Photodynamic Inactivation of *Streptococcus mutan* Bacteria
with Photosensitizer *Moringa oleifera* Activated by Light Emitting Diode (LED)

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Abstract. A Photodynamic Inactivation (PDI) research has been done as a method of photodynamic in a medical treatment that combine light with photosensitizer. The light energy absorption by the photosensitizer molecules will produce reactive oxygen species that cause biological damage. Photoinactivation has done by combining blue LED light and exogenous porphyrin photosensitizer *Moringa oleifera* leaf extract to a major cause of dental caries disease, *Streptococcus mutan*. The goal of this research is to analyze a maximum potential of LED exposure angle to inactivate the bacteria added by 20% concentration of *Moringa oleifera* leaf extract photosensitizer. The wavelength of blue LED light used is 450.00 ± 0.21 nm in 100% Pulse Width Modulation (PWM). Total Plate Count (TPC) is used as a method to determine the loss of bacteria viability in unit CFU/ml. The statistical test result show an angle that potentially inactivate the bacteria by the exposure of two groups treatment with six variation of angle and an exposure time is 90° angle which has different meaning with another variation (p < a = 0.05), that means the best angle to use is perpendicular which has a percentage result of absorbent media for 180 second duration is (30,301 ± 4,231)% of bacterial death without photosensitizer and increased to (46,742 ± 1,667)% bacterial death with a photosensitizer.

1. Introduction

Dental caries is a disease of the teeth and mouth that damages the structure of the tooth so that it causes cavities due to excessive accumulation of normal bacteria *Streptococcus mutan* that interact with saliva and food scraps in the mouth [1] According to Riskesdas research in 2007 and 2013, the number of patients with active dental caries increased from 23.2% to 25.9%. Treatment of dental caries with antibiotics has been widely carried out. However, some research reports that the administration of certain antibiotics causes a case of *Streptococcus mutan* resistance, so another method is needed, namely Photodynamic Inactivation (PDI) [2]. Photodynamic Inactivation is one of the in vitro approaches to inactivation of microorganisms [3]. The combination of certain photosensitizers and light on PDI will cause photoinactivation in bacteria [4].

The photoinactivation mechanism involves the photosensitization process which depends on the suitability of the light spectrum and the photosensitivity absorption spectrum [5]. Photosensitisers absorb photon light so that the electron configuration is unstable (excited), in this state the molecule is able to interact with oxygen which causes the oxygen molecule to become unstable. Excited oxygen
will interact with the surrounding biological system to get a stable state. The interaction between excited oxygen and biological systems such as cells will damage the system [6]. The photophysical process of absorbing light (photons) by molecules described above is a quantum phenomenon. Only light with certain wavelengths and discrete that can be used for PDI [7].

One of the light sources used for photoinactivation and in the photosensitiser absorption spectrum is Light Emitting Diodes (LEDs). The color of the light produced by an LED depends on the material and the semiconductor conditions that are inserted [8]. LED is widely used in PDI because of several advantages, namely easier in assembly [9] and does not produce photothermal effects due to heat emitted in small amounts [10]. In various studies, the use of LEDs in both endogenous and exogenous porphyrins showed success in the potential for inactivation. The unique ability of porphyrins to absorb specific light with certain wavelengths is used for photoinactivation of microorganisms.

The study was developed using porphyrins derived from *Moringa oleifera* chlorophyll with chlorophyll content of 6,890 μg / g [11]. *Moringa oleifera* leaves were selected based on the Photosensitizers Photosensitisers are selective, efficient, chemically stable, have a broad spectrum of absorption wavelengths, are easily soluble, non-toxic and toxic.

Research conducted by Astuti (2019) [12] states that there is lysis in the bacterium *Staphylococcus aureus* due to irradiation using a 430 nm blue LED and an energy dose of 75 for 30 minutes with an energy density of 135 J / cm2. Other research using a 408.6 nm purple laser 61.2 J / cm2 energy has the potential to photoinactivate the *Streptococcus mutan* bacteria by 42.11% [13]. Research conducted by Setiawatie (2016) [9] uses a 453 nm LED with a variation of quantum yield (4, 09; 7,73; 12,28; 16,38; 20,48) J / cm2 can inactivate 77.2% *Alfalfa Medicago sativa L* chlorophyll for ROS production against the inactivation of A.acticomycetacmonitans.

