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Analysis of the Changes Power Output and Energy Dose to Green Laser Against OD and MDA Values After Photoinactivation at *Candida Albicans* and *Staphyloccocus Epidermidis* Associate Biofilms

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Abstract. *Photodynamic Therapy* (PDT) or *Photodynamic Inactivation* (PDI) to kill the pathogenic microbe like *Candida albicans* and *Staphylococcus epidermidis* has been more researches. The photoinactivation mechanism utilizes the activation process of sensitizer molecules by light so that an excitation process occurs and gets some reactive compounds such as singlet oxygen. The absorption of a number of the photon energy of light which is called the radiation energy dose which depends on the power output of the light source over a certain period of time. This study aims to compare the effect of photoinactivation on the biofilm associated *C. albicans* and *S. epidermidis* after exposure 60 to 300 seconds with two different output power a laser. The light instrument had been designed to use a microcontroller with a green laser completed of the display unit of output power. The results showed that PDI treatment with green laser was able to reduce biofilm cells up to 66.23% (P1 treatment) and 71.37% (P2 treatment). The results of the *malondialdehyde* (MDA) test showed that the highest value in the PD5 group was (0.389±0.004) nmol/mL. This result is lower than other research because the photoinactivation against microbes associate biofilm is more difficult than a single microbe.

INTRODUCTION

The development of research in the field of biophotonics that utilizes light in cancer therapy to its application to inhibit the growth of pathogenic microbes has been increasingly widespread. This field in the world of health is known as *Photodynamic Therapy* (PDT) or specifically related to microorganisms known as *Photodynamic Inactivation* (PDI) or *Photoantimicrobial Chemotherapy* (PACT). The PDI mechanism is in line with the principle of the interaction of light with matter, where when a number of photon energy hits a material, a series of processes known as light-based processes appear, namely photophysics, photochemistry, and photobiology.

Photophysics includes absorption and excitation events (electronic transition), photochemistry includes chemical reaction processes, and the formation of radical compounds, especially *Reactive Oxygen Species* (ROS). Photobiology is an event of the destruction of bacterial cells through the inactivating cell metabolism so that further lysis and death occur [1–2]. The toxicity and reactivity of ROS compounds can damage cell membranes, inhibit cell division systems, and damage cell DNA chains. Damaged cell membranes allow photosensitizers to be transferred into cells, thereby damaging cell organs such as lysosomes, mitochondria, and nuclei [3].

One of the light sources often used in the PDI is Lasers because it is very suitable for superficial and interstitial PDI applications. Laser has unique characteristics that can produce monochromatic light, very narrow bandwidth,

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and coherence. Lasers produce collimated light with a small (sub-millimeter) dot size. Therefore, lasers can be effectively coupled with optical fibers for endoscopic or interstitial use [4–5].

No less important, component that should be attended to in PDI is the existence of photosensitizer agents which give impact to ROS product during radiating. The main property should have a photosensitizer are purity chemical, high quantum yield, toxic in the dark, easy soluble for removing. Research in recent years has shown that the potential development of chlorophyll as a photosensitizer from plants such as papaya leaves [5–6], suji leaves [7], alfalfa leaves [8] has succeeded in killing pathogenic microbes by more than 80%. The previous study was against the use of chlorophyll extract of papaya leaves which were applied to *Candida albicans* biofilm in 2017-2019 [5–6,9].

This study aims to determine how the dose of laser irradiation energy is affected by comparing the two laser output power values during the exposure period when applied to PDI. The test indicators of these two parameters were analyzed through the inhibitory effect resulting from the PDI treatment on *C albicans* and *S epidermidis* associate biofilms by calculating the percentage of inactivation and MDA levels.

MATERIALS AND METHODS

The PDI device used in this research is based on a microcontroller to regulate the amount of laser output power. The green laser (532 nm) was set at output power P_1 = 0.280 mW and P_2 = 0.305 mW, with exposure times of 60, 180 and 300 seconds. The radiating area of the laser corresponds to the surface of the test sample in the container, which is 0.38 cm^2. The radiant energy was calculated to refer intensity that absorbed chlorophyll, using equation (1).

$$A = \log\left(\frac{1}{\mathrm{T}}\right) \tag{1}$$

$$\%\mathrm{A} = (1 - \mathrm{T}) \times 100\% \tag{2}$$

$$\mathrm{I_{absorp}} = \%\mathrm{A} \times \mathrm{I_{lasers}} \tag{3}$$

$$\text{Radiant Energy} = \mathrm{I_{absorp}} \times \mathrm{t} \tag{4}$$

C albicans and *S epidermidis* cultures taken from the collection of the Microbiology Laboratory of the Faculty of Medicine Muslim Indonesia University Makassar were grown in the form of biofilms using Nutrient Broth 8% glucose media. The container for growing the biofilm was adjusted according to the test method carried out. A microplate 96-well was used for biofilm testing using the XTT assay method, while an Eppendorf tube was used for biofilm testing using the TBARS assay method.

