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# Antimicrobial photodynamic of blue LED for activation of curcumin extract (*curcuma longa*) on *staphylococcus aureus* bacteria, an in vitro study

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**Abstract**. *Staphylococcus aureus (S.aureus)* is a normal skin flora. In abnormal conditions, these bacteria can cause mild skin infections such as acne vulgaris and cellulitis folliculitis that attack the respiratory system causing severe infections such as pneumonia and empyema occur. Systemic treatment with antibiotics for a long time can cause resistance. Antimicrobial photodynamic therapy (aPDT) is a therapy that uses light sources, photosensitizer (PS) agents and oxygen. This study aims to determine the effectiveness of blue LED (400-450) nm to activate exogenous PS of curcumin extract in *S.aureus* in vitro. To determine the antibacterial effect of curcumin extract (*Curcuma longa*) on *S.aureus* bacteria with LED radiation, the distribution of samples was divided into four treatment groups, Group (S – K –) control group without any treatment, group (S– K +) control group with curcumin extract, group (S + K–) group with LED irradiation without curcumin extract, group (S + K +) laser irradiation group with curcumin extract. Bacterial growth is measured using Elisa Reader and Total Plate Count Method. The results showed that the LED energy density 16.19 J / cm2 was able to increase the percentage of bacterial mortality by (91.49 ± 0.01) % with curcumin extract and (44.88 ± 0.18) % without curcumin extract. So LED irradiation is able to activate curcumin extract to increase the percentage of *S.aureus* bacterial death.

## 1. Introduction

*Staphylococcus aureus* (*S.aureus*) is one of a pathogenesis bacteria causing several infections on human ranging from minor to serious illness when the number of bacteria is beyond the limits. It is a common floral that they live on skin, nose, throat, and other organs in humans [1]. The growth of *S.aureus* is at the temperature 7 – 48℃ and optimum on 37℃ acting as aerobic bacteria [2]. This bacteria has a high antibiotic resistance and can survive the immune system [3] making *S.aureus* bacteria live longer in the body. The longer they live causing the more serious disease. The case of disease caused by *S. aureus* bacteria tend to increase every year in various countries [2], [4], [5], the level of disease caused by *S. aureus* bacteria also increase along with the difficulties in treatment.

An alternative therapy treatment, called antimicrobial photodynamic (aPDT), is carried to eliminate the number of bacterial colonies without harming the safe cells [6]. Photodynamic reaction involves aPDT therapy which needs the specific wavelength of light that can react the photochemical

processes [7]. aPDT has three main components; light source (L), photosensitizer (Ps), and reactive oxygen singlet (ROS), a photochemical reactions between L and Ps will produce cytotoxic substance called ROS [8]–[12]. aPDT is effective therapy because the irradiation of L can be focused on the target area with Ps around it, so only the target that will be damaged by the photochemical reactions and the healthy tissue will be safe [12]. Several studies have shown that aPDT therapy is an effective and beneficial treatment to reduce the colonies of bacteria [13]–[15]. In the treatment on skin, aPDT has been reported to reduce several colonies of bacteria, such as *S.epidermis*, *P.acnes*, and *S.aureus* [16].

One of the main components in aPDT is a chemical agent called photosensitizer. An ideal photosensitizer used on skin therapy has to be safe and effective to reduce the number colonies of bacteria without damaging the normal cells and should not generate the chemical reaction after irradiated by L [17]. An internal enzyme on *S.aureus*, porphyrin is an endogenous photosensitizer [18]. Adding an exogenous photosensitizer can increase the reduction number colonies of bacterial. An active compound from extraction of Curcuma longa is curcumin that have several advantages such as anti – carcinogenic, antimicrobial, anticancer, and anti-inflammatory agent [19]. Curcumin extract (K) is a substance from extraction of *Curcuma longa* that has been reported to be an effective exogenous photosensitizer [20], [21]. Having the peak of absorption around 300 – 500 nm, indicates suitable to use violet or blue light as safe L beam [22].

