Biomedical & Pharmacology Journal, September 2022. Vol. **15**(3), p. 1729-1735

Phytochemical Screening and Bacterial Activity of *Hylocereus polyrhizus* Britton and Rose Peel against *Staphylococcus epidermidis* and Staphylococcus aureus

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https://dx.doi.org/10.13005/bpj/2511

(Received: 28 July 2022; accepted: 02 September 2022)

Hylocereus polyrhizus Britton & Rose fruit is a tropical plant that is popular and widely cultivated due to its qualities and advantages and high nutritional content. Some substances in Hylocereus polyrhizus Britton & Rose are plant sources rich in nutrients and minerals, namely vitamin B complex and vitamin C, protein, fat, carbohydrates, fiber, flavonoids, niacin, pyridoxine, cobalamin, phenolics, betacyanins, polyphenols, and carotenoids. This study aimed to assess the bacterial activity of the 96% ethanol extract of Hylocereus polyrhizus fruit peel against the growth of Staphylococcus epidermidis and Staphylococcus aureus using the well diffusion method. Maceration was used as an extraction method, and a phytochemical screening procedure was carried out according to the process from Harbone. The bacterial activity was conducted by the well diffusion method. Phytochemical screening yields from 96% ethanol extract of Hylocereus polyrhizus fruit peel showed the presence of alkaloids, steroids, flavonoids, terpenoids, tannins, polyphenols, and saponins. The findings of the bacterial activity test of 96% ethanol extract of Hylocereus polyrhizus fruit peel with a concentration of 20%, 60%, and 80% (b/v) indicated intense antibacterial activity against Staphylococcus epidermidis and Staphylococcus aureus.

Keywords: Antibacterial; Hylocereus polyrhizus fruit peel; Staphylococcus aureus; Staphylococcus epidermidis; Well diffusion.

Many infections caused by *Staphylococcus aureus* and *Staphylococcus epidermidis* those 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. However, *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* that 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] Like *Staphylococcus epidermidis*, *Staphylococcus aureus* is a significant cause of infection worldwide.[5] These bacteria are mainly found on the skin, glands, mucous membranes, and wounds. Furthermore, those are generally the cause of sore throat, central nervous system infection, and lung with clinical manifestations like impetigo, scalded skin syndrome, pneumonia, and sepsis.[1,5] The study states that around 40–62% of antibiotics are misused, among others, for diseases that do not require antibiotics. The existence of antibiotic resistance encourages exploration to find alternatives, one of which is by using natural ingredients. One alternative natural ingredient that can be used as an antibacterial is (*the Hylocereus polyrhizus*) plant.[6]

Hylocereus polyrhizus Britton & Rose fruit is one of the fruits of the Cactaceae family originating from America, which has begun to be widely developed in Indonesia. *Hylocereus polyrhizus* Britton & Rose fruit is a tropical plant that is much favored by the public because it has efficacy and benefits and high nutritional value. The types of dragon fruit cultivated are white-fleshed (*Hylocereus undatus*) fruit, red-fleshed *Hylocereus polyrhizus* fruit, super red-fleshed *Hylocereus costaricensis* fruit, and yellow-skinned with white flesh *Selenicereus megalanthus* fruit.[7] *Hylocereus polyrhizus* fruit is a plant source rich in vitamins and minerals, namely vitamin B complex, and vitamin C, niacin, cobalamin, betacyanins protein, fat, carbohydrates, fiber, flavonoids, polyphenols, and carotenoids.[8] Part of the *Hylocereus polyrhizus* fruit, 30-35%, is the fruit's peel. *Hylocereus polyrhizus* fruit peel compounds contain saponins, terpenoids, tannins, and alkaloids.[9] Saponin compounds can inhibit the function of cell membranes by impairing membrane permeability, resulting in damaged or destroyed cell walls. At the same time, terpenoids bond with porins, transmembrane proteins, on the outer membrane of bacterial cell walls by molding a robust polymeric bond, causing damage to porins. This porin is the entrance and exit of compounds. As a result of the damaged pore structure, it will reduce the 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 alkaloids themselves have a mechanism to predispose to the peptidoglycan matter components in bacterial cells, causing the cell wall layer to be incompletely formed and leading to cell death.[10] *Hylocereus polyrhizus* Britton & Rose fruit is efficacious as an antioxidant, antibacterial, and a source of natural pigments.[11] Amalia showed that red dragon fruit peel was proven to have antibacterial activity on *S. aureus* (Gram-positive), containing secondary metabolites, alkaloids, and terpenoids.[12] This finding is reinforced by research by Khalili which showed that red dragon fruit peel extraction using disc diffusion agar had antibacterial activity on *S. epidermidis and S. aureus* (gram-positive) bacteria.[13] 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 inhibitor zone of 9.6 mm at a value of 60 mg/ml.[14] Romero, on the ethanolic extract of the *Hylocereus polyrhizus* fruit peel, stated that the extract had bacterial activity against *E. coli* with an inhibition zone of 28.24 mm during 24-hour incubation using the disc diffusion method.[15]

The considerable potential *for Hylocereus polyrhizus*, but exploration is still rare regarding its potential, and it is essential to conduct research to assess the concentration of 96% ethanol extract of *Hylocereus polyrhizus* Britton & Rose fruit peel which can hinder the growth of *Staphylococcus epidermidis* and *Staphylococcus aureus* bacteria by using the well diffusion method.

