A Comparative Study of *Hylocereus Undatus* (White Dragon Fruit) Foliage and Peel for Antioxidant Activity and Phenolic Content

Nur Mardhiyyah Azizuddin[1], Ayub Md Som[1,3], Norizan Ahmat[2] and Hairul Amani Abdul Hamid[2]

[1]*Faculty of Chemical Engineering,* Universiti Teknologi MARA, 40450 Shah Alam
[2]*Faculty of Applied Sciences,* Universiti Teknologi MARA, 40450 Shah Alam
[3]*Accounting Research Institute,* Universiti Teknologi Mara, 40450 Shah Alam

***Abstract*—*Hylocereus undatus* foliage is believed to have high antioxidant compared to peel of *Hylocereus undatus*, which already known to contain high total phenolic content and antioxidant activities. Total phenolic content and antioxidant activity for two different solvent extractions namely; chloroform and methanol were done through Folin-Ciocalteu method and DPPH free radical scavenging assay. In determining total phenolic content, the results show that methanol extraction (30.30 ± 0.0065 mg GAE/100 g extract in foliage; 45.815 ± 0.0233 mg GAE/100g extract in peel) gives high total phenolic content than chloroform extraction (5.92 ± 0.0148 mg GAE/100 g extract in foliage; 18.89 ± 0.0055 mg GAE/100g extract in peel). However, in DPPH scavenging assay, methanol extraction (88.81 ± 0.0012 % in foliage; 97.42 ± 0.0061 % in peel) has high scavenging activity compared to chloroform extraction (38.30 ± 0.0080 % in foliage; 18.71 ± 0.0068 % in peel) which shows that antioxidant activity in chloroform solvent extraction is higher compared to methanol solvent extraction. When compared between foliage and peel, it shows that peel contain more antioxidants than foliage. This experiment has proved that *Hylocereus undatus* foliage has same potential as *Hylocereus undatus* peel in scavenge free radical in human body.**

Keywords*— *Hylocereus undatus, total phenolic content, antioxidant activity, DPPH scavenging effect

I. INTRODUCTION

Dragon fruit plant that is locally known as pitahaya fruit is one of the cactus families, *Cactaceae* [1]. It is a native fruit from Mexico and Central South America [2]. The best climate condition for dragon fruit plantation is dry, tropical or subtropical with annual rainfall ranges from 22 to 50 inches per year. The flowers of dragon fruits with diameter up to 30 cm only can bloom twice in a month, around 1st and 15th days of the lunar calendar [3]. Research on cultivation of dragon fruit shows that this plant can only produce about four to six cycles of fruits per year and the fruits are harvested when they are fully expanded and matured as their skins form 85% of red colour [1].

Nowadays, *Hylocereus undatus* have drawn more attention around the world due to their sensorial properties and economic importance. As in many other vegetables and fruits such as tomato, green kiwi, strawberry, citrus fruit and lemon, *Hylocereus undatus* is also high in antioxidant that helps to reduce many degenerative diseases such as arthritis, arteriosclerosis, cancer, heart diseases, inflammation and brain dysfunction. It is also rich in fibre and vitamins which can help digestive system, prevent colon cancer and diabetes, remove toxic substances such as heavy metal and helps to control the cholesterol level and blood pressure [4].

An antioxidant is a phytochemical compound or can be referred as bioactive compound. There are many types of chemical structures and functions of phytochemicals in fruits and vegetables, and one of it is phenolic compounds. Phenolic compound plays an important role in contributing to the overall antioxidant activity. Phenolic compounds have the potency to fight against reactive oxygen species (ROS) or known as free radical species by inhibiting the initiation of free radical, breaking their chain reactions and suppressing the formation of free radicals such as superoxide ion, hydroxyl radical, singlet oxygen and hydrogen peroxide. This reactive free radical species can damage cell body if it is present in a high level of quantity [5]. However, this free radical species may play important roles in some of the biological processes such as the intracellular killing of bacteria by leucocytes (white blood cells) and some cell signaling processes [6].

Instead of natural antioxidants, some industries still use synthetic antioxidants as food and cosmetics preservatives. BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), PG (propyl gallate) and TBHQ (tert-butylhydroxyquinone) are some of the synthetic antioxidants commonly used which are highly toxics. There are many effects of these antioxidants on human health such as cytotoxic effects towards monocryptic leukemia cells resulting in apoptosis and DNA damage. It will also give adverse effects on major organs such as kidney, liver and lungs. Hence, only appropriate and legal concentration of synthetic antioxidant must be used in food to prevent any negative effect on health. It will be much better if we can replace the high antioxidant foods with the natural antioxidants found in fruits and vegetables.