The blue high power LED (S) have several advantages i.e. a low cost instrument and spreading at low heat, so the damage on dermis can be minimized [23]. S is an instrument of light source that has a broadband wavelength and the energy from S beam is not as high as laser [24]. For a skin therapy, the used of S is very suitable. The key of success in APDT is the corresponding to wavelength of L and maximum wavelength absorbance of Ps that can lead to photo – physical processes before any photochemical reaction can be held [9].

According to the urgency of the resistance of bacteria and advantages of aPDT therapy, this research will analyze aPDT therapy using L in the form of S with different of energy densities given by variation of the exposure times referring to previous studies i.e. 30s, 60s, 90s, 120s, 150s and 180s [7], and combining with K at concentration 0,15%. The purpose of this study is to determine the effectiveness of S to activate exogenous Ps on each treatment by in–vitro design.

## 2. Materials and Methods

### *2.1. Bacterial Strain and Culture Methods*

This research used a sample strain of pure culture bacteria of Staphylococcus aureus ATCC 25923 obtained from the Surabaya Center for Health Laboratory. Bacteria cultured using Tryptone Soy Broth (TSB) media. The growth of bacteria was measured using Elisa Reader.

### *2.2 Extraction of Curcuma longa*

Curcumin extract (K) was obtained from extraction of dry *Curcuma longa* rhizomes by maceration method using ethanol ($C_2H_6O$) 96% as solvent for 1 gr:10 ml [25]. The filtration of maceration results were added by malt dextrin as much as 15% from the filtration of mass as filler before evaporated using rotary evaporator. K in the solid form can be obtained by dry it using oven at temperature 40℃. The solvent of K is obtained by dissolving the solid form of K on distilled water and was sealed inside dark room restoration place at room temperature before used.

### 2.3 *Light Source*

A convex lens was added so the beam of blue high power LED (S) can be more focused. The irradiations were carried out with wavelength ranging from 400 – 450 nm and the wavelength peak on (417.10±0.05) nm. Intensity of the S beam was (135.03 ± 0.01) mW $cm^{-2}$ for (0.0314±0.0063) $cm^2$ of beam area. The energy density value was calculated by multiplying light source intensity by irradiation time.

*2.4 Sample Treatments*

The concentration 0.15% of K was an ideal photosensitizer tested by using antibacterial test on disk diffusion test. There were about 56 samples of *S.aureus* bacteria and each samples consist of 50 µl planktonic suspensions at 6.06 x $10^9$ CFUml$^{-1}$. Each samples was plated on petri dishes and divided into 4 groups. The first group S − K− is a control variable with no treatment applied, the second group S− K+ (adding the concentration 0.15% of K), the third group S + K− (S indicates this group applying different energy densities i.e. 4.05, 8.09, 12.14, 16.19, 20.24, and 24.29 Jcm$^{-2}$) and the last group S + K+ (adding concentration 0.15% of K and combined with difference energy density applied). Each group has 4 replications. The samples on group S − K+ and S + K+ may have different value of CFU ml$^{-1}$, but the value proved by statistical analysis was not significantly different. Each samples was grown on petri dishes for 24 hours on *Tryptone Soy Agar* (TSA) media at 37℃ through inside the incubator before the next visual counting of bacterial colonies. *Total Plate Counting* (TPC) method was used to collect the data followed by transformed into log CFU ml$^{-1}$.

*2.5 Statistical Analysis*

The reduction percentage of bacterial colonies was counted by subtracting the number of colonies before treatment and after treatment. The result analyzed by one way ANOVA and Tukey's test at $p < 0.05$. Furthermore, the significant changing between treatment sample and control variable can be determined by exceeding of 95%.

## 3. Result and Discussion

*3.1 Absorbance of Curcumin Extract*

A solution of curcumin extract (K) was prepared and dissolved in distilled water. From antibacterial and disk diffusion tests, showed that K with concentration 0.15% has no effect of bacterial killing, so this concentration can be used as an ideal photosensitizer. The absorbance of K is presented using Spectrophotometer Genesys 30 as shown in figure 1.

