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The Antibacterial Effects of *Hylocereus polyrhizus* Fruit Extracts against Selected Bacteria

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ABSTRACT

In recent years, the growing number and active spread of antibiotic resistance have become a major concern globally. This forces the need to discover, analyse and develop new kinds of antibiotics, especially among plants. There are still limited data on the extracts from Hylocereus polyrhizus fruit as antimicrobials. In this study, the disc diffusion and broth-microdilution methods are used to investigate the antimicrobial activity of methanol and ethanol extracts of Hylocereus polyrhizus flesh towards selected bacteria (Staphylococcus epidermidis, Staphylococcus aureus, Proteus mirabilis, Bacillus cereus, Pseudomonas aeruginosa, and Escherichia coli). The methanolic extract possesses better antimicrobial properties. The methanolic extract of H. polyrhizus showed significant antimicrobial activities against all Gram-positive bacteria, and one of the Gram-negative bacteria, which is better compared to ethanolic extract. The range of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are 125mg/mL to 250mg/mL. In conclusion, this study shows that H. polyrhizus could be used as an alternative for the pre-existing antimicrobial agent.

Keywords: *antibacterial; Hylocereus polyrhizus; extract; skin; pathogen.*

1. INTRODUCTION

Nature has always been the main resource that caters to every human need ranging from shelters, foods, tools, and in this case, medicines. In 1985, The World Health Organization (WHO) estimated that almost 65% of the world population primarily depended on plant-derived traditional medicines for their primary healthcare [1]. The antimicrobial properties and antioxidants of plant and fruit extract have become the foundation of many uses like pharmaceuticals, food preservatives, natural therapies, and medicine [2]. The antimicrobial properties of plant extracts are connected to phenolics, alkaloids, terpenoids, lectin, essential oils, polypeptide, and others. The extract with the most abundant antimicrobial compound would be the phenolic group compounds [3]. Fruits are rich sources of nutrients and energy, have vitamins, minerals, fiber, and numerous other classes of biologically active compounds [4]. Moreover, many fruit extracts have been proven to be successful at killing or inhibiting the growth of bacteria [5].

In recent years, the growing number and active spread of antibiotic resistance have become a major concern globally [6]. Unceasing misuse and overuse of drugs by the public contributeto the emergence of antibiotic resistance [7]. Bacteria become immune to the same drug designed to treat and heal the disease they cause. Resistance will always be inevitable due to drug

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selection pressure even if antibiotics are prescribed and consumed aptly. This forces the need to discover, analyse and develop new kinds of antibiotics [8]. Natural sources are the new alternative that is used in producing drugs as a treatment for diseases. Despite the array of synthetic drugs out there, plants usually become the main choice among the natural sources when formulating new antibiotics to counter the resistance in infective agents.

Hylocereus polyrhizus (H. polyrhizus) or *Hylocereus costaricensis* [9] is a species of the *Cactaceae* family that is known as the Red Pitaya or dragon fruit. They are mainly cultivated in the subtropical and tropical regions worldwide and originated from Central and Nothern South America [10]. Previously, a study was conducted on the antioxidant properties of the Pitaya flesh and peel [11]. The study suggested that the red-purple pigment of the *H. polyrhizus* fruit might protect against oxidative stress-related disorders. The red dragon fruit also contains betacyanin alkaloid, which is the red-pigmented betalain and possesses antibacterial contents [12]. The antibacterial activities of alkaloids are exhibited through intercalating into the cell walls and DNA of bacteria [13]. Another study also showed that the extracts of red dragon fruit peels can heal wounds with effective antimicrobial properties when tested with chloramphenicol against *P. aeruginosa* [14].

Despite numerous studies done on the phytochemicals of the *Hylocereus* sp. plant, there are still limited data on the extracts from *H. polyrhizus* fruit. Thus, the study aims to determine the antibacterial activity of ethanolic and methanolic extracts of *H. polyrhizus* flesh against selected skin pathogens.

2. METHODOLOGY

2.1 Preparation of H. polyrhizus Fruit Extract

H. polyrhizus fruits were collected from the local market in Sepang, Selangor. The species of plant was identified by looking at a photograph on a website dedicated to plants [15]. The fruits were carefully washed 2-3 times with distilled water and dried with a soft cloth. The flesh was cut into tiny pieces and then ground into pulps with a mechanical blender. The samples were weighed, stored in a Schott bottle, and maintained at 4°C until ready to be used for extraction.

With some modification from [14], the extraction process was done using a ratio of 1:3 for *H. polyrhizus* flesh to solvent. 200 grams of *H. polyrhizus* flesh were macerated into 600 mL of 95% methanol and 95% ethanol in 1000 mL Schott bottles covered with aluminum foil. Both samples were kept at room temperature for 7 days with occasional shaking.