Hylocereus undatus is one of the main sources of plant-based antioxidant that is free from toxic and safe to be used. Several literatures only focus on the phytochemical screening and analysis of antioxidant present in peels and pulps of *Hylocereus undatus*, however there is no study carried out specifically on its foliage. Hence, the research on this foliage is carried out in order to investigate the presence of antioxidant capacity and phenolic content in the extract of *Hylocereus undatus* foliages and peels by using different solvent extractions.

II. METHODOLOGY

A. Materials

All chemicals such as chloroform, methanol, ethanol, gallic acid, sodium carbonate, 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) and Folin-Ciocalteu reagent were obtained from instrumentation lab in the Faculty of Applied Sciences in Universiti Teknologi MARA (UiTM), Shah Alam. *Hylocereus undatus* foliages were brought from dragon fruit farm in Sepang, Selangor while its fruits were brought from a CHECKERS supermarket in Shah Alam,

Selangor.

B. Procedure

Sample preparation: The foliages and peels of *Hylocereus undatus* were washed and clean with tap before they were cut and dried at 70ºC (foliage) and 60ºC (peel) for 24 hours by using drying oven (Gravity Convection, Fisher Scientific, United States). The dried sample were then ground and sieved to uniform particle size of 0.25mm by using Retsch Cutting Mill (SM 300, Retsch, United States)

Extraction and Isolation of Compound: The extraction was done by using a maceration technique. For chloroform extraction, 60 g of each foliages and peels powder were transferred into 100mL of volumetric flask separately and chloroform solvent was added up to the mark. The mixtures were then kept in the dark at room temperature for two days before filtered [7]. This method was repeated for three times before these samples were utilized again using the methanol solvent. The procedure for this solvent extraction was the same as the chloroform extraction. The extracted samples for both solvent extractions were evaporated by using Rotavap (Wilmad WG-EV311-V-PLUS,Amazon, United States) to eliminate the unwanted solvent.

Gallic Acid Calibration Curve: In 100mL conical flask, 0.5g gallic acid was dissolved with 10mL ethanol and it was then diluted to volume with deionized water. 0, 1, 2, 3 and 5mL of stock solutions were added into 100mL conical flask and they were then diluted to volume with water to obtain different concentrations of stock solution (0, 50, 100, 150, 250 and 500ppm). 0.25mL of different concentrations of gallic acid solutions were added into 25mL of conical flask with 1.3mL of 10-fold Folin-Ciocalteu reagent and 3.75mL of 7.5% sodium carbonate solution. The mixture was then diluted to volume with water and inverted for 20 times. These gallic acid stock solutions were kept for 30 minutes at room temperature before being measured by a visible spectrophotometer (GENESYS^{TM} 20, ThermoFisher, German) at 760nm against a blank sample [8].

Determination of Total Phenolic Content: In 25mL of conical flask, 0.25mL of extracted sample with 1.3mL of 10-fold Folin-Ciocalteu reagent and 3.75mL of 7.5% sodium carbonate solutions were mixed. The mixture was then diluted to volume with deionized water and inverted for 20 times. Then, it was kept at room temperature for 30 minutes before being measured by a spectrophotometer (GENESYS^{TM} 20, ThermoFisher, German) against a blank sample at 760nm [8].

Determination of Antioxidant Activity: The capability of DPPH free radical scavenging activity towards *Hylocereus undatus* foliages and peels extract was determined according to the method described with slight modifications [9]. In preparation of the blank sample, 0.28mL of DPPH solution (0.1mM, in 95% ethanol) was added into 10mL of conical flask and it was then diluted to volume with ethanol. In preparing the sample, 0.28mL of DPPH solution and 0.28mL of sample were added into 10mL of conical flask and the mixture was then diluted to volume with ethanol. The mixture was then inverted for several times and incubated in the dark room for 30 minutes at room temperature. The absorbance was measured against the blank sample by using a spectrophotometer (GENESYS^{TM} 20, ThermoFisher, German) at 517 nm. The radical scavenging activity was measured as a decreased in the absorbance of DPPH and was calculated by using the following equation;

$$\text{Scavenging effect (\%)} = [1 - (A_{sample} - A_{control})] \times 100\%$$

