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A comparative study on foliage and peels of *Hylocereus undatus* (white dragon fruit) regarding their antioxidant activity and phenolic content

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Abstract

Hylocereus undatus foliage is believed to contain antioxidants similar to its peel. Numerous studies have been conducted to determine the total phenolic content (TPC) and antioxidant activity on the *Hylocereus undatus* pulps and peels; however, similar studies on its foliage have yet to be investigated. In this study, *Hylocereus undatus* foliage and peels were extracted using two different solvents namely; chloroform and methanol through Folin-Ciocalteu method and Diphenyl-1-Ipicrylhydrazyl (DPPH) free radical scavenging assay for TPC and antioxidant activity, respectively. As for TPC, results revealed that the peels gave higher TPC in both methanol (48.15 mg GAE/100g extract) and chloroform (18.89 mg GAE/100g extract) extractions than foliage (30.3 mg GAE/100g extract and 5.92 mg GAE/100g extract, respectively). However, when a comparison was made between foliage and peels in terms of its scavenging effects in DPPH assay, the

peels contained more antioxidants (18.71%) than foliage (38.3%) in the chloroform solvent extracts. This study shows that *Hylocereus undatus* foliage has a similar antioxidant activity as its peels and is potentially a natural antioxidant in food applications.

Keywords: Food science, Food analysis

1. Introduction

A dragon fruit plant, known locally as pitahaya fruit is a member of the cactus family, *Cactaceae* (Ruzlan et al., 2008). It is a native fruit from Mexico and Central South America (Mello et al., 2015). The best climate condition for planting dragon fruit is dry, tropical or subtropical with an annual rainfall ranging from 22 to 50 inches per year. The flowers of the dragon fruits have a diameter of up to 30 cm and can only bloom twice in a month, around the 1st and 15th day of the lunar calendar (Halimoon and Hasan, 2010). Research on cultivation of the dragon fruit shows that this plant can only produce about four to six cycles of fruits per year, and that these fruits are harvested when they are fully expanded and matured which is when their skins turn 85% red (Ruzlan et al., 2008).

Nowadays, *Hylocereus undatus* or white-flesh with red peel dragon fruits have drawn more attention around the world due to their sensorial properties and economic importance. As in many other vegetables and fruits such as tomatoes (Elbadrawy and Sello, 2016), green kiwis (Halimoon and Hasan, 2010), strawberries (Panico et al., 2009), citrus fruits and lemons (Zou et al., 2016); the *Hylocereus undatus* are also high in antioxidants that help to reduce many degenerative diseases such as arthritis, arteriosclerosis, cancer, heart diseases, inflammation and brain dysfunction. This fruit is also rich in fibre and vitamins which can help relieve the digestive system, prevent colon cancer and diabetes, remove toxic substances such as heavy metal and help to control the cholesterol level and blood pressure (Susanti et al., 2012).

An antioxidant is a phytochemical compound commonly referred as a bioactive compound. There are many types of chemical structures and functions of phytochemicals in fruits and vegetables, and one of them is the phenolic compounds. The phenolic compounds play an important role in contributing to the overall antioxidant activity. These phenolic compounds have the potency to fight against reactive oxygen species (ROS) or better known as free radical species, by inhibiting the initiation of free radicals, breaking their chain reactions and suppressing the formation of free radicals such as superoxide ion, hydroxyl radical, singlet oxygen and hydrogen peroxide. These reactive free radical species can damage the body of cells if they are present in high quantities (Park et al., 2008).

