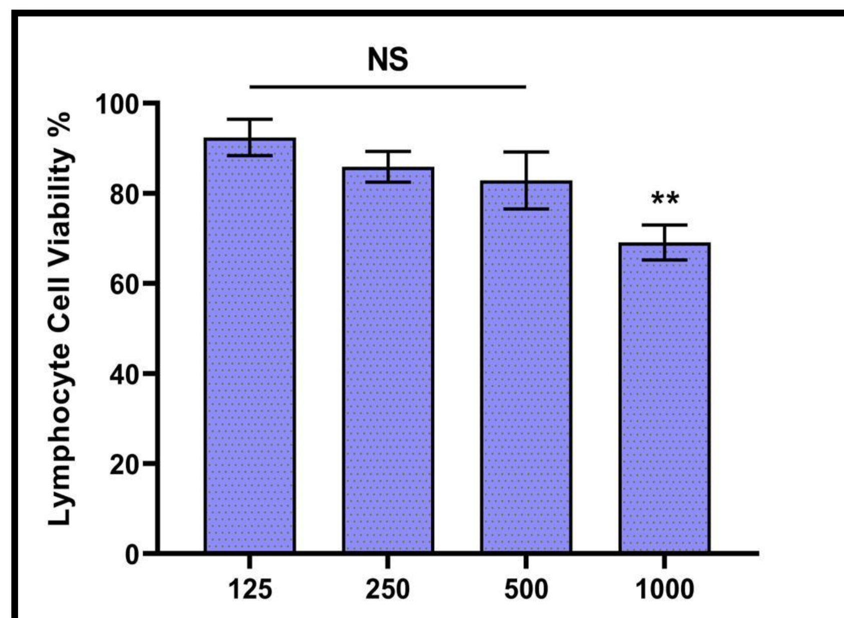

Fig. 15. Cell survival curves (mean±SD%) of human lymphocytes after treatment with different concentrations of ZnONPs (1000, 500, 250, 125, and 62.5 µg/mL) determined by MTT in vitro at 37°C and 5% CO2 for 24 hr.

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DISCUSSION

Enterococcus faecium isolates have been identified in gums of healthy people . White, smooth, large or small, convex, creamy, and smooth round colonies with perfect margins surrounded by clear space appeared after cultivation on MRS agar, as described previously [34]. The synergistic properties of the BLIS suspension improved its antibacterial activity, as it acted as an inhibitory substance and antimicrobial agent. [35]. on other hand bacteriocin of *P. fluorescens* and Citrus limon effects against Propionibacterium acnes and *S.epidermidis* in acne patients [36]. Conversely, employing plant extracts with antibacterial properties against isolated bacteria can have detrimental effects. This study compared the effects of MRS Acin, nisin A, and vancomycin on food-source-isolated *Staphylococcus aureus* bacteria that cause biofilm development_[37]. This study agreed with studies that confirmed that the disk diffusion method is the best method, as it showed that the filter paper disk diffusion test provides several advantages in contrast with other methods, includes ease of application, affordability, and the capacity to evaluate a big quantity of microbes and antibiotics. The results are easily understood. [38]. The factors affecting the antimicrobial activity of BLIS, with maximum inhibitory substance production and bacterial growth of *E. faecium*, were found at a pH of 5. [39]. The optimal incubation time for lactic acid bacteria to produce the highest zone of inhibition was 72 hours. The incubation period is also another important parameter because lactic acid bacteria need time to grow and produce lactic acid. [40]. Yeast extract was the optimal nitrogen source for the induction of inhibitory substances compared with other carbon sources, such as glucose, which was the best carbon source when compared with sucrose. [41]. The preliminary confirmation of the formation of ZnO-NPs was determined by visual observation of the reaction mixture. It was suggested that the biological substances released into the supernatant by the bacteria and functional groups on the bacterial cells were responsible for the reduction of zinc to ZnO -NPs. Markus et al. [42]. ZnO-NPs in the range of 200–400 nm were obtained from a ZnO NP solution. In this study, a noticeable absorption peak was found at 267 nm, indicating the success of the biosynthesis of zinc oxide NPs from *E. faecium*. This result is consistent with the presence of nanoparticles with UV RNs between 200 and 400 nm. [43]. Utilizing a scanning probe microscope, an AFM inquiry of the ZnO-NPs has been carried out to determine and describe the nanoparticle distribution. The mean square roughness and estimated the size of particles were calculated. The nanoparticles of zinc oxide can be produced by microorganisms in a variety of forms, sizes, functions, and behaviors. These variations could be caused by the pathway of synthesis, the digestive enzymes that bacteria use, temperatures, and other biological components. [44]. Fourier transform infrared spectroscopy is a characterization method to determine the the element. The FTIR series of photosynthesized zinc nanoparticles falls in the wavenumber range of 500–4400 cm1 [45]. The cytotoxicity assay no significant reduction in the viability of human lymphocytes treated with green synthesis ZnO nanoparticles. According to [46], The intrinsic capacity of an ingredient to kill organisms is demonstrated by in vitro cytotoxicity tests, which additionally offer concentration ranges for sensible and safe administration. Given these results, bacterocin purification seems to be an exciting alternative for the current physical and chemical approaches linked to environmental toxicity.

CONCLUSION

Given the widespread issue of antibiotic resistance, using BLISs as an antibiotic alternative can help minimize the use of antibiotics. *E. faecium*, which is isolated from dental root canals and has antibacterial qualities, is a possible substitute for antibiotics. A straightforward, low-cost, environmentally friendly, high-throughput, and green synthesis process was successfully used to create ZnO-NPs, which showed remarkable antimicrobial agents a variety of bacterial species. ZnO-NPs can therefore be employed as antibacterial agents in the environment.

ETHICAL DECLARATIONS

DECLARATION OF COMPETING INTEREST.

The authors declare no conflict of interest regarding the publication of this paper

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