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# INVESTIGATION OF THE ANTIBACTERIAL ACTIVITY OF GRAM POSITIVE AND GRAM NEGATIVE BACTERIA BY 405 NM LASER AND NANOPARTICLES

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## Abstract

The aims of current study to estimate the effectiveness of Silver Nanoparticles (AgNPs) and 405 nm Diode laser irradiation with power of 100mW on the growth of Gram-positive Methicillin Resistant *Staphylococcus aureus* bacteria (MRSA) and Gram- negative *Pseudomonas aeruginosa*. UV-Vis spectrophotometer was used to confirm the monodispersity of Silver Nanoparticles and Titanium dioxide Nanoparticles. Various concentrations of AgNPs (7.5, 15, 30, 60, and 120 µg/ml) and Titanium dioxide (20, 40, 80, and 160, 320 µg/ml) were used to determine Minimum Inhibitory Concentration (MIC) with three different laser exposure times of (5, 10 and 15 minutes). The results showed that the MIC of laser enhanced AgNPs and $TiO_2$ against *Pseudomonas aeruginosa* were 30 µg/ml and 40 µg/ml respectively and that for $TiO_2$ combined with laser against *S .aureus* was 80 µg/ml. Laser Irradiation experiments showed that the number of CFU/ml of *S. aureus* was significantly reduced with increasing exposure time. The blue Laser irradiation enhanced the bacterial activity of AgNPs and $TiO_2$ by owing to its photothermal effect that can be regarded as an effective antibacterial approach against methicillin resistant *S .aureus* (MRSA) and *Pseudomonas aeruginosa*.

***Key words:*** Silver, *S .aureus*, Laser, Antibacterial activity, Nanoparticles.

## Introduction

Multidrug resistance *Staphylococcus aureus* can cause burn and wound infections (Phillips *et al.*, 1989). *Pseudomonas aeruginosa* is another type of morbid pathogenic agents that present in the environment, for instance in soil, plants, and water (Costerton, 1994). Traditionally, antibiotics have been utilized to destroy bacteria, however, the wide variety of human bacterial and fungal burn wound pathogens, especially nosocomial isolates made it not very efficient (Clark *et al.*, 2003; Altoparlak *et al.*, 2004). Therefore, it became important to search for alternative treatments for infections (Coates *et al.*, 1997; Ross *et al.*, 2003; Rudramurthy *et al.*, 2016). or resistance to the penicillin drugs in the 1950s, resulted from indiscriminate use of drugs (Chambers and DeLeo, 2009). Having most of the therapeutic routs inappropriate, made it important to look for other treatments, especially those use the nanomaterials which are special candidates for most applications in science and technology that can replace conventional materials (Azam *et al.*, 2009). Silver, gold, titanium dioxide and zinc Nanoparticles with different optical properties have been approved as bacteriostatic and bacteriostatic agents (Lansdown, 2006; Zhou *et al.*, 2012).

AgNPs are known to have unique and attractive physical, chemical, and biological properties. In accordance with that, to explain their potential applications and properties, extensive research was carried out to investigate their applications as anticancer agents, antimicrobial agents, wound dressings, water treatment, and electronic devices (Lansdown, 2006; Abdel-Fattah and Ali, 2018), It has been shown that silver (Ag) has strong antimicrobial effects and has been used in medicine on a wide range of applications (Brennan *et al.*, 2015).

Noble metal Nanoparticles significantly differ from bulk materials by their unique properties. These special properties of Nanoparticles can be accounted for their small size and large specific surface area to volume ratio. Metal Nanoparticles find several applications in electronics, catalysis, photonics, and diagnostic biological

probes (Alkire *et al.*, 2008). Titanium dioxide Nanoparticles have gained attention for their antimicrobial activity against different types of bacteria owing to their oxidizing property (Chen *et al.*, 2010).

