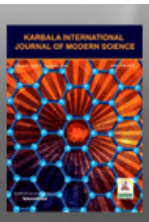

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Synthesized Zinc Nanoparticles via Pulsed Laser Ablation: Characterization and Antibacterial Activity

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Keywords

Laser ablation; ZnNPs; Nanotechnology; Plasmon resonance; Antibacterial activity

RESEARCH PAPER

Synthesized Zinc Nanoparticles Via Pulsed Laser Ablation: Characterization and Antibacterial Activity

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Abstract

The pulsed laser ablation of a metallic target in the liquid (PLAL) is a modern and good method for creating a variety of nanomaterials that have piqued the interest of researchers in the last decade. It is used in this work to prepare zinc nanoparticles and zinc oxide nanoparticles (ZnPNs and ZnO NPs) using Nd: YAG laser with the two wavelengths (532 nm) and (355 nm) using the energies (600 mJ) and (500 mJ) respectively, and the number of the pulse (500, 600, 700, 800, and 900 Pulses); for each wavelength used in this work. The properties of the prepared NPs were studied by diagnosing them by (UV−Vis, FTIR, AFM, XRD, SEM, and EDX) techniques. Then the antibacterial activity of the prepared nanoparticles was tested against two types of Gram-positive bacteria (*Streptococcus mutans* & *Staphylococcus aureus*) and two types of Gram-negative bacteria (*Pseudomonas aeruginosa* & *Escherichia coli*) isolated from the oral cavity. The finding showed that ZnNPs made using the PLAL approach could be employed to kill pathogenic and hazardous bacteria due to having antibacterial activity.

Keywords: Laser ablation, ZnNPs, Nanotechnology, Plasmon resonance, Antibacterial activity

1. Introduction

The attention of modern sciences is directed towards nanotechnology. This technique offers a huge range of new applications, including agricultural production, food processing, and the medical fields [1]. Nanoparticles are used in physical, biological, and chemical fields [2], where the size and shape of colloidal metal NPs play a substantial role in a variety of applications [2,3] such as the preparation of magnetic material [4], electronic devices, wound healing [2,5], antimicrobial [4,5], and the preparation of biocomposites [2,5]. Metal NPs are typically formed by reducing salts of metal found in a solution or by forming metal atom aggregates by heating or vaporizing a metal in a vacuum or an inert gas [6]. Metal colloids have optical, catalytical, and electromagnetic properties that vary depending on particle size and shape [2,7]. And one of the unique properties of metallic NPs is surface plasmon resonance (SPR) [6]. When exposed to certain light energies, free electrons collectively oscillate on the metal surface, resulting in absorption and scattering which depend on wavelength; metal NPs can highly absorb the light in the zone of the visible spectra by producing collective vibration of bands of conduction in powerful resonance with fixed light frequencies [8]. The excitation of the plasmon enhances the electromagnetic field in the surrounding environment and produces measurable changes in NPs that respond in the optical field [9]. Plasmonics is the localization and manipulation of an electromagnetic wave propagating along with a metal-dielectric interface [10]. The metal-based nanoparticles can be used in human health care [11]. One of the various metallic NPs studied is zinc metal and its oxides [12,13], where zinc nanoparticles (ZnNPs) have been used in a variety of industries [12,14], including the agriculture field, chemistry, textile and food processing, electronics,

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and medicine [12,15]. Zinc and its oxide are easy to prepare, chemically stable, and non-toxic [16,17]. ZnNPs and ZnO NPs are distinguished by their compositional, morphological, and crystalline properties. The reduction to nano-scale size affects the material properties in terms of mechanics, electrics, chemistry, structure, optics, and morphology allowing it to interact with bio cell molecules and assist the physical transmission of an NP inside the inner structure of cellular. They are being environmental-friendly and considered appealing for use in a variety of applications such as optical coatings, solar cells, electric devices, antibacterial, photocatalysts, gas sensors, and active medium in UV semiconductor lasers [1]. They have been prepared and tested for use in veterinary and human medicine due to their antibacterial activity for a wide range of bacterial species [12]. Because of the nano dimension of functional components of living cells, the application of nanotechnologies in biomedical applications is a pressing issue today. One of the most promising avenues is the use of ZnNPs in molecular diagnostics, drug delivery, and the development of new pharmaceutical preparations [18]. The activity mechanism of antimicrobial is binding to the negatively charged bacterial cell wall, resulting in cell envelope destabilization and altered permeability [19]. Several mechanisms have been proposed to explain the experimental finding, including the production of reactive oxygen species (ROS) due to the presence of the NPs, adhesion on the cell membrane, NP-induced oxidative stress which lead to damage of the membrane cell wall, and the NPs' penetration through the membrane cell wall [20]. The laser ablation method can produce high-purity nanoparticles without the use of a toxic chemical mixture, which is a simple and low-cost preparation process [21–25], It is a physical method of top-down production based on the principle of subdividing bulk precursors of metal ions into metal atoms [26,27]. Laser ablation is a promising method for producing metal colloids and NPs. It provides necessary benefits for biological applications of producing NPs without contamination, as well as the processing setup costs, are very low [28,29]. The PLAL is a modern and good method for creating a variety of nanomaterials that have piqued the interest of researchers [30–33]. The importance of the laser-assisted pulsed method in a liquid environment lies in its success in the synthesis of different sizes and shapes of nanoparticles that are used in many applications [34–36]. The nonequilibrium growth process is caused by plasma produced by laser ablation of a target in solution, which has extremely high pressure and temperature, and it is the method's most distinctive feature [30], It is one of the important methods for preparing ZnNPs [37,38] as well as ZnO NPs [39,40]. The response of materials to light is affected by the power of the laser beam, as well as the temperature of the beam. Continuous laser irradiation of material results in a series of reactions such as heating, melting, boiling, and plasma formation [41], which increases with increasing laser power [42]. In addition to melting, boiling, and vaporization; the response of materials to a laser beam in terms of thermal effects includes phase-explosion of nucleation, as well as some mechanical effects such as deformation and stress in materials [41]. Fig. (1) shows the absorption process within matter as a function of increasing power. The thermal diffusion depth of the laser pulse (D) can be determined by the following relationship:

$$D = (4N\tau_{las})^{1/2} \quad (1)$$

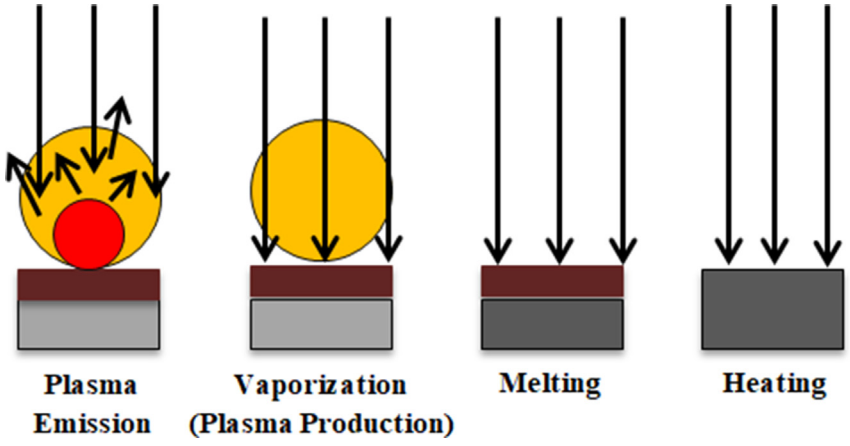

Fig. 1. Absorption process within matter as a function of increasing power.