III. RESULTS AND DISCUSSION

A. *Determination of Total Phenolic Content*

Extraction is one of the main techniques to obtain a total phenolic content from plant materials. Efficiency of this extraction is affected by the chemical nature of the plant materials, method of extraction used, size particle of the samples, types of solvent used and the presence of interfering substances [10]. The percentage yield of extraction depends on the polarity of the solvent, pH, temperature, time for extraction and the sample composition [11]. In this study, the extractions of *Hylocereus undatus* foliages and peels were carried out through a maceration method with uniform particle size of 0.25mm at room temperature and it was immersed for 2 days by using chloroform and methanol solvents. Table 1 shows the percentage yield of sample extraction from chloroform and methanol solvent;

Table 1: Percentage yield of sample extraction

Sample	**Yield of chloroform extract (%)**	**Yield of methanol extract (%)**
Foliage	28.51	47.11
Peel	3.90	23.56

From the Table 1, it shows that the percent yield of extract increases with the increase of the polarity of solvent in which chloroform has the polarity index of 4.1 which is lower than methanol that has the polarity index of 5.1 [12]. Methanol extract indicates the highest yield for both foliages (47.11%) and peels (23.56%) compared to chloroform extract for foliages (28.51 %) and peels (3.90 %). This is because chloroform is used in solvent extraction to extract non-polar molecules while a primer alcohol, methanol consists of polar region (-OH group) and non-polar hydrocarbon chain that can extract both polar and non-polar molecules.

Phenolic compound is the secondary metabolites and important antioxidant component that are found in the plants and fruits which act as antimicrobial, antimutagenic, anticancer and anti-inflammatory to human health due to its bioactivity. Phenolic acid, such as gallic acid and polyphenols such as flavonoids are some of the typical phenolic compounds that are highly correlated with antioxidant activity [1]. By referring to the chemical structure of the phenolic compound, it consists of electron donating groups at the ortho and para positions of phenols, which may enhance antioxidant and radical scavenging activity. Folin-Ciocalteu reagent that is used to determine total phenolic contents depends on the basic mechanism of oxidation and reduction reactions of antioxidant compounds [13]. The reactions between these compounds and Folin-Ciocalteu reagent will show blue colour solution that indicates the amount of total phenolic content in the crude extract. The darker the blue colour of the solution, the higher the amount of total phenolic content present in the extracts.

In this present study, total phenolic content of foliage and peels of *Hylocereus undatus* is expressed in milligram of gallic acid per gram of extract (mg GAE/g extract). It is determined from regression equation of calibration curve ($y = 0.001x + 0.0334$, $R^2 = 0.9902$) as shown in the Figure 1 and the calculated result of total phenolic content of chloroform and methanol crude extract is presented in the Table 2 and Figure2.

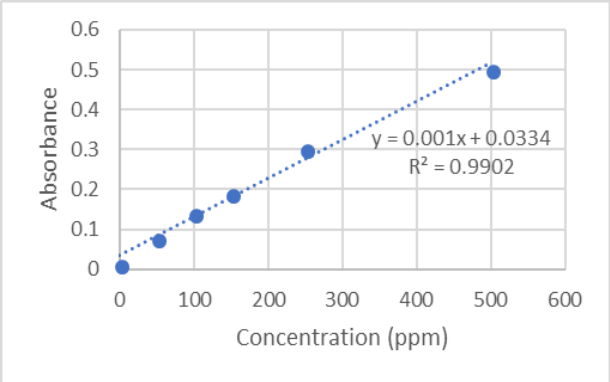

Figure 1: Calibration curve of gallic acid concentration

Table 2: Total phenolic content (TPC) of foliage and peels of *Hylocereus undatus*

Sample	Total phenolic content (mg GAE/100g extract)
Foliage chloroform extraction	5.92 ± 0.0148
Peel chloroform extraction	18.89 ± 0.0055
Foliage methanol extraction	30.30 ± 0.0065
Peel methanol extraction	45.815 ± 0.0233

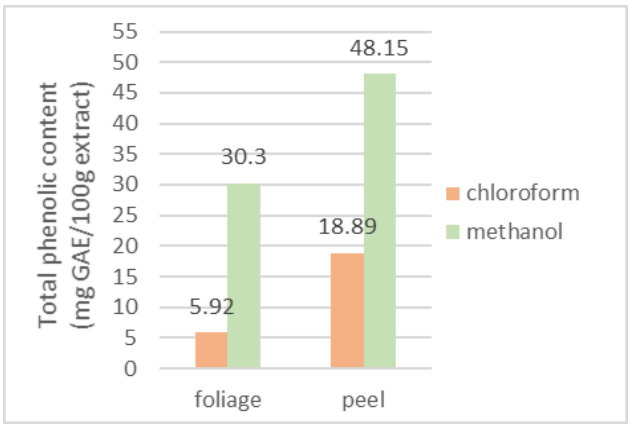

Figure 2: Comparison of total phenolic content of chloroform and methanol crude extracts

