Kütahya Dumlupınar University
Institute of Graduate Studies

Journal of Scientific
Reports-A
E-ISSN: 2687-6167
Number 47, June 2021

RESEARCH ARTICLE

GREEN SYNTHESIS of SILVER, ZINC, and CERIUM NANOPARTICLES USING THERMOPHILIC ANOXYBACILLUS SP. ST7 STRAIN and INVESTIGATION of THEIR VARIOUS BIOLOGICAL ACTIVITIES

Serpil GONCA

[1]Mersin University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Mersin, serpilgonca@mersin.edu.tr , ORCID: 0000-0002-8544-1184

Received Date:14.12.2021 *Accepted Date:22.12.2021*

ABSTRACT

The antioxidant activities of AgNP, ZnNP and CeNP synthesized extracellular from thermophilic *Anoxybacillus* sp. ST7 were evaluated by DPPH scavenging activity and ferrous chelating activity. The highest DPPH and ferrous chelating activities of AgNP, CeNP, and ZnNP at 200 mg/L concentration were 93.59% and 88.08%, 73.04% and 78.25%, and 77.47% and 82.96%, respectively. Also, the nanoparticles demonstrated significant DNA cleavage activity. The antimicrobial capabilities of NPs were researched in micro-dilution methods and it was observed that Gram +ve bacteria were more susceptible to nanoparticles. The nanoparticles showed more effective microbial cell inhibition viability activity toward *E. coli*. Also, NPs showed important biofilm inhibition activity toward *S. aureus* and *P. aeruginosa*.

Keywords: *Thermophilic bacteria, nanoparticles, antioxidant, antimicrobial activity, biofilm inhibition, microbial cell viability*

1. INTRODUCTION

Nanotechnology can be defined a science which investigates at the molecular level (in the range of 1 to 100 nm). Nanotechnology can create materials which have completely new physical, the emergence of structures with chemical and biological properties. Nanomaterials exhibits superior and new features with size-dependent compared to larger particles of the same materials [1], [2]. Nanoparticles can show superior properties by providing certain conditions because they are remarkable very small in size compared to the volumetric structures of materials. For example, the conductivity of the nanostructure that even if a single atom was added to the structure, can be changed completely can change [3]. The properties of nanoparticles are very superior because their shape and morphology can be controlled as well as their size [4].

The NPs are currently utilized in various objects such as from, electronics, cooking vessel to aerospace industry and renewable energy. So far, numerous synthesis techniques that are categorized onto bottom-up or top-down way have been developed for nanoparticles synthesis [5], [6]. Green synthesis is one of the methods that allows nanoparticles to be synthesized using environmentally friendly methods. The term of green nanotechnology can be defined as the production of nanoparticles from living cells which are environmentally friendly with low toxic substance content. This term

JSR A
Journal of Scientific Reports

within the scope of nanotechnology refers to easy-to-apply, harmless methods that reduce the waste product problem [7]. Green plant extracts and microorganisms are used within the scope of green nanotechnology. Although many living things are used as extracts, *Azadirachta indica*, *Acalypha indica*, *Camellia sinensis*, *Jatropha curcas* and *Aloe vera* can be given as an example for green plant extracts [8]. It was reported by Nematollahi [9] that plants and plant products were cheap and renewable resources for nanomaterial production. In addition, the use of microorganisms' extracts can be considered as an alternative way to physical and chemical methods.

There are various reports on the biological applications of the nanoparticles synthesized in the present study. The antioxidant capabilities of cerium oxide NPs for varies reactive species have been indicated in *in-vitro* and *in-vivo* studies with cerium oxide NPs displaying anti-inflammatory and bio-mimetic antioxidant properties [10]. Moreover, cerium oxide NPs have also been shown to have antimicrobial activity [11]. It has indicated in previous studies that silver nanoparticles exhibit biological activities such as antimicrobial activity, biofilm inhibition activity and anticancer activity [12], [13], [14]. Besides, zinc oxide NPs showed varied biomedical implementations in the areas of drug delivery systems, bio-imaging, tissue engineering, and can also be used as antioxidant, antibacterial, and antidiabetic agents [15].

The aim of this study, synthesized AgNP, ZnNP, and CeNP nanoparticles using thermophilic *Anoxybacillus* sp. ST7 bacterium extracts. The produced NPs were characterized by FTIR, FE-SEM, SEM-EDX, and XRD analyses. The antioxidant capabilities of the produced NPs were evaluated by DPPH scavenging activity and ferrous chelating activity. Moreover, DNA nuclease and biofilm inhibition activity were also tested.

2. MATERIALS AND METHODS

2.1. Materials

Cerium nitrate (Ce(NO3)3·6 H2O) and zinc sulfate ($ZnSO_4$) were obtained from Sigma Aldrich. Silver nitrate (AgNO3) was purchased from Merck. Also, all utilized chemicals were of analytical reagent grade. Distilled water (DW) used in all tests was obtained from purification system.

2.2. Nanoparticles Synthesis

10 mM silver nitrate, cerium nitrate, and zinc sulfate solutions were prepared for the synthesis of nanoparticles. 300 mL of each solution were added into the 100 mL bacteria extracts and were left to react for overnight at 70 ^{o}C. The prepared nanoparticles were washed with deionize water a few times and dried for 24 h at 80 ^{o}C.

2.3. Nanoparticles Characterization Methods

The synthesized nanoparticles were characterized by FTIR, XRD, FE-SEM and EDX. Perkin Elmer 400 model FT-IR spectrometer was used to obtain infrared spectrums. X-ray diffraction spectrums were obtained by using X-ray diffractometer (BRUKER AXS D8 ADVANCE). The electron microscopy images and energy dispersive X-ray analysis graphs were obtained using a field-emission scanning electron microscope (ZeissGemini 500).

2.4. DPPH Activity

The scavenging capability of AgNP, ZnNP, CeNP upon the DPPH radical was studied with the way expressed by Ağırtaş et al. [16]. A 250 µL of AgNP, CeNP and ZnNP solutions prepared at three various concentrations and were added to the test tubes separately. Then, 1,0 mL of DPPH was joined

JSR A
Journal of Scientific Reports

to the mixtures and incubated to 30 min at room temperature. Trolox and Ascorbic acid were used as controls and the above-mentioned protocol was performed for them as well. Methanol was used as a blank solution. When the incubation period was over, the reaction mixture was measured at 517 nm in the spectrophotometer. Finally, the scavenging capability was calculated with the equation (1):

$$Capacity\ (\%) = \left(\frac{Abs(control) - Abs(sample)}{Abs(control)} \right) \times 100 \quad (1)$$

$Abs_{control}$ is control absorbance value, Abs_{sample} is the absorbance value of the test compounds and DPPH after 30 min.

2.5. Ferrous Chelating Activity

The ferrous chelating ability of AgNP, ZnONPs and CeNP was evaluated by Dinis method [17]. The AgNP, ZnONP and CeNP prepared at different concentrations were treated with $FeCI_2$ for 2 min. Later ferrozine was added to the reaction mixtures. The reaction mixtures were then incubated in the dark for 10 minutes and a spectrophotometer was used to determine the ferrous chelating ability. Subsequently, the absorbance measured at 562 nm. Later the percent of chelating ability was calculated with equation (2):

$$Metal\ Chelating\ Effect\ (\%) = \left(\frac{Abs(control) - Abs(sample)}{Abs(control)} \right) \times 100 \quad (2)$$

$Abs_{control}$ is the absorbance value of the control reaction, Abs_{sample} *is* represents the absorbance value acquired in the existence of compounds or EDTA.

2.6. DNA Cleavage Activity

The DNA cleavage activity of the AgNP, ZnONP and CeNP were evaluated using agarose gel electrophoresis method. pBR322 were used as a target DNA. Different concentrations of AgNP, ZnONP and CeNP were mixed with the pBR322 DNA. Subsequently, obtained mixture incubated at 37 °C for 60 min. Subsequently, the reaction mixtures were loaded into gel. Later, electrophoresis process started. Untreated genomic DNA was utilized as a negative control. The gel were imagined via a transilluminator.

2.7. Antimicrobial Activity

Microdilution way was studied to appraise the antibacterial activity of the AgNp, CeNp and ZnONP. The tested microbial strains were Gr +ve [Enterococcus faecalis, Enterococcus hirae and Staphylococcus aureus], Gr –ve [Pseudomonas aeruginosa, Escherichia coli, and Legionella pneumophila subsp. pneumophila] and fungal strains [Candida parapisilosis and Candida tropicalis]. The green synthesized nanoparticles solutions were firstly prepared in 96 well plates and a serial two fold dilutions were done from 1024 to 1 mg/mL. Then, the microbial strains which was prepared 0.5 McFarland Scale were added to the microplate-wells and incubated at 37 ℃ for 24 h. When 24 h was over, antimicrobial activity was evaluated with MIC values described as the lowest concentration that inhibits microbial growth.