XTT assay Test

Cell viability test using XTT assay method, biofilm was stained with 40 L XTT 1 mg/mL, 2 L menadione 10 mg/mL, and 158 L sterile PBS then incubated in the dark for 2 hours at 37°C. Total of 100 L aliquots of XTT staining were transferred to a new microplate and analyzed using an ELISA reader on a 490 nm filter. The value shown from the ELISA reader measurement is the optical density value based on the brightness level of the XTT staining results. The fewer the number of surviving cells, the clearer the stain will be.

TBARS assay Test

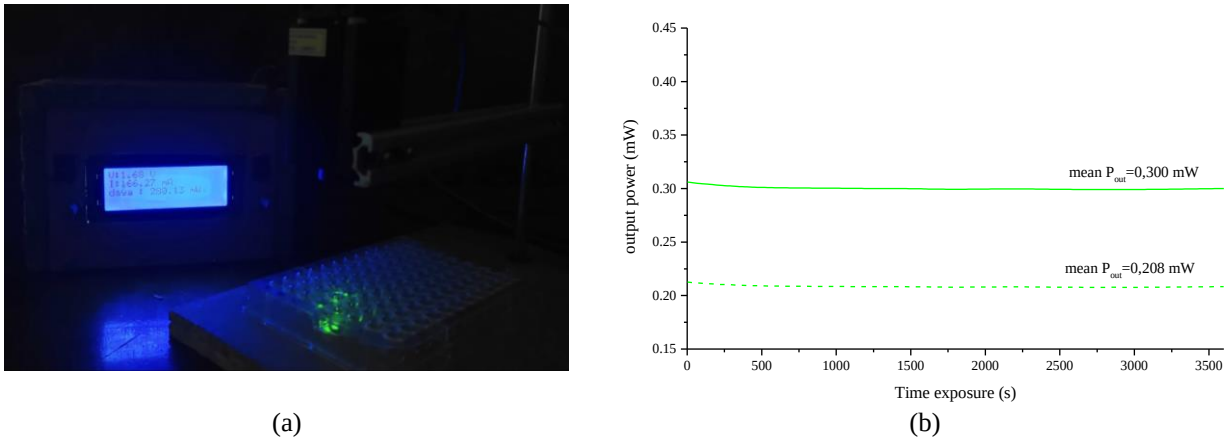
Testing the levels of MDA using the TBARS method [10], as much as 0.5 mL of PDI-treated biofilm filtrate which has been centrifuged at 40C, 10 minutes mixed with 0.5 mL of sterile PBS, 1 mL of 20% TCA, and 1 mL of 0.67% TBA and homogenized. The homogenate was heated in an 80C water bath for 15 minutes, cooled at room temperature for 60 minutes, and finally centrifuged for 15 minutes. The absorbance of the filtrate was read at a wavelength of 532 nm and the MDA content was converted after a standard curve was made using a standard MDA compound, namely TEP (1,1,3,3-tetraethoxypropane). The indicator solution is pink if it contains radical compounds. MDA levels are calculated based on the standard curve equation.

RESULTS AND DISCUSSION

Determine of radiant energy

The output stability of the laser light source is strongly influenced by fluctuations in voltage and current changes. A microcontroller device is needed to adjust the intensity of the laser light that reaches the sample surface, through the potentiation setting on the device. One of the stability tests carried out is to measure the change in laser output power for 1 hour of irradiation.

Figure 1 gives an idea that during the exposure within 3600 seconds. The stability test results show that the laser output power is relatively stable for one hour of exposure with the indicated potential values of 0.300 mW and 0.208 mW respectively. The curve of laser output power during expose shows the constant value, it is mean if the time exposes up to 300 s, their output power is still stable.



(a) (b)

FIGURE 1. Instrument and stability output curve of green laser output power during a hour exposure (a) instrument of microcontroller, (b). Stability curve of power output

Based on the output power of the green laser and spectrum absorbance of Chlorophyll papaya extract, the radiant energy can be calculating, which is fully presented in Table 1.

