Ultraviolet (UV) Activation Effect on Antibacterial Agents of Red Betel (*Piper Crocatum*) Extract to *Streptococcus mutans*

Ultraviolet (UV) Activation Effect on Antibacterial Agents of Red Betel (*Piper Crocatum*) Extract to *Streptococcus mutans*

Suryani Dyah Astuti[1]*, Rio Dysan Tirtana[1], Amalia Fitriana Mahmud[1], Amiliyatul Mawaddah[1], Abdurachman[2], Moh. Yasin[1]
[1]Department of Physics, Faculty of Sciences and Technology, Airlangga University, Surabaya, Indonesia, 60115
[2]Department of Anatomy, Faculty of Medicine, Airlangga University, Surabaya Indonesia 60115

E-mail: suryanidyah@fst.unair.ac.id

Abstract. Ultraviolet (UV) radiation has caused negative effect on biological environment. As non–moving living things, plants have their own response to reduce the negative effect by increasing flavonoid accumulation, such as red betel (*Piper crocatum*), which help the plant's protection from bacteria. In Indonesia, red betel has been used as a herb treatment, especially in oral treatment. This study aims to optimise the antibacterial effect of red betel extract on oral bacteria, *Streptococcus mutans,* by irradiating UV. *Kirby-Bauer* method of disk diffusion test was used on this study by showing the bacteria's diameter of inhibition zone. The samples distribution was devided into two groups, group (L–R+), control group, only used red betel extract without UV irradiation and group (L+R+) treatment combination of UV irradiation and red betel extract. The results showed that group L+R+ had better antibacterial effect on *Streptococcus mutans,* proven by the bigger inhibition zone, than group L–R+. UV irradiation for 12 hours showed the best result on each treatment with diameter of inhibition zone (15.00 ± 0.05) mm for 10 watt and (15.96 ± 0.05) mm for 15 watt. So, it is proven that UV irradiation can increase the antibacterial effect of red betel extract.

1. Introduction

Sunlight is the biggest source of energy and used by living things on earth, for example in this modern era, sunlight has its role to contribute to power plant as solar cell [1]. Not only always helpful, sometimes sunlight can stimulate negative biological effect caused by ultraviolet (UV) radiation [2]. Sunlight produce UV radiation for wavelength 100–400 nm. Recent human activity has damaged ozone layer and caused global warming. Being damaged, ozone layer could not protect the earth perfectly from UV radiation [3].

As non–moving living things, plants have their own response to reduce negative effect caused by UV radiation by increasing flavonoid accumulation, especially on their vacuole [4]. Flavonoid is a phenolic subtance produced by plants which has antioxidants, antidiabetic, anticancer, antiseptic and anti-inflammatory agents [5]. Flavonoid is able to denaturate bacterial's protein and causing lysis, so the bacteria can no longer live [6]. Red betel (*Piper crocatum*) is an example of plant that has flavonoid [5]–

[8]. Since the leaves of red betel contains flavonoid, used as a traditional treatment in Indonesia, especially in oral treatment [9]–[11].

Oral issues, such as caries and gingivitis, caused by the number of bacterial colonies forming dental plaque [10]. One of a pathogenesis bacteria causing several infections on periodontal is *Streptococcus mutans.* This gram-positive bacterium lived as anaerobic bacteria and has a significant contributor in tooth decay [12]. To prevent the negetive effect of this bacteria, Indonesian common folk has a traditional treatment to chew the betel leaves [9]. Another studies has also proven that the extract of red betel leaves has a good antibacterial agents preventing oral issues [11], [13], but in order to optimise the antibacterial effect, additional treatment is needed.

This study used irradiation of UV from 200–400 nm. UV–B irradiation (315–280 nm) to wheat plant has caused a decrease in total chlorophyll, carotenoid, flavonoid levels also their biomass and harvest [14], [15]. Wheat plant that has enough water and nutritions can overcome and protect itself from phisiological damage by decreasing photosynthesis pigment degradation and increasing accumulation of UV absorber which is flavoid [16]. The balance between damages caused by UV radiation and benefit for increasing flavonoid will be worthwhile as flavonoid production method [5], [8].

2. Materials And Method

2.1 Bacterial Strain

This study used a sample strain of pure *Streptococcus mutans* bacteria obtained from The Departement of Periodontitics, Faculty of Dentistry, Universitas Airlangga. The bacteria strain cultured in *Tryptone Soy Broth* (TSB) media, incubated for 24 hours at 37℃ on anaerobic environment [12].

