

# Antibacterial Activity of Methanol Extract of Red Dragon Fruit Peel ( *Hylocereus polyrhizus*) against Methicillin-Susceptible *Staphylococcus aureus* (MSSA) ATCC 25923 and Methicillin Resistant *Staphylococcus aureus* (MRSA) In Vitro

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## Abstract

**Background:** *Staphylococcus aureus* is one of the most virulent *Staphylococcus* which could cause systemic broad-spectrum infections and developed resistant strains. Red dragon fruit (*Hylocereus polyrhizus*) peel has a broad antibacterial activity against both gram-negative and positive bacteria, including *Staphylococcus aureus*. **Objective:** To determine and compare the antibacterial activity of methanol extract of red dragon fruit peel against Methicillin-Susceptible *Staphylococcus aureus* ATCC 25923 and Methicillin-Resistant *Staphylococcus aureus*. **Method:** Well diffusion test method was performed on 5 concentrations: 100%, 75%, 50%, 25%, and 0%, dissolved in DMSO 10% where at a concentration of 100% contains 1g/mL extract. The inhibition zone elicited as clear zone around the well then measured and analyzed with SPSS using One-Way ANOVA and Post Hoc LSD, to determine the significance of each concentration. **Results:** The mean diameter of the inhibition zone observed towards Methicillin-Susceptible *Staphylococcus aureus* was  $9.5333 \pm 0.26822$  -  $15.4167 \pm 0.22048$ , while bigger zone was found in Mueller-Hinton plate with Methicillin-Resistant *Staphylococcus aureus* which is  $12.30 \pm 0.20817$  -  $17.80 \pm 0.25166$  mm. **Conclusion:** This study showed that methanol extract of red dragon fruit peel has antibacterial activity against *S. aureus*, and shows higher antibacterial activity against Methicillin-Resistant *Staphylococcus aureus* than Methicillin-Susceptible *Staphylococcus aureus*.

**Keywords:** Antibacterial activity, *Hylocereus polyrhizus*, Methicillin-Resistant *Staphylococcus aureus*, Methicillin-Susceptible *Staphylococcus aureus*, Red dragon fruit peel

## Introduction

Multidrug-Resistant Organism (MDROs) are defined as microorganisms, predominantly bacteria, that are resistant to one or more classes of antimicrobial agent. MDROs infection are increasing worldwide, including in US, where more than 2.8 million antibiotic-resistant infections occur in the United States each year, and more than 35,000 people die as a result<sup>1</sup>. Thus,

impose a global public health threat and require a major concern in the medical fields

Massive and inadequate use of broad-spectrum antibiotics contributed to accelerate the emergence of resistant bacterial strains, one of which is Methicillin Resistant *Staphylococcus aureus* (MRSA). Although the incidence of *Staphylococcus aureus* infection has been stable in the last decade, the prevalence of MRSA infection is fluctuated<sup>2</sup>. A review studies shows that MRSA contributed between 13 and 74% of *S. aureus* infection. While it is difficult to determine the prevalence of *S. aureus* in East and South Asia, and Western Pacific. Several studies and national surveillance data indicates

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*S. aureus* as a significant pathogen, with MRSA incidence ranging from 2.3 to 69.1%<sup>3</sup>. In addition, CDC reports MRSA was estimated to cause 323,700 cases and 10,600 deaths incident in US in 2019<sup>1</sup>. Infections caused by MDROs are closely related to escalation of mortality and morbidity, as well as increased duration of hospital stay and costs, thus often emerge as a new problem for patients and families<sup>3,4</sup>. Currently, the choice of antibiotics which was used as treatment for MRSA infections are flucloxacillin, vancomycin or teicoplanin, in addition, there are also newer drug options such as Linezolid and Daptomycin<sup>5</sup>.

Indonesia has a vast variety of biodiversity, this could be an aptitude key to solve this problem, one of which is through exploration of natural antibiotics. Therefore, it is necessary to study antibacterial activity of natural ingredients to determine its potential and effectiveness as antibiotics, one of which can be derived from the red dragon fruit (*Hylocereus polyrhizus*) peel.