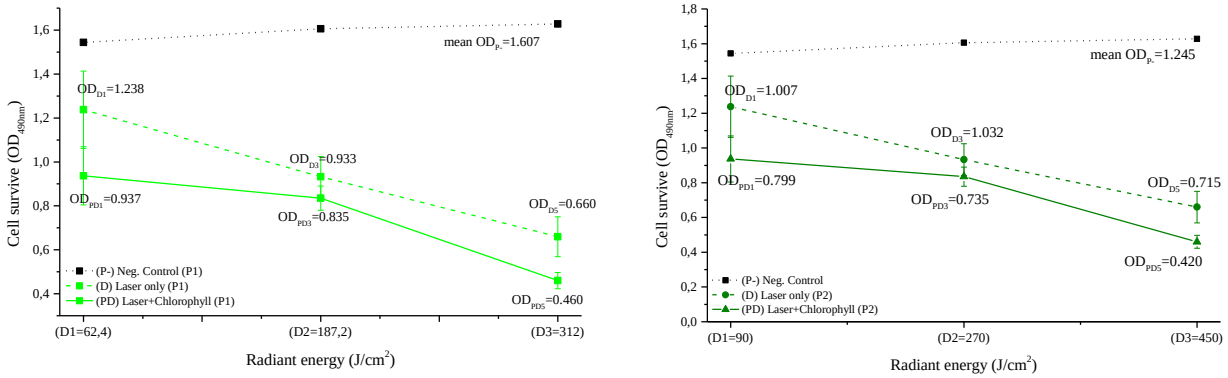
TABLE 1. The radiant energy of laser

No	Exposure area (cm^2)	Output power laser (mW)	Time exposure (s)	Radiant Energy (J/cm^2)
1.	0.38	0.208	60	62.4
2.			180	187.2
3.			300	312
4.		0.300	60	90
5.			180	270
6.			300	450

Table 1 contains the calculated values for the radiation energy dose from the three variations in the duration of exposure. An increase in output power of 100 mW provides an irradiation energy factor of 50% of the radiant energy. In writing the symbols D1, D2, and D3 on the graph the photoinactivation results correspond to the irradiation dose with an exposure duration of 1, 3, and 5 minutes namely 62.4, 187.2, and 312 J/cm^2 (for power output 0.208 mW) respectively. While the power output is 0.300 mW, the radiant energy value are 90, 270, and 450 J/cm^2, respectively.

Optical density analysis of XTT test after PDI

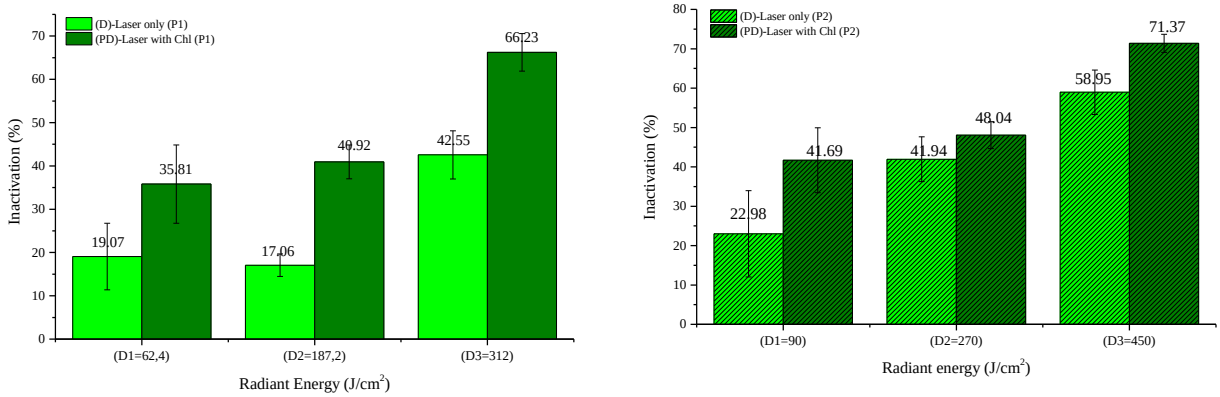
The result of PDI laser with P_1 power (at Figure 2a) shown the average value of *optical density* (OD) for the negative control group was OD_{P-}=1,607 decreased after being given laser treatment without or with chlorophyll extract, for radiant energy of 312 J/cm^2 is OD_{D5}= 0.660 (without chlorophyll) and OD_{D5}=0.460 (with chlorophyll). For the laser treatment with P_2 power (Figure 2b), the average value of OD for the negative control group of OD_{P-}=1,245 decreased after being given laser treatment without or with chlorophyll extract, for radiant energy of 450 J/cm^2 obtained OD_{D5}=0.715 (without chlorophyll) and OD_{D5}=0.420 (with chlorophyll).



