

## Research Article

Gold nanoparticles (AuNPs) synthesized via laser ablation serve as photosensitizers for the photodynamic inactivation of *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteriaAndi Hamim Zaidan<sup>a</sup>, Suryani Dyah Astuti<sup>a\*</sup>, Karwan Wasman Qadir<sup>b</sup>, Suhailah Wasman Qader<sup>c</sup>, Ahmad Khalil Yaqubi<sup>a</sup>, Sahda Vania<sup>a</sup>, Winarno Winarno<sup>a</sup>, Deny Arifianto<sup>d</sup>, Yunus Susilo<sup>e</sup>, Ardiansyah Syahrom<sup>f</sup><sup>a</sup>Department of Physics, Faculty of Science and Technology, Universitas Airlangga, 60115, Surabaya, Indonesia<sup>b</sup>Department of Physics, College of Education, Salahaddin University-Erbil, 44002 Erbil, Kurdistan Region, Iraq<sup>c</sup>Medical Analysis Department, Faculty of Applied Science, Tishk International University, 44001 Erbil, Kurdistan Region, Iraq<sup>d</sup>Department of Engineering, Faculty of Vocational, Universitas Airlangga, 60286, Surabaya, Indonesia<sup>e</sup>Faculty of Engineering, Dr Soetomo University, Surabaya 60118, Indonesia<sup>f</sup>Department of Applied Mechanics and Design, Faculty of Mechanical Engineering, Universiti Teknologi Malaysia, 81310, Johor Bahru, Malaysia

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## ABSTRACT

Bacterial infections pose a serious problem, often necessitating the use of antimicrobials in substantial quantities. Furthermore, microbial resistance has significantly escalated alongside the rapid adaptation of bacteria to antimicrobial agents. This research aims to explore alternative solutions for diminishing *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria through photodynamic therapy. Gold nanoparticles (AuNPs) were synthesized using a laser ablation technique and applied as photosensitizers at concentrations of 10.0, 15.0, and 21.5 µg/mL. A 405 nm blue diode laser was employed for photodynamic inactivation (PDI) against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Antibacterial efficacy was evaluated by quantifying bacterial reduction through Total Plate Count (TPC) in CFU/mL and confirmed using the disc diffusion assay. The research results indicated that adding 21.5 µg/mL of AuNPs photosensitizer at a volume of 50 µL led to a notable reduction in bacterial colonies. The most effective reduction in *Staphylococcus aureus* colonies was achieved with 150 s of laser exposure at an energy level of 2.87 J/cm<sup>2</sup>, reducing (80.3± 5.39)%. Similarly, the optimal reduction in *Pseudomonas aeruginosa* colonies occurred with 180 s of laser exposure at an energy level of 3.44 J/cm<sup>2</sup>, reducing (78.70±4.50)%. Blue laser irradiation at 405 nm combined with 21.5 µg/mL AuNPs effectively reduced *Staphylococcus aureus* by (80.37 ± 5.39)% in 150 s and *Pseudomonas aeruginosa* by (78.70 ± 4.50)% in 180 s, demonstrating its potential as an alternative antibacterial therapy.

## 1. Introduction

One of the severe challenges in the health sector is the increase in antibiotic resistance by pathogenic microorganisms such as *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (Hanora et al., 2016). Antimicrobial resistance occurs when microorganisms become insensitive to antimicrobial drugs that can inhibit them. Resistant organisms can survive the effects of antimicrobial drugs such as antibiotics, antivirals, and more. As a result, standard treatment becomes ineffective in treating infections. *S. aureus* is a pathogenic bacterium that can cause infections in humans (Pidwill et al., 2021). Almost everyone has experienced a *S. aureus* infection with varying degrees of severity, ranging from mild food poisoning or skin infections to serious ones that can be life-threatening (Chen et al., 2022). *S. aureus* is a Gram-positive bacterium with the potential to act as a pathogen (Cheung et al., 2021). Its presence in colonies significantly impacts the spread of infection. These bacteria can cause various diseases, from skin disorders to severe conditions

such as multi-organ failure (Frieri et al., 2017). Additionally, *S. aureus* is the leading cause of several diseases, including pneumonia, respiratory tract infections, cardiovascular infections, and nosocomial bacteremia (Garini et al., 2021). Some bacteria are able to form biofilms that protect the bacteria from adverse environments, making them more resistant to antibiotics (Høiby et al., 2010; Fakhry et al., 2024)

Infection by *P. aeruginosa* is also a severe problem for patients in hospital settings who suffer from cancer, cystic fibrosis, or burns, with a fatality rate that can reach 50% (Sathe et al., 2023). *P. aeruginosa* is a Gram-negative, rod-shaped, opportunistic pathogenic bacterium that is the main cause of nosocomial infections, with the ability to form biofilms on growth media. Biofilms often make it more difficult to treat infectious diseases (Mulcahy et al., 2014). These bacteria can appear in pairs or sometimes as individuals forming short chains. Due to its simple nutritional requirements, *P. aeruginosa* quickly breeds in various environments, especially the humid environment of hospitals (Alhede et al., 2014). These bacteria can be found in burn wounds, ear infections, and postoperative wounds (Jensen et al., 2010). *P. aeruginosa*

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is a frequent cause of infection in individuals with weakened immune systems (Pang et al., 2019).

These bacteria can cause serious diseases, such as pneumonia, chronic lung infections, ulcerative keratitis, urinary tract infections, and bacteremia in patients with burns (Reynolds et al., 2021). Antibiotics are used in the treatment of *P. aeruginosa* (Alves et al., 2014). A key element in *P. aeruginosa* pathogenicity is biofilm development. The bacteria become more resilient to immunological reactions and medications as a result of this biofilm (Bouillaguet et al., 2010). Unfortunately, *P. aeruginosa* may become resistant to many types of antibiotic drugs, even while the infection is being treated, making it difficult to choose the right medications (Escudero et al., 2021; Kwiatkowski et al., 2018).

