Exploring the phytochemical and antioxidant potential of *Hylocereus polyrhizus* peel extract using biochemical approach

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**Abstract.** Red dragon fruit or *Hylocereus polyrhizus* is one of the most popular fruits in Indonesia. Besides being consumed directly, *H. Polyrhizus* processed into various forms of processed food products such as jams, syrups, sweets, tea, and functional drinks. Unfortunately, massive quantities of solid waste, including *H. polyrhizus* peel produced every year, continues to increase from year to year. Their disposal led to severe environmental issues. Whereas, *H. polyrhizus* peels are abundant in beneficial secondary metabolites compound especially flavonoid and phenolic. The presence of flavonoid and phenolic content provides many benefits in the development of natural medicines, especially as antioxidants. However, the research related to exploring antioxidant potentials of *H. polyrhizus* peel is still very limited. This study aimed to explore the phytochemical of *H. polyrhizus* peel and their role as a natural-antioxidant agent. *H. polyrhizus* peels were extracted through a maceration method using 96% of ethanol as their solvent. A total phenolic essay is determined by the method of Folin-Ciocalteu reagent using gallic acid as a reference. AlCl$_3$ reagent is used to analyse the flavonoid content by comparing with quercetin. Antioxidant activity was done by DPPH and ABTS free radical scavenging methods. The total phenolic and flavonoid content of *H. polyrhizus* peel extract (HPPE) at 107.35 ± 8.02 mg GAE/g and 108.82 ± 12.69 mg QE/g respectively. Furthermore, antioxidant activity of HPPE showed IC$_{50}$ value at 136.20 ± 0.70 μg/ml μg/ml with DPPH methods and 390.70 ± 1.25 μg/ml μg/ml with ABTS methods. Based on this recent study, HPPE has a moderate antioxidant activity by reducing free radicals in dose dependent manner.

1. **Introduction**

Red dragon fruit or *Hylocereus polyrhizus* is not a native fruit of Indonesia. It natively comes from Mexico, North America and South America [1]. Last few decades, *H. polyrhizus* began to be widely cultivated in Indonesia. The climate and soil texture conditions in Indonesia are very suitable for the development of *H. Polyrhizus*. *H. polyrhizus* was first cultivated in Jember, Malang, and Pasuruan, Indonesia [2]. *H. polyrhizus* is one of the most popular fruits in Indonesia. Thus, the development of the dragon fruit-based food industry continues to grow rapidly. Apart from being consumed directly *H. polyrhizus* is generally processed into various types of processed foods such as jams, functional drinks, and tea [2-3].

In addition to the pulp, the anthocyanins contained in *H. polyrhizus* are commonly used as natural coloring agents in the food and textile industry [3]. Moreover, *H. polyrhizus* peels are abundant in beneficial secondary metabolites compounds especially flavonoid and phenolic. *H. polyrhizus* peel also
contains several vitamins and other secondary metabolites such as alkaloids, terpenoids, thiamine, niacin, pyridoxine, cobalamin, phenolic, carotene, and phytoalbumin. These whole secondary metabolites contained in \textit{H. polyrhizus} peel are synergistically responsible for many pharmacological activities that have a positive impact on human health, specifically antioxidant, anti-aging and antimicrobial [1-5]. Unfortunately, there is very limited research related to the antioxidant properties of \textit{H. polyrhizus} peel. Furthermore, massive quantities of solid waste, including \textit{H. polyrhizus} peel produced every year, continues to increase from year to year. The food processing industry is one of the biggest contributors to this type of waste. Their disposal led to severe environmental issues [6].

Based on their low utilization aspect and the high beneficial aspect, it is deemed necessary to explore more deeply about the utilization of \textit{H. polyrhizus} peel as a natural-based antioxidant agent. Excessive amounts of free radicals accumulated in the human body can induce cells and tissue damage and end up to apoptosis. This accumulation of free radicals contributes to many diseases such as cancer, cardiovascular disease, premature aging, and many others [7-15]. Hence, this recent study aimed to explore the potentials of \textit{H. polyrhizus} peel extract for their antioxidant properties. In this regard, this study will be very important for increasing the economic value of \textit{H. polyrhizus} peel.