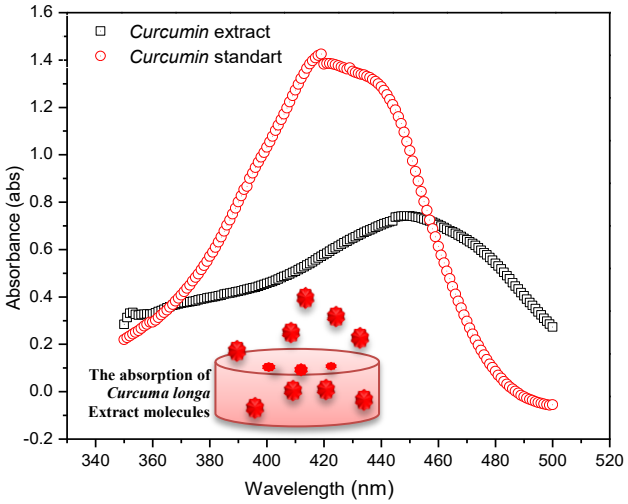


Figure 1. Curcumin extract absorbance test result along with curcumin standard as control variable. The inserted image is graphically molecules absorption process.

The curcumin extract is obtained from extraction of dried *Curcuma longa* rhizomes that has the same peak of wavelength absorbance 423 nm. The difference values of absorbance between standard curcumin and curcumin extract are caused by different substance in each solutions. Curcumin extract that used has substances more than curcumin. The peak value of curcumin extract absorbance is 0.742 abs on 423 nm. The L used in this experiment has wavelength peak on 417.10 nm giving the curcumin

extract absorbance 0.713 abs. The maximum absorbance peak both curcumin extract and standard curcumin showed at different range wavelength. The curcumin extract as red curve has higher absorbance value than standard curcumin, we assumed it's due to the molecules size of curcumin extract smaller than standard curcumin which significantly affected to absorption process. Using the law of Lambert – Beer the transmittance (*T*) then absorption percentage can be determined about 80.64 %. This value convinced us that curcumin extract is ideal candidate compare with standard curcumin.

*3.2 Antimicrobial Photodynamic using Blue High Power LED*

In this work we used blue high power LED with beam diameter around (0.0314±0.0063)cm$^2$, as the smaller beam diameter the higher energy density produced. The convex lens will adjust the beam light on focus position and the emitted light must not give extremely heat to the bacteria. The applied of beam temperature characterization is important matter related to the emitted beam quantity must be still in acceptable state and must not affected to the number of bacteria. Multi-meter Digital Constant 89 was used to measure the beam temperature for 100 seconds long. The average beam temperature was set around 27,200 ( ± 0,031) ℃. This temperature is acceptable for antimicrobial photodynamic process because this irradiating temperature suitable within the range of *S.aureus* growth temperature. In this work we used power meter OMM-6810B-220V to obtain the wavelength peak related with blue high power LED energy. As shown in figure 2 the wavelength peak occurred around (430.10±0.05) nm with energy intensity (3.44±0.01) mW respectively, while the S beam energy density was (135,03 ± 0,01) mW cm–2. The photo-chemical reaction occurred when the irradiation time more than one second with light source energy density range around mW [12]. The inserted image in figure 2 was the stable point for the blue high power LED by the time rising the LED energy remains constant and stable.