(a) (b)

FIGURE 2. The profile of cell survive reduction after PDI to associated biofilm *C. albicans* and *S. epidermidis* use the green laser and chlorophyll papaya leaf extract (a). P_1 treatment and (b). P2 treatment Conclusion

In line with a previous study using ZnPc (0.25 mg/mL) activated by a GaAlAs laser (λ=660 nm, radiant energy 26.3 J/cm^2, and times exposure 285 s) on *C. albicans* biofilms, an inactivation effect of 30 % was obtained for the laser group and 55 % for the laser with ZnPc group. For the laser group with the addition of a photosensitizer [11]. Another study stated that *C. albicans* biofilms, which are eukaryotic cell types, are more difficult to inactivate than gram-positive and gram-negative microbes with an inactivation effect of 45 % for *C. albicans* biofilm cells compared to other microbes, 55 % for *S. aureus* biofilm cells, and 50 % for *S. mutans* biofilm cells [12]. Photoinactivation treatment of *C. albicans* with a diode laser (λ=660 nm, power 40 mW) with photosensitizer methylene blue 150 µg/mL, optimum effective at a dose of 180 J/cm^2 with inactivation of 78 % compared to a radiant energy of 60 J/cm^2 of 62 % and 120 J/cm^2 by 42% [13].



(a) (b)

FIGURE 3. The profile of inactivation after PDI to associated biofilm *C. albicans* and *S. epidermidis* use the green laser and chlorophyll papaya leaf extract (a). P_1 treatment and (b). P_2 treatment

According to Figure 3, the comparison is shown with some categories such as different output power (P_1 and P_2), radiant energy variation (D1, D2, and D3), and treatment with or without chlorophyll extract. Figure 3a, in laser only group, the inhibitory effect at the lower radiant energy (62.4 J/cm^2) was 19.07 %, has increasing to 42.55 % when the radiant energy also was increased (312 J/cm^2). As well as for the photosensitizer combination laser groups, the inhibitory effect starts from 35.81 % up to 66.23 %. When the output power was changed to 0.300 mW (figure 3b), the inhibitory effect occurs has been higher than the output power of 0.208 mW. For the laser only groups, the percent inactivation in the range (22.98 – 58.95) % had significant increases when the biofilms were added with chlorophyll up to 71.37 %.

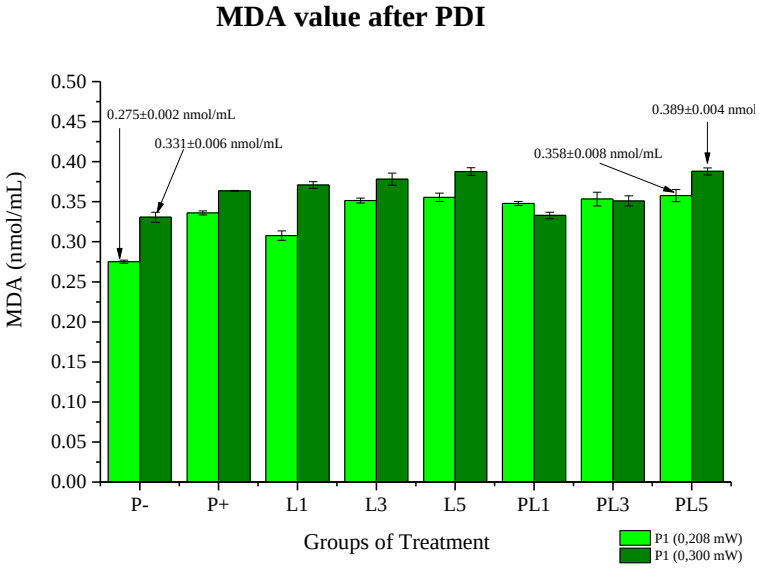


FIGURE 4. MDA level after PDI mechanism to associated biofilms *S.epidermidis – C. albicans*

In Figure 4, it can be seen that the MDA values for each main group have increased at a higher radiant energy, the main groups in question were the laser group only and the laser group with chlorophyll. The difference in MDA values for higher output power (0.208 mW) on average produces a greater value than lower output power (0.300 mW). The most significant difference in MDA values was seen in the laser treatment group with the addition of chlorophyll, where the MDA value when the radiant energy was 62.4 J/cm^2 and 312 J/cm^2 (P_1=0.208 mW) and 90 J/cm^2 and 450 J/cm^2 (P_2=0.300 mW), the values obtained are 0.275±0.002 nmol/mL and 0.331±0.006 nmol/mL, 0.358±0.008 nmol/mL and 0.389±0.004 nmol/mL, respectively.

CONCLUSION

The effectiveness of PDI mechanisms using a diode laser light source is highly dependent on the output power and the applied radiation dose. The increase of power output gave the inactivation effect was high respectively for P_1 and P_2 about 66.23% and 71.37% respectively.

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