Original Article

Antibacterial activity of *Hylocereus polyrhizus Britton* & Rose peel against *Staphylococcus epidermidis* bacteria

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ABSTRACT

Red dragon fruit (*Hylocereus polyrhizus Britton* & Rose) is a typical tropical plant that is popular and widely cultivated due to its qualities, advantages, and high nutritional content. Some of the substances in *Hylocereus polyrhizus Britton* & Rose are plant sources that are rich in nutrients and minerals, including protein, fat, carbs, fiber, flavonoids, thiamin, niacin, pyridoxine, cobalamin, phenolic, betacyanins, polyphenols, and carotenoids, as well as vitamins B1, B2, B3, and C. This research aimed to specify the antibacterial activity of the 96% ethanol extract of *Hylocereus polyrhizus* fruit peel against the growth of *Staphylococcus epidermidis* bacteria using the well diffusion method. Maceration was used as the extraction method and a phytochemical screening procedure was carried out according to the procedure from Harbone. The bacterial activity was conducted by well diffusion method. Phytochemical screening results from 96% ethanol extract of *Hylocereus polyrhizus* fruit peel showed the presence of alkaloids, steroids, flavonoids, terpenoids, tannins, polyphenols, and saponins. The outcomes of the antibacterial activity test of 96% ethanol extract of *Hylocereus polyrhizus* fruit peel with a concentration of 20%, 60%, 80%, and 100% (b/v) indicated that there was intense antibacterial activity against *Staphylococcus epidermidis*.

Keywords: Antibacterial, *Hylocereus polyrhizus*, *Staphylococcus epidermidis*, Well diffusion

Introduction

Many infections caused by *Staphylococcus aureus* and *Staphylococcus epidermidis* rank first among the causes of commensal infectious agents, the leading causes of nosocomial diseases worldwide, especially in developing countries. *Staphylococcus epidermidis* can generally be isolated from the human epithelium and colonize the axilla, head, and nasal cavity. This bacterium is part of the microflora, but now *Staphylococcus epidermidis* has emerged as a pathogenic strain that causes infection [1]. According to Jawetz [2], *Staphylococcus epidermidis* is a species of bacteria from the genus *Staphylococcus* which is known to cause opportunistic infections (attacking individuals with a weak immune system) such as patients with Acquired Immunodeficiency Syndrome (AIDS), critical patients, drug users (narcotics), newborns, and long-term hospitalized patients. Some of the characteristics of these bacteria are facultative, coagulase-negative, catalase-positive, gram-positive, cocci-shaped, and 0.5 – 1.5 µm in diameter.

Antibiotics are the therapy of choice for treating diseases caused by bacteria [3, 4]. The study states that around 40–62% of antibiotics are misused, among others, for diseases that do not require antibiotics. Beside that, the development of resistancy among antibiotics groups are facilitated by virulances factors, which maight be cured with peptides as antibiofilm activity against pathogenic bacteria. The existence of antibiotic resistance encourages exploration to find alternatives, one of which is by using natural ingredients. The red dragon fruit plant (*Hylocereus polyrhizus*) is an alternative natural component that could be utilized as an antibacterial [5, 6].

One of the fruits of the Cactaceae family with American origins, the red dragon fruit (Hylocereus polyrhizus Britton & Rose), has started to spread across Indonesia. The red dragon fruit (*Hylocereus polyrhizus* Britton & Rose) is a tropical plant which much favored by the public because it has efficacy and benefits

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and high nutritional value. White-fleshed dragon fruit (Hylocereus undatus), red-fleshed dragon fruit (Hylocereus polyrhizus), super red-fleshed dragon fruit (Hylocereus costaricensis), and yellow-skinned dragon fruit (Selenicereus megalanthus) with white flesh are the varieties of dragon fruit that are grown [7]. A plant source of vitamins and minerals, red dragon fruit (Hylocereus polyrhizus) is high in vitamin B1, B2, B3, and vitamin C, as well as protein, fat, carbs, fiber, flavonoids, cobalamin, phenolic, betacyanins, and other secondary metabolite [8, 9]. Secondary metabolites in Hylocereus polyrhizus Britton & Rose fruit peel work as antibacterial in various ways. For example, saponin compounds can inhibit cell membranes' function by impairing membrane permeability, resulting in damaged or destroyed cell walls. At the same time, terpenoids react with porins (transmembrane proteins) on the exterior membrane of bacterial cell walls by forming a potent polymeric bond, rendering damage to porins (reducing the level of permeability of the bacteria's cell wall), which will eventually cause the cells of the bacteria to experience a lack of nutrients so that the growth of the bacteria becomes inhibited or even dies. The tannin compounds can weaken the cell wall, interfering with cell permeability. The peptidoglycan constituent parts in bacterial cells can be invaded by the alkaloids themselves through a process that results in the defective formation of the cell wall layer and cell death [10].

