# Antioxidant Activity of *Hylocereus Undatus* Foliage Using Different Methods

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**Abstract**

*Hylocereus undatus* or commercially known as white dragon fruit contains high antioxidant activity. The *Hylocereus undatus* foliage is believed to have high antioxidant activity compared to pulps and peels. Antioxidant activity was extracted by two different solvents namely methanol and chloroform using Ferric Reduction Antioxidant Power assay (FRAP). In determining antioxidant activity, the results show that the yield of methanol extract (44.44%) is higher than chloroform extract (22.22%). A comparison was made between findings from this study with the previous studies. The results revealed that the antioxidant activity obtained from methanol extract (59.05%) and chloroform extract (20.58%) using FRAP assay were comparable to the methanol extract (88.81%) and chloroform extract (38.30%) using DPPH assay based on the previous study.

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## 1.0 Introduction

Plants, fruits and vegetables have their own antioxidants. The antioxidants from these plants were studied to develop natural antioxidant formulations for food, cosmetic and other applications (Miliauskas et al., 2004). It is also believed that the risks of degenerative diseases mainly cardiovascular diseases and cancer can be decreased by increasing the intake of natural antioxidants that is rich in food (Pérez-Jiménez et al., 2008). Based on Gilles (2002), the three significant classes of plant chemicals are terpenoids, phenolic metabolites and alkaloids. Phenolic compounds are the most important plant chemical for dietary applications as it is the most extensively researched, nowadays. The dietary phenolic has three most important groups which are flavonoids, phenolic acids and polyphenols (King & Younge, 1999). There are two types of phenolic acids which are hydroxybenzoic acid and hydroxycinnamic acids, while hydrolysable tannins and condensed tannins are under polyphenols. The great concerns for further applications in natural antioxidants, functional foods and nutraceuticals have led to discovering, not only new but safe antioxidants from natural sources. One of the methods that have been mainly used in exploring antioxidant compounds in plants is phytochemical screening.

In terms of ease of use, efficiency and widely applicable, solvent extraction is the most frequently used as the preparation extract of the plant materials. Nevertheless, extraction yield and antioxidant activity also depend on the solvent used for extraction. Chemical characteristics and polarities vary according to different antioxidant compounds. Hence, the presence of various antioxidant compound may or may not be soluble in particular solvent (Turkmen et al., 2006). Solvent such as ethanol, methanol, acetone, ethyl acetate and the aqueous mixtures of these chemicals are the most suitable solvent used broadly for the extraction of phenolics from plant materials.

Dragon fruit is also known as pitaya or *Hylocereus* which comes from the *Cactaceae* family. This fruit is round in shape and scales-like texture. It has juicy and delicate pulp with numerous tiny soft edible seeds and highly nutritious (Choo & Yong, 2011). *Hylocereus* is being cultivated on a large scale in Malaysia and heavily marketed in the Southeast Asian countries from

which the fruit is highly favourable. According to Abidin et al. (2014), it is also well established as a new crop in other countries such as Australia, China, Israel, Nicaragua, Taiwan and Vietnam. The commercial production of *Hylocereus* in Israel, Malaysia and Taiwan has produced 16, 000 – 27, 000 kg/ha. The peels of *Hylocereus* have always been discarded before it is being consumed as people only use its pulps to drink and eat. It is also found that the foliage of the plant is abundantly produced by dragon fruit farm but has no particular usage. Hence, previous researchers have determined the antioxidant properties of peels and foliage of the *Hylocereus* to ensure its peels and foliage have high content of antioxidant for other purposes such as pharmaceutical. In previous years, Sim & Khing (2011) studied the antioxidant and antiproliferative activities of *Hylocereus polyrhizus* (red pitaya) and *Hylocereus undatus* (white pitaya) grown in Jeju Island, Korea. They reported that the pulps and peels of *Hylocereus undatus* had higher bioactivity index values that those of *Hylocereus polyrhizus*.