One of the most significant applications of Silver Nanoparticles is, using them as antimicrobial coatings, and in biomedical devices. Silver Nanoparticles constantly release low-level silver ions to avoid bacterial infection (Yacamán *et al.*, 2001).

The objective of this study was synthesizing Ag Nanoparticles by a simple process, without hazard wastes. Further, different concentrations of Ag and $TiO_2$ Nanoparticles were used to investigate their antibacterial activity against gram positive and gram negative bacteria.

## Materials and Methods

### Materials

Polyvinylpyrrolidone (PVP), nutrient broth, agar, and $TiO_2$ were purchased from Sigma Aldrich, Mannitol salt, and molar Hinton agar.

### Bacterial isolates

The bacterial isolates used in this study were obtained from the Department of Biotechnology / College of Science / University of Baghdad; they were most antibiotics resistant bacterial isolates Gram-negative bacterium (*Pseudomonas aeruginosa*) and Gram-positive bacterium Methicillin resistant *Staphylococcus aureus* (MRSA) were used to investigate the combined

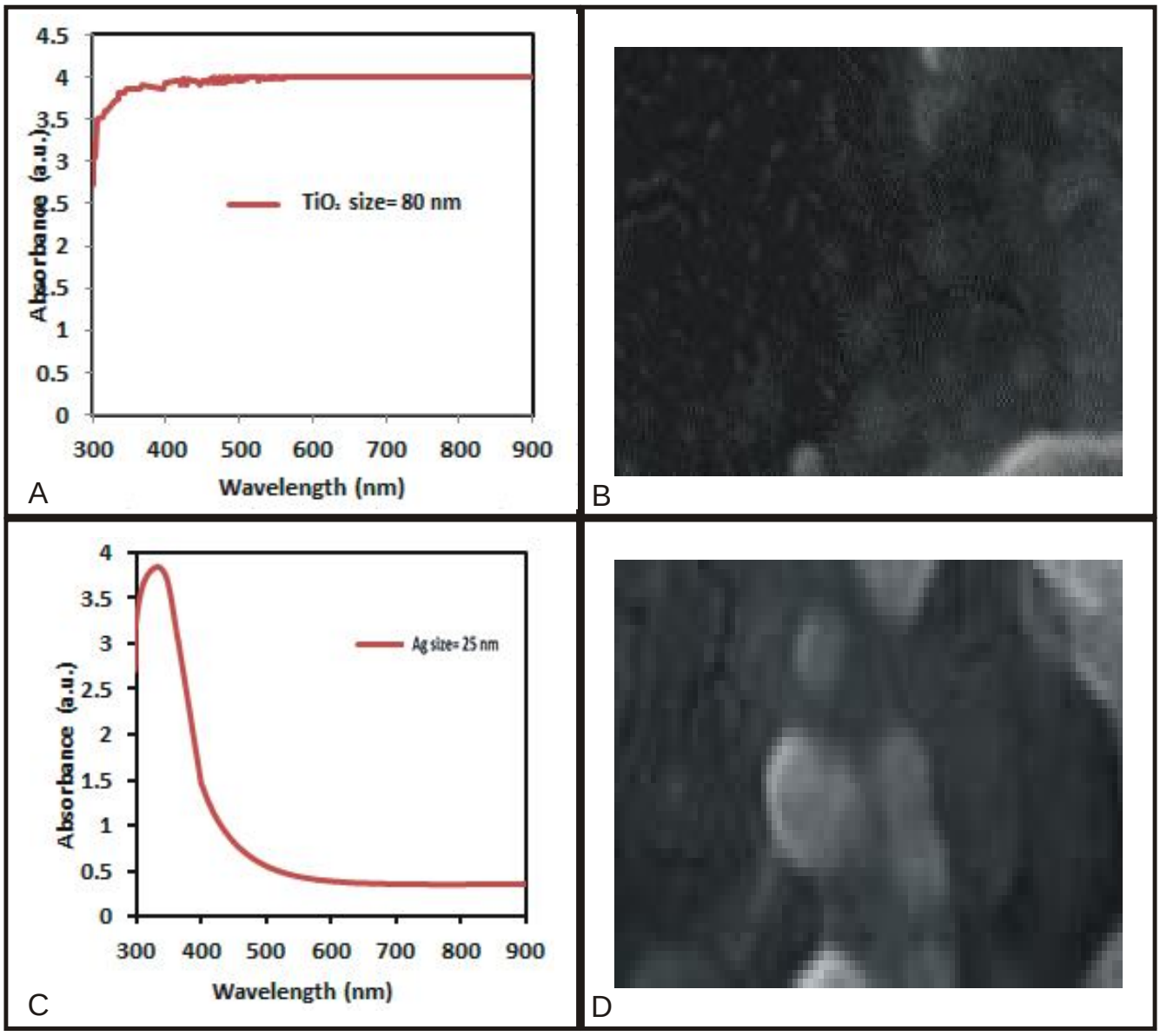


**Fig. 1:** Characterization of Silver and Titanium dioxide Nanoparticles. (A) $TiO_2$ Nanoparticles absorption spectrum. (B) TEM images of $TiO_2$ Nanoparticles showed the spherical shape with average size 80 nm. (C) Absorption spectrum of Silver Nanoparticles. (D) TEM images showed spherical shape of prepared Silver Nanoparticles with average size 25 nm.

and separate antibacterial activity of prepared Ag Nanoparticles and $TiO_2$ NPs with and without laser diode. The selected isolates were grown on Cetrimide agar for *P. aeruginosa* and on Mannitol salt agar for *S. aureus* and incubated at 37°C for 18 hours. A single colony was selected from media plate and inoculated into 5 ml of broth media then incubated for overnight at 37°C.

