

# The Antibacterial Activity of Red Dragon Fruit Peel (*Hylocereus polyrhizus* Britton & Rose) Methanolic Fraction Against *Staphylococcus epidermidis* and *Propionibacterium acnes*

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## ABSTRACT

Acnes can be caused by *Propionibacterium acnes* and *Staphylococcus epidermidis*. Red dragon (*Hylocereus polyrhizus* B & R) fruit peels is one of natural materials which is assumed to possess an antibacterial activity. The purpose of this study was to determine the antibacterial activity of red dragon fruit peels methanol fraction (RDFPMF) against *S. epidermidis* and *P. acne* by disc diffusion Kirby-Bauer method. Red dragon fruit peels were maceration in chloroform and fractionation in methanol. Clindamycin 4µg/disk was used as positive control and DMSO 10% was used as negative control. This research showed that the RDFPMF has antibacterial activity against *P. acnes* which the average of inhibition zones obtained from the concentrations from 100, 50, and 25 mg/mL respectively were  $10.5 \pm 0.50$  mm,  $10.0 \pm 0.00$  mm, and  $8.5 \pm 0.50$  mm. The RDFPMF has no inhibition zone toward *S. epidermidis*. The red dragon fruit peels methanol fraction has antibacterial activity on *P. acnes* by disc diffusion method (Kirby-Bauer test).

**Keywords:** Antibacterial, RDFPMF, *Staphylococcus epidermidis*, *Propionibacterium acnes*

## I. INTRODUCTION

Acne is a chronic skin disease caused by an inflammation in the sebaceous gland. Although it is not a serious disease, if not being cured, the acne can disrupt appearances and activities of people, reduce their confidence, or even become

depression (Hafez *et al.*, 2009). The development of acne involves two microorganisms, such as *Staphylococcus epidermidis* and *Propionibacterium acnes* (Leyden, 2001).

Acne therapy prescribed by doctors are antibiotics, such as clindamycin,

erythromycin and tetracycline which are in-long-term use will be risky to cause a resistance, an organ damage, and immune hypersensitivity (the sensitivity of immune in liver) (Swanson, 2003). Besides that, it is also common as drugs such as benzoyl peroxide, resorcinol, retinoid, and azelaic acid have side effects (irritation) (Oprica, 2004). This circumstance drives to conduct the development of the natural antibacterial research on plants that exist in Indonesia (back to nature), such as the skin of red dragon fruits.

Red dragon fruit (*Hylocereus polyrhizus*) is one of the fruits of the Cactacea family coming from Latin America and widely developed in Indonesia. The red dragon fruit contains lycopene, a natural antioxidant known to counter cancer and heart disease, and also decrease blood pressure (Zainoldin, 2012). Not only the meat of dragon fruit is beneficial, but also the skin has antioxidant and antibacterial potential because it contains  $\beta$ -Amyrin,  $\alpha$ -Amyrin, octacosane,  $\gamma$ -sitosterol, octadecane, 1-tetracosanol, heptacosane, campesterol and high betalains. Those components can function as antioxidants and cytotoxic activity (Luo *et al.*, 2014; Nurliyana *et al.*, 2010). The red dragon fruit peel is efficacious as an antioxidant, antibacterial and a source of natural pigments (Jamilah *et al.*, 2011; Ridwan, 2012; Azeredo, 2009).

The research conducted by Ridwan (2012), showed that the red peel of fruits showed an antibacterial activity on *S. aureus* containing saponins, alkaloids, tannins, phenolics, flavonoids, triterpenoids, steroids, and glycosides. Khalili (2012) showed that the red dragon fruit peel extraction using methanol has antibacterial activities on Gram-positive, *S. epidermidis* and *S. aureus*.

The test of antibacterial activities in a red dragon fruit peel were indicated as the extracting process only and still need more tests and researches held along the fractionation process. This study will use methanol fraction with disc-diffusion method of Kirby-Bauer. This research is expected to provide information concerning the potential skin of red dragon fruit as an alternative to natural antibacterial therapy against acne-causing bacteria, *P. acnes* and *S. epidermidis*.

## II. METHOD

### A. Materials

Materials used in this research are, the pericarp of red dragon fruit was taken from ripped dragon fruits, which are identified by all-rounded red pericarp. Chloroform (Merck), methanol (Merck), dimethyl sulfoxide (DMSO) (Merck), distilled water (Merck), clindamycin HCl 150 mg (Kimia Farma), the media of Mueller-Hinton Agar (MHA) oxid,

Mueller-Hinton - Blood Agar (MH-Blood Agar) oxoid and Mc Farland 0.5. The tools utilized in this research are separating funnel (IWAKI), rotary evaporator (BUCHI-R-100), ose needle, a petri dish Normax, and calipers, incubators (Mettler in 100), and autoclaves (All American).