The skin of the red dragon fruit (Hylocereus polyrhizus) is valuable as an antibiotic, antioxidant, and source of natural colors [11]. Ridwan demonstrated that the red dragon fruit peel, which contains saponins, alkaloids, tannins, phenolics, flavonoids, triterpenoids, steroids, and glycosides, was proved to have antibacterial action against S. aureus (Gram-positive).

This finding is reinforced by research by Khalili, which showed that red dragon fruit peel extraction using methanol had antibacterial activity on *S. epidermidis and S. aureus* (Gram-positive) bacteria [4]. Research by Maulana with a sample of *Hylocereus polyrhizus* fruit peel extract using the disc diffusion method showed that dragon fruit peel had an inhibitory power against the growth of *S. pullorum* bacteria which was characterized by an inhibition zone of 9.6 mm at a concentration of 60 mg/ml. Using the disc diffusion technique, Romero reported that the ethanolic extract of the fruit peel of Hylocereus polyrhizus showed antibacterial activity against E. coli with an inhibition zone of 28.24 mm during 24-hour incubation [5]. In line with those findings, antibacterial candidate is invented from natural resouces such as bacterial and algae resouces [12-14].

Therefore, it is crucial to carry out a study to establish the concentration of 96% ethanol extract from red dragon fruit peel (Hyrocereus polyrhizus Britton & Rose), using the well diffusion method, that might suppress the development of Staphylococcus epidermidis bacteria.

Materials and Methods

The 96% ethanol extract of *Hylocereus polyrhizus* Britton & Rose fruit peel taken from Pamekasan, Madura Island, pure culture suspension of *Staphylococcus epidermidi* (BBLK)*s*, NA media (Nutrient Agar) (Merck), 0.5% DMSO (Merck), chloramphenicol (to test antibacterial activity) (Bratachem). The tools used in this research include analytical balance (Ohaus), glass jar (Pyrex), rotary evaporator (Heidolph), grinding tool, tweezers, a set of glass tools (Pyrex), cotton swab, vortex (Thermo), petri dish (Duran), incubator (Memmert), Laminar Air Flow (LAF) (Biobase), and gloves (Sensi).

Extraction of Hylocereus polyrhizus Britton & Rose

The fruit peel powder of Hylocereus polyrhizus Britton & Rose was extracted using the maceration process with a solvent ratio of 1:10 for 3x24 hours. At a temperature of 40^0 C, a rotary evaporator concentrated the maceration filtrate until a thick extract was produced.

Phytochemical screening

Identification of saponins, triterpenoids, and steroids was made by foam test and color reaction. The foam test was carried out by weighing the extract as much as 0.3 g in a test tube and adding 10 mL of water, shaking vigorously for about 30 seconds. The color reaction was shown by weighing 0.3 g of the extract dissolved in 15 mL of ethanol, separated into three parts, and put in an equal number of test tubes. The tube I was controlled and tube II, was added three drops of anhydrous acetic acid and one drop of concentrated H_2SO_4, then gently shaken and observed for color changes, tube II added 1-2 mL of concentrated H_2SO_4 through the wall of the test tube. Polyphenols and tannins were identified by weighing the extract as much as 0.3 g plus 10 mL of hot distilled water, stirring until room temperature, adding 3-4 drops of 10% NaCl, stirring, and filtering. The obtained filtrate was divided into three equal parts. The tube I was controlled, tube II added a few drops of $FeCl_3$ solution (polyphenol), and tube II added $FeCl_3$ solution and gelatin (tannin). The flavonoid group of compounds was identified by weighing the extract as much as 1 g and shaking it with 3 ml of n-hexane many times until the n-hexane was colorless. The washed extract was dissolved in 15 mL of ethanol and divided into three equal parts. Tube I as a control, tube II added 0.5 mL of concentrated HCl and observed the color change that occurred, then heated on a water bath and observed the color change again, tube III was added with 0.5 mL of concentrated HCl and four pieces of magnesium and diluted with distilled water and added with 1 mL butanol. The identification of the alkaloid group compounds was carried out by weighing the extract as much as 0.3 g plus 5 mL of 2 N HCl, heated over a water bath for 2-3 minutes while stirring, and after being cold, added 0.3 grams of NaCl, stirred evenly and then filtered. The filtrate was added with 5 ml of 2 N HCl and divided into three equal parts. The tube I was controlled, tube II was