JSR A
Journal of Scientific Reports

2.8. Biofilm Inhibition activity

The biofilm inhibitory behavior of the green synthesized AgNP, ZnONP and CeNP by using thermophilic *Anoxybacillus* sp. ST7 was examined in 24-well plates based on crystal violet (CV) staining. The bacteria tested in the evaluation of biofilm formation inhibition of nanoparticles were *S. aureus* and *P. aeruginosa*. Bacterium cultures were grown for one night before starting the testing phase. Bacterial strains were inoculated to well plates with $2.9x10^8$ CFU/mL. Bacterial strains incubated at 37 ℃ for 72 h in well plates containing with three concentrations of synthesized nanoparticles at 125, 250 and 500 mg/L. Following, the plate's wells were drained and it cleaned two times with DW. The plates were left to dry for 30 min in the oven set at 80°C to dry. Subsequently, CV dye added into the well to stain biofilm formations for 45 min. CV was then lifted and the plates were washed slowly. The washing of wells was done twice. Ethanol was then added and it was waited for 15 minutes for the absorbed CV to be recovered. Spectrophotometer was used to determine biofilm inhibition and its absorbance was measured at 595 nm. Just *S. aureus* and *P. aeruginosa* containing wells were used as positive controls. Biofilm inhibition activity of NPs was calculated according to the equation (4).

$$Biofilm\ \ Inhibition\ (\%) = \left(\frac{Abs(control) - Abs(sample)}{Abs(control)} \right) \times 100 \qquad (4)$$

2.9. Bacterial Viability Test

E. coli was utilized to investigated the bacterial cell viability inhibition properties of Ag, ZnO and Ce nanoparticles. Firstly the bacteria was inoculated in Nutrient broth, and then it was incubated to 24 hours at 37°C. When the 24 h was over, *E. coli* was centrifuged for five minutes at 5000 rpm. The microbial residue was then cleaned with 0.9% NaCl to take culture media. The cleaned bacteria was suspended into 10 mL of 0.9% NaCl. This was used for cell viability test. Later bacteria was reacted with Ag, ZnO and Ce nanoparticles at 3 different concentrations (125, 250 and 500 mg/L) to 90 minutes at 37°C. The time was finished, the mixtures were diluted in different proportions and inoculated in NB agar and left to incubate at 37 °C for 24 hours. The same process was performed with control which was not included the NPs. Eventually, the colonies were counted and the bacterial cell viability calculated using with equation (3).

$$Cell\ viability\ (\%) = (A_{control} - A_{sample} / A_{control})\ x\ 100 \qquad (3)$$

3. RESULTS AND DISCUSSION

3.1. Nanoparticles Characterization

Structure, crystalline nature and morphology of the silver, cerium and zinc nanoparticles were carried out by using XRD, FE-SEM, and EDX analysis. The formation of Ag NPs, $Ce_2O(CO_3)_2.2H_2O$ NPs and ZnO NPs biosynthesized were demonstrated via XRD spectrum. (Fig. 1). The XRD peaks for 2θ at 33.4°, 46.3, 55.2°, 64.7°, and 77.9° correspond to 111, 200, 142, 220, and 311 planes for silver (JCPDS card number 04-0783). FE-SEM images of the Ag NPs showed that the particles had an average size of 20 nm with spherical shape (Fig. 2A). Formation of Ag NPs was also proved by SEM-EDX analysis (Fig. 2B). According to X-ray diffraction data for cerium, 2θ at 20.5°, 30.2°, and 38.3° proved the formation of $Ce_2O(CO_3)_2.2H_2O$ NPs (Fig. 3). Formation of the $Ce_2O(CO_3)_2.2H_2O$ NPs was also proved by FE-SEM and SEM-EDX analysis (Fig. 4A and 4B). The results showed that $Ce_2O(CO_3)_2.2H_2O$ NPs had a particle size lower than 100 nm was successfully synthesized (Fig.

JSR A
Journal of Scientific Reports

4A). Fig. 5. shows the XRD spectrum of the ZnO by two strong amorphous maxima. Formation of the ZnO NPs was studied with FE-SEM and SEM-EDX analysis (Fig. 5A and 5B). The results proved that ZnO NPs had a particle size lower than 100 nm was successfully synthesized (Fig. 5A and 4B).

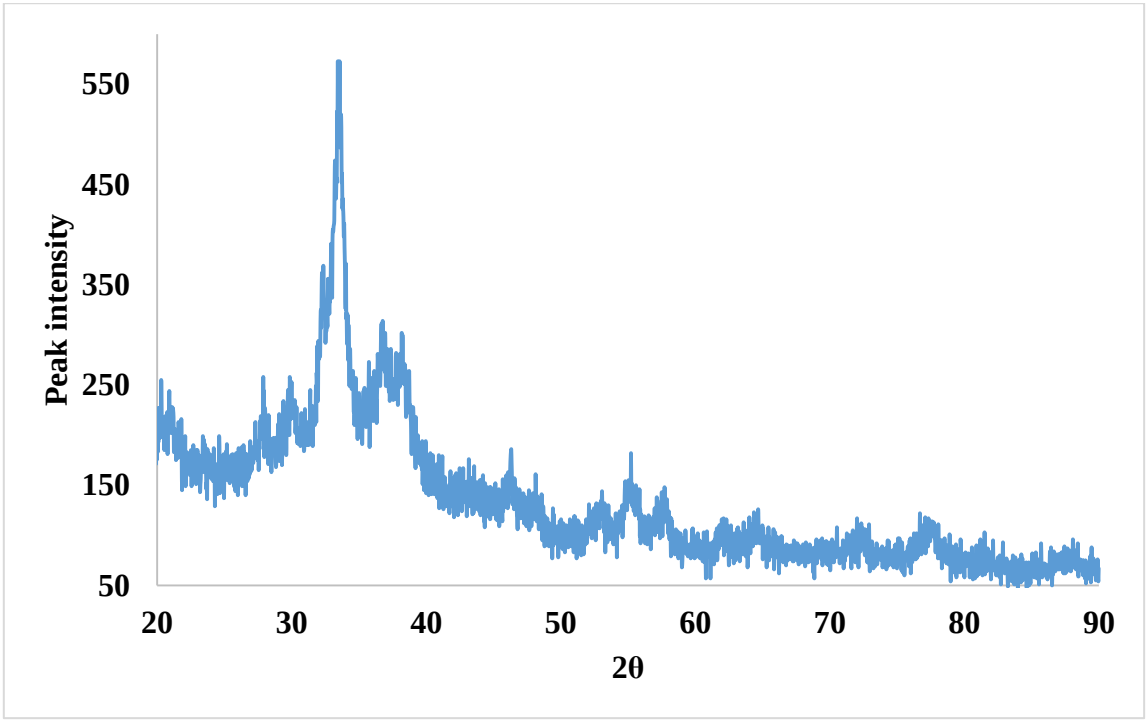


Fig. 1. XRD spectrum of Ag NPs.

JSR A
Journal of Scientific Reports

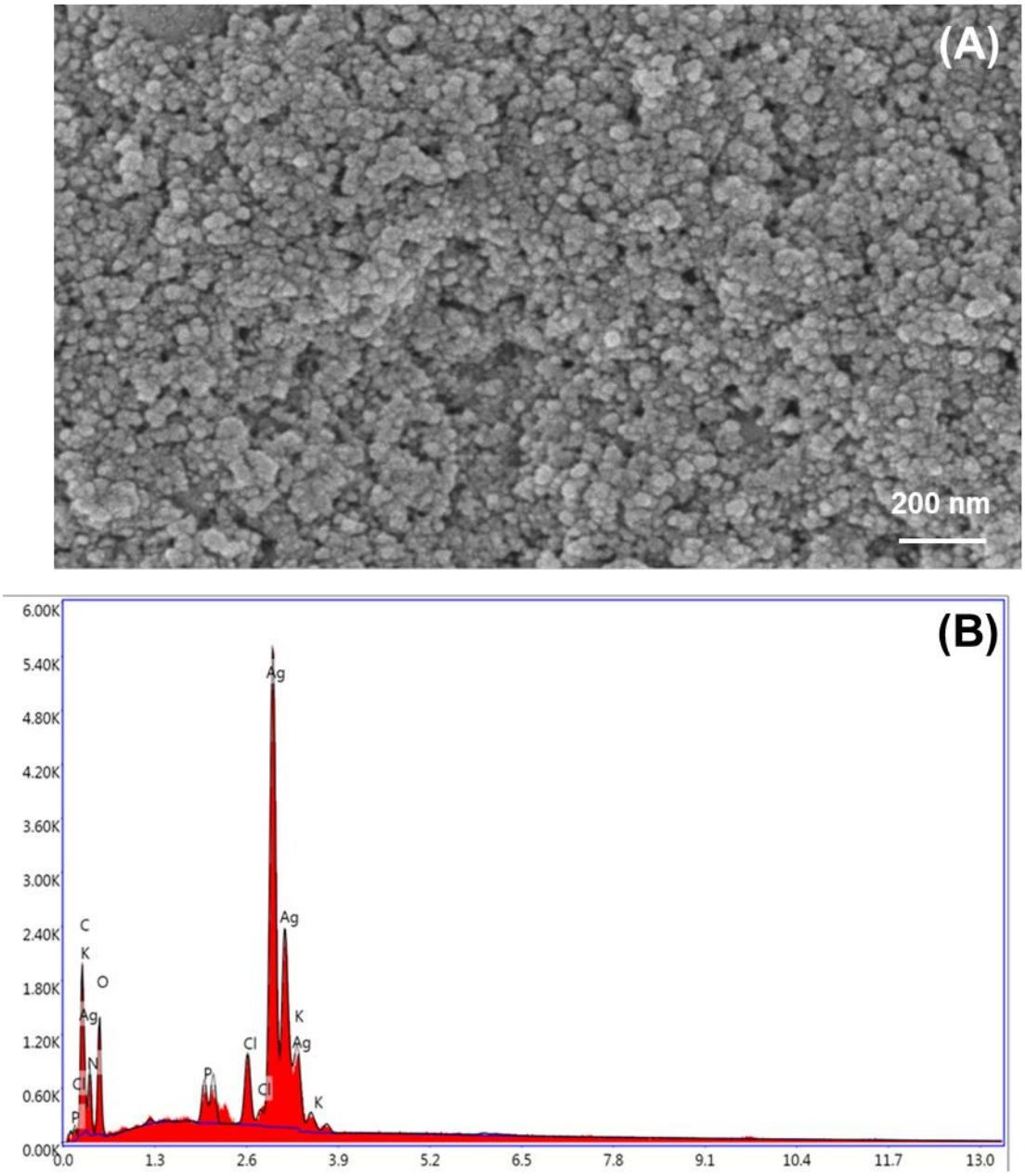


Fig. 2. FE-SEM image and SEM-EDX analysis of Ag NPs.