Photodynamic Therapy (PDT) is a biophotonics technique that uses light to inhibit the growth of pathogenic microbes (Polat et al., 2021). It involves a light source, photosensitizer, and oxygen. Visible light activates the photosensitizer, which becomes unstable and excited by absorbing incoming light (Didi et al., 2024; Astuti et al., 2022). PDT is a safer treatment than conventional antibiotics due to its lack of antibiotic resistance and few side effects (Permatasari et al., 2023). Studies have shown the effectiveness of diode lasers in inactivating pathogenic microbes, and natural extracts as photosensitizers (Astuti, S.D et al., 2020). Clinical trials have also shown the effectiveness of PDT and diode laser Photobiomodulation (Astuti, S.D et al., 2021).

PDT employing photodynamic inactivation (PDI) offers an alternative approach to harness nanoparticle antibacterial properties (Piksa et al., 2023). Various studies using nano photosensitizers have been conducted on bacteria (Mardianto et al., 2020), fungi (Rozykulyyeva et al., 2020), and biofilms (Astuti, S.D et al., 2019). Nanotechnology is an intriguing interdisciplinary field encompassing physics, chemistry, and biology, with nanoparticles being a crucial component (Yaqubi et al., 2024; Astuti et al., 2019). Any substance with a size between 1 and 100 nm is regarded as a nanoparticle (Biel et al., 2010). Gold nanoparticles (AuNPs) may be synthesized using a variety of techniques, which fall into two categories: top-down and bottom-up (Siddiqui et al., 2023). The initial bulk material is reduced to the nanoscale size using the top-down method (Patil et al., 2023; Hassan et al., 2022); laser ablation is one top-down synthesis technique (Semaltianos, 2010).

Laser ablation is a method for producing nanoparticles by irradiating metal in a liquid solution, forming plasma peaks that yield nanoparticles (Khan et al., 2022). This method is unique in its ability to synthesize stable nanoparticles without stabilizing or additional agents (Katas et al., 2019; Radhi et al., 2024). Studies have tested the antibacterial activity of AuNPs utilizing the bioreductors, *Lignosus rhinocerotis* and chitosan, and laser ablation is a common method for creating metal colloids (Narband, 2009; Lin et al., 2021).

According to the research, AuNPs produced by laser ablation exhibited antibacterial efficacy against *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, and *S. aureus*. The 405 nm wavelength activates endogenous photosensitizers in bacterial cells, leading to reactive oxygen species (ROS) generation. The intrinsic "dark" toxicity of AuNPs and blue light activation are likely responsible for bacterial death. Further improvements are needed to increase photoreactivity or combine AuNPs with external photosensitizers.

This study aims to increase the effectiveness of bacterial photoinactivation by combining a gold nano-photosensitizer with diode laser photodynamics. The novelty of this research lies in the innovative application of AuNPs synthesized via laser ablation as enhancers for the effectiveness of PDI against *S. aureus* and *P. aeruginosa*. Unlike conventional photosensitizers, which often require chemical stabilizers or external agents, laser-ablated AuNPs are produced in a pure colloidal state, free from residual reducing or capping agents, resulting in higher stability and biocompatibility. Their clean surface chemistry allows stronger interaction with bacterial cell walls and more efficient light absorption, enhancing ROS generation under 405 nm blue diode laser irradiation. Consequently, laser-ablated AuNPs not only eliminate the need for potentially toxic stabilizing agents but also provide superior photoreactivity and stability compared to traditional organic or chemical photosensitizers. This study demonstrates the potential of AuNPs to improve PDI outcomes against antibiotic-resistant pathogens, offering a sustainable and advanced strategy for combating multidrug-resistant infections.

## 2. Materials and Methods

### 2.1 Synthesis of gold nanoparticles

The synthesis preparation entails a physical method, with 0.1 g of Antam gold (Au) as the material. The procedure involves placing the gold into distilled water and positioning it directly beneath the laser. The software connected to the Nd: YAG device is then configured with a frequency of 20 kHz, a wavelength of 1064 nm, and a power setting of 50 W. The gold under the laser is focused onto a single laser point at 34 cm, initiating the ablation process. Ablation continues for 1 min until cherry red colored nanocolloids are formed (Wender et al., 2011).

To characterize the synthesized nanoparticles, the size distribution and polydispersity index (PDI) were analyzed using a Dynamic Light Scattering (DLS) particle size analyzer, providing direct evidence of the spherical shape and nanoscale dimensions of the AuNPs.

### 2.2 Bacterial culture

To culture *S. aureus* and *P. aeruginosa*, tryptone Soy Broth (TSB) was used. After that, the cultured samples were incubated for 24 h at 37°C.

### 2.3 Laser source

The light source employed to activate the photosensitizer is a blue diode laser with a specific wavelength of 405 nm. Laser characterization was conducted across four groups, including laser wavelength, laser output diameter, power (intensity) over time and distance, and measurement of laser output temperature. AA Thorlab PM100 Power meter was utilized to characterize the wavelength and laser power to characterize the wavelength and laser power. To measure the diameter of the laser output, the laser beam was directed towards a block of millimeter paper, and the diameter was subsequently measured using a caliper. Additionally, laser temperature was measured using a thermogenic.

### 2.4 Anti-bacterial activity test

The antibacterial test serves as a crucial stage to evaluate the ability of AuNPs to inhibit the growth of *S. aureus* and *P. aeruginosa* bacteria. The method employed in this test was disc diffusion, commonly used to measure the sensitivity of bacteria to antibacterial. In a petri plate, 50 µL of bacterial culture in TSB was applied as an inoculant to Tryptic Soy Agar (TSA) medium. Then, 10 µL of AuNPs were deposited onto a 5 mm paper disc and put in the petri plate after they solidified. The petri dish was then incubated at 37°C for 24 h.