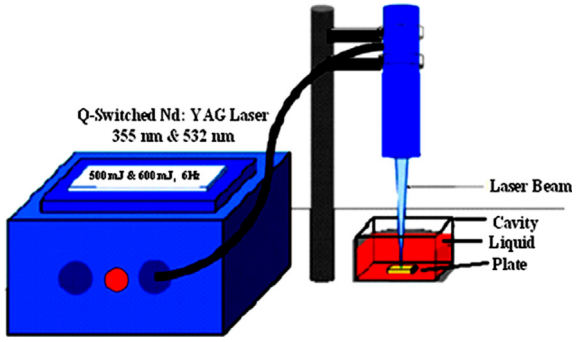

Fig. 2. Schematic diagram of PLAL for NPs synthesized.

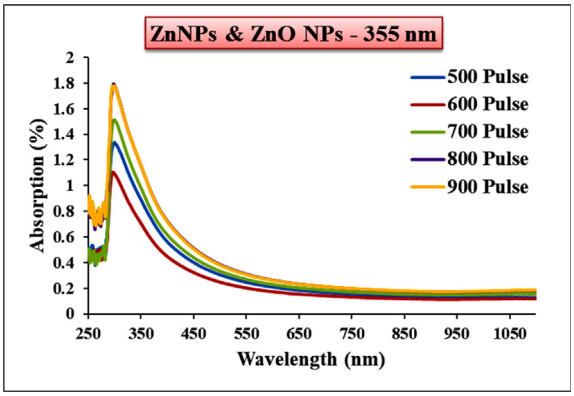

Fig. 3. UV–Vis absorption spectra at 355 nm.

where (D) represents the thermal diffusion depth, (N) represents the material thermal diffusion, and (τ_{las}) is the duration of the laser pulse [43].

The position of a metal colloidal solution's plasmon depends on the type of liquid environment used in the laser ablation process. Under the same laser beam parameters, the plasmon peak of metal NPs is affected not only by the type of liquid but also by the chemical concentration of the liquid; the plasmon peak width can also be changed by changing the wavelength of ablation laser light [41]. In this work, we used the laser ablation method to prepare ZnNPs and study their physical properties and antibacterial activity.

2. Materials and methods

ZnNPs were prepared using pulsed laser ablation technique in liquid (PLAL) using a pure zinc plate with dimensions (1 cm, 1 cm, & 2 mm) and placed in (5 mL) of ethanol of (99.99%) purity in a beaker and at a distance (5 cm) from the laser source, where Q-switched Nd: YAG laser was used with a rate of repetition (6 Hz), the wavelengths (355, 532 nm) and energies (500, 600 mJ) respectively, as its schematic shown in Fig. (2), and the number of pulses was used (500, 600, 700, 800, and 900 Pulses) for each wavelength. Then the structural and optical analyses of the samples prepared using XRD, FESEM, EDX, AFM, UV–Vis, and FTIR techniques were carried out. Then the antibacterial activity of the prepared NPs was tested against two types of Gram-positive bacteria (*Streptococcus mutans* and *Staphylococcus aureus*), as well as two types of Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) isolated from the oral cavity were done.

The bacteria were used after they were obtained ready (isolated, classified, and grower). These steps can be summarized as follows: collecting them, then isolating and classifying them in standard ways to know their types, and growing them on nutritious culture media, taking sterile methods and maintenance conditions into account in all steps, then freezing them until use. The prepared NPs were then tested to know their ability to inhibit the planted bacteria in an agar medium in Petri dishes, using the drilling method, placing the solution containing the particles in the holes, and incubating the dishes in the incubator for (24) hours.

3. Results & discussion

3.1. UV–vis absorption analysis

It is one of the most widely used techniques for the identification of various substances such as transition metal ions, organic compounds, and biological molecules[1]. The first evidence of a nanosynthesis happening was the color of zinc colloidal solutions changed from colorless to a yellowish-brown color and then the gradations of this color according to the different number of pulses used. Initial characterization of ZnNPs and ZnO NPs was performed by UV–Vis analysis. It was noticed as in Fig. (3) when using the wavelength (355 nm) and energy (500 mJ) of the Nd: YAG laser that the maximum absorption range was (295–297 nm), whereas when using the wavelength (532 nm) and energy (600 mJ) the maximum absorption was at (297–300 nm) as in Fig. (4), where the maximum absorption represents the SPR of NPs.

The two figures show that the shorter wavelength has generated a higher absorbance with a higher value which indicates the presence of a high concentration of prepared NPs in solutions, this is consistent with previous studies such as [7], also, the

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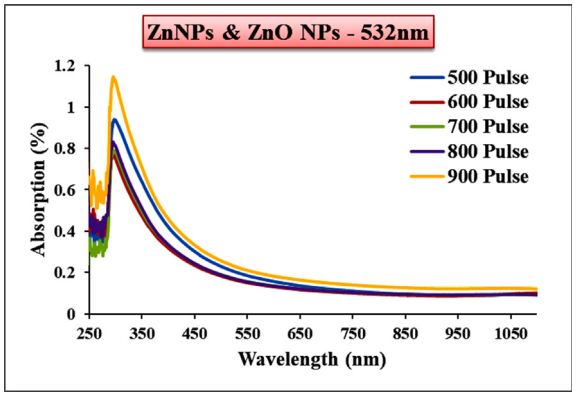

Fig. 4. UV–Vis absorption spectra at 532 nm.

used wavelengths led to a wider and uniformity in the width of the absorption peaks, by increasing the number of pulses, and the higher SPR peak with an increase in the pulses is attributed to a more efficient redistribution of NPs due to the longer period of exposure and the accumulation of an effective electric around the NPs, as in Ref. [44].

3.2. XRD analysis

Fig. (5) represents the XRD patterns obtained of the prepared ZnNPs; one of them refers to the sample prepared at the parameters (355 nm–500 mJ – 900 Pulse) and the other two belong to the samples prepared at (532 nm–600 mJ – 800 Pulse) and (532 nm–600 mJ – 900 Pulse), and we notice in all the resulting patterns the appearance of several intensities and peaks that belong to ZnNPs with a hexagonal crystal structure (the fitted XRD pattern was compared to the standard pattern (COD Card Number [96-901-1600])). We also notice the appearance of several peaks representing zinc oxide NPs, which are corresponding to (ZnO – COD Card Number [96-101-1260]). In this figure, the apparent crystal systems, angles, and Miller's index were indicated.

3.3. SEM analysis

SEM analysis of the prepared ZnNPs was made to determine the morphology of the NPs. Figs. (6)–(8) show that the prepared particles which were ablated in ethanol are nanospheres, and the particle size variation was widely distributed. Also, the particle size distribution, indicates that the NPs sizes generally range between (33.6 and 43.9) nm. From the figures, we note that the higher energy and higher wavelength of the laser led to better uniformity and alignment of the nanoparticles, and also, the higher number of pulses led to the clarity of the spherical shape of the prepared NPs.

From the EDX analysis, the elemental compositions of the prepared NPs are shown in Figs. (9)–(11), with present a long peak indicating the presence of zinc in the prepared sample.