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 also highly toxic (Kahl, 1984). 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 antioxidants must be used in food to prevent any negative effects on our health. It will be much better if we can replace these highly toxic synthetic antioxidants 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. Previous research focused on the use of both pulps and peels of *Hylocereus undatus* (Ruzlan et al., 2008; Choo and Yong, 2011; Susanti et al., 2012; Mello et al., 2015; Cheah et al., 2016; Romero et al., 2017) and the super red dragon fruit or *Hylocereus costaricensis* (Fidrianny et al., 2017) as natural antioxidants so as to determine their total phenolic content and antioxidant activity; however, very limited research is carried out on the foliage. As it is a natural resource available in abundance, the cost of using foliage as plant-based antioxidants is less compared to synthetic antioxidants. Furthermore, *Hylocereus undatus* foliage is environmentally friendly and not harmful to human health as it does not leave any toxic residue on human health and living things (Som et al., 2007; Idris et al., 2012; Som and Wahab, 2018). Hence, this present study is carried out to compare total phenolic content (TPC) and antioxidant activity between the foliage and peels of *Hylocereus undatus* by using different solvent extractions.

2. Material and methods

2.1. Material

All chemicals such as chloroform (R & M Chemicals, Selangor, Malaysia), methanol and ethanol (HmBG Chemicals, Selangor, Malaysia), gallic acid (Sigma-Aldrich Chemicals Company, USA), sodium carbonate (System Chemicals, Malaysia), 2,2-Diphenyl-1-picrylhydrazyl or DPPH (Sigma-Aldrich Chemicals Company, USA) and Folin-Ciocalteu reagent (R & M Chemicals, Selangor, Malaysia) were obtained from the instrumentation laboratory at the Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), Shah Alam, Selangor. The *Hylocereus undatus* foliage were taken from a dragon fruit farm in Sepang, Selangor while the fruits were bought from a CHECKERS supermarket in Shah Alam, Selangor.

2.1.1. Sample preparation of plant material

The foliage and peels of *Hylocereus undatus* were washed and cleaned with tap water before they were cut and dried at 70 °C (foliage) and 60 °C (peel) for 24 hours by using a drying oven (Gravity Convection; Fisher Scientific, USA). The dried samples were then ground and sieved to uniform particle size of 0.25 mm by using a Cutting Mill (Model SM 300; Retsch, USA).

2.2. Methods

2.2.1. Extraction of *Hylocereus undatus* foliage and peels

The extraction was done by using a maceration technique. For chloroform extraction, samples of 60 g of each foliage and peels powder were transferred into a 100 mL 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 being filtered (Choo and Yong, 2011). This method was repeated for three times. Final readings were recorded by taking the average from the three repeated samples and standard deviation (S) was recorded for each attempt. The same procedure was used for methanol extraction. The extracted samples for both solvent extractions were evaporated by using a rotary evaporator (Model Wilmad WG-EV311-V-PLUS; Amazon, USA) to eliminate the unwanted solvent.

2.2.2. Gallic acid calibration curve

In a 100 mL conical flask, a sample of 0.5 g gallic acid was dissolved with 10 mL ethanol and the mixture was then diluted to the required volume with deionized water. A varied volume of stock solutions from 0, 1, 2, 3, 4 and 5 mL were added into a 100 mL conical flask and they were then diluted to the required volume with water to obtain different concentrations of stock solutions i.e. at 0, 50, 100, 150, 250 and 500 ppm, respectively. Samples of 0.25 mL of different concentrations of gallic acid solutions were added into a 25 mL of conical flask with 1.3 mL of 10-fold Folin-Ciocalteu reagent and 3.75 mL of 7.5% sodium carbonate solution. The mixture was then diluted to the required volume with water and inverted 20 times. These gallic acid stock solutions were kept for 30 minutes at room temperature before being measured by a visible spectrophotometer (Model GENESYS™ 20; ThermoFisher, Germany) at 760 nm against a control sample (Seruga et al., 2011). This method was repeated for three times and final readings were recorded by taking the average from the three repeated samples. Standard deviations (S) were also calculated for each trial.