added with Mayer reagent, and tube III was added with Wagner reagent.

Staphylococcus epidermidis ATCC 25922 Bacteria Suspension Preparation

The bacterial suspension was placed by ± one ose of *Staphylococcus epidermidis* into a tube containing 5 mL of 0.9% NaCl solution until the turbidity was the same as McFarland's 0.5.

Antibacterial activity test with the well diffusion method

Antibacterial activity testing was conducted using the well diffusion method or the well method with Nutrient Agar (NA) media. The NA base layer solution was first put into a petri dish and then continued with the NA seed layer solution to which 10 µL of the *Staphylococcus epidermidis* suspension had been added. The solidified NA media was made with holes using a 6 mm diameter perforator. The well was filled with 20 µL of 96% *Hylocereus polyrhizus* Britton & Rose ethanol extract with 20.000 ppm, 60.000 ppm, and 80.000 ppm. The same thing was done for the positive control treatment using chloramphenicol and the negative control using 0.5% DMSO solution. Next, it was incubated at 37°C for 24 hours, observed, and measured the inhibition zone formed.

Results and Discussion

The peel samples of *Hylocereus polyrhizus* Britton & Rose obtained by the drying process were aerated for 2-3 days, this aims to avoid damage to the secondary metabolites contained in the fruit peel of *Hylocereus polyrhizus* Britton & Rose [11, 15]. After drying the sample is reduced in size because the size of the material can affect the extraction efficiency. The large size of the simplicia will complicate the contact of the solvent with the components to be separated so that reducing the size of the simplicia will increase the surface area and the solvent penetrates more effectively [16, 17].

The maceration process was used to extract the fruit peel powder of *Hylocereus polyrhizus* Britton and Rose such that the components of the compound that are not heat resistant did not decompose. Furthermore, the maceration technique is a method whose equipment and methods are simple and affordable, and it allows for the extraction of a wide range of chemicals through the use of cold extraction [18]. The maceration extraction process was submerged in a solvent with *Hylocereus polyrhizus* Britton & Rose fruit peel powder. Stirring and re-maceration are used to improve the extraction's efficacy. The premise of maceration extraction is the binding or dissolving of the active component based on its solubility in a solvent (Like Dissolves Like). The solvent will enter the cell via the plant cell wall, causing the cell's contents to dissolve in the solvent because of the difference in concentration between the solution within and outside the cell. A low-concentration solvent will be introduced to replace the high-concentration solution (diffusion process). Until the fluid concentration within and outside of the cell is equal, this occurrence will continue [19]. The rendement ethanol extract of 96% peel of *Hylocereus polyrhizus* Britton & Rose obtained 7.74%. The yield value shows how large the amount of content extracted by the solvent is in percent (%) [15]. As for the factors that affect the extraction results with the maceration method and quality and duration, other factors also influence such as solvent concentration, type of solvent, temperature, extraction technique used, and the ratio of solvents [20].

Based on the results of phytochemical screening **(Table 1)** on a 96% ethanol extract of *Hylocereus polyrhizus* Britton & Rose fruit skin is positive for secondary metabolites namely alkaloids, flavonoids, polyphenols, steroids, terpenoids, and saponins, each of which has an antibacterial mechanism of action [9, 10]. As a result, antibacterial activity was evaluated against the development of *Staphylococcus epidermidis* bacteria in this investigation utilizing the good diffusion method. Creating a clear zone around the well indicates a microorganism's susceptibility to specific chemicals as in **Table 2** [21].