After 7 days, both solutions were filtered with cotton wool and once again using Whatman No. I filter paper. The filtrate was then transferred into a flask and was concentrated using a rotary evaporator under reduced pressure at 40°C. The crude methanolic and ethanolic extracts of *H. polyrhizus* fruit were transferred into Schott bottles and stored at 4°C until further use.

Ethanolic and methanolic extracts of *H. polyrhizus* fruit with concentrations of 1000mg/mL were prepared using 10% dimethyl sulphoxide (DMSO). A 1000mg of both extracts respectively, were added to 1ml 10% DMSO to obtain the concentration of 1000mg/ml.

2.2 Evaluation of Antibacterial Activities

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2.2.1 Bacterial Culture, Identification, and Confirmation

The tested bacteria from the stock culture were separately cultured onto blood agar and nutrient agar at 37°C for 18-24 hours. The morphologies of the colonies were recorded, and each colony was isolated with a sterilized wire loop. The proliferated colonies were used for confirmation test; Gram stain, biochemical set, and cultured on differential agar. A total of 6 types of bacteria were selected in this study for the antimicrobial susceptibility testing (AST), minimum inhibition concentration (MIC), and minimum bactericidal concentration (MBC) tests. The selected bacterial strains consist of gram-positive (*Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 25923), *Proteus mirabilis* (ATCC 35659), and *Bacillus cereus* (ATCC 11778)) and gram-negative bacteria (*Pseudomonas aeruginosa* (ATCC 14149), and *Escherichia coli* (ATCC 25922)).

2.2.2 Antimicrobial Susceptibility Testing (AST)

The disc diffusion method was used for the determination of the inhibition zone, 3-5 colonies of each microorganism from the nutrient agar plate were suspended in Mueller-Hinton broth (MHB) and incubated at 37°C. The turbidity adjustment of the broth was performed visually by comparing it with 0.5 McFarland standard on a white background in the presence of enough light. A sterile cotton swab is used to streak the MHB of inoculum of the tested bacteria on Mueller-Hinton agar plates respectively. Four discs (ethanolic fruit extract, methanolic fruit extract, positive and negative control) were placed onto each plate. The standard antibiotics used as positive controls were gentamicin (30 µg), tetracycline (30 µg), and chloramphenicol (30 µg), while 10% DMSO was selected as a negative control. The plates were incubated at room temperature of 37°C for 18-24 hours. The zones of inhibition were then measured to obtain the antimicrobial activity and the tests were done in triplicate.

2.2.3 Determination of the Minimum Inhibition Concentration (MIC) by broth microdilution method

The MIC for the extract was evaluated according to the method described by [17] with minor modification using a 96-well microtiter plate. The first to the ninth well of 96 microtiter plate were pipetted with 100 µL of bacterial suspension of suspension CFU/mL based on log phase of each organism and 100 µL of specified dilution of ethanol and methanol extract in triplicate. Positive growth control (MHB with bacteria), and negative control (broth only) were dispensed into the last two remaining wells. For 18-24 hours, the plate was incubated aerobically at 37°C. The appearance of turbidity in solution from each well is compared with that in the positive growth control and the MIC value was recorded as the lowest concentration of extract that fully inhibits growth. The experiment included a negative control to ensure that no contamination occurred.

2.2.4 Determination of the Minimum Bacterial Concentration (MBC)

MBC is defined where no growth of bacteria is observed from the lowest concentration resulting from MIC. This is decided by subculturing the contents of the wells aseptically from the MIC outcomes for an antimicrobial-free individual bacterium agar, as indicated in [18]. MBC test is conducted to validate the result of the MIC test. It is done by streaking each well's aliquot, including positive and negative control over MHA. After incubating aerobically at 37°C for 18-24 hours, the dilution with 99.9% of the organisms dead was determined as the MBC.

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2.3 Data Analysis

The inhibition zone values were expressed as mean ± standard deviation (SD) of three replicates. Paired sample T-test was performed in this study to compare the antibacterial effect between methanolic and ethanolic extracts against each tested bacteria. *P* values less than 0.05 were considered statistically significant.

3. RESULT AND DISCUSSION

3.1 Antimicrobial Susceptibility Testing (AST)

The mean of inhibition zones diameter of the ethanolic and methanolic extracts of *H. polyrhizus* against the selected bacterial strains in triplicate are shown in Table 1.

Table 1: The inhibition zones (mm) of *H. polyrhizus* extracts against selected bacterial strains.