2.2.3. Determining total phenolic content (TPC)

In a 25 mL conical flask, 0.25 mL of extracted sample with 1.3 mL of 10-fold Folin-Ciocalteu reagent and 3.75 mL of 7.5% sodium carbonate solutions were mixed. The mixture was then diluted to the required volume with deionized water and inverted 20 times. Then, it was kept at room temperature for 30 minutes before being measured by a spectrophotometer (Model GENESYS™ 20; ThermoFisher, Germany) against a control sample at 760 nm (Seruga et al., 2011). The same experiment was run for three times so as to achieve the average final reading based on the repeated samples together with its calculated standard deviation (S) for each run.

2.2.4. Determining antioxidant activity

The capability of DPPH free radical scavenging activity towards *Hylocereus undatus* foliage and peels extract was determined according to the method described with slight modifications (Panico et al., 2009). In preparation of control sample (A_{control}), a 0.28 mL of DPPH solution (0.1 mM, in 95% ethanol) was added into a 10 mL of conical flask and it was then diluted to the required volume with ethanol. In preparing the test sample (A_{sample}), 0.28 mL of DPPH solution and 0.28 mL of the sample were added into a 10 mL of conical flask and the mixture was then diluted to the required volume with ethanol. The mixture was then inverted several times and incubated in the dark room for 30 minutes at room temperature. The absorbance was measured against the control sample by using a spectrophotometer (Model GENESYS™ 20; ThermoFisher, Germany) at 517 nm. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated by using the following equation:

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

This method was also repeated for three times so as to achieve the average final reading with its calculated standard deviation (S) recorded for each attempt.

3. Results and discussion

3.1. Total phenolic content of *Hylocereus undatus* foliage and peels

Extraction is one of the main techniques to obtain a TPC from plant materials. Efficiency of this extraction is affected by the chemical nature of the plant materials, method of extraction used, particle size of the samples, types of solvent used and the presence of interfering substances (Stalikas, 2007). The percentage yield of extraction depends on the polarity of the solvent, pH, temperature, time for

extraction and the sample composition (Diem Do et al., 2014). In this study, the extractions of *Hylocereus undatus* foliage and peels were carried out through a maceration method with uniform particle size of 0.25 mm 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 using chloroform and methanol solvent.

From the table, it shows that the percentage yield of extract increases with the increase of the polarity of solvent in which chloroform has a polarity index of 2.7 which is lower than methanol which has a polarity index of 5.1 (Katz et al., 1998). The methanol extract indicates the highest yield for both foliage (47.11%) and peels (23.56%) compared to chloroform extract in which the foliage recorded only at 28.51 % while the peel was at 3.90 %. This is because chloroform is used in solvent extraction to extract non-polar molecules while a primer alcohol, i.e. methanol consists of polar regions (-OH group) and a non-polar hydrocarbon chain that can extract both polar and non-polar molecules.

The phenolic compound is the secondary metabolite and an important antioxidant component that is found in 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 (Ruzlan et al., 2008). When 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 antioxidants 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 (Verzelloni et al., 2007). The reactions between these compounds and Folin-Ciocalteu reagent resulted in a blue solution that indicates the amount of total phenolic content in the crude extract. The darker the blue of the solution, the higher the amount of total phenolic content present in the extracts.

In this study, the TPC 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 the regression equation of the calibration curve ($y = 0.001x + 0.0334$, $R^2 = 0.9902$). The calculated results of TPC for both chloroform and methanol crude extracts are presented in Fig. 1.

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

As shown in Fig. 1, the results from the extraction of foliage and peels of *Hylocereus undatus* for both chloroform and methanol solvent extractions indicate that the peels have high phenolic content compared to foliage. By comparing the solvent used in the extraction, it is found that methanol solvent has high total phenolic content for both foliage (30.30 ± 0.0065 mg GAE/100g extract) and peels (48.15 ± 0.0233 mg GAE/100g extract) compared to chloroform solvent that has lower total phenolic content for both foliage (5.92 ± 0.0148 mg GAE/100g extract) and peels (18.89 ± 0.0055 mg GAE/100g extract). This is because phenolic, including a simple phenol 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 (Halimoon and Hasan, 2010).