Based on research that has been successfully conducted previously shows that the success of photoinactivation is determined by the suitability of wave lengths with porphyrin absorption spectrum and irradiation angle used, therefore this study focuses on the effect of angles and exposure of LEDs on the activation of photosensitisers from *Moringa oleifera* leaf extract.

2. Material and Methods

2.1. *Moringa oleifera* Leaf Sample Extraction

A total of 15 grams of *Moringa oleifera* leaves were extracted and then the yellow pigment content was separated from chlorophyll. The chlorophyll deposition obtained will then be tested for UV–VIS chlorophyll spectrum, an antibacterial test, and a toxicity test.

2.2. LED Performance Test

This test is carried out to determine the accuracy of the LED before use. The wavelength of the LED used is 450.00 ± 0.21 nm. The irradiation temperature which is considered stable is 29°C to avoid the photothermal effect when the test is carried out, the irradiation distance to be used is 2.5 cm with a given power of (0.226 ± 0.001) mW.

2.3. Establishment of Mc.Farland Standards and Bacteria Dilution Standards

Mc. Farland standard was carried out after the bacterial culture of *Streptococcus mutan* to determine the number of bacterial colonies through the cup count method, so that the number of bacteria in CFU / ml was obtained. The number of bacteria that grows is calculated using TPC (Total Plate Count), the number of colonies of 30-300 becomes the standard reference dilution factor which will then be irradiated

2.4. Exposure of LEDs to Streptococcus mutans with the addition of Moringa Leaf Extract Photosensitizer

Before irradiation, the chlorophyll *moringa oleifera* leaves are diluted with distilled water as much as 1 ml and put into 4 ml bacterial culture, this is done because *Moringa oleifera* leaf extract can absorb light optimally, the bacteria and photosensitizer are crushed for 2 hours before irradiated. Irradiation is
carried out at a maximum 100% PWM (intensity of 100 mW / cm²) at a distance of 2.5 cm accompanied by a control group without exposure. Irradiation is carried out with 6 time variations (0.5 minutes; 1 minute; 1.5 minutes; 2 minutes; 2.5 minutes; 3 minutes) and 6 angular variations (15°, 30°, 45°, 60°, 75°, 90°) so that there are 36 treatment groups. After irradiation with various treatments, then performed plating and incubation 24 hours at 37 °C, to further count the number of bacteria after irradiation.

3. Result and Discussion

3.1. Moringa oleifera Leaf Extraction Results

*Moringa oleifera* leaves that have been extracted are then carried out UV-Vis chlorophyll spectrum test which aims to determine the ability of chlorophyll absorption of 20% concentration to light, from the test results obtained information on the value of absorbance to the wavelength of visible light, UV-Vis absorption spectrum measurements carried out in the wavelength range 200-800 nm. The absorbance plot of the wavelength (Figure 1) shows the 2 highest absorption spectrum peaks of 2.337 ± 0.006 at a wavelength of 415.0 ± 0.3 nm and 1.106 ± 0.004 at a wavelength of 670.0 ± 0.3 nm which results are close to chlorophyll wavelength literature a. So it was concluded that the isolated chlorophyll contained chlorophyll a. According to the research of Setiawatie [9] chlorophyll a can absorb light until the electrons are excited and support the photoinactivation process. Based on the results of antibacterial potential tests (Table 1), no chlorophyll inhibition zone was found for bacteria at all concentrations. Toxicity tests conducted to obtain information on chlorophyll concentration of 20% also showed no significant difference between the two treatment groups as presented in table 2.