JSR A
Journal of Scientific Reports

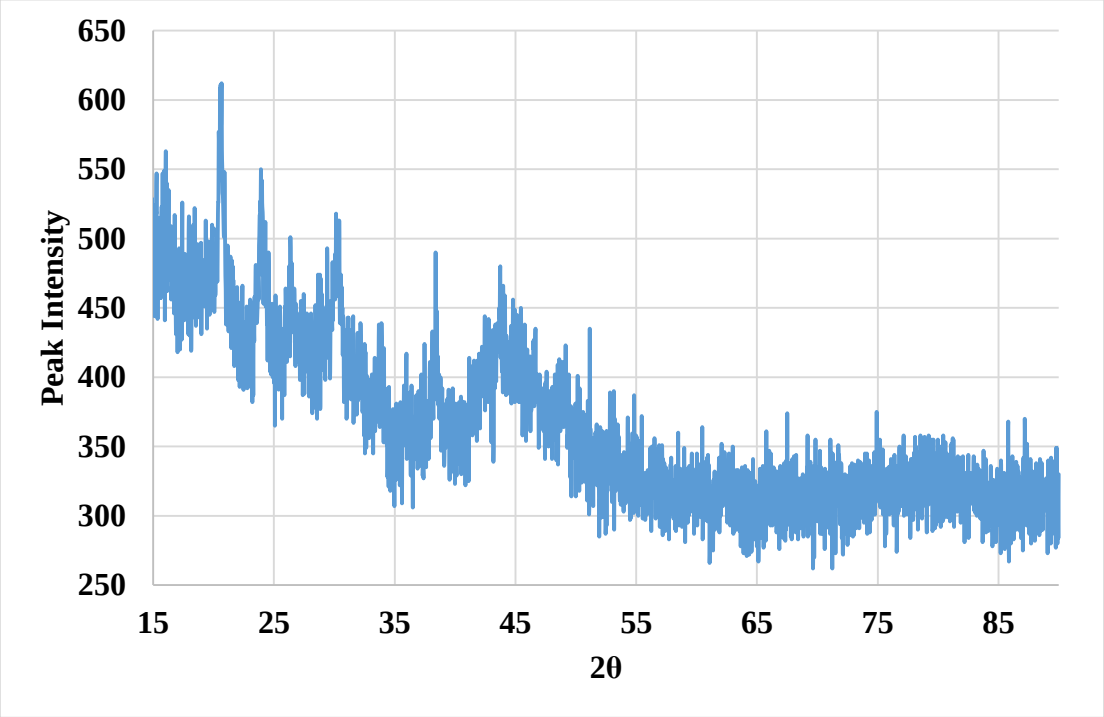


Fig. 3. XRD spectrum of $Ce_2O(CO_3)_2.2H_2O$ NPs.

JSR A
Journal of Scientific Reports

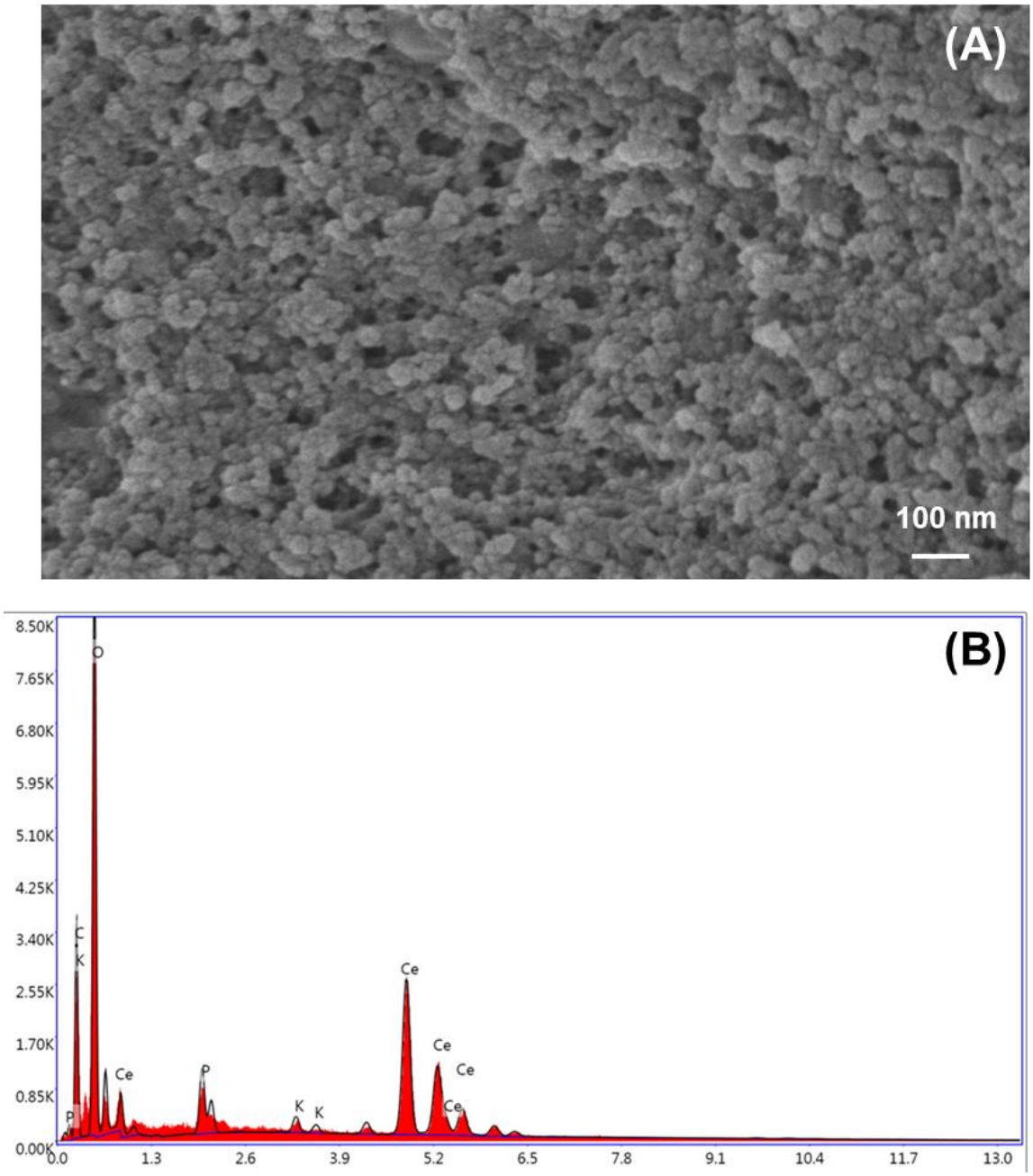


Fig. 4. FE-SEM image and SEM-EDX analysis of $Ce_2O(CO_3)_2.2H_2O$ NPs.

JSR A
Journal of Scientific Reports

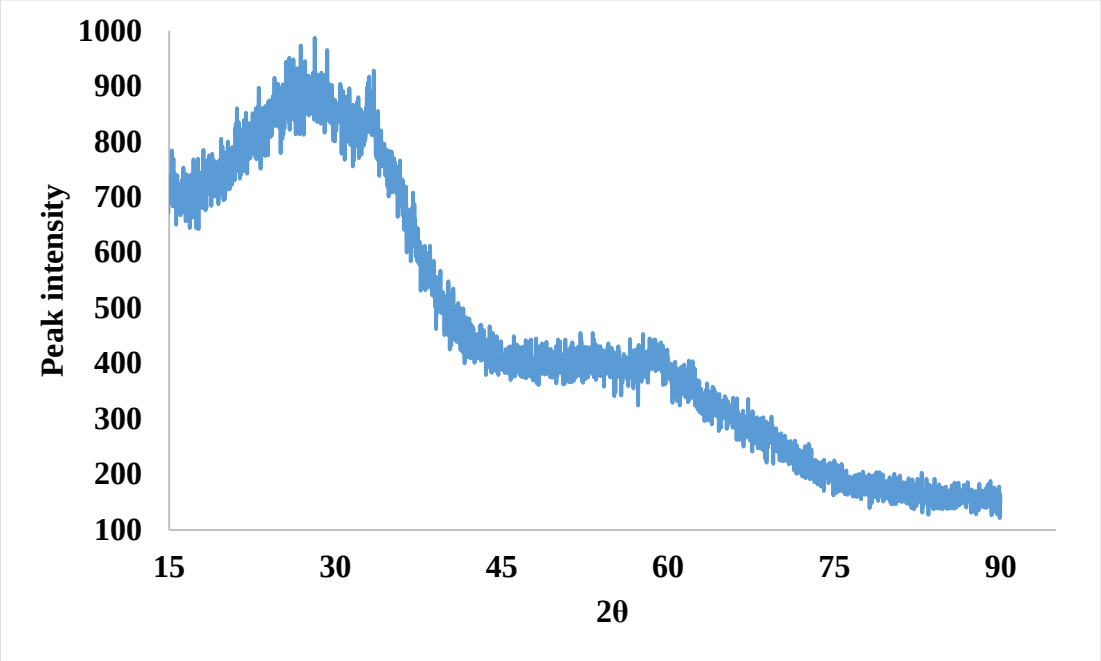


Fig. 5. XRD spectrum of ZnO NPs.