However, based on the research done by using ethanol as solvent extraction, the total phenolic content of *Hylocereus undatus* peels is 36.12 mg GAE/100g (Ruzlan et al., 2008) which is lower than methanol extraction from this study. This may be due to the difference of sources and maturation stage of fruits used in the experiment. Previous researches have also been done on the total phenolic content in different solvent extractions by using pulps of *Hylocereus undatus* as their samples. It is found that the phenolic compound is higher in ethanol extraction (179.348 ± 0.02 mg/L) followed by methanol extraction (160.87 ± 0.03 mg/L) and distilled water extraction (157.609 ± 0.25 mg/L) (Halimoon and Hasan, 2010).

3.2. Antioxidant activity of *Hylocereus undatus* foliage and peels

Free radical compounds 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 to generate more stable compounds (Bhuiyan et al., 2009). Different types of cells in the human body compose of many different

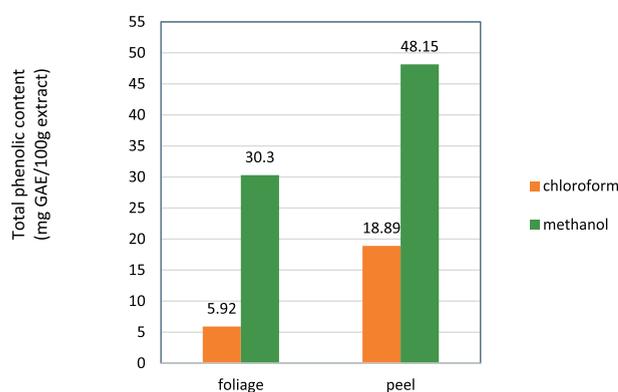


Fig. 1. Comparison of total phenolic content of chloroform and methanol crude extracts.

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 (Halliwell, 1989). However, when this weaker bond splits, it will try to attack nearest electrons from another molecule. The attacked molecules that lose their electrons will form free radicals of longer chains. Once the process has started, it can cascade and result in the disruption of leaving cell (Jesberger and Richardson, 1991).

In our body, the 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 radicals in our body compounds (Bhuiyan et al., 2009). If these free radicals are not scavenged by cellular constituents, it can lead to diseases such as cancer, arteriosclerosis, ageing and cerebrovascular disease.

The molecule 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical that acts as a 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 are present in the crude extracts react to the stable free radicals 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 activities for both chloroform and methanol crude extracts are presented in Fig. 2.

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 solvents, the percentage of free radical scavenging activity in peels must be lower than foliage. This is because these phenolic contents react with DPPH free radicals which result

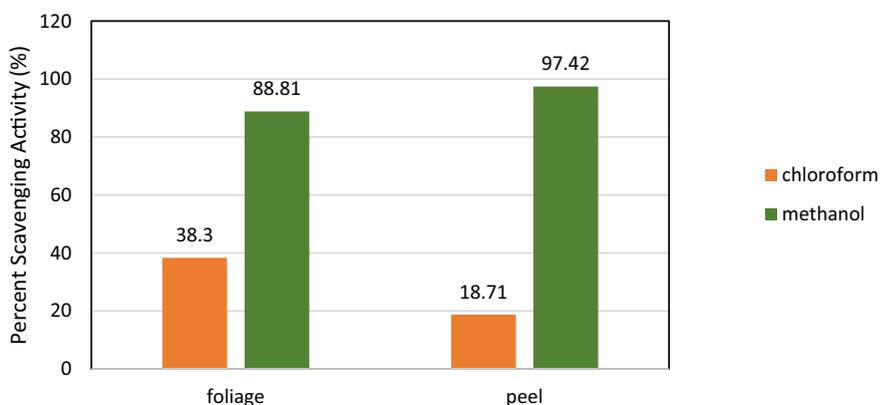


Fig. 2. Comparison of radical scavenging activity (%) of chloroform and methanol crude extracts.

in high percentage of inhibition to form DPPH. Thus, the colour of the extracted peel solution with the presence of DPPH free radicals was changed from violet to light yellow. In comparison between chloroform and methanol solvents, the methanol extracts showed a high radical scavenging activity for both foliage ($88.81 \pm 0.0012\%$) and peels ($97.42 \pm 0.0061\%$) compared to the chloroform extracts of both foliage ($38.30 \pm 0.0080\%$) and peels ($18.71 \pm 0.0068\%$).