### 2.5 PDI treatment

Four groups are created from the samples that are receiving treatment. The first group is T0, the control group, which has not been exposed to laser light. Samples of *S. aureus* (A1 and A2) in the second group are subjected to a 405 nm laser at different AuNP concentrations and periods. *P. aeruginosa* samples (A3 and A4) in the third group are subjected to a 405 nm laser at different AuNP concentrations and periods. Bacteria in groups A2 and A4 are also exposed to AuNP-derived photosensitizer (PS) and incubated for 20 min prior to being exposed to radiation for 90, 120, 150, and 180 min. The treated samples are then cultivated on TSA medium and incubated at 37°C for a whole day. A Quebec colony counter is used to count the number of bacterial colonies after incubation. This procedure makes it possible to assess how well the therapies work against *S. aureus* and *P. aeruginosa*.

### 2.6 Statistical analysis

To ascertain the impact of each component and the interplay among them, a two-way ANOVA Factorial test was used for the statistical analysis using IBM SPSS. The data must be uniformly distributed and have a normal distribution in order for a two-way ANOVA Factorial design to examine the impact of two variables. The null hypothesis ( $H_0$ ) was rejected when the p-value was less than the significance

level ( $\alpha = 0.05$ ), i.e.,  $p < 0.05$ . Subsequently, the Tukey method post hoc test was performed to examine the differences between sample factors. It is important to note that the  $p < 0.005$  for the results to be considered significant. The outcome of this test will aid in determining the radiation method with optimal potential for inactivating *S. aureus* and *P. aeruginosa*.

### 3. Results

AuNPs were characterized using physical methods, specifically laser ablation, employing a 0.1-g Antam gold (Au) metal plate. Ablation was performed utilizing an Nd: YAG laser device set at a frequency of 20 kHz, a wavelength of 1064 nm, and a power of 50 W. The ablation process involved directing the laser spot at the gold metal plate, after which the gold was ablated for 1 min until a cherry red color formed.

The AuNPs used in this study had concentrations of 10.0  $\mu\text{g/mL}$ , 15.0  $\mu\text{g/mL}$ , and 21.5  $\mu\text{g/mL}$ . After the formation of the AuNPs, dilution to achieve various concentrations was performed. Subsequently, the successfully synthesized AuNPs were characterized to examine the absorption spectrum, particle size, and stability of the AuNPs observed from day 1 to day 7.

UV-VIS spectrophotometric characterization was conducted using wavelengths of 325-1100 nm. This characterization aimed to determine the ability of AuNPs to absorb light. An absorbance spectrum, with wavelength on the x-axis and absorbance on the y-axis, is used to display the characterization findings. Fig. 1(a) shows that the AuNPs' UV-VIS findings fall within the wavelength range of 523 nm.

Fig. 1(b) illustrates the variations in particle size distribution by displaying the PSA findings for AuNPs at concentrations of 10  $\mu\text{g/mL}$ , 15  $\mu\text{g/mL}$ , and 21.5  $\mu\text{g/mL}$ . The nanoparticles show a limited and homogeneous size range at 10  $\mu\text{g/mL}$ , but at 15  $\mu\text{g/mL}$ , the size distribution somewhat expands, perhaps because of more interactions. At 21.5  $\mu\text{g/mL}$ , the distribution broadens further, indicating possible aggregation at higher concentrations. These results demonstrate how increasing AuNP concentration impacts particle uniformity and size.

The stability of AuNPs was assessed over days 1 to 7 of storage. Stability measurements were conducted by visually observing AuNPs for any color changes. Additionally, wavelength spectrum measurements were performed to assess the stability of the absorbed waves. In Table 1, the comparison of AuNPs indicates no agglomeration after the storage period, indicating the absence of larger particles. Furthermore, the color change of AuNPs was not very significant, suggesting that the color of the ablated AuNPs remained stable.

The absorbance wavelength spectrum was measured using UV-VIS spectrophotometry. After storage for 1 week, the wavelength changed by 1-4 nm, as shown in Fig. 2. The position of the maximum wavelength ranged from 525-521 nm. A shift in the position of the maximum

wavelength indicates a difference in particle size. However, the shift in position remains relatively constant.

Fig. 3(a) shows the viability of *S. aureus* treated with AuNPs at 10.0, 15.0, and 21.5  $\mu\text{g/mL}$ . Bacterial viability decreases as AuNP concentration increases, with 21.5  $\mu\text{g/mL}$  showing the strongest antibacterial effect.

Fig. 3(b) shows the percentage reduction of *P. aeruginosa* treated with AuNPs at concentrations of (a) 10.0  $\mu\text{g/mL}$ , (b) 15.0  $\mu\text{g/mL}$ , and (c) 21.5  $\mu\text{g/mL}$ . The reduction increases with higher AuNP concentrations, with 10.0  $\mu\text{g/mL}$  showing a moderate effect, 15.0  $\mu\text{g/mL}$  achieving a significant reduction, and 21.5  $\mu\text{g/mL}$  demonstrating the highest antibacterial efficacy. This indicates a concentration-dependent antibacterial activity of AuNPs against *P. aeruginosa*.

The two-way ANOVA factorial test was the statistical method used to ascertain how laser energy affected bacterial mortality. With a significance level of  $p = 0.617 > \alpha = 0.05$ , the results of the normality test for the data from the two treatments demonstrated that the data were normally distributed. The findings of the two-way ANOVA factorial test revealed a significant difference in each temporal variation associated with laser energy, with a p-value of  $p = 0.000 < \alpha = 0.05$ . The treatment groups that produced significant variations in the interaction outcomes between each sample were then identified using post hoc testing. The test results indicated that exposure to a concentration of 21.5  $\mu\text{g/mL}$  for 150 s yielded significant results. At that time, the death of *S. aureus* bacteria was at a percentage death rate of  $80.37 \pm 5.39\%$ . Table 2 shows the results of statistical analysis on *P. aeruginosa* bacteria.

50  $\mu\text{L}$  of AuNPs was added to the *P. aeruginosa* bacterium to perform laser therapy. As shown in Figs. 4(a, b), bacteria were exposed to radiation for varied durations of 90, 120, 150, and 180 s, which led to a reduction in bacterial colonies. The Two-way ANOVA factorial test was the statistical method used to ascertain how laser energy affected bacterial mortality.