2.2 Extraction of Red Betel (Piper crocatum) Leaves

The leaves extract of red betel (*Piper Crocatum*) obtained by cleaning the leaves first and then drying it using furnace for 40^0C. Dried leaves were pounded and mashed using blender. Methanol as maceration solvent of betel powder was used for 24 hours. Maceration result then strained using filter to separate filtrate of red betel and its solvent as red betel extract [17].

2.3 Light Source

As the source of light, UV irradiation on this study are T8 Blacklight/05 (UV-A) G13 15 watt, 2 watt of radiation, and wavelength of 360 nm and TL-10W/05 (UV-A) lamp Fluorescent Philips, 1,15 watt of radiation, and wavelength of 365 nm. The intensity are 9.16 W/m and 15.92 W/m. The power electricity used on this study are 10 watt and 15 watt.

2.4 Samples Treatments

The sample that used on this study was the culture of *Streptococcus mutans* grown in *Tryptone Soy Agar* (TSA), incubated for 24 hours at 37℃ on anaerobic environment [12]. After incubation, the sample was given the paper disk that has been soaked by 10 µl of red betel extract. The treatments of red betel extract on *Streptococcus mutans* was devided into two groups, group L–R+ (control; without UV irradiation) and group L+R+ (UV irradiation for 4, 8, and 12 hours irradiation combining to 10 watt and 15 watt power electricity). Each treatments was repeated four times for replications. After treatments, the diameter of inhibition zone then measured using calipers.

2.5 Antibacterial Test

Kirby-Bauer plat method was used on this study as antibacterial test [18]–[20]. This test aimed to determine productivity of antibacterial by measuring diameter of inhibition zone around paper disk. Calipers with 0.05 accuracy was used to measure the diameter. The percentage of inhibition zone then calculated using formula as follows.

$$Percentage\ of\ Antibacterial\ Effect = \left(\frac{Treatment\ Diameter - Control\ Diameter}{Treatment\ Diameter}\right) \times 100\%$$

2.6 Data Analysis

This study resulted on the diameter of inhibition zone of *Streptococcus mutans* and the relation between inhibition zone percentage to UV irradiation. The data was analyzed using Microsoft Excel 2016 and Origin Pro 9.0. Statistical analysis was done using One way anova at $p < 0.05$.

3. Result and Discussion

This study was done as laboratorical experiment using red betel extract from red betel leaves as seen on figure 1. Spectrophotometer UV–Vis was used to identify the type of flavonoid and oxygenation scheme in red betel extract by observing the absorbance of the extract [21], [22]. Hydroxyl group of phenol on flavonoid can be determined by adding sliding reactor to the solvent and observing the peak of absorbance. This determination was using methanol as solvent [17]. Maximum spectrum has two maximum peak on 240–280 nm (band II) dan 300–550 nm (band I).

Figure 1. Red betel powder and red betel extract

From UV–Vis spectrophotometer result, it was known that the maximum wavelength for antibacterial subtance of red betel extract on 367 nm, 536 nm, and 488 nm. Flavonol and chalcone were two subtances that are identified from absorption on UV irradiation, while anthocyanin was identified from absorption on visual light irradiation. These three subtances are flavonoid that has antibacterial effect [6], [8].

Table 1. Type of subtance and spectrum of light on red betel extract

Maximum Wavelength (λ_{max})	Type of Subtance
367 nm	Flavonol
	Chalcone
536 nm	Anthocyanin
488 nm	Anthocyanin

Photophysics process was involved when UV irradiation was held on this study. The basic principle on light absorption by *Stark-Einstein* law is that each molecule can only absorb one photon at a certain time [23]. The arsorbed photon can trigger an electron excitation on a molecule. Valence of

electron at the lowest orbital commonly excited and pushed away from nucleus which has positive charge as far as the number of energy of absorbed photon.

In this study, the photon absorption by red betel extract subtance can also be transmitted. The subtance that absorb the photon energy from UV irradiation is the antibacterial subtance, flavonoid. After absorbing the photon from UV irradiation at a certain wavelength, the molecule of flavonoid has a different energy from ground state by excitation [24].