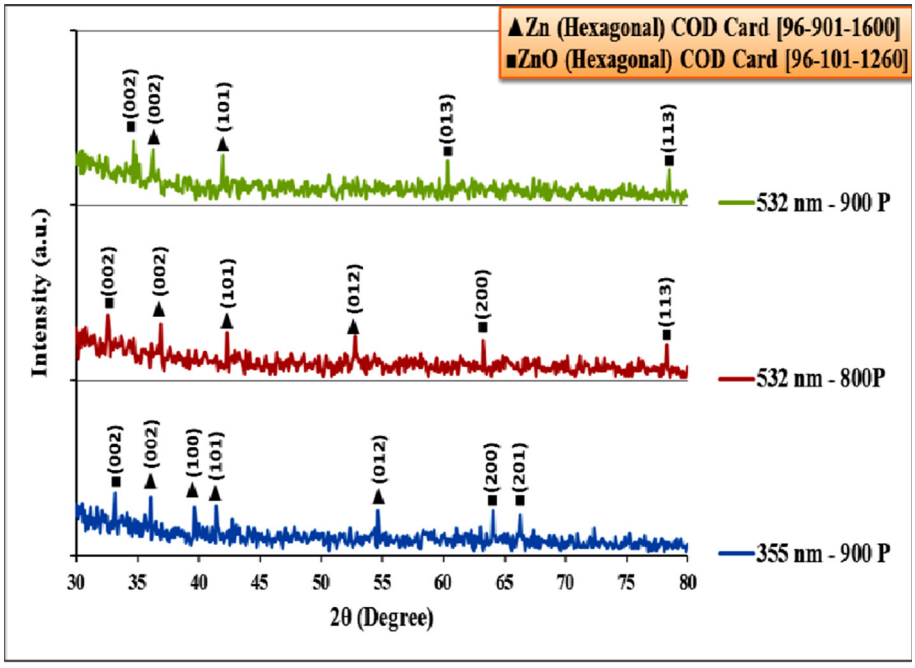

Fig. 5. XRD patterns of ZnNPs.

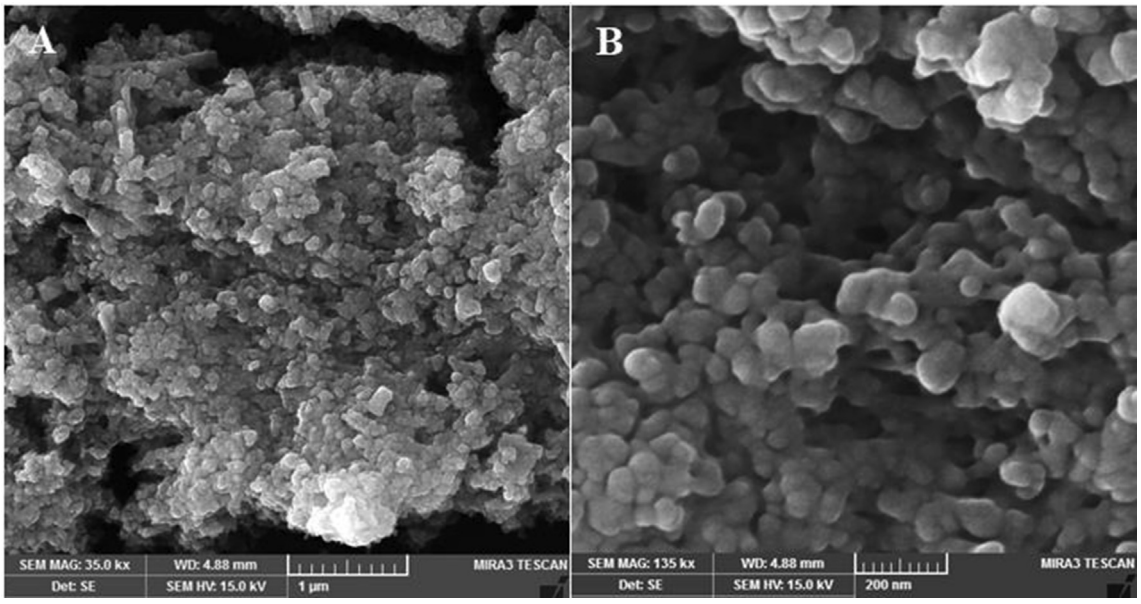

Fig. 6. SEM images of ZnNPs at 355 nm-500 mJ-900 P zoom (A) 1µm (B) 200nm.

3.4. AFM analysis

From Figs. (12)–(14) it has been noticed that the samples prepared have regular and dense surfaces. The grain size of the prepared ZnNPs at (355 nm–500 mJ – 900 P) was equal to (72.32 nm). And the grain size at (532 nm–600 mJ – 800 P) was (75.1 nm), while the increase in the number of pulses at (532 nm–600 mJ – 900 P) led to an increase in the grain size to (95 nm), so, it can be said that the surface topography analysis showed that increasing the number of laser pulses leads to an increase in the grain size.

3.5. FTIR analysis

Figs. (15 and 16) represent FTIR analysis of ZnNPs obtained at (355 nm–500 mJ) and (532 nm–600 mJ)

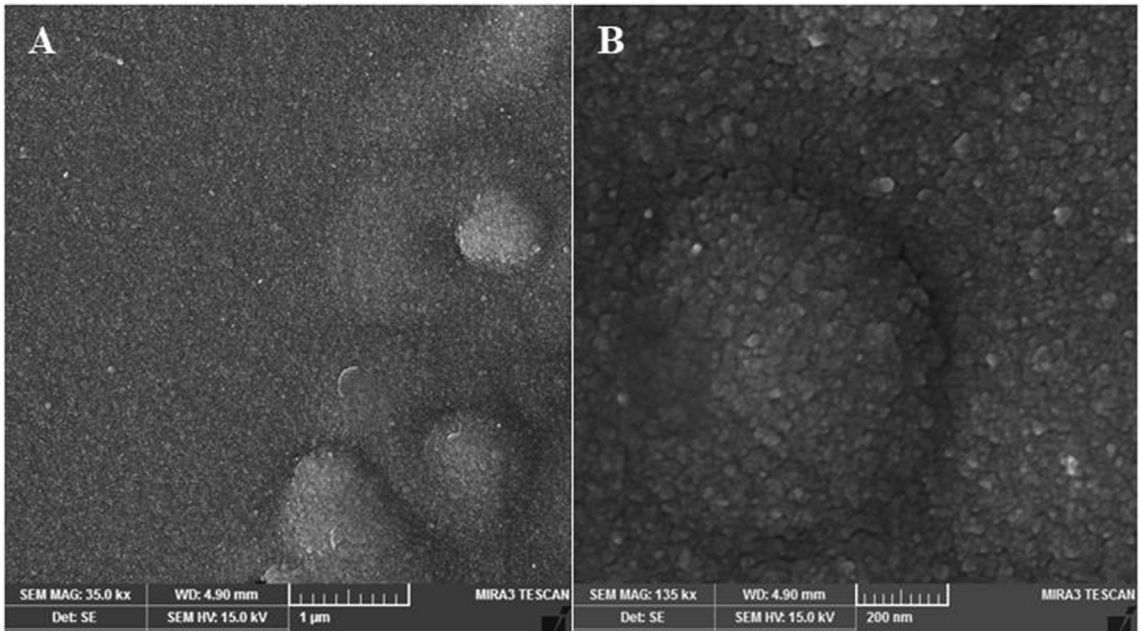

Fig. 7. SEM images of ZnNPs at 532 nm-600 mJ-800 P zoom (A) 1µm (B) 200nm.