With a significance level of  $p = 0.743 > \alpha = 0.05$ , the results of the normality test for the data from the two treatments demonstrated that the data were normally distributed. The findings of the two-way ANOVA factorial test revealed a significant difference in each temporal variation associated with laser energy, with a p-value of  $p = 0.000 < \alpha = 0.05$ . The treatment groups that produced significant variations in the interaction outcomes between each sample were then identified using post hoc testing. According to the test findings, exposure to a dose of 21.5  $\mu\text{g/mL}$  for 150 s had noteworthy outcomes. At that time, the death of *P. aeruginosa* bacteria was at a percentage death rate of  $78.70 \pm 4.50\%$ .

Table 3 shows the statistical analysis of *P. aeruginosa* bacterial death percentages under varying AuNP concentrations, exposure times, and their interaction. Higher AuNP concentrations (10.0, 15.0, and 21.5  $\mu\text{g/mL}$ ) and longer exposure times (90, 120, 150, and 180 s) significantly increased bacterial mortality ( $P = 0.00$ ). The interaction of both factors

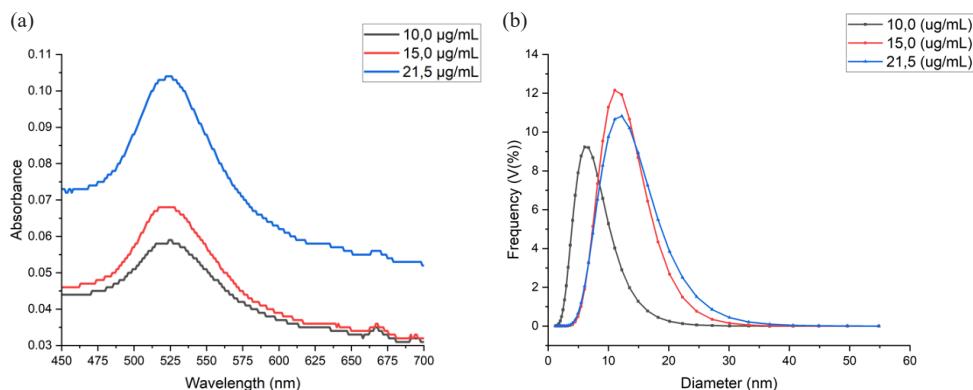

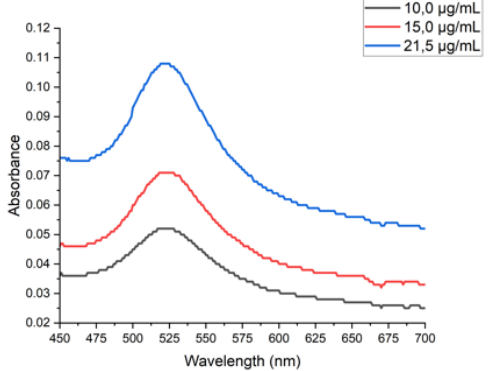

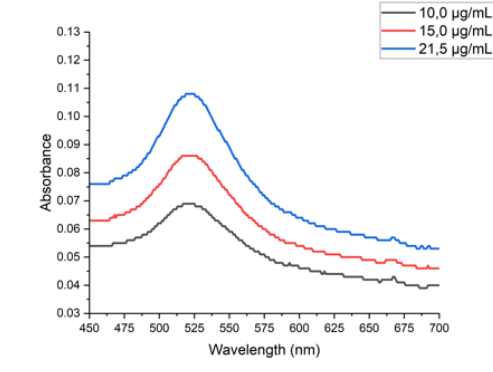

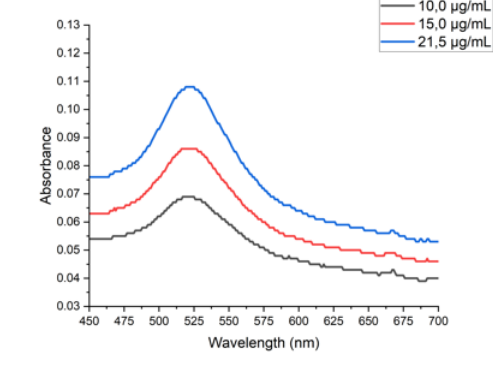

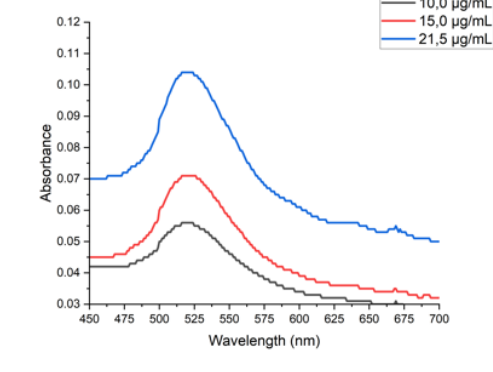
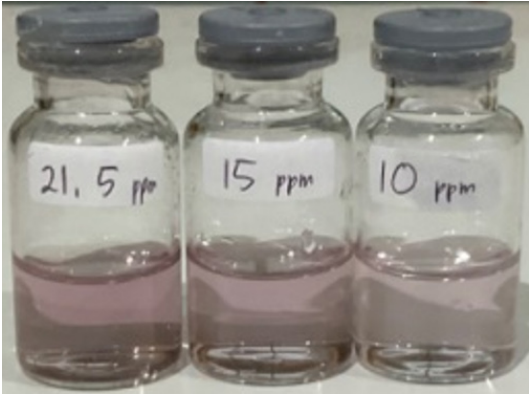
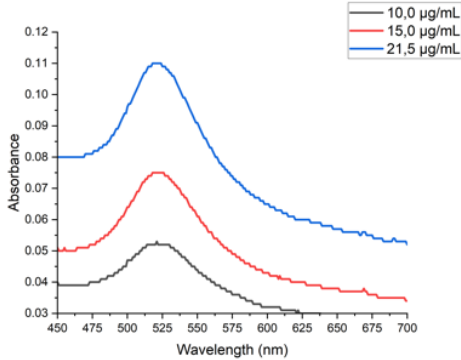
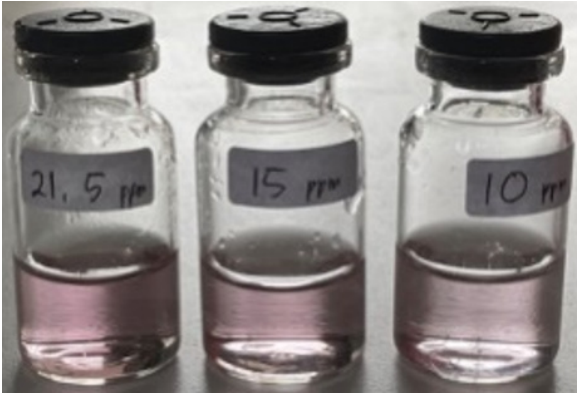
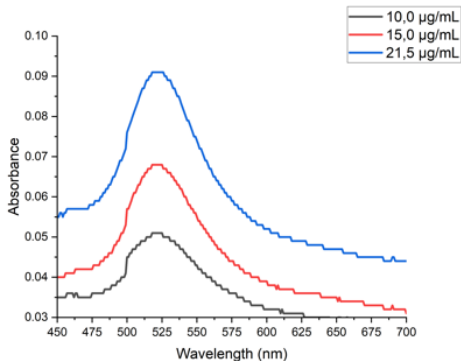

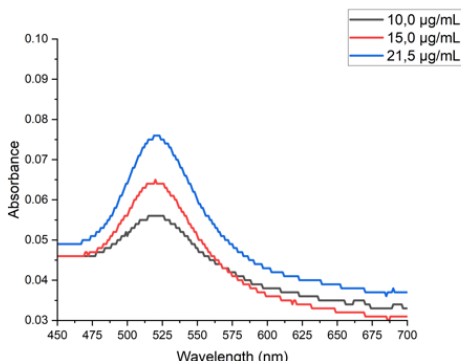


Fig. 1. (a) UV-Vis absorbance spectra of AuNPs synthesized by laser ablation at 10.0, 15.0, and 21.5  $\mu\text{g/mL}$ , exhibiting localized surface plasmon resonance (LSPR) peaks around  $\sim 523$  nm. The absorbance intensity increased with concentration, indicating higher nanoparticle yield. (b) Particle size distribution measured by PSA (DLS), expressed as mean size  $\pm$  SD ( $n = 3$ ). AuNPs at 10.0  $\mu\text{g/mL}$  displayed the most uniform size distribution, whereas 21.5  $\mu\text{g/mL}$  showed a tendency toward larger particle aggregation. Statistical significance was determined by one-way ANOVA with Tukey's post hoc test ( $p < 0.05$ ,  $p < 0.01$  compared with 10.0  $\mu\text{g/mL}$ )