JSR A
Journal of Scientific Reports

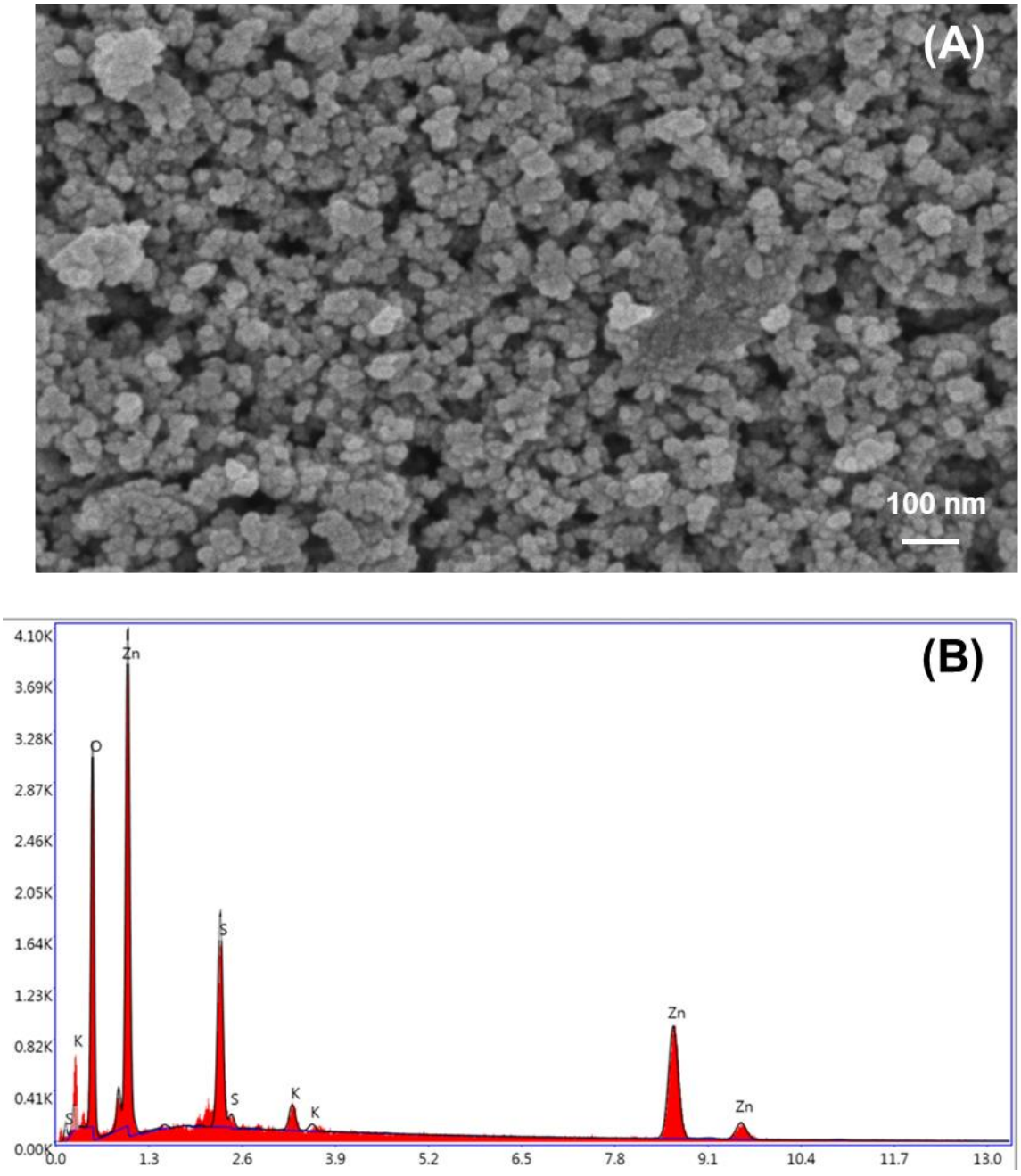


Fig. 6. FE-SEM image and SEM-EDX analysis of ZnO NPs.

JSR A
Journal of Scientific Reports

3.2. DPPH Radical Scavenging Activity

The antioxidant activity of synthesized nanoparticles by using thermophilic bacteria was determined by using DPPH assay (Fig. 7). The free radical scavenging activity of nanoparticles tended to increase with increasing its concentration, also new synthesized NPs had a significant inhibitory activity against the DPPH radicals. According to antioxidant activity results new synthesized AgNP, CeNP and ZnONP were showed the efficient free radical scavenging potential (Fig. 7). DPPH activity order at all concentrations was AgNP>ZnONP>CeNP. When the concentration increased from 12.5 mg/L to 50 mg/L, the scavenging activities of AgNP, CeNP and ZnONP were increased from 73,75% to 88,06%, from 57,62% to 67,43% and from 63,63% to 71,38%, respectively. In addition, DPPH activities of AgNP, CeNP, ZnONP, Ascorbic acid and Trolox at a concentration of 200 mg/L were 93,59%, 73,04%, 77,47, 100% and 100%, respectively. In the present study, DPPH activity results show that DPPH activity was dose depended manner and importantly elevated with the concentration of nanoparticles. An increase in the antioxidant activity of nanoparticles with increasing concentration has also been reported in previous studies [18], [19]. The effect of NPs with antioxidant properties on DPPH is thought to be because of their hydrogen donating capabilities [20]. According to our DPPH activity results, it was determined that the synthesized nanoparticles had effective DPPH activity. In particular, AgNP exhibited activity close to the standards with 93% DPPH scavenging activity at 200 mg/L concentration. Farias et al. [21] evaluated antioxidant activity of CeNP, and they noticed that it showed an average antioxidant activity with 15,06%. In our results, 57,62% antioxidant activity was observed even at the lowest concentration (12.5 mg/L). Therefore, our results showed better results than the aforementioned study. Mahabadi et al. [22] biosynthesed of CeO_2 NPs and they evaluated its antioxidant activity. As a result of their study the maximum antioxidant activities were seem that at 100 µg/mL concentration with 69,8%. Our antioxidant activity results for the green synthesis CeNP had similar results for the aforementioned study. Ameen et al. [23] synthesized AgNPs using fungal extract and investigated their antioxidant activity. They found that its maximum DPPH activity was 78%. The antioxidant activity results of AgNP in the presented study are quite good compared to the mentioned study. Gur et al., [24] synthesized biogenic ZnO NPs and evaluated its antioxidant activity. They reported that DPPH activity of ZnO NP at 250 µg/mL concentration was 79,67%. In the study, 77,47% DPPH activity was observed at 200 mg/L concentration and it had similar results compared to the mentioned study. The new synthesized nanoparticles can be used as an antioxidant agent with effective DPPH activity, and as a result, it can protect normal cells from the harmful effects of ROS.

JSR A
Journal of Scientific Reports

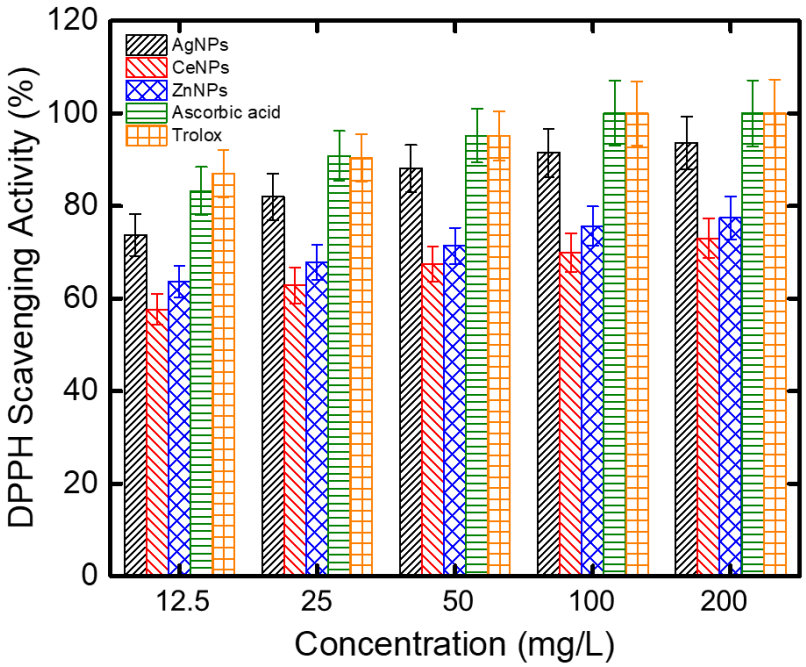


Fig. 7. DPPH scavenging activity of AgNP, CeNP and ZnONP.