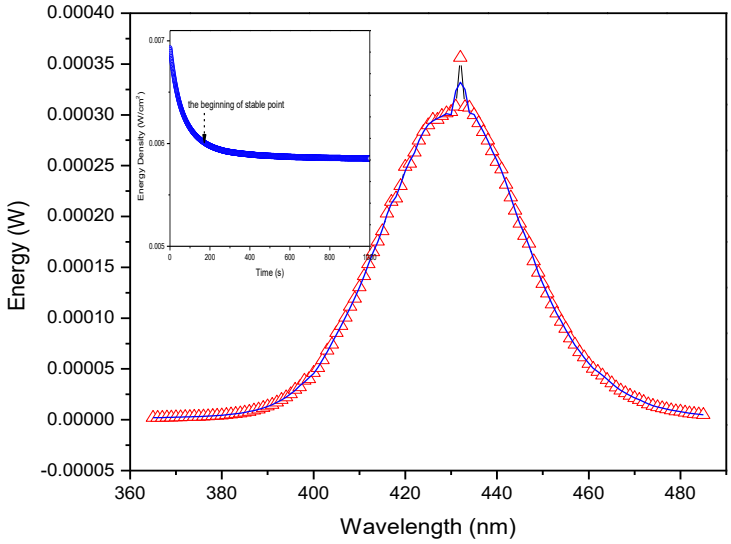


Figure 2. Blue high LED characterization. The inserted image is stability curve of LED source

The energy density of S beam with some different irradiation time were presented on table 1. The longest irradiation time (180 second) the highest energy density produced (24.29 Jcm$^2$). The energy density risen up significantly correspond with the increasing of irradiation time. It convince us that photo-chemical reaction process successfully occurred during inactivation of photodynamic therapy.

Table 1. The blue high LED output and its specification

| Wavelength peak (nm) | Intensity of blue high power LED (mW) | Diameter of blue high power LED ($cm^2$) | Irradiation time (s) | Energy density ($J\ cm^{-2}$) |
|---|---|---|---|---|
| 417.10 ± 0.05 | 135.03 ± 0.01 | 0.0314±0.0063 | 30.000 ± 0.005 | 4.05 |
| | | | 60.000 ± 0.005 | 8.09 |
| | | | 90.000 ± 0.005 | 12.14 |
| | | | 120.000 ± 0.005 | 16.19 |
| | | | 150.000 ± 0.005 | 20.24 |
| | | | 180.000 ± 0.005 | 24.29 |

The results shown 100% treatments sample group succeed to decrease the number of bacteria colonies compared to control variable group. The group sample S− K+ shown no significantly differences with control group due to the p value was obtained around p=0.072 by using independent statistical analysis test. The planktonic suspension of *S.aureus* was 4.70 x $10^9$ $CFUml^{-1}$.

Table 2. The analysis result of aPDT by using blue high power LED with curcumin extract

| Group | Energy Density ($J\ cm^{-2}$) | N | Percentage of bacterial colony reduction (%) | | One Way Anova Test | |
|---|---|---|---|---|---|---|
| | | | Average Value | DS of Measurement | Significance | Conclusion |
| S+ K− | $4.05^{(a)}$ | 4 | 16,50 | 9,72 | p = 0,005 | There are significant difference |
| | $8.09^{(a)(b)(c)}$ | 4 | 33,66 | 6,31 | | |
| | $12.14^{(a)(b)(c)}$ | 4 | 36,30 | 15,30 | | |
| | $16.19^{(c)}$ | 4 | 44,88 | 8,31 | | |
| | $20.24^{(a)(b)}$ | 4 | 20,79 | 10,67 | | |
| | $24.29^{(b)(c)}$ | 4 | 41,25 | 8,17 | | |
| | Total | 24 | 32,23 | 13,84 | | |
| S+ K+ | $4.05^{(a)}$ | 4 | 52,34 | 2,41 | p = 0,000 | There are significant difference |
| | $8.09^{(b)}$ | 4 | 60,74 | 2,96 | | |
| | $12.14^{(b)(c)}$ | 4 | 65,32 | 0,81 | | |
| | $16.19^{(e)}$ | 4 | 91,49 | 1,39 | | |
| | $20.24^{(c)}$ | 4 | 66,81 | 4,05 | | |
| | $24.29^{(d)}$ | 4 | 74,89 | 3,15 | | |
| | Total | 24 | 68,60 | 12,76 | | |