**Table 1.**  
Comparison of physical properties and wave absorbance spectrum of AuNPs.

Days	Sample	Description	Absorbance spectrum
1		Red to purple	
2		Red to purple	
3		Red to purple	
4		Red to purple	

(Contd..)

Days	Sample	Description	Absorbance spectrum
5		Red to purple	
6		Red to purple	
7		Red to purple	

also showed a significant effect, with bacterial death ranging from 37.35% at 10.0 µg/mL and 90 s to 78.70% at 21.5 µg/mL and 180 s, confirming their combined impact on enhancing antibacterial efficacy.

The results showed that laser irradiation alone produced only a minimal reduction in bacterial viability for *S. aureus* and *P. aeruginosa*, less than 20%, which was not statistically significant compared with the untreated control group ( $p > 0.05$ ). In contrast, treatments combining AuNPs with laser exposure resulted in a concentration- and time-dependent antibacterial effect, with statistically significant reductions in bacterial viability compared with both the untreated control and the laser-only control groups ( $p < 0.01$ ).

#### 4. Discussion

The resistance of *S. aureus* and *P. aeruginosa* bacteria to antibiotics is a significant health concern (Mancuso et al., 2021). PDT, an alternative method, uses light and photosensitizers to inhibit pathogenic growth (Garini et al., 2021). AuNPs and blue diode lasers were created in this work by laser ablation at concentrations of 10.0 µg/mL, 15.0 µg/mL, and 21.5 µg/mL (Astuti, S.D et al., 2023). Laser ablation is an efficient

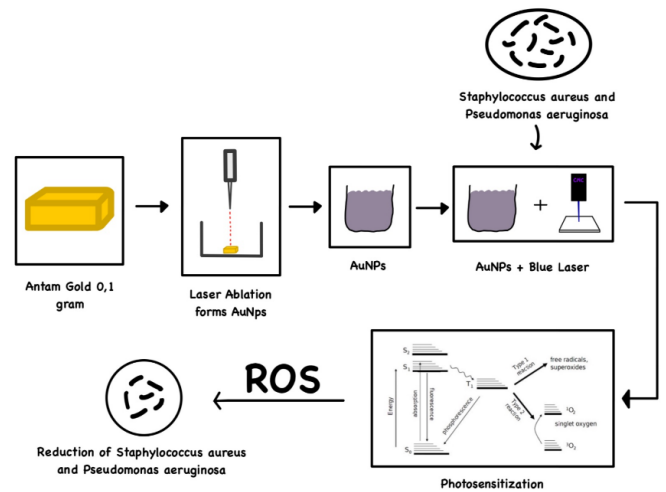
method for creating nanoparticles from various solvents, breaking down smaller fragments and removing them from larger materials (Fazio et al., 2020).