In the extraction of foliage and peels using chloroform solvents, it is proven that peels contain higher antioxidant than foliage since the percentage of free radical scavenging activity is lower in peels than in foliage. However, as shown in Fig. 2 by using methanol solvents, the antioxidant activity is lower in peels but higher in foliage. This is contrary with the results obtained from TPC experiments which showed a higher TPC in peels than in foliage. This situation is the same with the results when different solvent extractions were used.

Even though the methanol extraction for both foliage and peels has shown higher TPC than the chloroform extraction, the antioxidant activity of chloroform extraction is still higher compared to methanol extraction as confirmed through the DPPH assay. This occurrence might be due to some possible reasons. Firstly, it has been reported that reaction of DPPH with some phenols such as eugenol and its derivatives is reversible, hence it shows 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 a high redox potential than chloroform extracts (Halimoon and Hasan, 2010). Table 2 shows a comparison of Total Phenolic Content (TPC) and scavenging effect between this study and the previous works.

3.3. Recommendation for future works

It is recommended to have a further study on phytochemical screening and antioxidant activity of *Hylocereus undatus* foliage since there are many other specific

Table 2. Comparison of TPC and scavenging effect in peels of *Hylocereus undatus*.

Types of solvent	<i>Hylocereus undatus</i>				References
	Foliage		Peels		
	TPC (mg GAE/100g)	Scavenging effect	TPC (mg GAE/100g)	Scavenging effect	
Chloroform	5.92	38.30 %	18.89	18.71 %	This study
Methanol	30.30	88.81 %	48.15	97.42 %	
Ethanol	NA	NA	36.12	87.02 %	Ruzlan et al. (2008)

NA = Not Available.

antioxidant compounds present such as carotenoids, betalains and lutein. Further studies on these types of antioxidant activities could improve the nutritional values found in the *Hylocereus undatus* foliage. In addition, some further analyses are suggested and required so as to isolate and identify the main phenolic compounds present in each extract. Since this present study is very much preliminary in nature, further works need to be carried out in order to determine the total flavonoid content as well as phenolic and flavonoid acids.

4. Conclusion

This study shows that methanol extraction gives a higher total phenolic content (TPC) 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. Results from the extraction of *Hylocereus undatus* foliage and peels in both methanol and chloroform solvents indicate that the peels have higher total phenolic content (48.15 mg GAE/100g extract and 18.89 mg GAE/100g extract, respectively) compared to the foliage (30.3 mg GAE/100g extract and 5.92 mg GAE/100g extract, respectively). In DPPH assay, the percentage of radical scavenging 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, as well as the slow rate of reaction between DPPH radicals and the substrate molecules. In the extraction of foliage and peels using chloroform solvents, it is proven that peels contain a higher antioxidant than foliage since the percentage free radical scavenging activity of the peels is lower (18.71 %) in peels than in foliage (38.30 %). Since there is no previous study done in determining Total Phenolic Content and antioxidant activity for *Hylocereus undatus* foliage, it can be concluded that the foliage has a potential to be one of the natural antioxidants which could replace highly toxic synthetic antioxidants in the future.

Declarations

Author contribution statement

Ayub Md Som: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Norizan Ahmat: Analyzed and interpreted the data.

Hairul Amani Abdul Hamid: Conceived and designed the experiments.

NurMardhiyyah Azizuddin: Performed the experiments.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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