The bacterial colony reduction number for both sample group S+ K− and S+ K+ were summarized on Table 2. The clear significantly reduction of *S.aureus* occurred on both group with energy density around 16.19 J $cm^{-2}$, promoting reductions of 44.88% and 91.49% for sample group S+ K− and S+ K+ respectively. From these results, the increasing irradiation energy density will not always followed by increasing the bacterial photo-killing. When the energy density around 20.24 and 24.29 J $cm^{-2}$, this state indicating that the ideal energy density on antimicrobial photodynamic does not always the higher one.

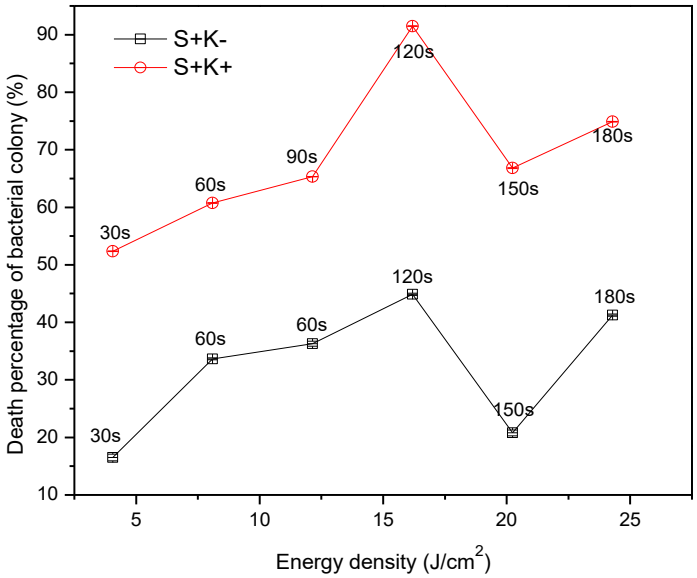


Figure 3. The death percentage of bacterial colony versus energy density of blue high LED

The effectiveness of curcumin extract on antimicrobial photodynamic therapy by using blue high LED was proved by 91.49% reduction of bacterial colonies. In other word the curcumin extract succeed as antimicrobial agent by increasing bacterial percentage of death as shown in figure 3. The death of bacterial colonies caused by the reaction of photo–physical, photochemical, and photobiology reactions in molecular cells [26]. Photo–physical process talks about the excitation of Ps molecules after absorbing the energy from L, the triplet state of Ps molecules can be reached by doing intersystem crossing [27]. This triplet state of Ps molecules that interact with water ($H_2O$) or oxygen ($O_2$) will produce radical particles, especially ROS [9]. The damage cytoplasmic membranes caused by ROS through peroxidation of lipid or protein will obstruct metabolism activity by cell lysis or inactivation of membrane transport system [28]. The blue high power LED has wavelength range from 400nm to 450 nm. This broadband light source irradiated to samples which consist porphyrin and some group samples also consist curcumin extract as endogenous and exogenous photosensitizer. Each of these photosensitizer has their own absorbance value and will generate photo–physical process when they have enough energy. By using this broadband light source, the more photon energy will be absorbed by both endogenous and exogenous photosensitizer as indicate the successfully of PDT process.

## 4. Conclusion

We successfully conduct the photodynamic therapy by using curcumin extract as effective exogenous photosensitizer along with blue high LED source as photo-inactivation. Sample group S− K+ presented non significantly differences of CFU $ml^{-1}$ compare to sample group S − K−, so that the K is an ideal photosensitizer. Both group S + K− and S + K+ have optimum irradiation time on 120 s, but

group S + K+ presented the decreasing of CFU ml–1 up to 91.49% while group S + K− can only decrease the CFU $ml^{-1}$ 44.88%. Irradiation time contributed significant affect to percentage of death of bacterial colony with optimum irradiation time around 120 second while the optimum energy density on PDT did not always the higher one.

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