A photophysical technique allows the best photosensitizer to absorb laser light (Saeed et al., 2019). The photosensitizer's electrons will be stimulated from their lowest state (S<sub>0</sub>) to their excited state (S<sub>1</sub>) when light is used as an energy source. By releasing fluorescence at the S<sub>1</sub> level, the photosensitizer may undergo a radiation transition and return to the S<sub>0</sub> state (Balachandran et al., 2022). While non-radiation transitions occur through internal conversion, vibrational relaxation, and intersystem crossing. When the photosensitizer is in an excited state, if there is an overlap with the triplet excitation state, a spin reversal will occur, so that it moves to the triplet excitation state. Furthermore, it will initiate a photochemical reaction (Yoon et al., 2013).

Photosensitizers require oxygen reactions through photochemical reactions to return from the T<sub>1</sub> state to the ground state S<sub>0</sub>. This reaction is divided into two types: type I and type II. Type I reactions occur in the transfer of electrons between photosensitizer molecules and other molecules around them, which causes the formation of radical ions. The radical ions formed then react with oxygen at the

**Table 2.**  
Results of statistical analysis on *P. aeruginosa* bacteria.

Treatment	Group	N	Bacterial death (%)		Factorial test	
			Average	SD	Significance	Conclusion
Concentration of AuNPs	10.0 µg/mL <sup>a</sup>	20	18.41	6.61	P=0.00	There is a significance difference
	15.0 µg/mL <sup>b</sup>	20	22.95	6.33		
	21.5 µg/mL <sup>c</sup>	20	30.41	5.38		
Total		60				
Time	90 s <sup>a</sup>	15	34.14	5.79	P=0.00	There is a significance difference
	120 s <sup>b</sup>	15	40.39	8.06		
	150 s <sup>c</sup>	15	49.55	6.75		
	180 s <sup>a,b</sup>	15	49.80	10.74		
Total		60				
Interaction	10.0 µg/mL 90s <sup>a</sup>	5	36.37	10.75	P=0.00	There is a significance difference
	10.0 µg/mL 120s <sup>a,b</sup>	5	46.00	10.86		
	10.0 µg/mL 150s <sup>c,d</sup>	5	69.39	5.19		
	10.0 µg/mL 180s <sup>a,b</sup>	5	42.26	6.22		
	15.0 µg/mL 90s <sup>a,b</sup>	5	46.44	8.06		
	15.0 µg/mL 120s <sup>a,b</sup>	5	50.83	5.23		
	15.0 µg/mL 150s <sup>c,d</sup>	5	72.66	7.47		
	15.0 µg/mL 180s <sup>a,b</sup>	5	46.57	6.26		
	21.5 µg/mL 90s <sup>a,b</sup>	5	50.18	8.31		
	21.5 µg/mL 120s <sup>b,c</sup>	5	57.87	5.11		
	21.5 µg/mL 150s <sup>d</sup>	5	80.37	5.39		
	21.5 µg/mL 180s <sup>a,b</sup>	5	51.53	6.44		
Total		60				



**Fig. 2.** Set-up and mechanism of bacterial inactivation.

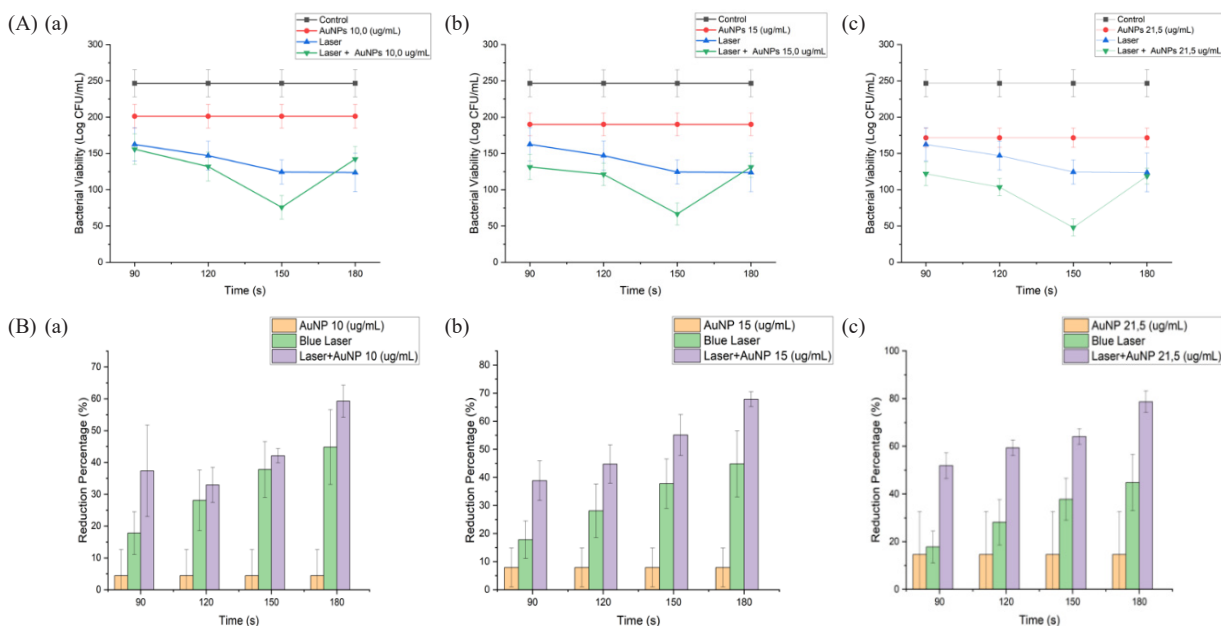
activity, cause cell lysis, or deactivate the membrane transport system (Huang et al., 2012).

Singlet oxygen and ROS are potent oxidants that can react with various types of molecules. Through photobiological processes, inactivation of cell membrane enzymes, increased membrane permeability, membrane disruption, and cell lysis can occur. Biological effects such as more cell necrosis can occur if the photosensitizer has a high enough concentration (Bae et al., 2022). Fig. 2 shows the setup and mechanism of bacterial inactivation.