2. Material and Methods

2.1 Sample collection

\textit{H. polyrhizus} peel that has been collected and separated from its pulp, washed under running water then drained to remove the remaining water that was still stuck to the surface of the peel. Clean dragon fruit peel was then cut into smaller pieces and dried at the controlled temperature (37°C \text{–} 40°C). Drying was carried out until a constant sample weight was obtained. Dried peel then blended until it becomes a powder.

2.2 Crude extract preparation

Extraction was carried out using the cold extraction method and adapted from Rofida and Nurwahdaniati (2015) [16]. Cold extraction was carried out by soaking the powder for 3 x 24h in an enclosed glass jar. Solvent used for extractions was 96% ethanol in the ratio of 1:10 (powder : ethanol). The supernatant from each repetition was collected into one container and concentrated with a rotary evaporator at a maximum temperature of 50°C to obtain crude extract.

2.3 Assessment of total phenolic content

Assessment of the total phenolic content was conducted by referring to the method that has been carried out by Prasedya et al. (2021). Approximately 20 μL (1 mg/mL) of \textit{H. polyrhizus} peel extract (HPPE) were put in a 96 well plate well and 100 μL of Folin-Ciocalteu's solution was added. Homogenized and incubated for 5 minutes at room temperature. To the same well, it was added sodium carbonate (re-incubate for 60 min). After 60 min, the absorbance was determined under UV-Vis Spectrophotometer (Multiskan GO Microplate Reader Thermo Fisher Scientific) at 725 nm. Total phenolic content is expressed in mg gallic acid equivalent per gram extract (mg GAE/g of EL) [17]

2.4 Assessment of total flavonoid content

A total of 25 μL of the sample was combined and mixed with 125 μL of aquades and sodium nitrite, the mixture was incubated for 5 minutes while continuously shaking to make it completely homogeneous. In the same well, 15 μL of 10% aluminum chloride (AlCl3) solution was added and re-incubate for 50 minutes. After 50 minutes, 1 M sodium hydroxide (NaOH) was added to each sample well. The entire test solution was read for absorbance with a UV-Vis spectrophotometer at a wavelength of 510 nm [17]
2.5 DPPH anti-scavenging assay
Sample with various concentrations (50 – 5000 μg/mL) was combined and mixed with DPPH solution in ratio 1:1. Then, incubate for 30 minutes (25°C, keep away from light exposure directly). After 30 min, the absorbance was determined under UV-Vis Spectrophotometer (Multiskan GO Microplate Reader Thermo Fisher Scientific) at 517 nm [17].

2.6 ABTS anti-scavenging assay
Sample with various concentrations (50 – 5000 μg/mL) was combined and mixed with ABTS solution in ratio 1:1. Then, incubate for 6 minutes at room temperature and keep away from light exposure. The absorbance of the sample was measured at a wavelength of 734 nm using UV-Vis spectrophotometer (Multiskan GO Microplate Reader Thermo Fisher Scientific) [17].

2.7 Statistical analysis
All experiments were carried out with 3 repetitions and data were presented with mean ± SD to evaluate the significant difference between every repetition. Data were further analyzed by one-way ANOVA and Tukey’s HSD posthoc with a 95% of confidence level. A significant value of <0.05 was considered statistically different. The IC50 value and graphs were generated with Graphpad Prism software (v9.1.10).

3. Results and Discussion
The extraction process by maceration method using 96% ethanol solvent causes the crude extract becoming dark green and 2.5% of rendement was obtained from the process. The total phenolic content of HPPE using the Folin-Ciocalteu reagent was analyzed using UV-Vis spectrophotometer. Phenolic compounds will react with the Folin-Ciocalteu reagent and form a blue compound complex with appropriate color intensity with the presence of phenolic compounds. The standard used is gallic acid which is a stable derivative complex of hydroxybenzoic acid [19]. Standard solution measurement results gallic acid obtained a calibration curve with the equation $y = 0.004x + 0.059$ ($R^2 = 0.996$). The total phenolic content is expressed as Gallic Acid Equivalent (GAE) because the specific chemical structure of the phenolic compounds present in HPPE is unknown. Based on this study, the total phenolic content of HPPE equals 107.35 ± 8.02 mg GAE/g, which means that in every gram of extract is equivalent with 107.35 mg gallic acid (Table 1.)