### **B. The Establishment of Methanol Fraction of the Red Dragon Fruit Leather**

The sample used in this research is 8 kg of red dragon fruit peels obtained from Mekar Sari farm in Segedong sub-district, Pontianak, West Kalimantan. The peels were cleaned in water, chopped, and moistly sorted. After that, dry and blend it until being a simplicia. When it has been a simplicia, macerating the simplicia by using chloroform for 7 days. The chloroform extract was fractionated into a liquid with methanol. Next, the fraction of methanol was concentrated using a rotary evaporator.

### **C. The Establishment of Various Concentration of Methanol Fraction of The Red Dragon Fruit Leather**

The stock solution of methanol fraction in red dragon fruit leather is 100 mg/ mL. It is prepared by dissolving 1000 mg of methanol fraction in 1mL DMSO, then adding 10 mL of distilled water.

Gradual dilution used to create a concentration in 50; 25 and 12.5 mg / mL.

### **D. The Test of Antibacterial Activity of Methanol Fraction of The Red Dragon Fruit Leather**

The sample of antibacterial activity was carried out with *S. epidermidis* and *P. acnes* was obtained by the Department of Microbiology, Medical Faculty, University of Indonesia. Tests were using the disc diffusion method (Kirby Bauer). The bacteria from the rejuvenating on media was inoculated by media of MHA which has been solid for *S. epidermidis* and MH-Blood Agar for the bacteria of *P. acnes*. Then, drop 20  $\mu$ L methanol fraction of red dragon fruit leather with various concentrations on paper discs (6 mm). After that, the sample was incubated in an incubator for 24 hours and measured the diameter of the inhibitory zone or a clear zone with calipers. The positive control was clindamycin in 4 $\mu$ g /disk and the negative control was 10% DMSO.

## **III. RESULT AND DISCUSSION**

The antibacterial activity test is a technique to measure how the potential or the concentration of a compound which can give an effect on microorganisms. The test of antibacterial activity of methanol fraction in red dragon fruit peels was performed by using Kirby-Bauer disc

diffusion; a method to determine the antimicrobial sensitivity.

The test result of an antibacterial fraction of methanol, negative control, and positive control are in table I. The result showed inhibitory zone on *P. acnes* bacteria concentration of 100 mg/mL was  $10.5 \pm 0.50$  mm; 50 mg/mL was  $10 \pm 0.00$  mm; and 25 mg/mL was  $8.5 \pm 0.50$  mm; clindamycin 4 µg/disk as a positive control was  $8 \pm 0.00$  mm; 10% DMSO as a negative control has no inhibition zone. Meanwhile, the result of research on the antibacterial

testing of methanol fraction in red dragon fruit peels on *S. epidermidis* bacteria did not show an inhibition zone in each concentration.

*P. acnes* and *S. epidermidis* have different characteristics and endurances. The growth (lag phase) of *P. acnes* is slower than *S. epidermidis* (Marbun *et al.*, 2021). The growth of *S. epidermidis* on the media was faster than the sample penetration from the paper disc, so the methanol fraction could not inhibit the growth of *S. epidermidis*.

**Table I.** The Result of Inhibitory Zone Diameter

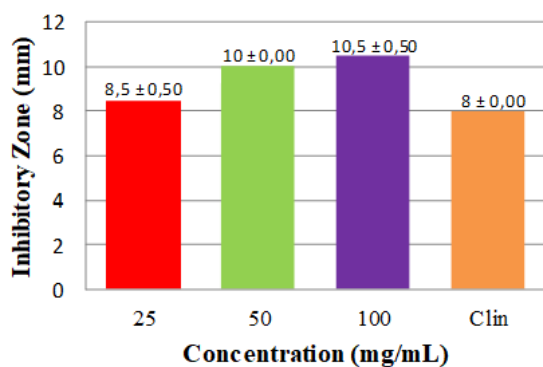
The Bacteria	Concentration (mg/mL)	Inhibitory Zone (mm)		Mean ± S.E
		Repetition 1	Repetition 2	
<i>Propionibacterium acnes</i>	100	11	10	$10,5 \pm 0,50$
	50	10	10	$10 \pm 0,00$
	25	8	9	$8,5 \pm 0.50$
	Clindamycin	8	8	$8 \pm 0,00$
	DMSO 10%	-	-	-
<i>Staphylococcus epidermidis</i>	100	-	-	-
	50	-	-	-
	25	-	-	-
	Clindamycin	24	24	$24 \pm 0,00$
	DMSO 10%	-	-	-

Notes: - = no inhibitory zone

According to the results on Figure 1, it can be seen that the inhibitory zone produced by methanol fraction in red dragon fruit peels toward *P. acnes* is getting bigger as the increase of concentration. Then, it can be assumed that there is relationship in the same line between the concentration and the result of the inhibitory zone. This is related to compounds of the concentration dissolved

in the fraction, the more fractional concentration increased, the more compounds of the concentration dissolving the bacterial growth be risen. Furthermore, it can be implied that the inhibitory zone obtained from various concentrations of methanol fraction in red dragon fruit peels is better than the positive control of clindamycin 4 µg/disk.