The chemical structure of flavonoid shown in figure 2. Oxygen is an unstable atom which has two free electron spins. This atom will become reactive and produce free radical. This free radical will react to molecule around, including electron from UV irradiation. This reaction will go on and on, the more free radical produce, the new electron pair will form.

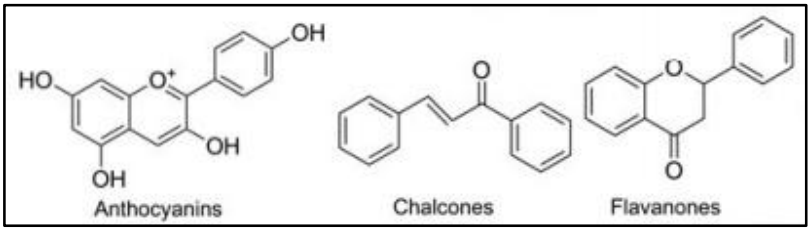

Figure 2. Flavonoid Chemical Structure [25]

Antibacterial test of red betel extract in this study used Kirby-Bauer method by measuring diameter of inhibition zone of *Streptococcus* [18]–[20]. This method is a principle method using diffusion or molecular transfer from higher to lower concentrations [20]. In general, diffusion is generated by molecular turbulance, molecular collisions then caused scattering phenomena [4].

The colonies of *Streptococcus mutans* kept growing on TSA media [12]. This grown number of colonies forced bacteria to penetrate red betel extract zone. Since the red betel had antibacterial effect, the colonies that forced to penetrate will be died. This processes made the inhibition zone occured.

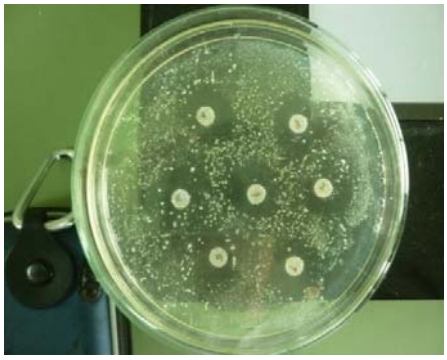

Figure 3. Diameter of inhibition zone

Six different treatments on *Streptococcus mutans* using UV irradiation vary in power and irradiation time was held to determine diameter of inhibition zone. The diameter of inhibition zone showed in figure 3. The better antibacterial effect proven by the bigger diameter. Group L–R+ has smaller diameter than group L+R+, which is (10.35 ± 0.03) mm. The study results of group L+R+ showed on table 2.

Table 2. A conclusion table of UV irradiation

Power of electricity (W)	Time of irradiation (hour)	Diameter of inhibition zone (mm)	One way anova test	
			Significance	Conclusion
10	4[(a)]	14.25 ± 0.08	$p = 0.798$	There are no significant difference
	8[(a)]	14.50 ± 0.15		
	12[(a)]	15.00 ± 0.07		
15	4[(a)]	15.40 ± 0.05	$p = 0.452$	There are no significant difference
	8[(a)]	15.45 ± 0.01		
	12[(a)]	15.96 ± 0.05		

[(a)]Superscript from Tukey test

Table 2 presented that the longer UV irradiation treatment, the wider diameter of inhibition zone become. From one way anova statistical analysis, the study results showed no significant difference since $p>0.05$. The tukey test also showed the same superscripts, meaning that the time of UV irradiation did not give a significant difference to each treatments.

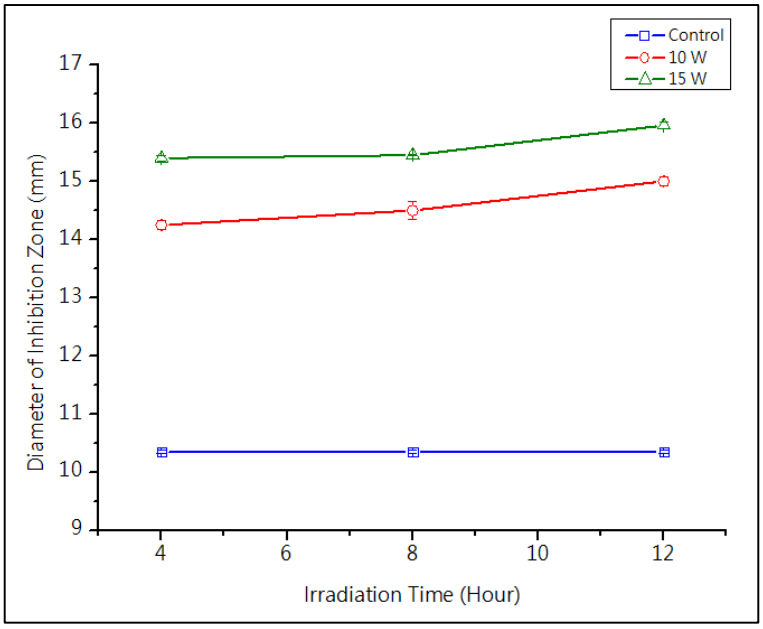

Figure 4. The relation between diameter of inhibition zone to UV irradiation power and time