3.3. Ferrous Ion Chelating Activity

Ferrous ion chelating method was also used to investigate the antioxidant activity of the synthesized nanoparticles. The ferrous chelating activity of AgNP, CeNP and ZnONP were demonstrated in Fig. 8. EDTA was used as standard. As can be clearly seen in the figure, the chelating activity of nanoparticles increased in parallel with the concentration. The chelating activity of AgNP, ZnONP and CeNP increased from 67,90% to 81,91%, 36,86% to 75,23%, and 29,94% to 70,40%, respectively, when the concentration increased from 12,5 to 100 mg/L. The chelating activity of AgNP, ZnONP and CeNP at 200 mg/L were 88,08%, 82,96% and 78,25%, respectively. When the metal chelating activities of the green synthesized nanoparticles were compared, the chelating activities were EDTA>AgNP> ZnONP>CeNP at the all tested concentrations. AlSalhi et al. [25] synthesized AgNPs using plant derived and studied in vitro antioxidant activity such as DPPH, ferrous ion chelation activity. They noticed that AgNPs showed ranged from 21,90 to 82,49% chelation activity at 10–100 µg/mL concentration. Our results displayed similar results with the aforementioned investigation. Soren et al. [26] synthesized ZnO NPs by polyol and aqueous method and they reported that they showed ferrous chelating ability. The surplus of free radicals in the body causes oxidative stress, and when the body's antioxidative system is weakened, biomolecules are damaged. Molecules with antioxidant properties extinguish these surplus free radicals in the body [26]. Therefore, the antioxidant behavior of the synthesized nanoparticles is important in order to protect our body from diseases caused by oxidative stress. According to result of metal chelating activity green synthesized and characterized AgNP, ZnONP and CeNP can be used as a metal chelating agent after further studies.

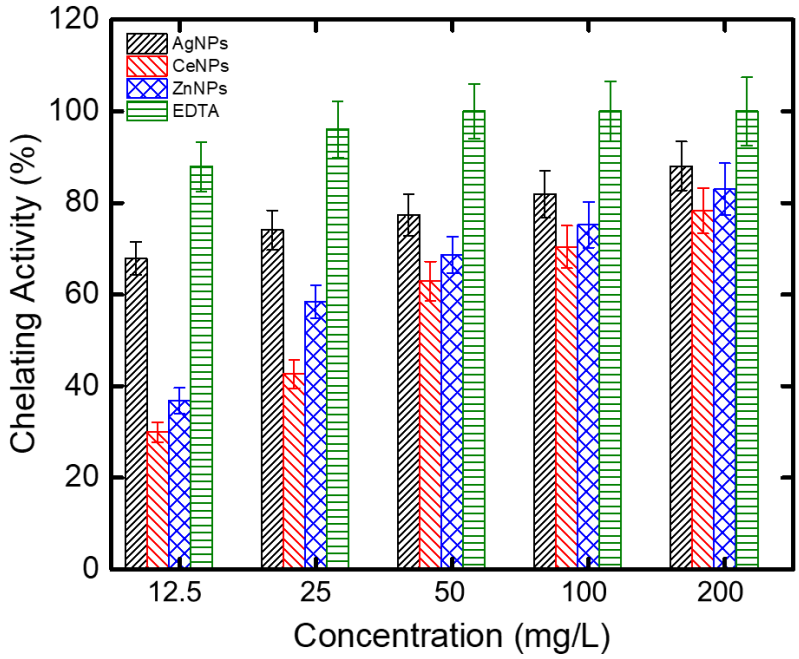


Fig. 8. Ferrous chelating activity of AgNP, CeNP and ZnONP.

3.4. DNA Cleavage Ability

In order to investigate the DNA nuclease effect of new AgNP, ZnONP and CeNP, pBR322 DNA was used as a goal. The efficiency of nanoparticles was tested by agarose gel electrophoresis. Electrophoresis gel image of the study is given in Fig. 9. As can be seen in the Fig. 9, single strand break occurred in supercoiled DNA for all studied three concentrations with the all synthesized nanoparticles. There was a transition from Form I to Form II (Lane 2-4, Lane 5-7 and Lane 8-10). DNA cleavage study results clearly revealed the effect of synthesized NP's on DNA. When we considered our DNA fragmentation results and other results including antimicrobial, cell viability and biofilm inhibition activity, we can be concluded that nanoparticles may have been exhibited antimicrobial activity by also acting on DNA molecules. DNA cleavage efficacy of nanoparticles has been demonstrated in previous studies. Gonca et al. [27] synthesized AgNPs using *Verbascum thapsus* leaf and the green synthesized AgNP demonstrated DNA cleavage activity. De et al. [28] reported that ZnONPs synthesized by the green synthesis method showed 55% DNA nuclease activity towards *E. coli* DNA. Generally, the results suggest that extracellular green synthesized AgNP, ZnONP and CeNP mediated-thermophilic *Anoxybacillus* sp. ST7 can be used as chemical nuclease for medicine industries after further studies.

JSR A
Journal of Scientific Reports

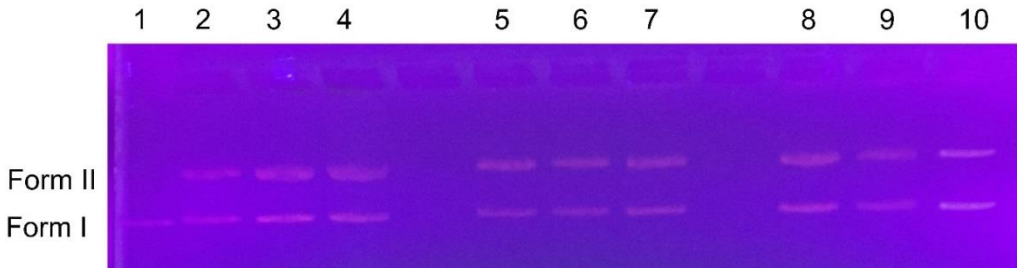


Fig. 9. DNA cleavage activity of AgNP, ZnONP and CeNP (Lane 1, pBR 322 DNA + 50 mg/L *Anoxybacillus sp.* ST7 mediated-AgNP; Lane 2, pBR 322 DNA + 100 mg/L of *Anoxybacillus sp.* ST7 mediated-AgNP*;* Lane 3, pBR 322 DNA + 200 mg/L *Anoxybacillus sp.* ST7 mediated-AgNP; Lane 5, pBR 322 DNA + 50 mg/L *Anoxybacillus sp.* ST7 mediated- CeNP; Lane 6, pBR 322 DNA + 100 mg/L of *Anoxybacillus sp.* ST7 mediated- CeNP; Lane 7, pBR 322 DNA + 200 mg/L *Anoxybacillus sp.* ST7 mediated- CeNP; Lane 9, pBR 322 DNA + 50 mg/L *Anoxybacillus sp.* ST7 mediated- ZnONP; Lane 10, pBR 322 DNA + 100 mg/L of *Anoxybacillus sp.* ST7 mediated- ZnONP; Lane 11, pBR 322 DNA + 200 mg/L *Anoxybacillus sp.* ST7 mediated- ZnONP).

3.5. Antimicrobial Activity

Diseases caused by microorganisms is a public health problem. Especially, as a result of improper utilise of antibiotics the emergence of drug-resistant microorganisms is the important concern all over the world. NPs have been noticed in the studies as a promising improvement of health produces. In the present study, antibacterial activity of new synthesized nanoparticles was studied against Gram +ve and Gram -ve bacterium and fungal strains by micro dilution method. Antimicrobial activity results are presented in Table 1. The results displayed the MIC value of AgNP, CeNP and ZnONP against selected microorganisms was varied and this variability depends upon the bacterial strains. The AgNP had strong antibacterial activity compared to CeNP and ZnONP. The MIC values of AgNP were 16 mg/L against *E. coli, E. hirae* and *E. fecalis*, 32 mg/L against *L. pneumophila* subsp. *pneumophila, S. aureus, C. parapisilosis*, and *C. tropicalis*, and 64 mg/L against *P. aeruginosa*. The MIC values of ZnO were found as 64 mg/L against *E. fecalis*, 128 mg/L against *E. coli, E. hirae, S. aureus, C. parapisilosis*, and *C. tropicalis*, 256 mg/L against *P. aeruginosa* and 512 mg/L against *L. pneumophila* subsp. *pneumophila*. When the MIC values for CeNP were examined, it was observed that it had a weaker antimicrobial effect compared to the other new synthesized two nanoparticles. According to our results, we can indicate that all three nanoparticles were more effective on Gram +ve bacteria than Gram -ve and fungi. Al-Otibi et al. [29] were synthesized silver nanoparticles using *Malva parviflora* and they reported that the biosynthesized AgNPs effectively reduced the mycelial growth of various fungus. Sharmila et al. [19] synthesized ZnO NPs using *Tecoma castanifolia* and evaluated its antibacterial activity against *E. coli*, *B. subtilis*, *S. aureus*, and *P. aeruginosa*. They reported that ZnO NPs exhibited good antibacterial activity towards all the studied bacterial strains. Similarly, Wang et al. [30] showed that coated CeO_2 NPs remarkably inhibited with 55.14% the growth of *P. aeruginosa*. Also, they expressed that PAA (polyacrylic acid)-coated CeO_2 NPs could be potential newly materials for various antibacterial implementations. Moreover, ceria NPs have been extensively studied for a diversity of potential implementations in different area, including nanomedicine [31]. Bellio et al. [31] investigated the use of CeO_2 NPs as an adjuvant to increase the effect of antibiotics, and as a result, they reported that the antibacterial capability of beta-lactam antibiotics towards *K. pneumoniae* increased when combined with CeO_2 NPs. In another study, Kumar et al. [32]

JSR A
Journal of Scientific Reports

synthesized CeO_2 NP using secondary plant metabolite Tannic acid. They reported that synthesized CeO_2 NPs showed good antimicrobial activity against *B. subtilis* and *E. coli* and indicated that the toxicity of CeO_2 NP could be due to the generation of reactive oxygen species (ROS). In the presented study, it can be said that the antimicrobial activities of the synthesized NPs may be due to the generation of ROS. According to the results, also it may use in biomedical and biotechnology areas in the improvemet of a newly nano biomaterial for clinical implementations as a drug candidate in future.