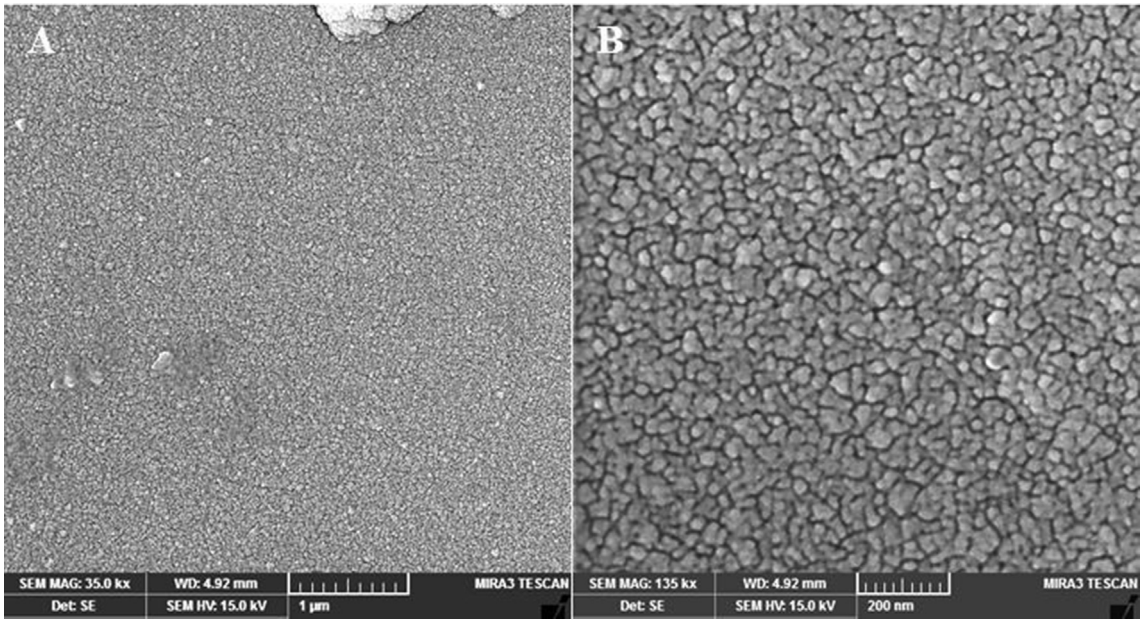

Fig. 8. SEM images of ZnNPs at 532 nm-600 mJ-900 P zoom (A) 1µm (B) 200nm.

respectively, where this analysis is utilizable for characterizing the surface chemistry of nanoparticles.

Spectra FTIR in both figures showed absorption peaks corresponding to the group of hydroxyl (O–H), alkane (C–H), and aliphatic amines (C–O) vibrations, in addition to appearing carboxylic acid (C=O) group in Fig. (16), also, in both figures the band which below (600 cm^{-1}) was in charge of the ZnNPs forming and its oxides. It can be seen from the two figures that the number of pulses affected the value of the absorption peaks, as well as the appearance and disappearance of some of them, as the number of pulses affected the concentration of solutions.

A peak was observed in the spectrum at (3300–3400 cm^{-1}), which can be attributed to (H–O–H) bending and (–OH) stretching vibrations

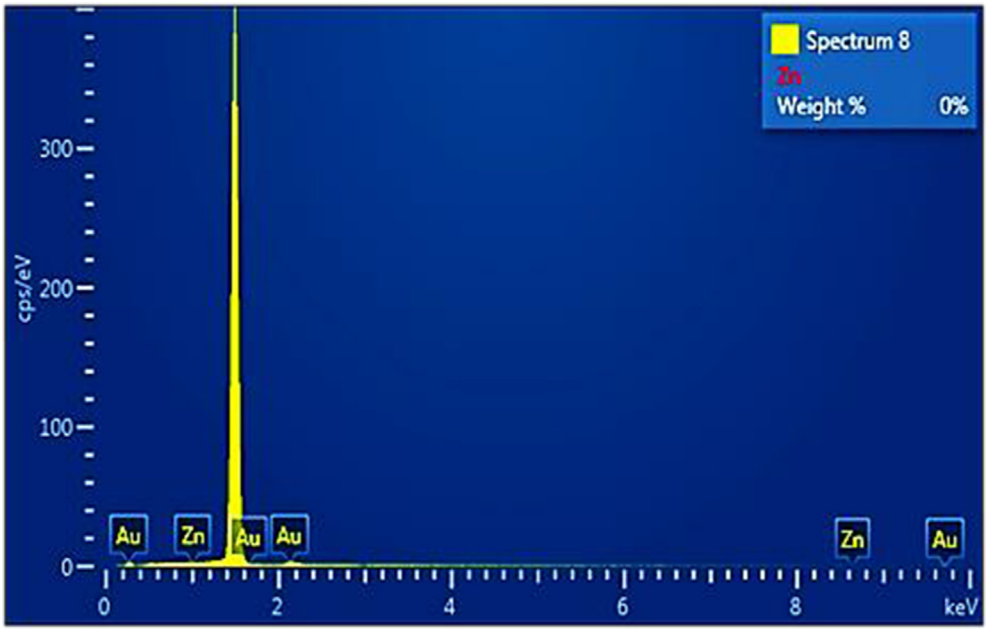

Fig. 9. EDX analysis at 355nm-900P.

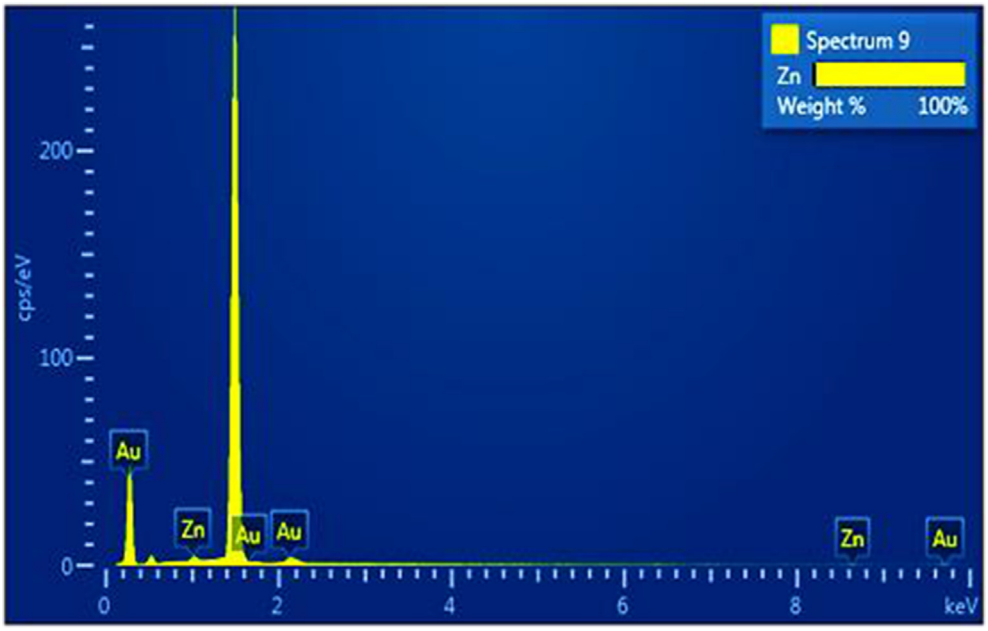

Fig. 10. EDX analysis at 532nm-800P.

that can be due to the presence of atmospheric water during analysis [45,46].

3.6. Energy gap (Eg)

As we noticed from the analysis of XRD, besides ZnNPs, zinc oxides NPs were formed in the samples prepared in this work, and to calculate the energy gap (Eg) for these oxides we plotted absorption as a function of wavelength as in Fig. (17 and 18) and we used the following equation [47]:

$$\text{Eg (eV)} = 1240 \,/\, \lambda_g \text{ (nm)} \quad (2)$$

where λ_g is the absorption edge calculated as the intersection of the absorption curve's tangent and