MATERIAL AND METHODS

Hylocereus polyrhizus Britton & Rose fruit peel taken from Pamekasan, Madura Island, 96% ethanol, pure culture suspension of *Staphylococcus epidermidis* and *Staphylococcus aureus*, NA media (Nutrient Agar) (Merck), 0.5% dimethyl sulfoxide (DMSO) (Merck), chloramphenicol (to test antibacterial activity) (Bratachem). The tools used in this research include analytical balance, glass jar, rotary evaporator, grinding tool, tweezers, a set of glass tools, cotton swab, vortex, petri dish, incubator, Laminar Air Flow (LAF), gloves, HCl 2 N (Merck), NaCl (Bratachem), H_2SO_4 (Merck),

HNO_3 (Merck), Toluene (Merck), n-Hexane (Merck), Chloroform (Merck), NH_4OH (Merck), $FeCl_3$ (Merck), methanol (Merck), glacial acetic acid were purchased from Merck, Mayer, and Wagner reagents, Dragendorf reagents, technical ethanol.

Extraction of *Hylocereus polyrhizus* Britton & Rose Fruit Peel

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

***Staphylococcus epidermidis* and *Staphylococcus aureus* Bacteria Suspension Preparation**

The bacterial suspension was made by placing ± 1 ose of *Staphylococcus epidermidis* and *Staphylococcus aureu*s inside a tube containing 5 mL of 0.9% natrium chloride solution until the turbidity was the same as standard Mc Farland's 0.5. The turbidity was measured by spectrophotometer (Shimadzu).[16]

Bacterial Activity Testing with the Well Diffusion Method

Bacterial 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 ìL of the *Staphylococcus epidermidis* and *Staphylococcus aureus* suspension had been added. The solidified nutrient agar media was made with holes using a 6 mm diameter perforator. The well was filled with 20 ìL of 96% *Hylocereus polyrhizus* Britton & Rose ethanol extract with 20% (20.000 ppm), 60% (60.000 ppm), and 80% (80.000 ppm). All procedures were conducted for the positive control treatment using chloramphenicol and the negative control using 0.5% DMSO solution. After that, it was incubated at 37°C for 24 hours, observed, and measured the inhibition zone that formed.

RESULTS AND DISCUSSION

The peel samples of *Hylocereus polyrhizus* Britton & Rose obtained by the drying process were aerated for 2-3 days, and this aims to avoid damage to the secondary metabolites contained in the fruit peel of *Hylocereus polyrhizus* Britton & Rose, and reduced the size of herbs.[17,18,19] The extraction of secondary metabolites occurred through the difference in concentration between the solution within and outside the cell, the solvent will enter the cell via the plant cell wall, causing the cell's contents to dissolve in the solvent.[20] A solution with a high concentration will be pushed out and substituted with a low-concentration solvent (diffusion process).[21] The rendement ethanol extract of 96% peel of *Hylocereus polyrhizus* Britton & Rose obtained 7.7434%. This value was affected by many factors like the maceration method and quality and duration, and other factors also influenced, namely solvent concentration, type of solvent, temperature, extraction technique used, and the ratio of solvents.[11,16]

Based on the results of phytochemical screening on a 96% ethanol extract of *Hylocereus polyrhizus* Britton & Rose fruit peel, positive for secondary metabolites such as alkaloids, flavonoids, polyphenols, steroids, terpenoids, and saponins, as listed in table 1, each of which has an antibacterial mechanism of action.[9,10,22] This is in line with the study by Amalia, which stated that the hexane fraction of dragon fruit peel contains alkaloids, polyphenols, flavonoids, saponins, terpenoids, steroids, and tannins.[12] Another study by Elfi showed that the methanolic extract of white dragon fruit peel contains triterpenoid compounds, alkaloids, flavonoids, and saponins.[23,24]