Figure 4 presented the relation between diameter of inhibition zone and UV irradiation time. From this study, the higher power that used to irradiation, the wider diameter of inhibition zone appeared. The comparation between group L–R+ and group L+R+ showed the percentage of antibacterial effect. As seen on figure 5, the percentage of antibacterial effect using 15 watt is better than 10 watt.

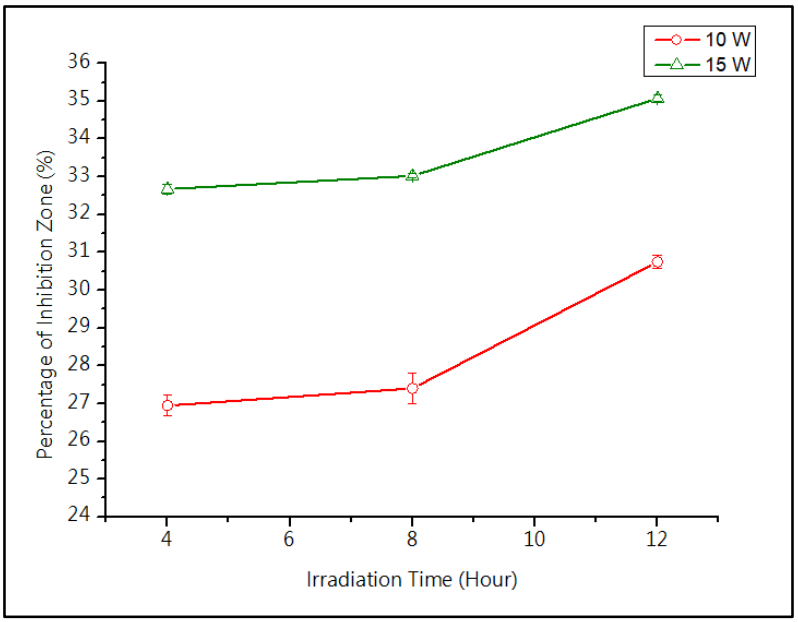

Figure 5. The percentage of inhibition zone

4. Conclusion

Red betel is one of herbal plant that has phenol in vary types and has antibacterial effect. From this study, the extract of red betel is proven to inhibit the number of *Streptococcus mutans*. With treatment combination of UV irradiation vary in power and time, it was proven that antibacterial subtance on red betel extract increased. UV irradiation for 12 hours showed the best result on each treatment with diameter of inhibition zone (15.00 ± 0.05) mm for 10 watt and (15.96 ± 0.05) mm for 15 watt. So, it is proven that UV irradiation can increase the antibacterial effect of red betel extract.

5. References

[1] Q. Tang, H. Zhang, B. He, and P. Yang, "Nano Energy An all-weather solar cell that can harvest energy from sunlight and rain," *Nano Energy*, vol. 30, pp. 818–824, 2016.

[2] M. Rivas, E. Rojas, M. C. Araya, and G. M. Calaf, "Ultraviolet light exposure , skin cancer risk and vitamin D production," pp. 2259–2264, 2015.

[3] L. I. N. E. T. Al, "Changes of the Tropical Tropopause Layer under Global Warming," pp. 1245–1258, 2017.

[4] W. Gao *et al.*, "Effects of Supplementary Ultraviolet-B lrradiance on Maize Yield and Qualities : A Field Experimentq," no. 1, pp. 127–131, 2004.

[5] M. Fadlilah, "BENEFIT OF RED BETEL (PIPER CROCATUM RUIZ & PAV .) AS ANTIBIOTICS," vol. 4, pp. 71–75, 2015.