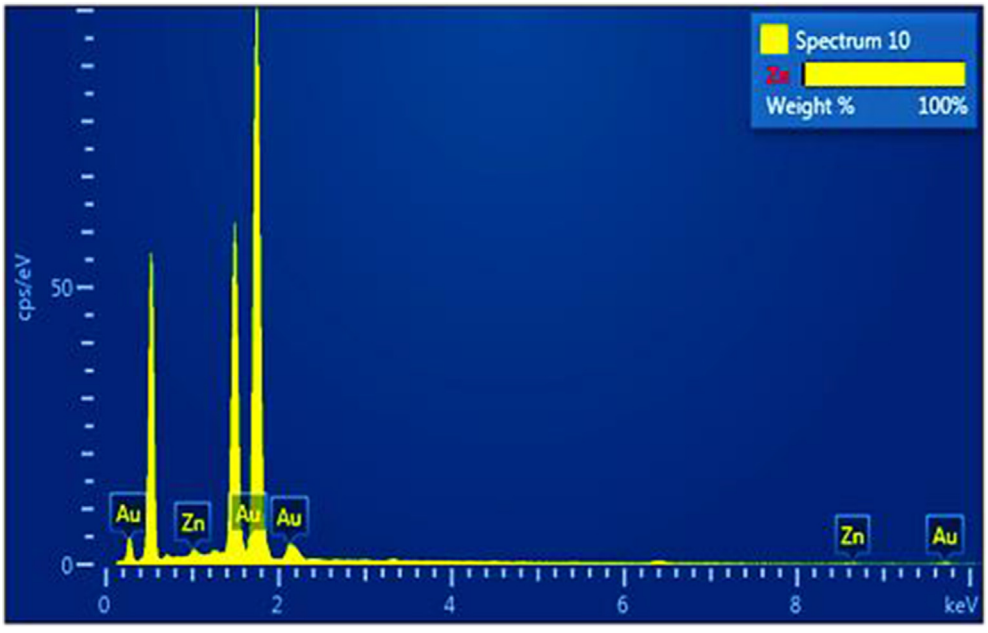

Fig. 11. EDX analysis at 532nm-900P.

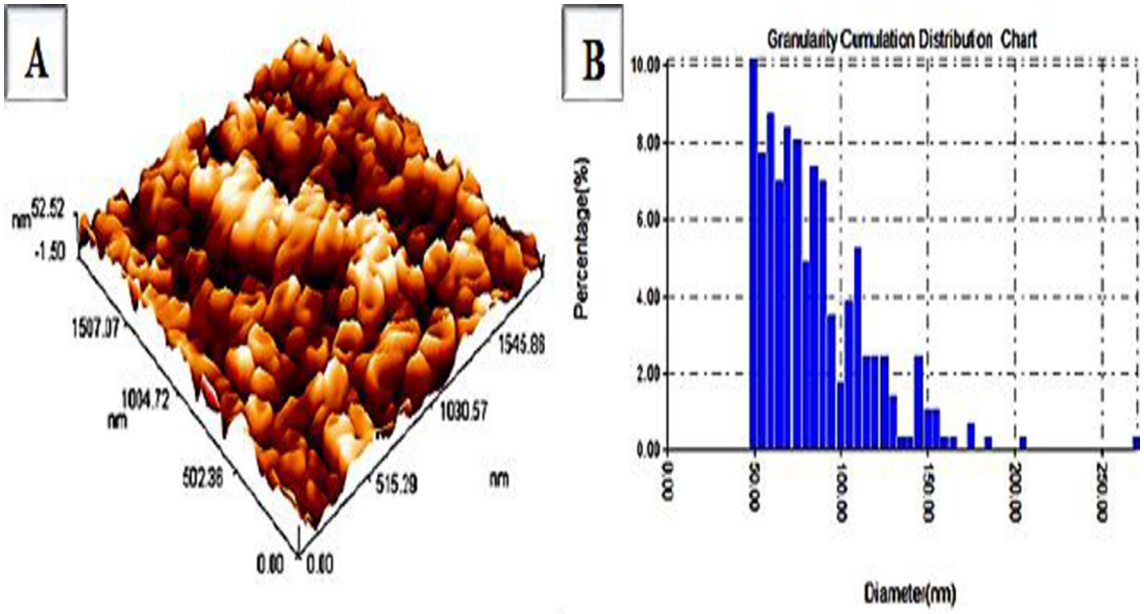

Fig. 12. AFM analysis at 355 nm-500 mJ-900 P (A) 3D image, (B) Granularity cumulative distribution.

the abscissa coordinate [48]. In general, we find that Eg at (355 nm) with fluctuating values between (1.88–2.1 eV) where the highest value was when the number of pulses (600 P), while its values, in general, increased by increasing pulses at (532 nm) and its range (2.07–2.34 eV) this is due to the regularity of the prepared particles morphology at this wavelength, according to SEM analyzes.

3.7. Antibacterial activity

The results of our study showed that the ZnNPs prepared at (355 nm) have a good inhibiting activity,

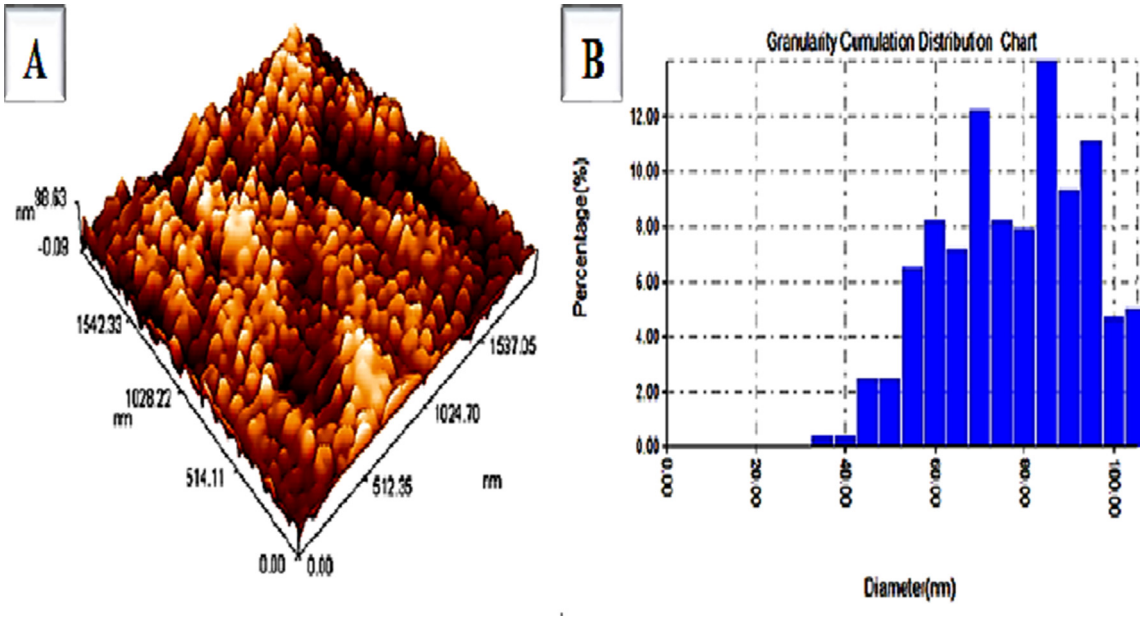

Fig. 13. AFM analysis at 532 nm-600 mJ-800 P (A) 3D image, (B) Granularity cumulative distribution.