**Determination of Minimum inhibitory concentration (MIC) as an antibacterial activity of Ag and $TiO_2$ Nanoparticles**

The antibacterial activities of Ag and $TiO_2$ Nanoparticles were evaluated against Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*P. aeruginosa*) by method of serial dilution through the minimum inhibitory concentration (MIC) determination in the broth culture. In this study, the twofold serial dilutions method according to Farokhzad and Langer, (2006) that used for the minimum inhibitory concentration (MIC) values determination. Serial dilution method was done twelve times giving concentrations of Silver Nanoparticles (7.5, 15, 30, 60 and 120 µg/ml) and twelve times for Titanium dioxide (20, 40, 80, 160 and 320 µg/ml). Then 1 mL of each serial Nanoparticles were added to the set of tubes except for that tubes of control. All tubes of controls and tested samples were incubated for 24 h., at 37°C with shaking 200 rpm. The values of MIC were taken as the lowest concentration necessary to stop the growth of the bacteria in the test tube after incubation (no turbidity is shown). After incubation, 1 mL of each assigned concentrations was transferred to a sterile Eppendorf and irradiated by 405 nm blue laser for (5, 10 and 15 minutes). The laser beam was defocused on the surface of the suspension at a distance of 19 cm and a beam diameter of 1 cm, using a convex lens. Thereafter, a sterile loop was used to inoculate and spread the solutions on Cetrimide agar for *P. aeruginosa* and on Mannitol salt agar for *S. aureus* in plates. The plates were incubated at 37°C for 24 h at the same conditions. plates of MIC were compared with control, the number of colonies was counted using a colony counter and the colony forming units (CFUs) were then calculated using the plate count method (Manna *et al.*, 2000; Xu *et al.*, 2006).