The results from the extraction of foliages and peels of *Hylocereus undatus* for both chloroform and methanol solvent extractions show that the peel has high phenolic content compared to foliage. By comparing with the solvent used in the extraction, methanol solvent gives high total phenolic content for both foliages (30.30 ± 0.0065 mg GAE/100g extract) and peels (45.815 ± 0.0233 mg GAE/100g extract) compared to chloroform solvent that has lower total phenolic content for both foliages (5.92 ± 0.0148 mg GAE/100g extract) and peels (18.89 ± 0.0055 mg GAE/100g extract). This is because, phenolics including simple phenols majority of which is phenolic acids are hydrophilic compounds with antioxidant activity *in vitro*. Since methanol consists of hydroxyl group (-OH), hence it can extract more phenolic compounds. As carbon chain increases across the alcohol homologous series, the extraction of phenolic compound will increase [3].

However, based on the research done by using ethanol as solvent extraction, total phenolic content of *Hylocereus undatus* peel is 36.12 mg GAE/100g which is lower than methanol extraction from this study [1]. This may be due to the difference of sources and maturation stage of fruits used in the experiment. Previous research has also been done on total phenolic content in different solvent extractions by using pulps of *Hylocereus undatus* as their samples. It is found that phenolic compound is higher in ethanol extraction (179.348 ± 0.02 mg/L) followed by methanol extraction (160.87 ± 0.03) and distilled water extraction (157.609 ± 0.25) [3].

B. *Determination of Antioxidant Activity*

A free radical compound such as Reactive Oxygen Species (ROS) have unpaired electrons at their outer shell that make them very unstable and quite reactive towards other molecules to combine with them in other to generate more stable compounds [14]. Different types of cell in human body compose of many different types of molecules. These molecules are joined together by chemical bonds. In normal situations, bonds will not breakdown to leave a molecule with unpaired electrons [15]. However, when this weaker bond splits, it will try to attack nearest electrons from another molecules. The attacked molecules that lose its electron will form free radicals of longer chains. Once the process has started, it can cascade and results in the disruption of leaving cell [16].

In our body, formation of free radicals occurs continuously as normal by-products of the oxygen metabolism during an oxidative phosphorylation of mitochondrial. Hence, mitochondrion is the source of free radical in our body [14]. If these free radicals are not scavenged by cellular constituents, it can lead to diseases such as cancer, arteriosclerosis, aging and cerebrovascular disease.

The molecule of 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical that acts as hydrogen radical scavenger. It has an unpaired valence electron at one atom of nitrogen bridge. The antioxidant activity in the extract can be measured by using DPPH assay. The antioxidants that present in the crude extracts will react with stable free radical from DPPH and they are then converted to α,α-diphenyl-β-picrylhydrazine with colour changes from violet to yellow due to the ability of antioxidant in donating hydrogen to DPPH. The results of free radical scavenging activity of chloroform and methanol crude extracts are presented in the Table 3 and Figure 3.

Table 3: Free radical scavenging for foliage and peels of *Hylocereus undatus*

Sample	Percent radical scavenging activity (%)
Foliage chloroform extraction	38.30 ± 0.0080
Peel chloroform extraction	18.71 ± 0.0068
Foliage methanol extraction	88.81 ± 0.0012
Peel methanol extraction	97.42 ± 0.0061

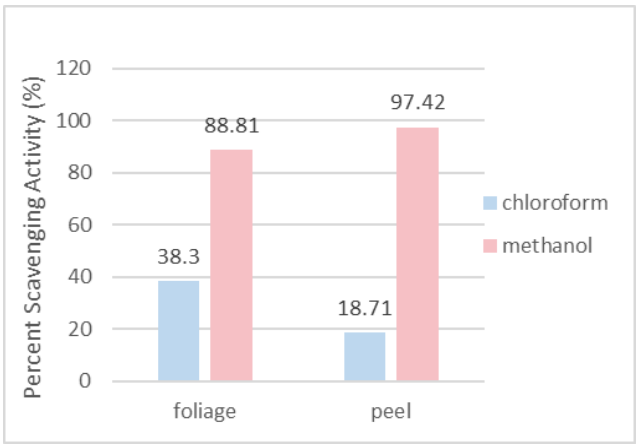

Figure 3: Comparison of radical scavenging activity (%) of chloroform and methanol crude extracts