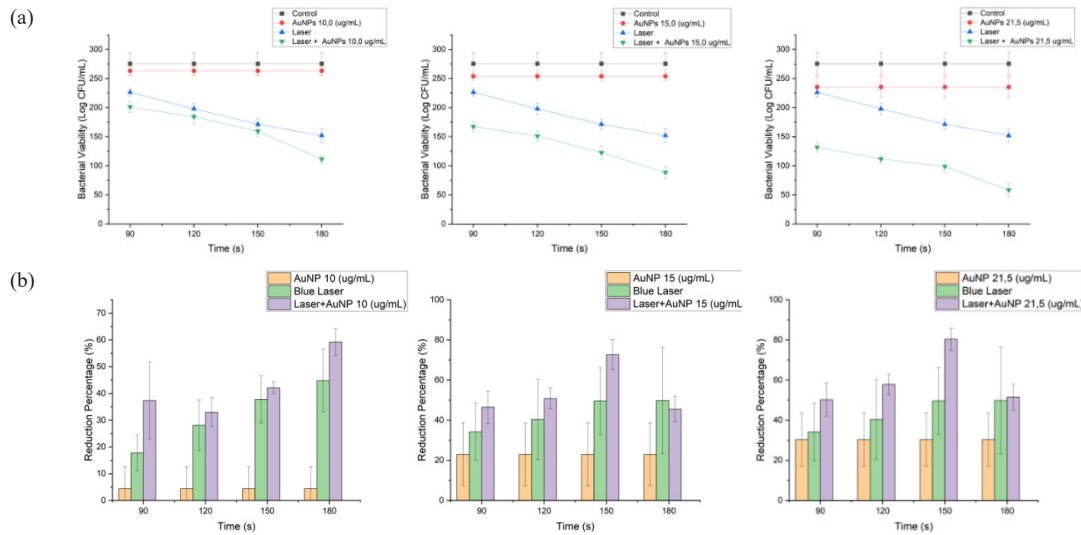
AuNPs reduce *S. aureus* and *P. aeruginosa* bacteria via a photosensitization process that happens when they absorb energy from the blue laser. The decrease of *P. aeruginosa* and *S. aureus* bacteria using AuNPs in this work shows that photodynamics utilizing a laser with a wavelength of 405 nm was more successful for *S. aureus* bacteria. The two bacteria were exposed to radiation for varied periods of 90, 120, 150, and 180 s in a dark room (Yaqubi et al., 2023).

The study compared the irradiation of *S. aureus* and *P. aeruginosa* bacteria using blue diode laser light and photosensitizers. *S. aureus* showed a significant death rate at 150 s, while *P. aeruginosa* showed

primary energy level (3O<sub>2</sub>) to produce ROS. Furthermore, in type II reactions, photosensitizers in the T1 state will transfer energy to 3O<sub>2</sub> to form singlet oxygen (1O<sub>2</sub>). These ROS can damage the cytoplasmic membrane through lipid or protein peroxidation, inhibit metabolic



**Fig. 3.** (A) Images of *Staphylococcus aureus* treated with AuNPs at three concentrations: (a) 10.0 µg/mL, (b) 15.0 µg/mL, and (c) 21.5 µg/mL. (B) Bacterial death (%) of *S. aureus* corresponding to the treatments in panel (A): (a) 10.0 µg/mL, (b) 15.0 µg/mL, and (c) 21.5 µg/mL. A clear concentration-dependent increase in bacterial death is observed, with 21.5 µg/mL showing the highest antibacterial effect. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test ( $p < 0.05$ ,  $p < 0.01$  vs. 10.0 µg/mL).



**Fig. 4.** (a) Viability of *Pseudomonas aeruginosa* treated with AuNPs at 10.0, 15.0, and 21.5 µg/mL, expressed as mean ± SD (n = 3). A marked decrease in bacterial viability was observed with increasing AuNP concentration, with the strongest effect at 21.5 µg/mL. (b) Percentage reduction of *P. aeruginosa* under the same treatments, expressed as mean ± SD (n = 3). Results demonstrated a clear concentration-dependent enhancement of antibacterial activity, confirming the effectiveness of AuNP-assisted PDT. Statistical significance was determined using one-way ANOVA followed by Tukey’s post hoc test (p < 0.05, p < 0.01 compared with 10.0 µg/mL).

**Table 3.**

Results of statistical analysis on *P. aeruginosa* bacteria:

Treatment	Group	N	Bacterial death (%)		Factorial test	
			Average	SD	Significance	Conclusion
Concentration of AuNPs	10.0 µg/mL <sup>a</sup>	20	4.43	2.97	P=0.00	There is a significance difference
	15.0 µg/mL <sup>b</sup>	20	7.92	2.52		
	21.5 µg/mL <sup>c</sup>	20	14.60	6.50		
Total		60				
Time	90 s <sup>a</sup>	15	17.79	2.43	P=0.00	There is a significance difference
	120 s <sup>a</sup>	15	28.10	3.45		
	150 s <sup>b</sup>	15	37.76	3.19		
	180 s <sup>c</sup>	15	44.81	4.27		
Total		60				
Interaction	10.0 µg/mL 90s <sup>a</sup>	5	37.35	14.36	P=0.00	There is a significance difference
	10.0 µg/mL 120s <sup>b</sup>	5	32.93	5.51		
	10.0 µg/mL 150s <sup>a,b,c</sup>	5	42.11	2.21		
	10.0 µg/mL 180s <sup>d,e</sup>	5	59.22	5.04		
	15.0 µg/mL 90s <sup>a,b</sup>	5	38.84	7.05		
	15.0 µg/mL 120s <sup>a,b,c</sup>	5	44.72	6.86		
	15.0 µg/mL 150s <sup>c,d,e</sup>	5	55.10	7.28		
	15.0 µg/mL 180s <sup>e,f</sup>	5	67.85	2.63		
	21.5 µg/mL 90s <sup>b,c</sup>	5	51.86	5.41		
	21.5 µg/mL 120s <sup>d,e</sup>	5	59.37	3.20		
	21.5 µg/mL 150s <sup>d,e</sup>	5	64.07	3.28		
	21.5 µg/mL 180s <sup>f</sup>	5	78.70	4.50		
Total		60				

a significant death rate at 180 s. The blue diode laser light source and its energy can influence bacterial inactivation, and adding a photosensitizer can enhance it further. AgNPs with grape seed extract and *Curcuma longa* extract reduced *S. aureus* by 87.01% and 81.67%, respectively. However, the study only achieved a maximum 50% reduction in *P. aeruginosa* for 300 s. The percentage reduction of *S. aureus* and *P. aeruginosa* showed significantly different results, possibly due to the choice of photosensitizers. The study also used antibacterial

activity tests to evaluate the likelihood of reducing bacteria (Rupel et al., 2021).