**Synthesis of AgNPs**

Silver Nanoparticles were prepared using laser ablation method with a slight modification of (Ganguly *et al.*, 2013) in presence of 18 mM polyvinylpyrrolidone (PVP). The fundamental emission of Nd-YAG laser was 1064 nm. Briefly, silver plate (99.99%; purity) has been immersed in quartz tube containing PVP solution. Laser irradiation lasted for 10 minutes with pulse energy of 12 ml, pulse duration of 8 nanoseconds and pulse repetition rate of 10 Hertz. Synthesis of Nanoparticles by laser ablation offers many advantages that include nontoxicity, chemical stability, reshaping ability and controlling the characteristics of generated Nanoparticles by varying the experimental parameters (Pal *et al.*, 2007).

**UV-visible spectrometer**

The UV-visible absorption spectra were measured at 200-1200 nm with UV-visible absorbance spectrometer (UV-1800 Shimadzu, Japan).

**Transmission Electron Microscopy (TEM)**

TEM analysis has been used to characterize the morphology of Nanoparticles. For TEM, a drop of synthesized colloidal solution of Nanoparticles has been placed on a carbon-coated copper mesh and dried at room temperature. The micrographs were monitored by TEM (CM120, Phillips Holland) which can be operated up to 120 KV.

**UV-Vis Spectra analysis**

The Ultraviolet-Visible Spectrophotometer (UV-1800

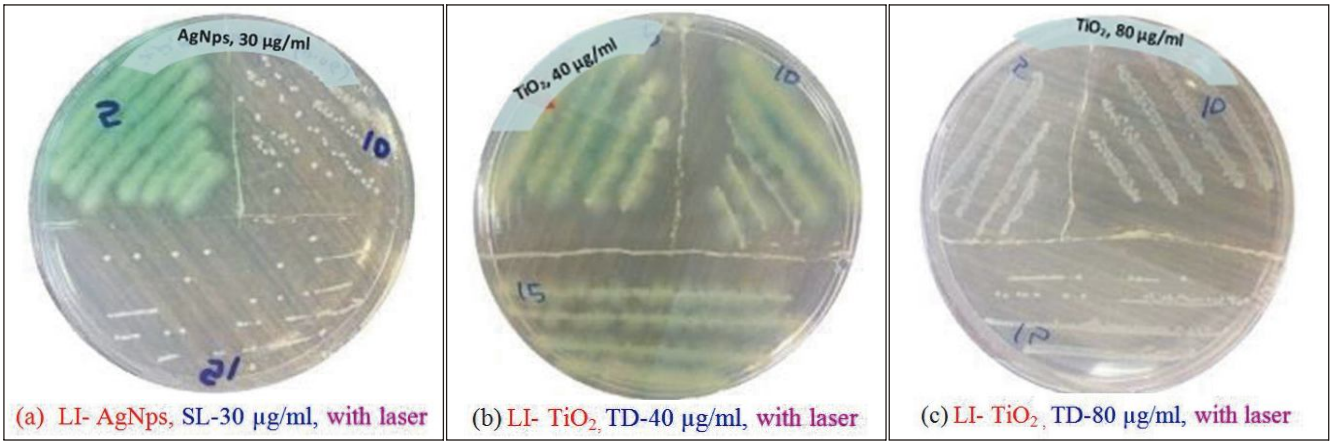


**Fig. 2:** Antibacterial activity of Laser induced (LI) (LI-AgNPs, and LI-$TiO_2$NPs) against *Pseudomonas Aeruginosa* (a and b first row) and MRSA (c second row) at different concentrations (Silver (SL) and Titanium Dioxide (TD)) and Laser exposure times (5, 10, and 15 min).

Shimadzu, Japan) was used to evaluate the characteristics of Nanoparticles as an easy method to give information about concentrations, sizes, and shapes of particles. Absorbance shift relay on the size (diameters) and the particles shape (Ganguly *et al.*, 2013). Spectra were monitored periodically as a function of wavelength within range from 190 to 1100 as shown in fig. 1. For sample analysis, each milliliter of the sample was diluted to two milliliters with distilled water. The UV-Vis spectra for the resulting diluents were monitored for all samples prepared at a resolution of 1 nm at room temperature.