The antibacterial activity of methanol fraction on *P. acnes* is assumed due to the activity of the compounds contained in the methanol fraction. Red dragon fruit peels contain flavonoids (Kim *et al.*, 2011), polyphenols (Kim *et al.*, 2011; Wu *et al.*, 2006), alkaloids (betalain and betacyanin) (Jamilah *et al.*, 2011), and terpenoids (Luo *et al.*, 2014). The dragon fruit peels mostly has terpenoids compounds ( $\alpha$ -Amyrin and  $\beta$ -Amyrin) (Luo *et al.*, 2014). However, the *Moringa Pregrina* contains  $\alpha$ -Amyrin and  $\beta$ -Amyrin (terpenoids compounds) proved that they have antibacterial activity (Tahany *et al.*, 2010).



**Figure 1.** The chart of methanol fraction and clindamycin toward *Propionibacterium acnes*

Terpenoids mechanism as antibiotics is reacted with porin (transmembrane protein) on the outer membrane of the bacterial cell walls, forming a strong bond of polymers that destruct porin. The broken porin which is the entry and the exit of the compounds will

reduce the permeability of bacterial cell walls that cause a bacterial cell on lack of nutrients, so the bacterial growth is inhibited or even death (Cowan, 1999).

On others plants, *Coscinium fenestratum* is containing alkaloids, has antibacterial activity on the *P. acnes* better than on the *S. epidermidis* (Kumar *et al.*, 2007). The previous research show that the compounds which may be involving in antibacterial activity has been implicated as betalain compounds (Geidam *et al.*, 2007; Yang *et al.*, 2004; Livermore *et al.*, 2002). Based on Valsaraj *et al* (1997) in his research, alkaloids and its derivatives also have an antibacterial activity on *S. aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) (Valsaraj *et al.*, 1997). The main alkaloids compounds that have role as an antibiotic, such as berberine and harmane with mechanisms of inhibiting DNA synthesis (Hopp *et al.*, 1976; Phillipson & O'Neill, 1987).

The working mechanism of alkaloids as antibiotics in general is by interfering structural components of the peptidoglycan in the bacterial cell, therefore the layers of the cell wall are not fully formed and the cell will be dead. In addition, alkaloids compounds consist of alkali clusters containing nitrogen which will react with amino acid compounds compiling the bacteria cell wall and bacterial DNA. This reaction affects the

structural change and the amino acid sequences that will cause changes in the balance of genetics on the DNA chain, then it will be defect and lead to a bacterial cell lysis which can cause the death cell in bacteria (Katzung, 2004). The example of alkaloids contained in the skin of red dragon fruit is betacyanins; it is isolated from *Bougainvillea glabra* which showed that it has an antibacterial activity on *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* with concentration at 40µg/mL (Napoleon *et al.*, 2013).

The mechanism of polyphenols compounds as antibacterial works by denaturing cell proteins and damaging the cell wall until the bacteria dies. Moreover, other mechanisms that can happen are precipitating proteins actively and breaking lipids in the cell membrane through mechanisms of dropping voltage on the cell membrane surface. The antibacterial activity of flavonoid compounds has the same mechanisms as phenol compounds, furthermore flavonoids work in bacteria by damaging the cytoplasmic membrane; it controls the entry of food ingredients or nutrients, if the cytoplasmic membrane is broken, the essential metabolites in the bacteria come out and food ingredients to generate energy cannot come inside, then there is an inability of bacterial cells to grow and there will be death (Pelczar &

Chan, 1986). One of flavonoid compounds that has antibacterial activity on *Staphylococcus aureus* is Auran, flavonols, and flavones compound (Arum *et al.*, 2012).

#### IV. CONCLUSION

Methanol fraction of red dragon fruit peels has antibacterial activity on *Propionibacterium acnes* and has not activity toward *Staphylococcus epidermidis* by disc diffusion method (Kirby-Bauer test). The result of the average diameter of inhibitory zone for each concentration of *P. acnes* in sequence of the concentration at 100; 50; 25 mg / mL is  $10.5 \pm 0.50$ ;  $10 \pm 0.00$ ;  $0:50 \pm 8.5$  mm.

#### ACKNOWLEDGEMENTS

We are much obliged for the bacteria lab of analyst health department of Politeknik Kesehatan Kementerian Kesehatan Pontianak to conduct the research in the laboratory.

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