Table 1. Phytochemical Screening of 96% Ethanol Extract of *Hylocereus polyrhizus* Britton & Rose peel

Test	Method	Result
Alkaloids	Dragendrof	+
	Mayer	+
	Wagner	+
Flavonoids	Willstater	+
Steroids	Salkwoski	+
Terpenoids	Liberman buchard	+
Tannins	Gelatine test	-
Polyphenol	$FeCl_3$	+
Saponins	Foam test	+

Table 2. Inhibition Zone Diameter Data 96% Ethanol Extract Hylocereus polyrhizus Britton & Rose Peel

The concentration of 96% Ethanol Extract to Hylocereus polyrhizus Britton & Rose peel ($x10^3$ ppm)	Inhibition zone (mm) Replicate 1	2	3	Average ± SD (mm)
20	11.00	11.58	11.16	11.24 ± 0.29
60	13.00	14.16	14.83	13.90 ± 0.92
80	15.00	15.33	14.83	15.05 ±0.25
100	17.83	17.50	18.75	18.02 ±0.64
Positive Control 0,1% of Chloramphenicol	49.25	49.75	49.83	49.61 ± 0.31
Negative control 0,5% of DMSO	0.00	0.00	0.00	0.00 ± 0.00

Antibacterial activity of 96% ethanol extract of *Hylocereus polyrhizus* Britton & Rose fruit peel can be observed from the formation of inhibition zones in **Table 2**. The test results of 96% ethanol extract of *Hylocereus polyrhizus* Britton & Rose fruit peel

with concentrations of 20.000 ppm, 60.000 ppm, 80.000 ppm, and 100.000 ppm indicate that the extract can inhibit the growth of *Staphylococcus epidermidis with inhibition zone values of* 11.24 ± 0.29 mm, 12.44 ± 0.67 mm,15.05 ± 0.25 mm, and 18.02 ± 0.64 mm. This finding above is the same as the previous research by Amalia, who found that the hexane fraction of red dragon fruit peel had inhibitory zones of 11.17 mm and 12.80 mm, respectively [22, 23]. Another study by Aulia used the methanol fraction of red dragon fruit peel extract against *Staphylococcus aureus* bacteria with five different concentrations and had an inhibition zone of 9.5333 ± 0.26822 - 15.4167 ± 0.22048 mm [24]. The extract has an antibacterial effect that prevents the growth of Staphylococcus epidermidis bacteria through various mechanisms, such as alkaloid compounds that can compromise the integrity of the peptidoglycan constituent components in bacterial cells is made possible by the presence of secondary metabolites. Since the bacterial cell wall contains peptidoglycan, the presence of this disruption will prevent the cell wall layer from forming entirely and result in cell death [25]. The flavonoid compounds have antibacterial activity with three working mechanisms: inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting energy metabolism [26]. The content of terpenoid compounds can damage bacterial cell membranes [21]. Damage to cell membranes might result from active antibacterial substances reacting with the membrane's active site or lipid contents being dissolved and becoming more permeable. Protein molecules and phospholipids make up the membrane of bacteria. Antibacterial substances can enter a cell with increased permeability and lyse the cell membrane or cause the cytoplasm of the bacterial cell to coagulate [1].

The active content in the 96% ethanol extract of *Hylocereus polyrhizus* Britton & Rose fruit peel can suppress the growth of *Staphylococcus epidermidis* bacteria, according to the mode of action of each chemical. The higher the concentration of 96 percent ethanol extract of *Hylocereus polyrhizus* Britton & Rose fruit peel, the higher the quantity of antibacterial active chemicals, resulting in a larger ability to inhibit the growth of *Staphylococcus epidermidis*. According to Brooks, the quantity of antibacterial concentration impacts a material's potential to prevent bacterial growth [25].

Conclusion

Based on the discoveries of this study, it can be inferred that the 96% ethanol extract of *Hylocereus polyrhizus* Britton & Rose fruit peel has a substantial inhibitory result on the development of *Staphylococcus epidermidis* bacteria at the test of intense concentration.

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