Photodynamic Inactivation for Phatogenic Bacteria: Adding Chlorophyll and Oxygen

by Basitha Febrinda Hidayatulail

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Photodynamic Inactivation for Pathogenic Bacteria:

Adding Chlorophyll and Oxygen

Basitha Febrinda Hidayatulail
Magister Biomedical Engineering of Postgraduate School
Airlangga University
Surabaya, Indonesia
bfebrinda@gmail.com

Mohammad Yasin
Physics Department of Science and Technology Faculty
Airlangga University
Surabaya, Indonesia
yasin@fst.unair.ac.id

Suryani Dyah Astuti
Physics Department of Science and Technology Faculty
Airlangga University
Surabaya, Indonesia
Suryani-d-a@unair.ac.id

***Abstract*—Photodynamic inactivation has been developed in reducing colony bacteria such as Staphylococcus aureus. Chlorophyll is known as a green pigment which can act as a photosensitizer. This research aimed to reduce colony using blue laser diode, chlorophyll and oxygen. Chlorophyll was produced from leaf extract of angsana (*Pterocarpus indicus*) and oxygen source obtained by medical oxygen. The treatments were divided to four groups, i.e. laser group, laser and adding chlorophyll group, laser and adding oxygen and laser and adding chlorophyll and oxygen. This result showed that 17.96% decrease at laser and adding chlorophyll group with time exposure of 40s.**

Keywords*—PDI, Staphylococcus aureus, chlorophyll, oxygen and blue laser.

I. INTRODUCTION

Skin infection can be caused by pathogenic bacteria, such as Staphylococcus aureus [1]. This bacteria is a Gram (+) and lives in noses, respiratory tract and skin. Antimicrobial drug therapy is usually used to reduce this bacteria. However, bacteria will form drug resistance caused by improper use of drug therapy [2]. New application of medical technologies should be developed to reduce drug-resistance of bacteria, such as photodynamic inactivation (PDI)[3].

PDI therapy uses light and light-sensitive molecules that produce toxic molecules to reduce bacteria colony [4]. Light-sensitive molecules are termed as photosensitizer, the peak spectra of the photosensitizer must be in the range of the light sources. The photo-physical process ensues when the light energy is absorbed by the photosensitizer [5]. This process causes the photosensitizer in ground singlet state to excitation singlet state through vibration or internal conversion. However, if it had enough energy, it could cross to triplet state by the intersystem crossing (ISC) process. It can produce toxic molecules through a photochemical process called reactive oxygen species (ROS). The important factors for PDI are compatibility between absorbance spectra of the photosensitizer with the light source wavelength, the value of photosensitizer and ROS. This could happen if the photodynamic energy dose is fulfilled. With the addition of oxygen, it increase the production of ROS during the photochemical process [6].

Chlorophyll is a pigment from green plants which is activated by light energy [7]. One such green plant is angsana (*Pterocarpus indicus*). Chlorophyll from the leaf extract can be used as photosensitizer [8]. Astuti et al. showed that using chlorophyll and blue diode laser in PDI *Streptococcus mutans* was able to decrease 78% of colony [9].

This research is aimed to study photodynamic inactivation to Staphylococcus aureus bacteria using blue laser diode, chlorophyll photosensitizer and adding oxygen.

II. MATERIALS AND METHODS

A. Light sources

Light source was a blue diode laser equipped by a control system managed by a microcontroller for controlling output power and exposure time. Wavelength and steady time measurement were shown in the result.

B. Chlorophyll

Photosensitizer used in this research is chlorophyll obtained by leaf extract of angsana (*Pterocarpus indicus*). This leaf was filtrated and washed by 99% acetone. Adding dioxane ($C_4H_8O_2$) and pure water formed precipitate of solution. This was strained to obtain the precipitate and washed by 99% acetone. This precipitate was diluted using diethyl ether ($C_4H_{10}O$) and added silica gel.

C. Oxygen source

Oxygen source was obtained by medical oxygen. This output was controlled by solenoid valve.

D. Bacterial Strain

Sample was Staphylococcus aureus strain (ATCC 28923) obtained in the Veterinary Faculty, Airlangga University. This bacteria was cultivated in TSB medium until it obtained optical density in 600nm 0.5 or equal ~ 10^6 CFU/mL.

E. Treatment

Treatment of sample was done in a completely dark room. Group samples were the laser group, laser and adding chlorophyll group, laser and adding oxygen group and laser and adding chlorophyll and oxygen group. Output power of laser was 16.97mW. Time exposure was 20s, 40s, 60s and 80s.

F. Statical Analysis

Using repeated samples, % decrease log CFU/mL of bacteria is:

$$\%decrease = \left| \frac{\sum apoptosis(\log CFU/\ mL)}{\sum \text{no Treatment } (\log CFU/mL)} \right| \times 100\% \quad (1)$$

Data were presented by mean of % decrease log CFU/mL ± standard error for every treatment. Each group would be analyzed by Kolgomorov-Smirnov test and ANOVA one way test with significance of p=0.05.

III. Results and Discussions

The first step measured a characterization of laser obtained output and the laser was measured by powermeter Thermolab PM100 (Germany) and monocromathor Jasco CT-100 (Japan). Steady time laser is shown in Figure 1 (a). Wavelength of laser is shown in Figure 1(b). Steady time of output power is time needed to obtain steady power of laser. From this measurement, steady time is about 800s. Wavelength of laser from measurement is 450nm, with FWHM 40nm.