Table 1. The MIC of studied microorganisms.

Microorganisms	Ag	Ce	ZnO
E. coli	16	1024	128
P. aeruginosa	64	512	256
L. pneumophila* subsp. *pneumophila	32	1024	512
E. hirae	16	512	128
E. fecalis	16	512	64
S. aureus	32	512	128
C. parapisilosis	32	1024	128
C. tropicalis	32	512	128

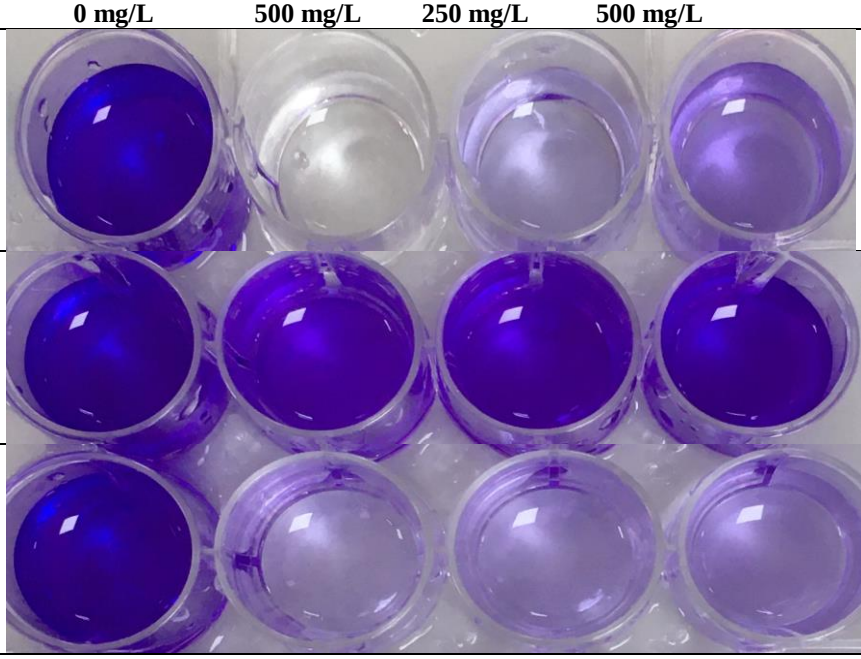
* mg/L

3.6. Biofilm Inhibition Capability

Biofilms are described as structured bacterial communities sticked to biological or abiotic surfaces. Biofilms have turn into even more important today because bacteria in biofilms have been shown to be more resistant to antibiotics than individual forms. Biofilm-associated infections affect many people worldwide and causing death. Hence, the need for more efficient compounds such as biogenic effective nanoparticles has received more attention [33]. Regarding the higher ability of *P. aeruginosa* and *S. aureus* to create a biofilm, these strains were selected for present study. The activities of various concentrations of new synthesized AgNP, CeNP and ZnONP nanoparticles on biofilm generation was appraised by monitoring the binding of the CV to attached cells in 24 well plates. As seen in Fig. 10 and 11, this directly reflects the efficacy of biofilm generation. In accordance with the results of biofilm formation inhibition against *S. aureus* (Fig. 10) and *P. aeruginosa* (Fig. 11), the percent inhibition order of nanoparticles at all studied concentrations was AgNP>ZnONP>CeNP. It was also observed that the biofilm inhibition activity was concentration dependent. When the concentrations increased from 100 to 250 mg/L, the percentage inhibitions of biofilm formation by AgNP, CeNP and ZnONP against *S. aureus* increased from 66,7% to 84.2%, from 2,29% to 5,4% and from 69,3% to 81,7% and also the percentage inhibition of biofilm formation by AgNP, CeNP and ZnONP against *P. aeruginosa* increased from 78,5% to 98,63%, from 3,65% to 8,54% and from 35,67% to 48,74%, respectively. The biofilm inhibition activity towards *S. aureus* and *P. aeruginosa* were found as 98,6% and 100% for AgNP, 12,8% and 15,98% for CeNP and 92,6% and 82,63% for ZnONP at 500 mg/L concentration, respectively. Zamanpour et al. [33] were synthesized silver nanoparticles by biosynthesis process. They reported that synthesized NPs indicated good biofilm inhibition activity towards *E. coli* and *P. aeruginosa*. Ishwarya et al. [34] reported that synthesized ZnO NP by green synthesis method inhibited bacterial biofilm formation of different bacteria including *E. coli, P. vulgaris, B. pumilis* and *B. licheniformis*. Altaf et al. [35] synthesized CeO_2 NPs using *Acorus calamus* aqueous extract and studied its antibiofilm activity towards various microorganisms containing *E. coli, S. aureus,* and *P. aeruginosa*. They reported that the biofilm

JSR A
Journal of Scientific Reports

formation of tested bacterium inhibited by more than 75% by the treatment with CeNPs. Rajivgandhi et al. [36] synthesized AgNPs and studied their antibiofilm inhibition activity against Methicillin-resistant coagulase negative Staphylococci (MR-CoNS). They reported that AgNPs could ensure a safer alternating to traditional antibiofilm agents against studied strain. Khan et al. [37] reported that ZnO, SnO_2 and CeO_2 NPs can be thinking as probable agents against Gr+ve bacterial infection. Biofilm is one of the new targets in the improving of newly antimicrobial entities, so the results we have obtained especially for Ag and ZnO nanoparticles are very valuable, and they can be utilized as biofilm inhibitory nanodrugs after further investigations.

	Concentrations			
Nanoparticles	**0 mg/L**	**500 mg/L**	**250 mg/L**	**500 mg/L**
AgNP				
CeNP				
ZnONP				

JSR A
Journal of Scientific Reports

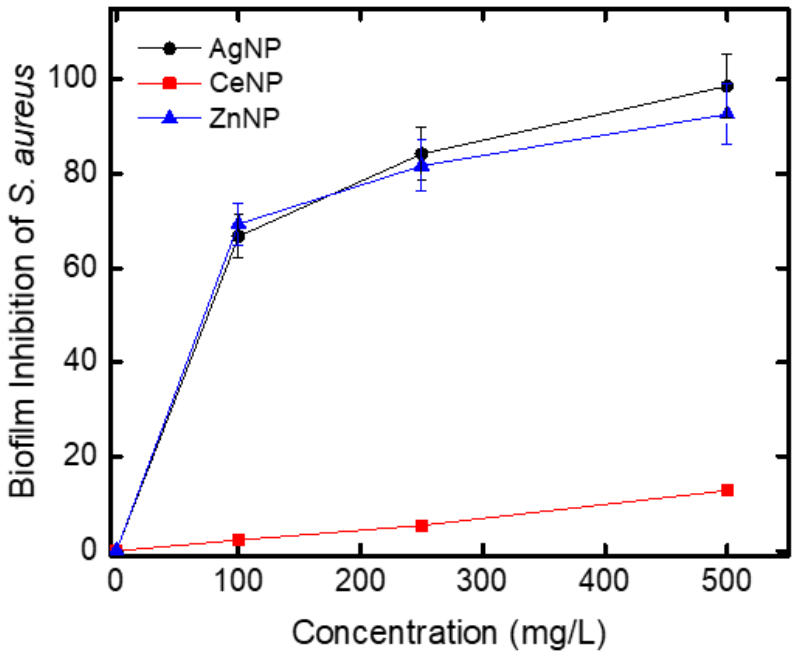
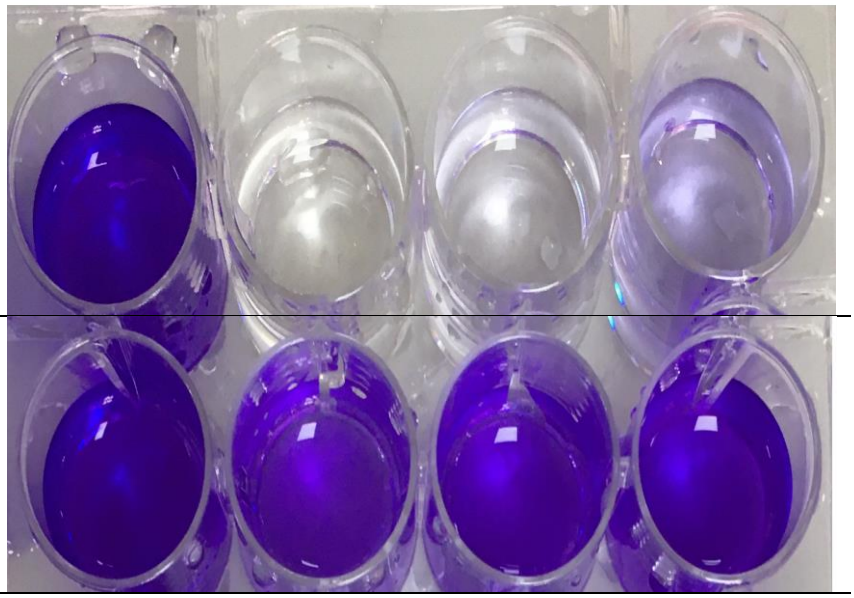
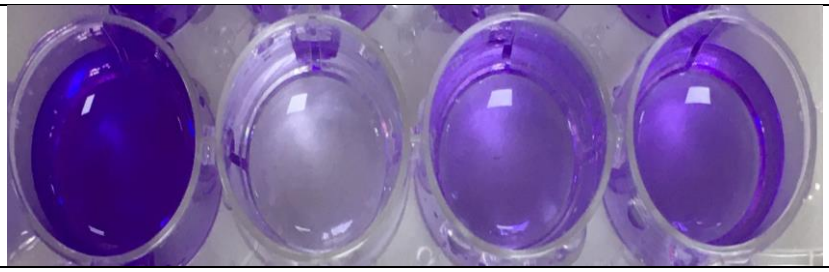