	Zone of inhibition in mm (Mean±SD)				
Organism	Positive control (Standard antibiotics)	Negative control (10% DMSO)	Methanolic extract (1000mg/mL)	Ethanolic extract (1000mg/mL)	P-value
S. aureus (ATCC 25923)	24.0±0.00 Tetracycline (30µg)	0.0±0.00	9.1±0.17	14.7±1.15	0.016
S. epidermidis (ATCC 12228)	28.0±0.00 Gentamicin (30µg)	0.0±0.00	10.0±1.00	8.1±0.17	0.083
B. cereus (ATCC 11778)	24.0±0.00 Chloramphenicol (30µg)	0.0±0.00	12.9±0.12	0.0±0.00	0.000
E. coli (ATCC 25922)	26.0±0.00 Chloramphenicol (30µg)	0.0±0.00	14.1±0.12	0.0±0.00	0.000
P. mirabilis (ATCC 35659)	22.0±0.00 Gentamicin (30µg)	0.0±0.00	0.0±0.00	15.7±0.58	0.001
P. aeruginosa (ATCC 14149)	21.0±0.00 Gentamicin (30µg)	0.0±0.00	0.0±0.00	0.0±0.00	-

The methanolic and ethanolic extracts of *H. polyrhizus* have a significant difference in antibacterial effect against all the tested bacteria except for *P. aeruginosa*. The methanolic extract of *H. polyrhizus* revealed the highest activity against *E. coli* (14.1 ± 0.12 mm), followed by *B. cereus* (12.9 ± 0.12 mm), *S. epidermidis* (10.0 ± 1.00 mm), and *S. aureus* (9.1 ± 0.17 mm). Although *P. mirabilis* was resistant to methanolic extract, it was susceptible to the ethanolic extract (15.7 ± 0.58 mm). Besides that, the ethanolic extract is also effective against *S. aureus* (14.7 ± 1.15 mm) and *S. epidermidis* (8.1 ± 0.17 mm). The gram-negative bacteria group was found to be more susceptible compared to gram-positive bacteria. This may be due to the existence of lipopolysaccharide as an important barrier to the gram-negative bacteria's permeability [19,20]. The result also showed that the growth of *P. aeruginosa* was not inhibited by any of the extracts. Previous reports [21,22] found that the existence of AmpC β-lactamase and an active efflux pump allows *P. aeruginosa* to be able to develop additional mechanisms of resistance to several classes of antimicrobial agents.

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Paired t-Test was chosen in this study to observe the significant difference between the antimicrobial activity of methanol and ethanol extracts of *H. polyrhizus* against *S. aureus, S. epidermidis, B. cereus, E. coli, P. mirabilis*, and *P. aeruginosa*. The statistical analysis indicated that there was a significant difference between both methanol and ethanol extracts against *S. aureus, B. cereus, E. coli*, and *P. mirabilis* as P value (< 0.05) indicates both extracts have different antimicrobial activity. However, there was no significant difference between both extracts against *S. epidermidis* since the P value (> 0.05) indicates it possessed similar antimicrobial activity. As for *P. aeruginosa*, paired t-Test cannot be done to compare methanol and ethanol extract of *H. polyrhizus* towards *P. aeruginosa* due to no zone of inhibition which indicates that no antimicrobial activity was present.

3.2 Minimum Inhibition Concentration (MIC) and minimum bactericidal concentration (MBC)

Based on Table 2, *S. epidermidis* was inhibited in the methanolic and ethanolic extracts at the MIC value of 125 mg/mL. This was followed by *S. aureus* which was inhibited in the extracts at the MIC value of 250 mg/mL and 125 mg/mL, respectively. Meanwhile, the methanolic fruit extract of *H. polyrhizus* was effectively inhibiting both *B.cereus* and *E.coli* at a concentration of 125 mg/mL. For gram-negative bacteria, *P. mirabilis* had a higher MIC value of 250 mg/mL in the ethanolic extract as compared to other strains.

Table 2: Mean of triplicate MIC & MBC values for *H.polyrhizus* extracts against selected bacterial strains.

Bacteria	MIC (mg/mL)				MBC (mg/mL)			
	Methanolic extract	Ethanolic extract	Positive control	Negative control	Methanolic extract	Ethanolic extract	Positive control	Negative control
S. aureus (ATCC 25923)	250	125	T	C	250	250	G	N
S. epidermidis (ATCC 12228)	125	125	T	C	125	500	G	N
B. cereus (ATCC 11778)	125	-	T	C	125	-	G	N
E. coli (ATCC 25922)	125	-	T	C	125	-	G	N
P. mirabilis (ATCC 35659)	-	250	T	C	-	250	G	N

Clear (C) = indicates the bacterial growth is inhibited or killed and Turbid (T) = indicates that bacteria are not inhibit.
Growth (G) = indicates growth of organism and No growth (N) = indicates organism inhibited or killed.