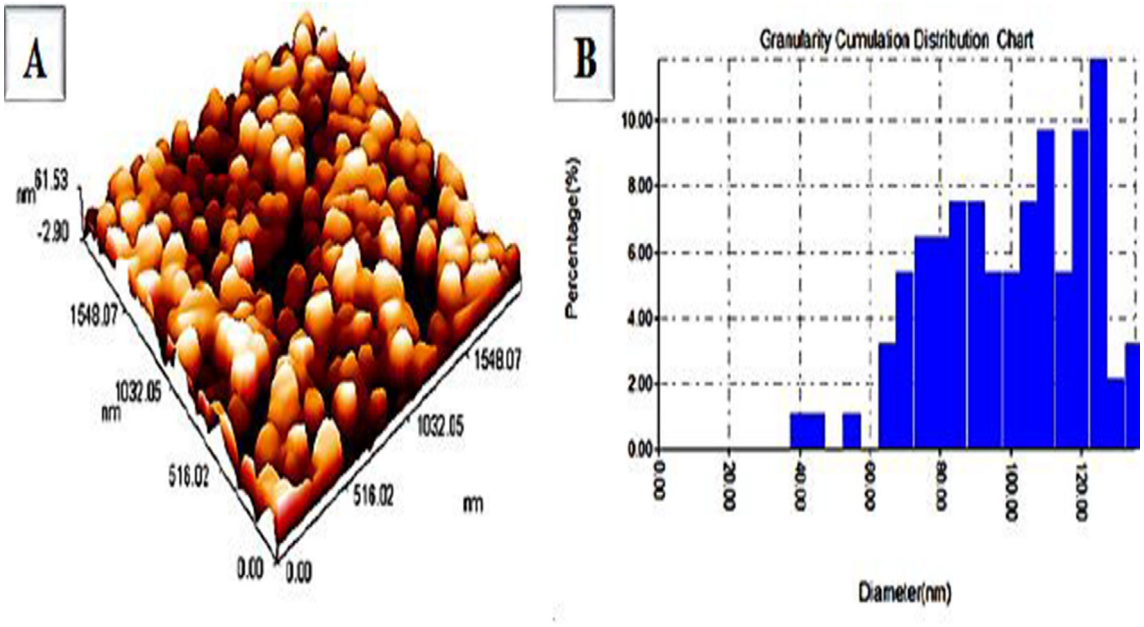

Fig. 14. AFM analysis at 532 nm-600 mJ-900 P (A) 3D image, (B) Granularity cumulative distribution.

while at (532 nm) do not have significant inhibiting activity and generally on all mentioned bacteria (*S. aureus*, *S. mutans*, *E. coli*, and *Pseudomonas*), this may be due to increased bacterial resistance proven by antibiotic sensitivity test (not put in this research). However, we can observe the bacteria inhibition regions resulting by using ZnNPs prepared by laser ablation for both the two wavelengths and for all mentioned pulses numbers as shown in Figs. (19 and 20).

The antimicrobial activity of prepared NPs was compared with the standard antibiotics by

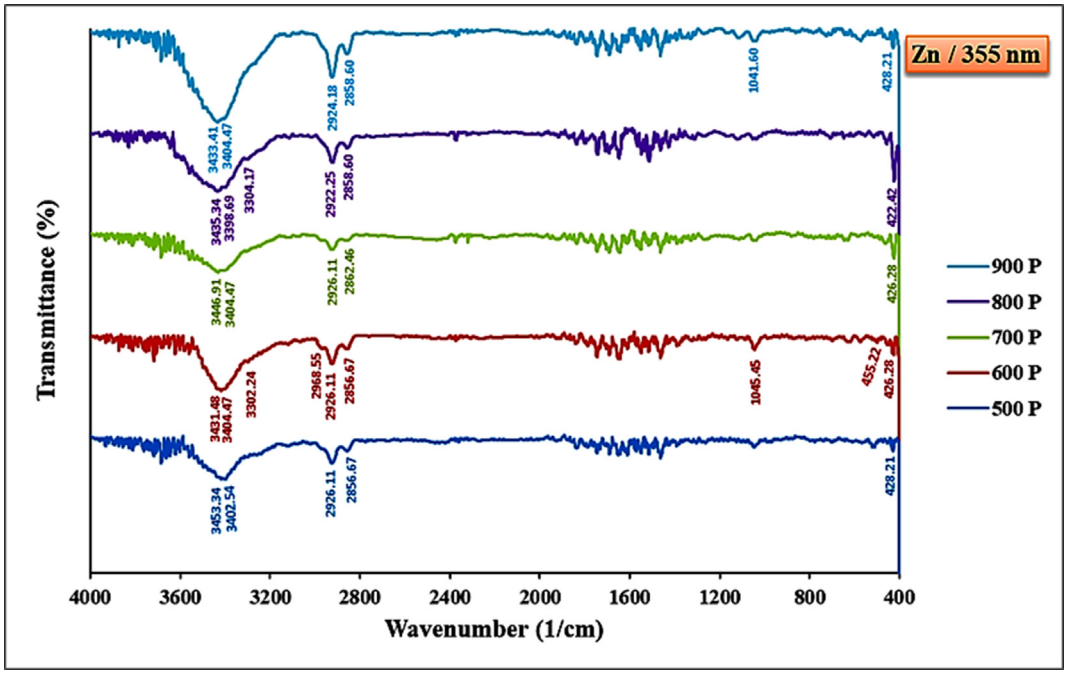

Fig. 15. FTIR analysis at 355 nm-500 mJ.

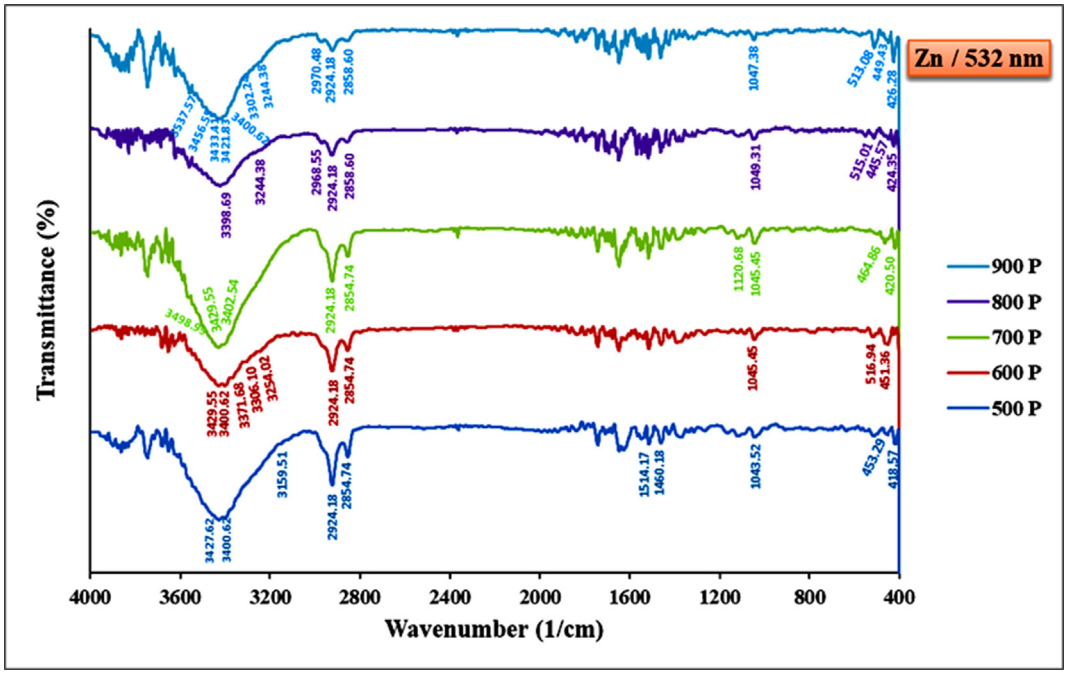

Fig. 16. FTIR analysis at 532 nm-600 mJ.

calculating the diameter of the inhibition zone as in previous studies [49,50]. From these two figures, we note that ZnNps affected bacteria in different proportions, and the particles prepared at the wavelength (355 nm) were more effective as they affected all species, while bacteria (*S. mutans*) were not affected by the particles prepared at (532 nm) at a relatively small number of pulses (500 and 600 P) where they did not show inhibition zones, also at this wavelength there was no affected on (*Pseudomonas*) at the less number of pulses (500 P).

It was observed that the antibacterial activity for gram-negative bacteria was more than that for gram-positive bacteria, the difference in the chemical composition and cell wall structure of bacteria may be caused this result [49]. The diameters of the bacteria inhibition areas were calculated as in Figs. (21 and 22).