The observed differences between *S. aureus* and *P. aeruginosa* can be explained by several mechanisms. Gram-positive *S. aureus* possesses a thick peptidoglycan layer but lacks an outer membrane, allowing easier penetration of ROS and nanoparticles. In contrast, Gram-negative *P. aeruginosa* has an additional outer membrane rich in lipopolysaccharides, which provides a protective barrier that reduces nanoparticle uptake and limits ROS diffusion. Furthermore, *P. aeruginosa* is known for efficient efflux pump systems and biofilm-forming capacity, both of which can decrease PDT efficacy. These structural and physiological defenses likely explain why longer irradiation times and higher nanoparticle concentrations were needed to achieve comparable bacterial reduction.

Gold nanomaterials have emerged as versatile agents in biomedical applications due to their unique physicochemical properties, including tunable optical absorption, high surface area, and strong biocompatibility. Beyond antibacterial activity, they have been widely investigated for imaging, targeted therapy, and cancer treatment. For example, AuNP-based hydrogels embedded with copper carbon dots have been reported as effective photothermal/photodynamic antibacterial platforms with strong clinical potential (Lv et al., 2024). Similarly, multifunctional gold nanoplatfoms have been developed for castration-resistant prostate cancer, where microwave-triggered photothermal chemotherapy demonstrated promising therapeutic efficacy (Liu et al., 2023). In diagnostic imaging, charged AuNPs have enabled precise target identification and segmentation in CT image-guided adaptive radiotherapy for hepatocellular carcinoma, further showcasing their medical versatility (Lai et al., 2024).

Within the antimicrobial field, the photothermal and photodynamic properties of AuNPs have been increasingly recognized as effective alternatives to antibiotics, particularly against multidrug-resistant pathogens. Their capacity to generate localized heat and ROS under light irradiation provides a dual mechanism for microbial inactivation. Unlike traditional photosensitizers, laser-ablated AuNPs present additional advantages, including high stability, absence of chemical stabilizing agents, and efficient energy transfer under blue diode laser activation, which we demonstrate in this study against *S. aureus* and *P. aeruginosa*.

When AuNPs' antibacterial activity against *S. aureus* and *P. aeruginosa* was examined, it was found that at 500 µg/mL, *S. aureus* had a more pronounced inhibitory zone. Similar patterns were seen in the % decrease of germs using AuNPs, with *S. aureus* being reduced more successfully than *P. aeruginosa*. The difference in membrane structure between the two bacteria is due to the lipopolysaccharide layer, which acts as a barrier to antibacterial compounds (Garcez et al., 2013).

Further research is needed to understand the specific concentrations needed for effective inhibition (Al et al., 2019). The death of bacteria after laser irradiation without photosensitizers is due to the ability of bacteria to produce endogenous photosensitizers in their cells that can absorb light energy and initiate photochemical reactions, producing ROS. The administration of nano photosensitizers can increase the absorbed energy, thereby increasing the amount of ROS formed and increasing the ability to reduce bacteria (Lavaee et al., 2021). However, the addition of AuNPs significantly amplified ROS generation, proving their role as efficient nano-photosensitizers. This represents the key novelty of our study: demonstrating that laser-ablated AuNPs enhance PDT against both Gram-positive and Gram-negative bacteria, overcoming some of the limitations reported in previous nanoparticle-based studies.

## 5. Conclusions

Blue laser irradiation with a specific wavelength of 405 nm induces bacterial cell death using the photosensitizer AuNPs and without AuNPs. Treatment using AuNPs resulted in maximum bacterial death of *S. aureus* at 49.80%, while for *P. aeruginosa*, it was 44.81%. The group that added 21.5 µg/mL AuNPs for *S. aureus* experienced a death rate of (80.37±5.39)% in 150 seconds with a laser energy density of 2.87 J/cm<sup>2</sup>. Meanwhile, *P. aeruginosa* experienced a death rate of 78.70 ± 4.50% in 180 seconds with a laser energy density of 3.44 J/cm<sup>2</sup>. AuNPs have been successfully utilized in photoinactivation because they can reduce bacteria through PDT. Additionally, AuNPs do not possess proven toxic properties as they cannot penetrate more gram-negative bacteria. AuNPs with a greater concentration (21.5 µg/mL) increased the effectiveness of killing *S. aureus* and *P. aeruginosa* bacteria.

## CRedit authorship contribution statement

**Andi Hamim Zaidan, Suryani Dyah Astuti:** Designed the project. **Suryani Dyah Astuti:** Supervised this study. **Andi Hamim Zaidan, Karwan Wasman Qadir, Ahmad Khalil Yaqubi, Sahda Vania, Winarno Winarno:** Performed the study and collected the data. **Deny Arifianto, Yunus Susilo, Suhailah Wasman Qader, Ardiansyah Syahrom:** Drafted the manuscript. **Andi Hamim Zaidan, Suryani Dyah Astuti, Ahmad Khalil Yaqubi:** Reviewed the manuscript. All the authors have read the manuscript.

## Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work presented in this paper.

## Declaration of Generative AI and AI-assisted technologies in the writing process

The authors confirm that there was no use of Artificial Intelligence (AI)-Assisted Technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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