Based on the theory of DPPH radical scavenging activity, the higher amount of antioxidant in the solution, the lower the percentage of free radical scavenging activity. Since total phenolic content is well extracted by peels in both types of solvent, hence percentage of free radical scavenging activity in peels must be lower than foliage. This is because, these phenolic contents will react with DPPH free radical which results in high percent of inhibition to form DPPH. Thus, the color of extract peel solution with the presence of DPPH free radical was change from violet to

light yellow. In comparison between chloroform and methanol solvents, methanol extracts give high radical scavenging activity for both foliage (88.81 ± 0.0012%) and peels (97.42 ± 0.0061%) compared to chloroform extraction of foliage (38.30 ± 0.0080%) and peels (18.71 ± 0.0068%) .

In the extraction of foliages and peels in the chloroform solvents, it is proved that peels contain high antioxidant than foliages since the percent of free radical scavenging activity is lower in peels than in foliages. Thus, produce higher amount of DPPH. However, in methanol solvents, antioxidant activity is lower in peels but higher in foliages. This is contrary with the results of total phenolic contents that shows high total phenolic contents in peels than in foliages. This situation is same with the results of different solvent extraction.

Even though methanol extraction for both foliages and peels have higher total phenolic content than chloroform extraction of foliages and peels, in free radical scavenging assay, the antioxidant activity of chloroform extraction is higher compared to methanol extraction. This situation occur may be due to some of the possible reasons: Firstly, it has been reported that reaction of DPPH with some phenols such as eugenol and its derivatives is reversible, hence give low antioxidant activity. Secondly, it may be due to the slow rate of reaction between DPPH and the substrate molecules. Thirdly, for the relatively low reducing power, it could be that certain phenols in methanol extracts have high redox potential than chloroform extracts [3]. The summary of the total phenolic content and antioxidant activity for both foliages and peels is shown in the Table 4.

Table 4: Comparison between total phenolic contents (TPC) and antioxidant activities of both foliages and peels in different solvent extractions

Test	**Chloroform extraction**		**Methanol extraction**	
	Foliage	**Peel**	**Foliage**	**Peel**
TPC (mgGAE/100g)	5.92 ± 0.0148	18.89 ± 0.0055	30.30 ± 0.0065	45.815 ± 0.0233
Scavenging Effect (%)	38.30 ± 0.0080	18.71 ± 0.0068	88.81 ± 0.0012	97.42 ± 0.0061

IV. Conclusion

This study was conducted to investigate the total phenolic content and antioxidant activity in both foliages and peels of *Hylocereus undatus* (white dragon fruit) by using different solvent extraction; chloroform and methanol solvent. In determining total phenolic contents, Folin-Ciocalteu reagent test was used while DPPH free radical scavenging assay test was used to determine antioxidant activity. For both tests, it shows that methanol extraction gives high total phenolic content than chloroform extraction. This is because chloroform solvent can only extract non-polar compound while methanol solvent can extract both polar and non-polar compounds. However, in DPPH free radical scavenging assay, antioxidant activity in chloroform extraction is lower than methanol extraction. This situation may due to some possible reasons such as reversible reactions that occur between DPPH and some phenols and because of the slow rate of reaction between DPPH radicals and the substrate molecules. Since there is no study done in determining total phenolic content and antioxidant of *Hylocereus undatus* foliage, it can be concluded that the foliage has a potential to be one of the natural antioxidants which can replace synthetic antioxidants that are toxics to living things.

It is recommended to have a further study on antioxidant activity in foliage of *Hylocereus undatus* since there are many other specifics antioxidant compounds instead of phenolic compounds, such as carotenoids, betalains and lutein. Further studies on this antioxidants activity can improve the nutritional value in the foliage.

Acknowledgment

First of all, I wish to thank Almighty God for giving me the opportunity to complete my thesis for final research project through this long journey despite facing challenges along the way. I would like to show my gratitude to my supervisor, Assoc Prof Dr. Ayub Md Som and the other collaborators namely; Assoc Prof Dr. Norizan Ahmat and Mrs. Hairul Amani Abdul Hamid from the Faculty of Applied Sciences that always advise me to improve my writing skills in order to form a good thesis. They also always make sure my progress is on schedule. I would also like to give a special thanks to lab staff from the instrumentation lab Faculty of Applied Sciences, En. Faizal and all my friends for helping me in completing this research. Finally, I feel grateful to thanks my parents that always give support to me and encourage me throughout duration of my study.

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