As a result, the antibacterial activity of 96% ethanol extract of *Hylocereus polyrhizus* Britton & Rose fruit peel indicated that the extract had potential bioactivity as an antibacterial against the *Staphylococcus epidermidis* and *Staphylococcus aureus* bacteria by using the well diffusion method as shown in table 2.[25,26] The test results of 96% ethanol extract of *Hylocereus polyrhizus* Britton & Rose fruit peel against *Staphylococcus aureus* at doses of 20.000 ppm, 60.000 ppm, and 80.000 ppm showed inhibition zones of 11.24 ± 0.29 mm, *13.90* ± 0.92 mm, 15.05 ± 0.25 mm, respectively. The data displayed 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.[12] 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.[37] The second finding was related to the antibacterial activity of *Hylocereus polyrhizus* Britton & Rose fruit peel against *staphylococcus epidermidis* bacteria with a concentration of 20.000 ppm, 60.000 ppm, and 80.000 ppm showing inhibition zones of 11.22 ± 0.34 mm, 13.42 ± 0.52 mm, 15.25 ± 0.23 mm. Furthermore, it follows research by Khalili, which stated that the inhibition zone produced by methanol extract of dragon fruit peel was 19.00 ± 0.43 mm.[13] Another study by Rahayu used red dragon fruit peel extract against *Salmonella pullorum* bacteria with concentrations of 100% and 50% ppm, and 60 ppm had inhibition zones of 10.34 mm and 8.57 mm, respectively.[16]

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	+

The presence of secondary metabolites allows the extract to have an antibacterial effect that inhibits the growth of *Staphylococcus epidermidis* and *Staphylococcus aureus* bacteria.[26,227] The secondary metabolite compound of the alkaloid can disrupt the integrity of the peptidoglycan components in bacterial cells wall. Peptidoglycan is a component of the bacterial cell wall, so the presence of this disturbance will cause the cell wall layer not to be completely formed and cause cell death.[28,29] These differences in inhibition zone could be influenced by the vary of factors such as secondary metabolites content, the solvent of extraction, extraction method, plant origin, plant age, and others. [19,20,21,37]

The flavonoid compounds have antibacterial activity with three working mechanisms: hindering synthesis of nucleic acid, hindering membrane cell function, and energy metabolism. The mechanism of action of flavonoids in inhibiting nucleic acid synthesis is carried out through ring B on flavonoids which have a primary function in the intercalation process or hydrogen bonding by accumulating nucleic acid bases that hinder DNA and RNA synthesis.[1,27,30] The second mechanism of flavonoids inhibiting membrane cell function occurred by involving the interactions between flavonoids and lipid bilayers, which include the interactions of membrane interface.[31] Reducing adenosine triphosphate (ATP) can be affected by F – type ATP synthase (atpD) via oxidative phosphorylation.[32] The content of

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) of *Staphylococcus epidermidis* (in triplicate)			Average ± SD (mm)	Inhibition zone (mm) of *Staphylococcus aureus* (in triplicate)			Average ± SD (mm)
20	11.00	11.58	11.16	11.24 ± 0.29	11.50	10.83	11.00	11.22 ± 0.34
60	13.00	14.16	14.83	13.90 ± 0.92	12.83	13.83	13.60	13.42 ± 0.52
80	15.00	15.33	14.83	15.05 ±0.25	15.60	16.00	15.58	15.25 ± 0.23
Positive Control 0,1% of Chloramphenicol	49.25	49.75	49.83	49.61 ± 0.31	45.60	45.40	45.70	4.56 ± 0.15
Negative control 0,5% of DMSO	0.00	0.00	0.00	0.00 ± 0.00	0.00	0.00	0.00	0.00 ± 0.00

terpenoid compounds can damage bacterial cell membranes.[25,35,36] The damage to the cell membrane can occur when active antibacterial compounds interact with the active site of the membrane or by dissolving lipid constituents and mounting their permeability.[1,28] Like others compounds, saponins are extracted chemicals that are efficient against gram-positive bacteria. Saponins' antibacterial action method involves terracing the permeability of the cell membrane, causing the membrane of bacteria to become unstable and resulting in cell hemolysis.[25,32,33]

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 active antibacterial chemicals, resulting in a larger capability to inhibit the growth of *Staphylococcus epidermidis and Staphylococcus aureus*. According to Brooks, the quantity of antibacterial concentration impacts a material's potential to prevent bacterial growth.[26,30,31]

Even though the extract showed great antibacterial activity against both bacteria, our study had limitations, and the study has not determined the minimum inhibitory concentration, which compound has the greatest concentration and did not conduct statistical analysis among those concentrations.

CONCLUSION

Based on the findings of this research, it can be concluded that the 96% ethanol extract of *Hylocereus polyrhizus* Britton & Rose fruit peel has a substantial inhibitory effect on the development of *Staphylococcus epidermidis* and *Staphylococcus aureus* bacteria at strong concentration.

ACKNOWLEDGMENT

The authors would like to express their gratitude to Lunardhi Susanto, Liza Yudhistira, and Ana Khusnul Faizah for their contributions to and construction of this article. We also appreciate the financial assistance the University of Hang Tuah in Surabaya provided.

Conflict of interest

The authors declare no conflicts of interest, financial or otherwise.

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