Fig. 10. Biofilm Inhibition of *S. aureus.*

Nanoparticles	Concentrations			
	0 mg/L	500 mg/L	250 mg/L	500 mg/L
AgNP				
CeNP				

ZnONP



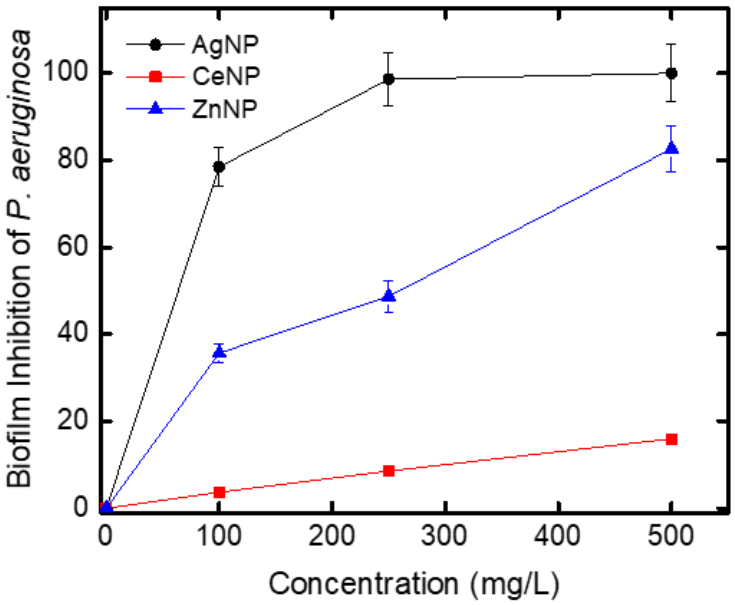
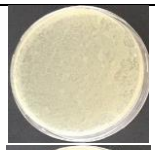
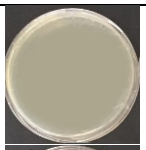
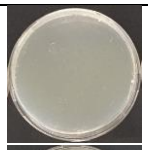
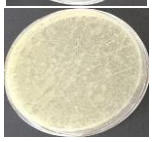
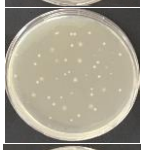
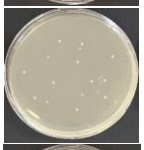
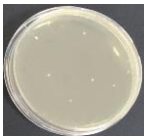
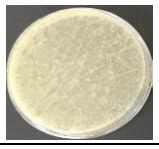
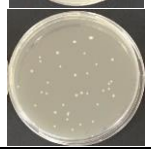
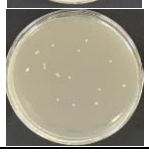
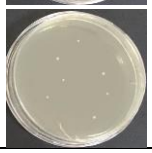


Fig. 11. Biofilm Inhibition of *P. aeruginosa*

3.7. Microbial Cell Viability

In the present study, we also investigated the *E. coli* cell viability activity. The related results of the cell viability inhibition are showed in Fig. 12. According to our results, all three nanoparticles perfectly inhibited the *E. coli* viability. AgNP, CeNP and ZnONP inhibited the *E. coli* cell viability as 99,99%, 85,12%, and 99,84%, respectively. It was also determined that green synthesized AgNP, CeNP and ZnONP displayed excellent bacterial cell viability inhibition as 100,00 % at the concentration of 500 mg/L. The antibacterial activity mechanism of AgNP, CeNP, and ZnONP in the study can be considered as ZnONP. It's known that antibacterial capability by NPs was mostly because of release of ions, creation of ROS and adhering to bacterial cell membrane. Due to the dissolution and accumulation of NPs, the bacterial membrane permeability changes, causing the proton motive force to disperse [38].

JSR A
Journal of Scientific Reports

Nanoparticles	Concentrations (mg/L) 0	125	250	500
Ag				
Ce				
Zn				

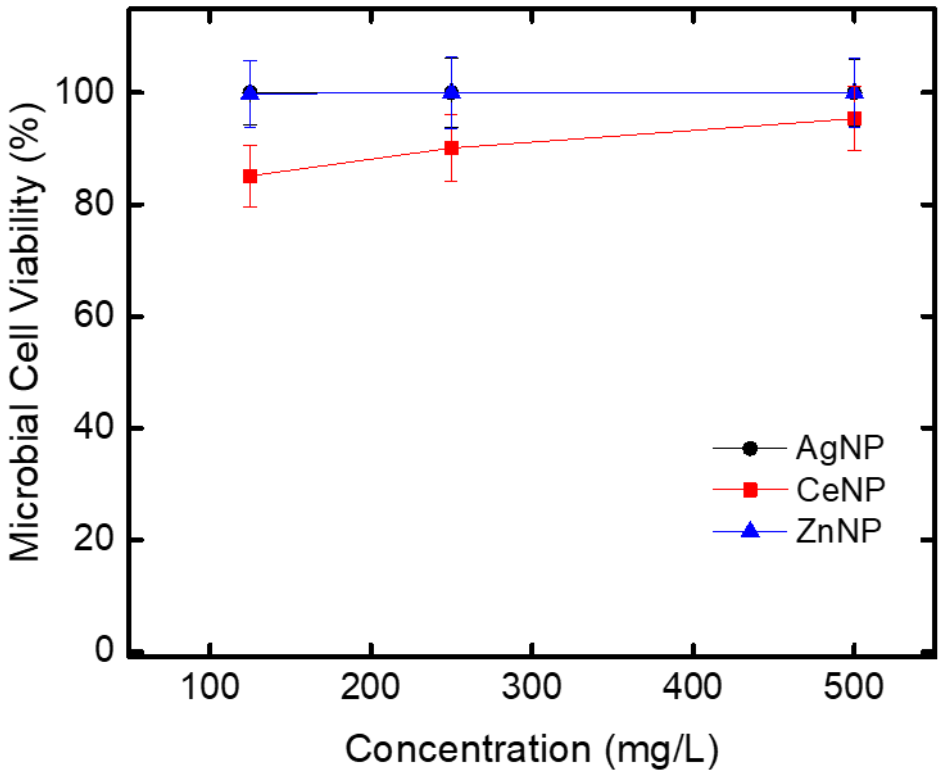


Fig. 12. Microbial cell viability.

4. CONCLUSION

In the presented study, the biological activity results of AgNP, ZnNP and CeNP synthesized extracellular from thermophilic *Anoxybacillus* sp. ST7 were also evaluated. In this context, antioxidant activity including DPPH and ferrous ion chelating activities, antimicrobial activity, DNA cleavage, cell viability and biofilm inhibition activity studies were conducted. When considering the pharmacological properties of nanoparticles, it is crucial to appraise all aspects including their medicinal features. It was determined that the synthesized nanoparticles had very effective DPPH and iron chelating activities. It exhibited effective chemical nuclease activity by creating a single strand break on *E. coli* plasmid DNA. Moreover, the nanoparticles were observed to be more effective on Gr +ve bacteria. It was observed that AgNP at 500mg/L concentration inhibited biofilm formation of *S. aureus* and *P. aeruginosa* close to 100%. In addition, highly effective cell viability results were determined. As a result, this study may supply beneficial details about the biomedical implementations of newly synthesis nanoparticles.

ACKNOWLEDGEMENT
I would like to thank Zelal Işık, and Erkan Yılmaz, Sadin Özdemir and Nadir Dizge for their contribution to the study.

REFERENCES

[1] Enderby, J. and Dowling, A., Nanoscience and nanotechnologies: Opportunities and Uncertainties, The Royal Society & The Royal Academy of Engineering Report, London, 2004.

[2] Ramsden J., Nanotechnology: An introduction, (ISBN: 978-0-08-096447-8) Elsevier, 2011.

[3] Lines M.G. (2008). Nanomaterials for Practical Functional Uses, Journal of Alloys and Compounds, 449, 242-245, 2008.

[4] Scenihr, The appropriateness of Existing Methodologies to Assess the Potential Risks Associated with Engineered and Adventitious Products of Nanotechnologies, Committee Opinion, 58-59, 2006.

[5] Ealias A.M., Saravanakumar M.P. A review on the classification, characterisation, synthesis of nanoparticles and their application, 14th ICSET-2017, IOP Conf. Series: Materials Science and Engineering 263 (2017) 032019 doi:10.1088/1757-899X/263/3/032019.