The fruit flesh from red dragon fruit has been widely studied both regarding its content and antioxidant activity, but dragon fruit peel which takes up to 30-35% of the fruit weight is often only disposed of as waste<sup>6</sup>. Whereas based on the results of the phytochemical component test<sup>7,8</sup>, dragon fruit peel contains several compounds which possess antibacterial activity, including phenol hydroquinone, flavonoids, triterpenoids, steroids, saponins, tannins and alkaloids. In addition, red dragon fruit peel also has proven to have a broad antibacterial activity, both towards gram-positive and gram-negative bacteria, one of which is against *Staphylococcus aureus*<sup>9</sup>.

## Materials and Methods

### Study materials:

Red Dragon Fruit (*Hylocereus polyrhizus*) was purchased from the local market in Sidoarjo, East Java, Indonesia. While Methicillin-Susceptible *Staphylococcus aureus* ATCC 25923 and Methicillin-Resistant *Staphylococcus aureus* was acquired from Laboratory of Microbiology, Faculty of Medicine, Universitas Airlangga.

### Extraction of Red Dragon Fruit Peel:

Red dragon fruit peel was washed under running water, then cut into small pieces and dried at room temperature. A total of approximately 300 grams of dried red dragon fruit peel were collected, then made into crude powder and extracted by maceration method.

The maceration process was achieved by submerging the simplicia powder in a maceration vessel together with a total of 1,250 mL of methanol. Maceration was carried out for 2 x 24 hours with occasional gentle stirring. After the first 24 hours, the simplicia powder was drained and filtered with filter paper, then submerged with methanol for the second time. The methanol extract obtained was further processed using a rotary vacuum evaporator, where the solvent will be evaporated in low pressure and temperature distillation to avoid decomposition of the thermo-labile compounds contained<sup>10</sup>.

Through the extraction process, 16.597 grams of thickened extract were obtained. One gram of the extract was then dissolved with 1 mL of 10% DMSO which was used as 100% concentration standard. The 100% solution was then diluted to a concentration of 75%, 50% and 25%.

### Evaluation of Antibacterial Activity using Well-Diffusion Method:

Bacteria planted on the Mueller-Hinton agar medium, aseptically beforehand. Agar plate inoculation was carried out by spreading bacterial inoculum evenly on the entire agar surface. The media then left to dry for 3-5 minutes at room temperature (25°C). Afterwards, 4-5 well were made per petri dish, and filled with 100 mL of red dragon fruit peel methanol extract with various concentrations<sup>11</sup>. The Mueller-Hinton agar was then incubated at 37° for 24 hours. In this study, triple replications were carried out for each concentration according to the calculation of Federer formula. Erythromycin disk of 15 mg was used for positive control for MSSA ATCC 25923 and vancomycin of 30 mg for MRSA. Meanwhile, DMSO 10% was used as negative control.

## Result and Discussion

Antibacterial activity evaluation on red dragon fruit peel was carried out using well diffusion method. The inhibition zone diameter is determined by calculating the clear zone around the well using calliper. Before

the results of the data were analysed using One-Way ANOVA and Least Significant Difference (LSD), the data were analysed using the Shapiro-Wilk test and Levene's test which shows that the data were distributed normally and homogeneous ( $p > 0.05$ ).

**Table 1 : Inhibition zone of methanol extract of red dragon fruit peel against MSSA ATCC 25923**

Concentration	Inhibition Zone (mm)			Mean $\pm$ SE (mm)
	1	2	3	
100%	15.75	15.00	15.50	15.4167 $\pm$ 0.22048
75%	14.60	14.00	14.65	14.4167 $\pm$ 0.20883
50%	11.60	11.55	11.50	11.5500 $\pm$ 0.02887
25%	9.75	9.85	9.00	9.5333 $\pm$ 0.26822
0%	0	0	0	0
Eritromisin 15mg	23.00	23.15	23.75	23.30 $\pm$ 0.22913

**Table 2 : Inhibition zone of ethanol extract of red dragon fruit peel against MRSA**

Concentration	Inhibition Zone (mm)			Mean $\pm$ SE (mm)
	1	2	3	
100%	18.1	18.00	17.30	17.80 $\pm$ 0.25166
75%	14.60	16.00	16.20	16.2167 $\pm$ 0.13017
50%	14.30	14.85	14.90	14.6833 $\pm$ 0.19221
25%	12.20	12.70	12.00	12.30 $\pm$ 0.20817
0%	0	0	0	0
Vancomycin 30 $\mu$ g	20.25	21.15	20	20.4667 $\pm$ 0.34921