**Visual observation and UV-Vis spectra analysis**

Detection of Silver Nanoparticles by UV-Vis spectroscopy was carried out by visual observation of the color changes of the reaction solutions. Noble metals exhibit distinctive optical properties due to the property of Surface Plasmon Resonance (SPR) (Bindhu and Umadevi, 2013). The Nanoparticles were found to be spherical and monodispersed; Characteristic Surface Plasmon Absorption band was investigated at 405 nm for the color change indicated the generation of Silver Nanoparticles fig. 1D.

## Results and Discussion

The treatment of *pseudomonas aeruginosa* with laser enhanced AgNPs for 5, 10 and 15 minutes was effective. The temperature of each treated sample was monitored during the experiment and kept below the 50°C, such as below the cell thermal damage threshold (Ahmad and Jaffri, 2018). Bacteriostatic effect was evaluated by Minimum Inhibitory Concentrations (MIC) and compared to untreated controls. NPs used have been found to be highly toxic to the strains of bacteria with increased antibacterial efficacy in dose-dependent manner. The antibacterial activities of AgNps, $TiO_2$ and laser enhanced AgNps and $TiO_2$ were studied. For *P. aeruginosa*, the MIC for AgNps was 60 µg/ml, while for laser enhanced AgNps the MIC on *P. aeruginosa* was recorded to be 30 µg /ml as shown in fig. 2a, Whereas, the MIC for $TiO_2$ against *P. aeruginosa* was 80 µg/ml and that for laser enhanced $TiO_2$ was 40 µg/ml as shown in fig. 2b. For *S. aureus*, the MIC for $TiO_2$ was 160 µg /ml and for laser enhanced $TiO_2$ was 80 µg /ml as shown in fig. 2c. The antimicrobial activity of $TiO_2$ and AgNPs has been investigated by several studies, but the potential hazards and possible mechanisms of antibiotic are not yet clear (Durán *et al.*, 2016; Velusamy *et al.*, 2016; Al-Sharqi *et al.*, 2019). While both *P.auerginosa* and *S.aureus* depict high sensitivity to the titanium dioxide Nanoparticles, the difference is less for *S.aureus* compared to *P.auerginosa*. The Nanoparticles antimicrobial activity was enhanced by the laser light by photothermal deterioration in combined approach (Akram *et al.*, 2016; Astuti *et al.*, 2017), AgNPs-laser, causing loss of cell membrane integrity in quick manner (Durán *et al.*, 2016; Gurunathan *et al.*, 2019). According to the proven results, it may be concluded that the laser exposure combined with AgNPs and $TiO_2$ Nanoparticles can be used as a local approach of effective antibacterial for both MRSA and *Pseudomonas aeruginosa*. In all cases we note the loss of bluish-green pigment of bacteria which consists of blue pyocyanine, this may be an evidence for decreasing the ferocity of *Pseudomonas Aeruginosa*. To our knowledge, this is the first study explaining such a detailed and systematic study of relative MIC values for the synergistic effect of blue laser and AgNps/$TiO_2$ against gram positive and gram negative bacteria. Gram negative bacteria have thinner cell walls while Gram positive ones have thicker cell walls (Morita *et al.*, 2013; Hamzah and Hasso, 2019). These differences in structures may determines the factors of laser irradiation penetration that includes the biological effects. In accordance with these findings, we suggest that the aspects of each bacteria need to be examined physiologically and morphologically. However, it is predicted that a consensus might be reached regarding the results in order to determine the best parameters standardization to use for each species.

The Gram positive bacteria, the cell wall made of a thick layer of peptidoglycan, consist of linear chains of polysaccharide crossed linked by short peptides, therefore, more solid structure forming, leading to the difficult penetration of AgNPs. Whereas, in Gram negative bacteria, the cell wall composed of thinner peptidoglycan layer (Shrivastava *et al.*, 2007).

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