![Graph of absorbance against wavelength of Moringa oleifera chlorophyll](image)

**Figure 1.** Graph of absorbance against wavelength of *Moringa oleifera* chlorophyll

| Table 1. Test results for antibacterial potential with variations in chlorophyll concentration |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Concentration Percentage | Chlorophyll + DMSO (ml) | Aquades (ml) | inhibition zone diameter (cm) |
| 20% | 0.10 | 0.40 | 0 |
| 25% | 0.12 | 0.37 | 0 |
| 30% | 0.15 | 0.35 | 0 |
| 35% | 0.17 | 0.32 | 0 |
| 40% | 0.20 | 0.30 | 0 |
| 45% | 0.22 | 0.27 | 0 |
| 50% | 0.25 | 0.25 | 0 |
3.2 Results of Streptococcus mutans Photoinactivation with LED exposure

Photoinactivation test on *Streptococcus mutans* bacteria was divided into 2 treatments namely using Moringa leaf photosensitizer and without using *Moringa oleifera* leaf photosensitizer, with various treatments that have been determined. The success of this study can be seen from the presentation of bacterial death to control. A graph of decreased bacterial viability is shown in Figure 2 and Figure 3.

### Tabel 2. Test result of photosensitizer toxicity

| Concentration Percentage | Mean of colony (CFU/ml) | Log CFU/ml | Error (±) |
|--------------------------|-------------------------|------------|-----------|
| 0%                       | 186,50                  | 373000     | 6,57      | 0,57      |
| 20%                      | 184,50                  | 369000     | 6,56      | 0,32      |

**Figure 2.** Decreased *Streptococcus mutans* bacterial viability of the LED treatment group

**Figure 3.** Percentage Increase in *Streptococcus mutans* bacterial mortality in the LED treatment group with photosensitizer

3.3 Results of photoinactivation of Streptococcus mutans with LED exposure and photosensitizer

Measurements made for the LED irradiation group on bacteria provide information that the angle and time are independent variables. The test results showed an increased percentage of *Streptococcus mutans* bacterial death at various angles and irradiation times with the addition of photosensitizer [14]. Comparison of the results of exposure tests on bacteria at a maximum angle of 90° and 3 minutes is shown in table 3.
Table 3. Comparison of the percentage of *Streptococcus mutans* bacterial deaths in the LED test group and the LED test group with the addition of photosensitisers

| Interaction                        | Percentage Increase in bacterial mortality (%) |
|------------------------------------|-----------------------------------------------|
|                                    | Treatment with LED | Treatment with LED + Photosensitiser |
|                                    | Mean     | SD | Mean     | SD |
| Irradiation with an angle of 90°   | 30.301%  | 4.231% | 46.742%  | 1.667% |
| in 180 seconds                     |          |    |          |    |

The results of bacterial inactivation research from 2 test groups showed that the addition of Moringa leaf photosensitizer in this study gave maximum results in bacterial inactivation than the test group without the use of photosensitizer.

The chlorophyll photosensitizer in this study plays a role as an LED light absorber used to illuminate bacteria so that it is expected that the use of chlorophyll photosensitizer LED light absorbed more optimally for bacterial inactivation. The suitability of the photosensitizer wavelength spectrum with the wavelength of the LED light becomes a concern for a photodynamic process. UV-VIS test results showed the peak of chlorophyll absorbance in the wavelength spectrum of blue light is still included in the range of FWHM LED wavelength spectrum so as to enable the photodynamic process.

Photodynamic success depends on the photophysical and photochemical interactions of the photosensitizer [15]. Photophysics as an initial stage of involvement between light and photosensitizer interactions with surrounding oxygen, causing radical Reactive Oxygen Species (ROS) to damage bacterial cells.

When irradiation occurs absorption by photosensitizer molecules which initially has a configuration at the basic level. When direct collisions of high-energy photos of light with molecules, photon energy will be absorbed to transition to higher energy levels [7]. This energy absorption process occurs with the principle of one molecule absorbing one photon [12]. It can be assumed that the amount of decreased viability of Streptococcus mutans bacteria shows the amount of reactive oxygen produced.

4. Conclusion
Chlorophyll Moringa leaf extract can be used as a photosensitizer has been tested is not antibacterial and non-toxic. LED light with a wavelength of 450.00 ± 0.21 is able to activate the photosensitizer molecule, this can be seen from the increase in the percentage of the number of bacterial deaths more than without using photosensitizer. The best angle obtained for bacterial inactivation is 90° perpendicular to the surface of the absorbent medium with an irradiation time of 3 minutes. The percentage of bacterial death was (30.301 ± 4.231)% in the LED treatment, and (46.742 ± 1.667)% in the LED treatment with photosensitizer incubation for 2 hours.

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