[6] S. Tu, N. Carolia, and K. Sw, "THE INHIBITION TEST OF RED BETEL LEAVES (Piper crocatum) TOWARDS Staphylococcus aureus AND Salmonella typhi," vol. 4, pp. 117–122, 2015.

[7] L. Novilia, U. Harahap, and P. A. Z. Hsb, "Asian Journal of Pharmaceutical Research and Development," vol. 5, no. 5, pp. 1–8, 2017.

[8] P. Ruiz, "Cytotoxic Activities of Fractions and Two Isolated Compounds from Sirih Merah (Indonesian red betel)," *Procedia Chem.*, vol. 13, pp. 79–84, 2014.
[9] E. Verawati, T. W. Widowati, B. Santoso, S. Rusdiana, P. Dewi, and R. Pambayun, "Microbial & Biochemical Technology Antibacterial Activity toward Streptococcus mutans and Antioxidant from Traditional Betel Chew Formulation of Indonesia," vol. 9, no. 6, pp. 316–320, 2017.
[10] B. Paridhi, U. S. Ashita, P. Swati, and N. G. Dilip, "An Invitro Study Of Determination Of Anti-Bacterial , Antioxidant , Anti- Inflammatory Potential Of Piper Betel Essential Oil," vol. 6, no. 2, pp. 37–44, 2015.
[11] P. Phumat, S. Khongkhunthian, P. Wanachantararak, and S. Okonogi, "Streptococcus mutans and Streptococcus intermedius," vol. 12, no. 3, pp. 133–141, 2018.
[12] S. D. Astuti, A. Zaidan, and E. M. Setiawati, "Chlorophyll Mediated Photodynamic Inactivation of Blue Laser on Streptococcus Mutans," vol. 120001, 2016.
[13] F. A. Bangash *et al.*, "Original Research Article," vol. 03, no. 04, pp. 639–646, 2012.
[14] M. Tevini and A. H. Teramura, "UV-B EFFECTS O N TERRESTRIAL PLANTS," vol. 50, no. 4, pp. 479–487, 1989.
[15] R. Tripathi, K. Rai, S. Singh, and M. Agrawal, "Role of supplemental UV-B in changing the level of ozone toxicity in two cultivars of sun fl ower : growth , seed yield and oil quality," *Ecotoxicology*, vol. 3, pp. 277–293, 2019.
[16] M. Singh, G. Bashri, S. Mohan, P. Vijay, and P. Singh, "Kinetin Alleviates UV-B-Induced Damage in Solanum lycopersicum : Implications of Phenolics and Antioxidants," *J. Plant Growth Regul.*, vol. 0, no. 0, p. 0, 2019.
[17] D. G. Kanjwani, T. P. Marathe, S. V Chiplunkar, and S. S. Sathaye, "Evaluation of Immunomodulatory Activity of Methanolic Extract of Piper betel," pp. 589–593, 2008.
[18] I. P. Online, S. C. Borty, K. Musabbir, B. Hafiz, M. Ali, and K. Begum, "Asian Journal of Medical and Biological Research Isolation , identification and antibiogram profile of bacteria isolated from dental caries patients of Mymensingh district of Bangladesh," vol. 1, no. 2, pp. 244–253, 2015.
[19] M. Shin *et al.*, "Cytotoxicity and Antimicrobial Effects of a New Fast-Set MTA," vol. 2017, 2017.
[20] P. Singh, R. Misra, and K. N. Prasad, "Reliability of Kirby-Bauer Disk Diffusion Method for Detecting Doripenem Susceptibility in Oxidase Positive Non-Fermenting Gram Negative Bacilli," vol. 6, no. September, pp. 395–397, 2016.
[21] A. Masek, E. Chrzescijanska, M. Latos, and M. Zaborski, "Influence of hydroxyl substitution on flavanone antioxidants properties," *Food Chem.*, 2016.
[22] Ş. Selin, E. Elhussein, M. Bilgin, J. M. Lorenzo, F. J. Barba, and S. Roohinejad, "Effect of drying method on oleuropein , total phenolic content , flavonoid content , and antioxidant activity of olive (Olea europaea) leaf," no. December 2017, 2018.
[23] E. J. Land, "Physical Properties of Excited," 1978.
[24] M. H. Niemz, *Laser-Tissue Interactions, Fundamentals and Applications , Third Enlarged Edition*, 3rd ed. Heidelberg: Springer-Verlag, 2007.
[25] J. Of and N. Science, "Journal of nutritional science," 2017.