[6] Baig, N., Kammakakam, I., Falath W. (2021). Nanomaterials: a review of synthesis methods, properties, recent progress, and challenges, Mater. Adv, 2, 1821–1871.

[7] Duncan, T.V. (2011). Applications Of Nanotechnology In Food Packaging And Food Safety: Barrier Materials, Antimicrobials And Sensors. J Colloid Interface Sci 363(1): 1-24.

[8] Kumar, A., Chisti, Y., Banerjee, U. (2013). Synthesis of metallic nanoparticles using plant extracts: Biotechnology Advances (31) 346–356.

[9] Nematollahi, F. (2015). Silver nanoparticles green synthesis using aqueous extract of Salvia limbata C. A. Mey. Research Paper. International Journal of Biosciences ISSN: 2220-6655 (Print), 2222-5234(Online) http://www.innspub.net Vol. 6, No. 2, p. 30-35.

[10] Stephen Inbaraj B and Chen BH. (2020) An overview on recent in vivo biological application of cerium oxide nanoparticles. Asian J Pharm Sci. 15(5):558–75.

[11] [11] Eka Putri G, Rilda Y, Syukri S, Labanni A, Arief S.(2021) Highly antimicrobial activity of cerium oxide nanoparticles synthesized using *Moringa oleifera* leaf extract by a rapid green precipitation method. J Mater Res Technol. 15 :2355–64.

[12] Cheon JY, Kim SJ, Rhee YH, Kwon OH, Park WH. (2019). Shape-dependent antimicrobial activities of silver nanoparticles. Int J Nanomedicine. 14: 2773–80.

[13] Estevez MB, Raffaelli S, Mitchell SG, Faccio R, Alborés S. (2020). Biofilm eradication using biogenic silver nanoparticles. Molecules. 25(9): 1-14.

[14] Ruiz AL, Garcia CB, Gallón SN, Webster TJ. (2020). Novel silver-platinum nanoparticles for anticancer and antimicrobial applications. Int J Nanomedicine. 15: 169–79.

[15] Mishra PK, Mishra H, Ekielski A, Talegaonkar S, Vaidya B. (2017). Zinc oxide nanoparticles: a promising nanomaterial for biomedical applications. Drug Discov Today. 22(12):1825–34.

[16] Ağırtaş MS, Karatas C, Özdemir S. (2015). Synthesis of some metallophthalocyanines with dimethyl 5- (phenoxy) -isophthalate substituents and evaluation of their antioxidant-antibacterial activities. Spectrochimica Acta Part A : Molecular and Biomolecular Spectroscopy. 135:20–4.

[17] Dinis T.C.P, Madeira V.M.C, Almeida L.M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers, Arch. Biochem. Biophys. 315; 161-169.

[18] Raghava S, Munnene Mbae K, Umesha S. (2021). Green synthesis of silver nanoparticles by Rivina humilis leaf extract to tackle growth of *Brucella* species and other perilous pathogens. Saudi J Biol Sci. 28(1):495–503.

[19] Sharmilaa G, Thirumarimurugan M, Muthukumarana C. (2019) Green synthesis of ZnO nanoparticles using *Tecoma castanifolia* leaf extract: Characterization and evaluation of its antioxidant, bactericidal and anticancer activities. Microchemical Journal. 45: 578-587.

[20] Shejawal KP, Randive DS, Bhinge SD, Bhutkar MA, Wadkar GH, Jadhav NR. (2020). Green synthesis of silver and iron nanoparticles of isolated proanthocyanidin: its characterization, antioxidant, antimicrobial, and cytotoxic activities against COLO320DM and HT29. J Genet Eng Biotechnol. 18(43), 1-11.

[21] Farias IAP, Santos CCL, Xavier AL, Batista TM, Nascimento YM, Nunes JMFF, Silva PMF, Menezes-Júnior RA, Ferreira JM, Lima EO, Tavares JF, Sobral MV, Keyson D, Sampaio FC. (2021). Synthesis, physicochemical characterization, antifungal activity and toxicological features of cerium oxide nanoparticles. Arab J Chem. 14 (1), 102888.

[22] Mahabadi AG, Mirzakhani A, Azizi A, Chavoshi S, Khaghani S.(2021). Extracts of *Pelargonium hortorum*: A natural and efficient fluid for fast and eco-friendly biosynthesis of CeO_2 nanoparticles for antioxidant and photocatalytic applications. Inorg Chem Commun. 127, 108553.

[23] Ameen F, Al-Homaidan AA, Al-Sabri A, Almansob A, AlNAdhari S. (2021). Anti-oxidant, anti-fungal and cytotoxic effects of silver nanoparticles synthesized using marine fungus *Cladosporium halotolerans*. Appl Nanosci. 1-9.

[24] Gur T, Meydan I, Seckin H, Bekmezci M, Sen F. (2022). Green synthesis, characterization and bioactivity of biogenic zinc oxide nanoparticles. Environ Res. 204 (Part A):111897.

[25] AlSalhi MS, Elangovan K, Ranjitsingh AJA, Murali P, Devanesan S. (2019). Synthesis of silver nanoparticles using plant derived 4-N-methyl benzoic acid and evaluation of antimicrobial, antioxidant and antitumor activity. Saudi J Biol Sci. 26 (5):970–8.

[26] Soren S, Kumar S, Mishra S, Jena PK, Verma SK, Parhi P. (2018). Evaluation of antibacterial and antioxidant potential of the zinc oxide nanoparticles synthesized by aqueous and polyol method. Microb Pathog. 119 :145–51.

[27] Gonca S, Arslan H, Isik Z, Özdemir S, Dizge N. (2021). The surface modification of ultrafiltration membrane with silver nanoparticles using *Verbascum thapsus* leaf extract using green synthesis phenomena. Surfaces and Interfaces. 26:101291.

[28] De A, Das R, Kaur H, Jain P. (In Press). Synthesis, physicochemical investigations, DNA cleavage activity of biogenic zinc oxide nanoparticles and their interaction with Calf-Thymus DNA. Mater Today Proc. 1–5.

[29] Al-Otibi F, Perveen K, Al-Saif NA, Alharbi RI, Bokhari NA, Albasher G, Al-Otaibi RM, Al-Mosa MA. (2021). Biosynthesis of silver nanoparticles using *Malva parviflora* and their antifungal activity. Saudi J Biol Sci. 28(4):2229–35.

[30] Wang Q, Perez JM, Webster TJ. (2013). Inhibited growth of *Pseudomonas aeruginosa* by dextran- and polyacrylic acid-coated ceria nanoparticles. Int J Nanomedicine. 8:3395–9.

[31] Bellio P, Luzi C, Mancini A, Cracchiolo S, Passacantando M, Di Pietro L, Perilli M, Amicosante G, Santucci S, Celenza G. (2018). Cerium oxide nanoparticles as potential antibiotic adjuvant. Effects of CeO_2 nanoparticles on bacterial outer membrane permeability. Biochim Biophys Acta-Biomembr. 1860(11):2428–35.

[32] Kumar KM, Mahendhiran M, Diaz MC, Hernandez-Como N, Hernandez-Eligio A, Torres-Torres G, Godavarthi S, Gomez LM. (2018). Green synthesis of Ce^{3+} rich CeO_2 nanoparticles and its antimicrobial studies. Mater Lett. 214:15–9.

[33] Zamanpour N, Mohammad A, Mashreghi M, Shahnavaz B. (2021). Bioorganic Chemistry Application of a marine luminescent *Vibrio* sp . B4L for biosynthesis of silver nanoparticles with unique characteristics, biochemical properties, antibacterial and antibiofilm activities. Bioorg Chem.; 114:1–12.

[34] Ishwarya R, Vaseeharan B, Kalyani S, Banumathi B, Govindarajan M, Alharbi NS, Kadaikunnan S, Al-anbr MN, Khaled JM, Benelli G. (2018). Facile green synthesis of zinc oxide nanoparticles using *Ulva lactuca* sea weed extract and evaluation of their photocatalytic, antibiofilm and insecticidal activity, J. Photochem. Photobiol. B Biol. 178:249–258.

[35] Altaf M, Manoharadas S, Zeyad MT. (2021) .Green synthesis of cerium oxide nanoparticles using *Acorus calamus* extract and their antibiofilm activity against bacterial pathogens. Microsc Res Tech. 84(8):1638–48.

[36] Rajivgandhi G, Maruthupandy M, Muneeswaran T, Anand M, Quero F, Manoharan N, et al. (2019). Biosynthesized silver nanoparticles for inhibition of antibacterial resistance and biofilm formation of methicillin-resistant coagulase negative Staphylococci. Bioorg Chem. 2019;89 (March):103008.

[37] Khan F, Lee JW, Pham DNT, Khan MM, Park SK, Shin IS, Kim YM. (2020). Antibiofilm Action of ZnO, SnO_2 and CeO_2 Nanoparticles Towards Grampositive Biofilm Forming Pathogenic Bacteria. Recent Pat Nanotechnol. 14(3):239-24.

[38] Basumatari M, Devi RR, Gupta MK, Gupta SK, Raul PK, Chatterjee S, Dwivedi SK. (2021). Musa balbisiana Colla pseudostem biowaste mediated zinc oxide nanoparticles: Their antibiofilm and antibacterial potentiality. Curr Res Green Sustain Chem. 4 (December 2020):100048.