It's still unclear whether a single mechanism or a combination of processes was responsible for cell death. One of them could be due to the interaction between ZnNPs and the cell wall of bacteria and the simultaneous permeation of Zn ions inside the cells

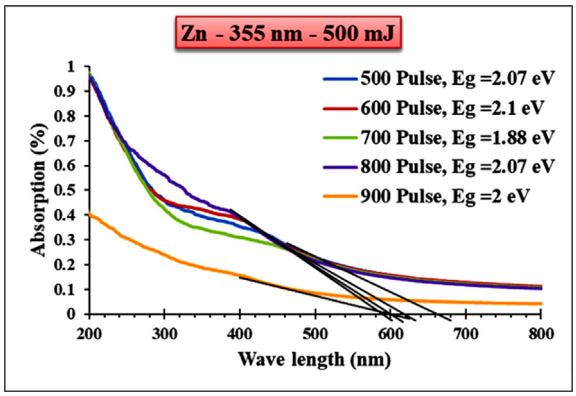

Fig. 17. Energy gap of Zn oxides NPs at 355 nm-500 mJ.

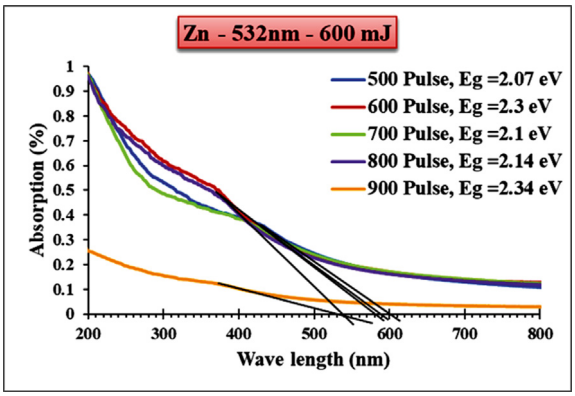

Fig. 18. Energy gap of Zn oxides NPs at 532 nm-600 mJ.

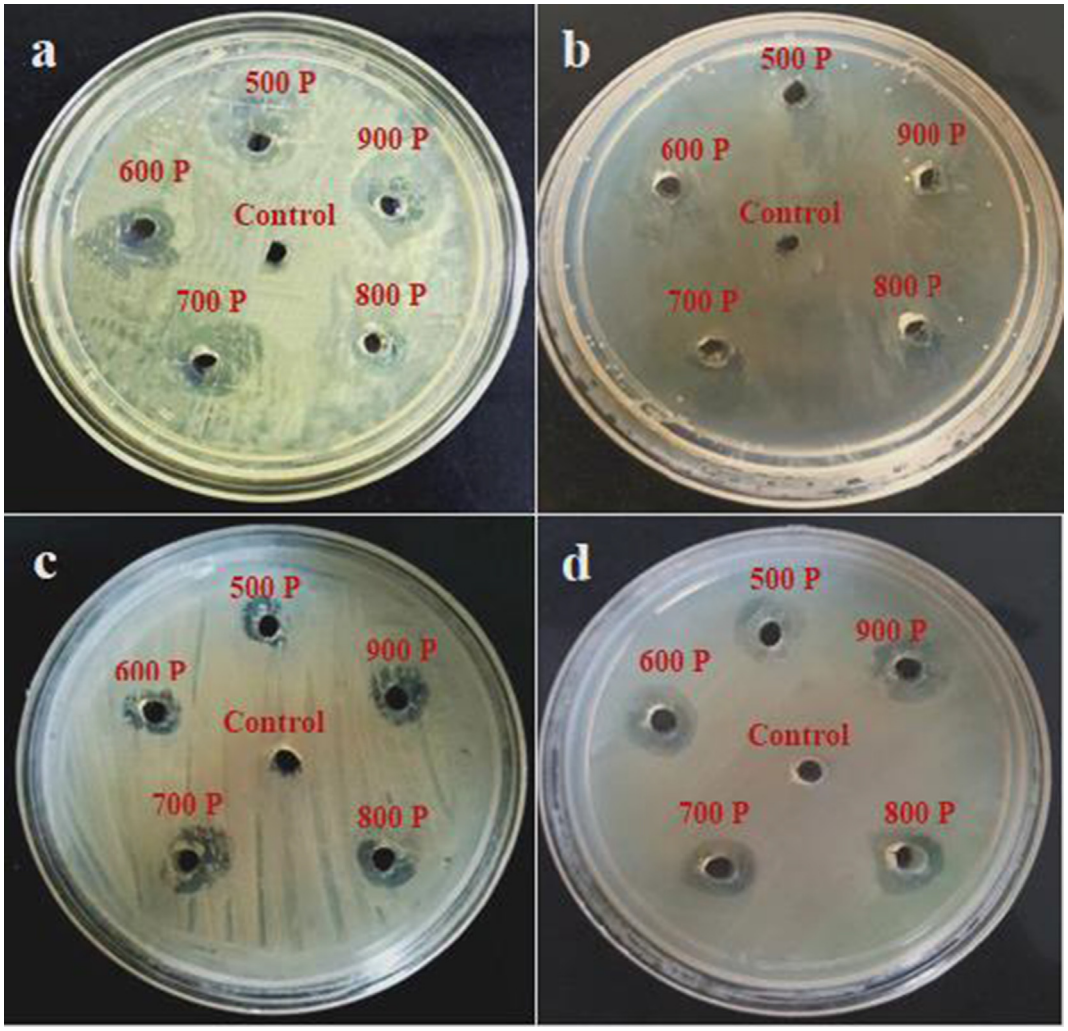

Fig. 19. Antibacterial activity at 355 nm-500 mJ (a) S. aureus, (b) S. mutans, (c) E. coli, (d) Pseudomonas.

of bacteria [51]. Generally, the antibacterial activity of NPs is primarily dependent on the electrostatic attraction between the negative charge of bacterial cell surfaces and the positive charge of NPs, and this attraction is critical for NPs' activity as a bactericidal agent [52]. Cell death is caused by mitochondrial damage, and the current result like previous studies shows that NPs led to DNA and mitochondrial damage, then led to cell death. This is due to the cellular toxicity of NPs on the bacteria, and this is added to the growing body of evidence regarding the potentially damaging effects of NPs, indicating that caution should be exercised in their widespread usage [53]. The mechanism of potential inhibitory could be explained by releasing ROS on the surface of the NPs, which bond to the surface and kills the bacteria through electrostatic forces. Furthermore, the free Zn^{2+} was hypothesized to partially contributed to an antimicrobial effect through the contact mechanically between ZnO surface and the bacteria [20]. The situations of the experiments during NPs synthesis including temperature, pH [20,54], and the material concentration determine the morphology and size distribution of NPs. Also from the previous experiments, the different shapes and sizes of metal NPs show different cytotoxicity [55]. Further, there is damage in DNA, the authors assumed that the extreme production of ROS by NPs results in direct DNA damage and therefore induces necrosis and then apoptosis [56,57].