Based on Table 1 and Table 2, it is known that an increase in concentration of the extract, resulted in an increase of the inhibition zone elicited. Meanwhile, the lowest inhibition zone is produced by a concentration

of 50% in MSSA and 25% in MRSA. Therefore, it can be concluded that the concentration of red dragon fruit peel extract is equivalent with the inhibition zone diameter elicited, both on MSSA and MRSA which is dose-dependent. The effect of DMSO 10% as solvent is

negligible as there was no inhibition zone observed in well diffusion method at a concentration of 0% which acted as negative control. Methanol extract of red dragon fruit peel at a concentration of 100% produced a higher inhibition zone against MRSA ( $17.80 \pm 0.25166$  mm) than towards MSSA ( $15.4167 \pm 0.22048$  mm) with significant difference, proven by Post Hoc LSD analysis ( $p < 0.05$ ). While at a concentration of 25%, the methanol extract of red dragon fruit peel was still able to produce inhibition zone with mean of  $9.5333 \pm 0.26822$  mm against MRSA, there was no inhibition zone observed in MSSA plate.

Antimicrobial activity performed by red dragon fruit peel is a result of active antibacterial compound possessed which could be divided into phenolic and non-phenolic compound. Phenolic compound affects *S. aureus* through multiple pathways, one of which was through protein denaturation that was achieved from hydrogen chain formed and bacterial protein reaction and resulted in enzyme inactivation and inhibition of bacterial metabolism<sup>9</sup>. Furthermore, phenolic compound could also inhibit cell wall synthesis and damage bacterial cell wall.

Quercetin which belongs to flavonoid, is one of the phenolic compounds which is worth mentioning because of its ability to inhibit *S. aureus* growth completely. Even though quercetin has low solubility and sensitive to oxidative degradation, this problem was counter-attacked by the presence of ascorbic acid which could help stabilize and enhance quercetin activity<sup>12,13</sup>. Red dragon fruit peel also consists of non-phenolic compounds, such as alkaloid, steroid and saponin whose also projects antibacterial activity in various mechanism. For example, alkaloid could inhibit cell wall synthesis and disrupt amino acid structure of bacterial DNA, while steroid and saponin will make an interaction with phospholipid membrane of bacteria and decrease cell wall integration which leads to cell death<sup>8,14</sup>.

Antibacterial activity could be classified based on inhibition zone produced, if the inhibition zone formed in the diffusion test is  $< 5$  mm in diameter, the antibacterial activity is considered as low, and the inhibition zone of 5-10 mm is considered as moderate, moreover 10-19 mm is considered as high and 20 mm or more is categorized

as very high<sup>15</sup>. If this classification is correlated with the data from this study, it can be stated that the methanol extract of dragon fruit peel shows high inhibitory activity against MSSA at concentrations of 50-100% and MRSA at concentrations of 25-100%.

Based on Post Hoc LSD analysis, the average diameter of inhibition zone produced by the methanol extract of red dragon fruit peel at a concentration of 50% against MRSA and 75% against MSSA did not have a significant difference ( $p > 0.05$ ), while the other concentrations showed a significant difference ( $p < 0.05$ ). In corresponds to those, the activity of methanol extract of red dragon fruit peel against MRSA at a concentration of 50% could be considered as equivalent to the extract activity in concentration of 75% against MSSA and the antibacterial activity of methanol extract of red dragon fruit peel is higher against MRSA than MSSA. Those statement is relevant to a study conducted by Chuah in 2014, where methanol extracts from *D. linearis* and *M. calabura* had higher antibacterial activity against MRSA than MSSA through diffusion, dilution and colorimetric tests using resazurin, however further research is needed to explain the mechanism on how an extract has a higher antibacterial activity against resistant organism than those that are susceptible<sup>16</sup>.

## Conclusion

In conclusion, methanol extract of red dragon fruit peel has shown its potential as a source of natural antibiotics against *S. aureus* in this study. Methanol extract of red dragon fruit peel was proven to have antibacterial activity against *S. aureus* and elicit higher antibacterial activity against MRSA than MSSA, with the highest average inhibition zone of  $15.4167 \pm 0.22048$  mm against MSSA and  $17.80 \pm 0.25166$  mm against MRSA.

**Ethical Clearance:** This experimental study protocol had been approved by the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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