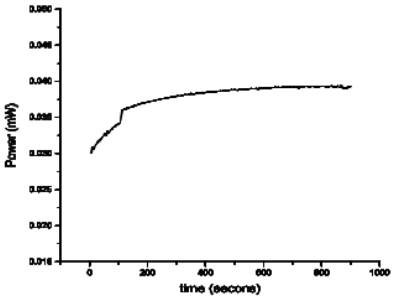

Figure 1. (a) Steady time of laser

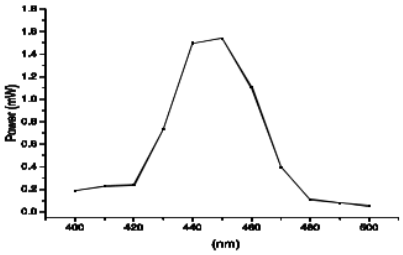

Figure 1. (b) Wavelength of laser measurement

Absorbance peak in chlorophyll is shown in Figure 2. The absorbance peak of chlorophyll was in 435nm and 471nm. Percentage of efficiency absorbance using absorbance spectroscopy equation showed 95.37%.

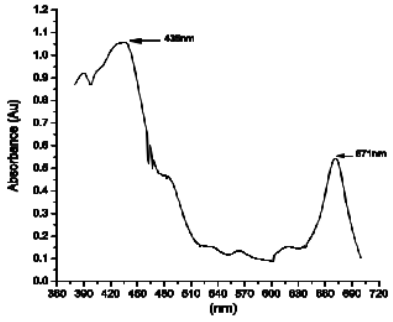

Figure 2. Absorbance of Chlorophyll

Statistical analysis was analyzed for each treatment. Laser group is show in Figure 5. They had normal distribution but there was no significant difference between them. Laser group in this research was able to decrease 8.27% log CFU/mL.

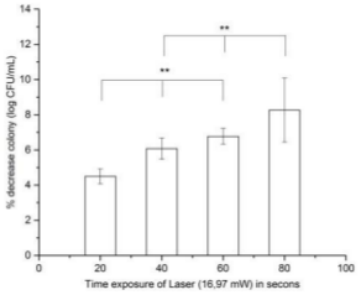

Figure 3. Result of laser group. Marker * and ** show significant level in the ANOVA one way p<0.05 and p>0.05, respectively

Laser and adding chlorophyll group had normal distribution and treatment had difference in time exposure 40s. Laser and adding chlorophyll group in this research was able to decrease 17.96% log CFU/mL.

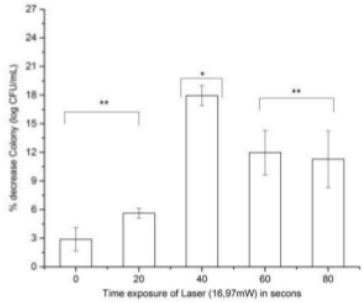

Figure 4. Result of laser and adding chlorophyll. Marker * and ** show significant level in the ANOVA one way p<0.05 and p>0.05, respectively

Laser and adding oxygen had normal distribution, but no significant difference. This treatment was able to decrease 11.89% log CFU/mL.

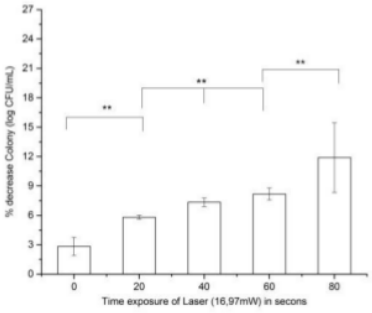

Figure 5. Result of laser and adding oxygen. Marker * and ** show significant level in the ANOVA one way test p<0.05 and p>0.05, respectively

Laser and adding chlorophyll and oxygen had normal distribution but there was no significant difference. This treatment

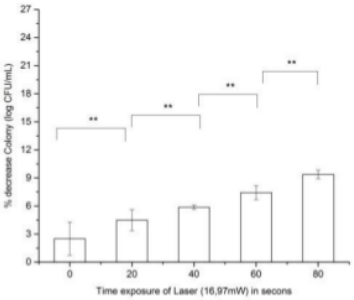

Figure 6. Result of laser and adding chlorophyll and oxygen. Marker * and ** show significant level in the ANOVA one way p<0.05 and p>0.05, respectively

Photodynamic inactivation was therapy using light and photosensitizer for producing reactive oxygen species (ROS). Peak absorbance spectra should appropriate with wavelength of light source. Absorbance of photosensitizer in bacteria was roughly in the blue region [10]. Light source was blue diode laser which had wavelength 450nm with FWHM 40nm. Laser group showed that irradiation laser was able to decrease colony bacteria.

The amount of photosensitizer was an important factor in the photodynamic process. One photosensitizer is chlorophyll which can be obtained from leaf extract [7]. Chlorophyll from *Pterocarpus indicus* was made as an

antimicrobial drug [11]. This showed that adding chlorophyll was able to decrease colony, although it was not different with no treatment colony. Photodynamic group with adding chlorophyll showed 17.96% log CFU/mL decrease of colony in exposure time 40s. It showed that adding photosensitizer in which absorbance spectra is similar to wavelength of light source would decrease colony.

Laser and adding oxygen group was still not enough to decrease more colony; however, one of the success factors in PDI is production of ROS [6]. Photosensitizer can transfer energy from light to oxygen during the photochemical process. Transfer energy made oxygen form singlet oxygen as well as toxic molecules. This group showed decreased colony, but less than laser and adding chlorophyll group. This is the same as laser and adding chlorophyll and oxygen. There is still no clear explanation.

IV. Conclusions

Photodynamic inactivation using chlorophyll and adding oxygen has been described. Adding oxygen was still not enough to decrease colony, so it shows that the amount of oxygen may be not be a factor in photodynamic therapy. It needs further information about the success factor in the photodynamic. Chlorophyll from leaf extract of Pterocarpus indicus was able to decrease colony, so it will be considered as a photosensitizer.

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