The total phenolic content in the extract depends on the polarity of the solvent used in the extraction. High solubility of phenolic compounds in polar solvents provide a high concentration of phenolic obtained in HPPE which is extracted using ethanol as polar solvent (Table 1.). Research conducted by Prasedya et al (2021) revealed that the higher total phenolic content, gives a higher free radical scavenging activity of DPPH respectively [17]. Phenolic compounds in several studies provide antioxidant activity due to their ability to reduce reactive oxygen. This is because the aromatic ring of phenolic compounds has several hydroxyl groups that act as hydrogen donors [18].

The total flavonoid content of HPPE was determined by the method of forming a complex between aluminum chloride with a keto group on the C-4 atom and a hydroxyl group on the C-3 or C-5 atoms that are neighbors of the flavone and flavonol groups. Quercetin was used as the standard for determination of flavonoid levels because quercetin belongs to the group flavonols which have a keto group at the C-4 atom and also hydroxyl groups on neighboring C-3 and C-5 atoms. Standard solution measurement results quercetin obtained a calibration curve with the equation $y = 0.005x + 0.068$ ($R^2 = 0.999$). The data in Table 1 shows that the HPPE contains high levels of flavonoids in the amount of 108.82 ± 12.69 mg equivalent to quercetin per gram (mg QE/g). The total flavonoid content obtained correlated with the phenolic content in HPP because they belong to the same class of compounds. Based on several previous studies, it is known that
flavonoid intake above 100 mg has antioxidant activity equivalent to 2-3 mg of beta carotene, 70-100 mg of vitamin C, 7-10 mg of vitamin E.

**Table 1.** Total phenolic and total flavonoid content of *H. polyrhizus* peel extract (HPPE)

| Result equivalent to standard | Documentation | Description |
|-------------------------------|--------------|-------------|
| Total phenolic content        | ![](image) | This figure shows the final product of Folin-Ciocalteu's reaction with the extract which forms a blue colored complex in 96 well microplate. The intensity of blue color is directly proportional to the phenolic content present in the extract. |
| Total flavonoid content       | ![](image) | This figure shows the final reaction product of aluminum chloride with a keto group reaction from the extract which forms a yellow to orange colored complex in 96 well microplate. The intensity of blue color is directly proportional to the flavonoid content present in the extract. |

The DPPH method is a commonly used method for testing antioxidant activity on natural ingredients or plant extracts. Antioxidants are electron donor compounds and have small molecular weights, but are able to inactivate the development of oxidation reactions, by preventing the formation of free radicals. Exploring antioxidant activity with the DPPH radical is based on the reduction of the purple color of DPPH radical compound to yellow due to the hydrogen transfer by antioxidant compounds so that the DPPH radical becomes stable [18]. The results obtained show that the HPPE gave a moderate inhibition with an IC50 value of 136.20 ± 0.70 μg/ml (Fig 1).

**Figure 1.** DPPH free radicals scavenging activity of HPPE. Antioxidant capacity of HPPE is significantly different compared to vitamin C (IC50 14.53 ± 2.02 μg/mL). Nevertheless, HPPE has moderate antioxidant activity by reducing DPPH free radicals in dose dependent manner [18]
Figure 2. ABTS free radicals scavenging activity of HPPE. Based on the IC$_{50}$ value, it showed that HPPE has an IC$_{50}$ value of 390.70 ± 1.25 μg/ml.

In this study, that was also used to determine the antioxidant activity of HPPE. This method uses the principle of inhibition, namely the sample is added to a free radical generating system and the effect of inhibition on the effect of free radicals is measured to determine the total antioxidant capacity of the sample [20]. The results obtained show that the HPPE gave a moderate inhibition with an IC50 value of 390.70 ± 1.25 μg/ml (Fig 2).

4. Conclusion
Based on this recent study that has been done it can be concluded that HPPE has a high potential to be developed as a natural-based antioxidant due to their abundance in flavonoid and phenolic content. HPPE has a total phenolic content of 107.35 ± 8.02 mg GAE/g and total flavonoids of 108.82 ± 12.69 mg QE/g. The results of the free radical scavenging test (DPPH and ABTS) showed a moderate inhibition capacity of HPPE.

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