MIC and MBC were done to exhibit the bactericidal properties of the extracts as they show the concentration at which the growth of bacteria is inhibited. For bactericidal effect, the methanolic fruit extract of *H. polyrhizus* kills *S. epidermidis*, *B. cereus*, and *E.coli* at the minimum concentration of 125mg/mL. An antibacterial agent is recognised as bacteriostatic when the ratio of MBC to MIC is >4 [23]. The MBC value of ethanolic extract against *S.*

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epidermidis was two times greater (500 mg/mL) than *S. aureus* and *P. mirabilis* (250 mg/mL). The MBC is typically equal to or higher than the MIC [24]. In this study, the overall data demonstrated that the MBC values were the same for *S. epidermidis, B. cereus,* and *E.coli* and were two times higher for *S. aureus* and *P. mirabilis* compared to the MIC values. However, the MIC and MBC tests for *B. cereus* and *E. coli* in ethanolic extract and *P. mirabilis* in the methanolic extract were not performed as no zone of inhibition was observed during the earlier test.

The methanolic and ethanolic extracts of *H. polyrhizus* showed effective antimicrobial activity, especially against the gram-positive bacteria compared to gram negative. This is because the gram-positive bacteria cell wall is only composed of peptidoglycan, teichoic acid with the absence of the outer membrane and porins protein. On the other hand, the chemical compositions of gram negative bacteria cell walls consist of lipopolysaccharide, lipoprotein, peptidoglycan, and porins proteins that are present at the outer membrane [25]. As a result, gram negative bacteria are more resistant to compounds or molecules from the extract that can permeate into the bacterium's inner membrane, causing membrane disruption owing to the cell wall's complex chemical makeup. Besides, gram negative bacteria have more lipids on their walls, making them less permeable than gram positive bacteria. The antibacterial activity of *H. polyrhizus* against the gram-positive and gram-negative bacteria show in this study is supported by previous reports where plant extracts had affected the cell wall of the bacteria such as *S. aureus, E. coli,* and *K. pneumoniae*. The treatment of the bacteria with methanolic and ethanolic extracts causes deformities in cells where they have become sticky and crumbled relative to control cells [26,27].

Our results are higher in comparison to other studies [14,20,28]. Exposure to light during the extraction process can cause the degradation of bioactive compounds which leads to a decrease in antimicrobial activity. Besides, the duration of storage of the crude extract may also play a role in affecting the result. When the storage of extraction is more than 5 days it can reduce the potential of antimicrobial activity by approximately 10-25% against bacteria [29]. These factors may have contributed to the higher MIC and MBC values when tested with respective extracts.

In this study, the extraction solvent of methanol and ethanol was chosen as they have high polarity index which is 5.1 and 5.2, respectively. Polar solvents are chosen because they are prone to recover more compounds or constituents from plant material. Antimicrobial activities and the ability to extract bioactive compounds from plants are influenced by the polarities of extraction solvent [30, 31] found that higher content of phenolic compounds is found in methanol due to the high solubility of polyphenols in the solvent. It is observed that methanol extract of *H. polyrhizus* shows great antimicrobial activity in comparison to the ethanol extract. According to [32], among the different solvent extracts studied, methanol and ethanol showed high degrees of inhibition, followed by other solvents such as petroleum, ether, and aqueous extract. This finding also was supported by other studies which have reported that methanol is a better solvent extract of the plant compared to other solvents such as aqueous [33]. However, ethanol has also been recognised as a good solvent for polyphenol extraction [34].

Furthermore, phytochemical compounds such as alkaloids, flavonoids, tannins, triterpenoids, essential oils, glycosides, phenols, and saponins are commonly associated with antimicrobial activity and fighting microbial resistance in medical plants [35]. Several studies showed that *H.*

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polyrhizus extract contains phytochemical compounds such as tannin, alkaloids, flavonoids, and phenolic acids that might be responsible for combating microbial infection [36].

4. CONCLUSION

The methanolic and ethanolic fruit extracts of *H. polyrhizus* showed effective antimicrobial activity, especially against the Gram-positive bacteria. It can be used as a treatment against skin pathogens given the efficacies against the bacteria tested. It is observed that methanolic extract of *H. polyrhizus* inhibits all Gram-positive bacteria in this study, which is better than ethanolic extract. The ethanolic extract inhibits *P. mirabilis* while methanolic extract inhibits *E. coli* among the Gram-negative bacteria. *P.aeruginosa* is the only test organism that had no inhibition zone from both extracts. The methanol extract inhibits the bacteria in this study at a lower concentration compared to ethanol extract which could be seen from the MIC and MBC test results. This suggests that the methanolic extract possesses better antimicrobial properties. The presence of betacyanins, flavonoids, and phenolic acids might also be linked with the antibacterial activities of *H. polyrhizus*.

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