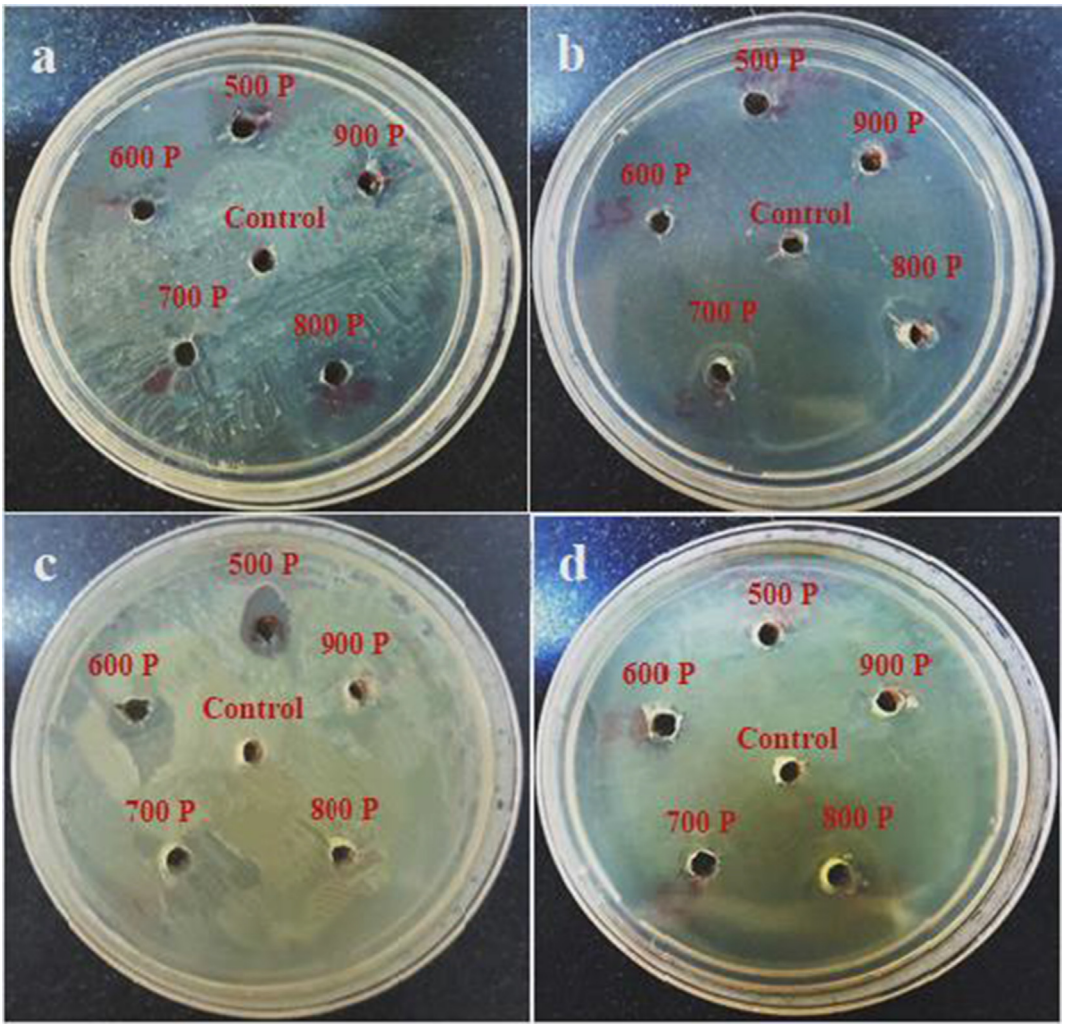

Fig. 20. Antibacterial activity at 532 nm-600 mJ (a) S. aureus, (b) S. mutans, (c) E. coli, (d) Pseudomonas.

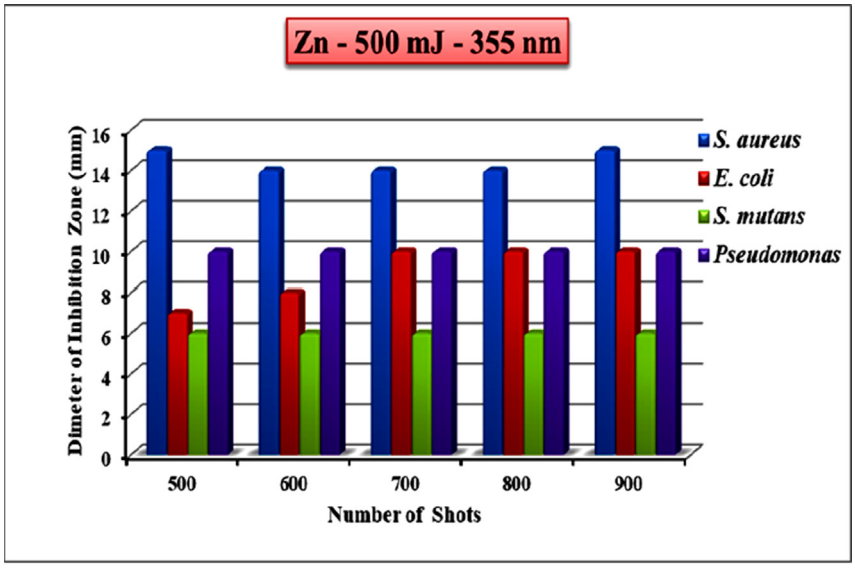

Fig. 21. Histogram of antibacterial activity at 355nm-500mJ data table show that the inhibition zones are calculated in mm.

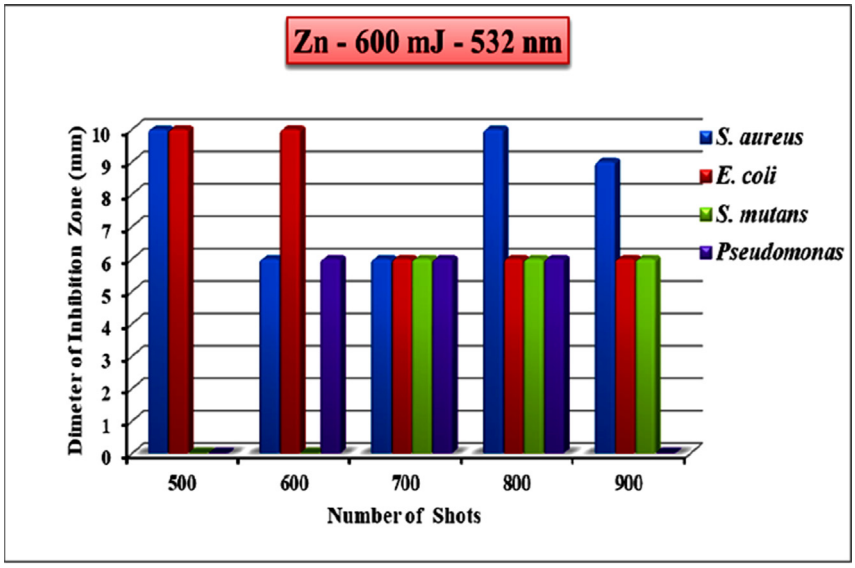

Fig. 22. Histogram of antibacterial activity at 532nm-600mJ data table show that the inhibition zones are calculated in mm.

4. Conclusions

This work investigated the ability to synthesize ZnNPs by the physical method that is PLAL, and one of its biomedical applications is its antibacterial activity. The results showed the possibility of obtaining ZnNPs using two wavelengths of the Nd: YAG laser (355 nm and 532 nm) in the process of ablation and with different numbers of pulses, by changing the color of the solution and analyzing the absorption spectrum as a preliminary proof of the nanosynthesis of the material, knowing that this laser treatment led to the formation of ZnO NPs as well, and the energy gaps were calculated for them, and it was found that they increase with the increase in the number of pulses at (532 nm). Also, FTIR analysis was conducted and it was found that the effective groups present in the prepared samples are (O–H, C–H, C–O, and C=O), in addition to observing bending and stretching vibrations that can be due to the presence of atmospheric water during analysis i.e. contamination by moisture. As for the structural analysis that was conducted, they included XRD, SEM with EDX, and AFM for selected samples, which were prepared at the highest number of pulses and gave the highest antibacterial activity for both two wavelengths used. From the photograph, spherical shapes with various sizes of the prepared NPs were successfully synthesized. In general, it can be said that the prepared samples at the wavelength (355 nm) are the most effective under the conditions of this work. The results obtained proved that pulsed laser ablation is an easy and fast method for nanometal synthesis and it can be a good alternative to other methods, and the NPs prepared with it have biomedical efficacy, however, additional studies may be needed to change the laser parameters and study other applications.

Conflict of interest

There is no conflict of interest.

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