## 2.0 Methodology

### *2.1 Instrumentation & Material*

The instruments used for the preparation of sample such as drying oven (Gravity Convection; Fisher Scientific, USA) and a Cutting Mill (Model SM 300; Retsch, USA) were located in the laboratory at the Faculty of Chemical Engineering, Universiti Teknologi MARA (UiTM), Shah Alam, Selangor. The solvent extraction was carried out using a rotary evaporator (Model Wilmad WG-EV311-V-PLUS; Amazon, USA). Spectrophotometer (Model GENESYS$^{TM}$ 20; ThermoFisher, Germany) and water bath are used for determining antioxidant activity.

Chemicals used are namely: chloroform (LiChrosolv), methanol, sodium phosphate buffer (pH 6.6) (R&M), potassium ferricyanide (R&M), trichloroacetic acid (R&M) and ferric chloride (R&M). The *Hylocereus undatus* foliage used in this research were taken from Mimi Dragon Fruit Farm, Sepang, Selangor.

### *2.2 Methods*

#### *2.2.1 Sample Preparation of Plant*

The foliage of *Hylocereus undatus* was washed and cleaned with tap water before their thorns were removed, and they were cut and dried at 70 $^{o}$C for a day by using a drying oven. They were then ground and sieved to a uniform particle size of 0.25 mm by using a Cutting Mill.

#### *2.2.2 Extraction of Hylocereus Undatus*

Maceration technique was used for the extraction. 50 g sample of foliage powder was transferred into a 250 mL of volumetric flask separately and 180 mL of chloroform solvent was added into the volumetric flask. Before the mixture was being filtered, it was kept in the dark at ambient temperature (20 $^{o}$C) for two days (Sim & Khing, 2011). The experiments were repeated for three times to get an average reading. The extracted sample was then evaporated by using a rotary evaporator to eliminate unwanted solvent.

#### *2.2.3 Ferric Reduction Antioxidant Power (FRAP)*

This assay is based on the procedure reported by Benzie & Strain (1998). 1 mL of different concentration of methanolic extracts of *Hylocereus undatus* was added into 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution in a 20 mL vial. The reaction mixture was then be shaken for 20 times and then incubated at 50 °C for 20 minutes using water bath. After the incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 minutes. 2.5 mL of the supernatant was then mixed with 2.5 mL of deionised water an 0.5 mL of 0.1% ferric chloride in another clean 20 mL vial. The vial was then wrapped with aluminium foil for 30 minutes. The solution was then read at 700 nm against the blank with reference to standard using UV-spectrophotometer. In this study, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were used as a reference standard. The reducing power of the samples were compared with the reference standard. This method was repeated with chloroform extract of *Hylocereus undatus.*

## 3.0 Results and Discussion

### *3.1 Extraction of Hylocereus Undatus Foliage*

Extraction is an important technique to obtain phenolic contents and it is continued with further assay to determine antioxidant activity in a plant material, *Hylocereus undatus* foliage. The method of extraction technique carried out in this study was a maceration technique involving two solvents; namely methanol and chloroform. The powdered *Hyloccereus Undatus* foliage with the particle size of 0.25 mm were immersed with the two solvents, respectively for two days in the dark at room temperature. The efficiency of the maceration extraction was affected by the plant's chemical nature, method of the extraction used, size of the particle samples, type of solvent used and presence of any interfering substances (Stalikas, 2007). Table 1 shows the percentage yield of extraction using methanol and chloroform solvent. The percentage yield of extraction depends on the polarity of the solvents used, pH, temperature, time for the extraction to complete, and the sample composition (Do et al., 2014).

From the table shown, the percentage yield of methanol extract is higher compared to the percentage yield of chloroform extract. This shows that the percentage yield of extract increases as an increase in the solvent polarity from which methanol has a polarity index of 5.1, whereas chloroform has a polarity index of 2.7 (Katz et al., 1998). Methanol consists of polar region with -OH group and a non-polar hydrocarbon chain which allows methanol to extract both polar and non-polar molecules, while chloroform is to extract only non-polar hydrocarbon. Hence, the methanol extract has the highest yield with 44.44% compared to chloroform extract with a yield of 22.22%.

**Table 1:** Percentage Yield of Sample Extraction

| Sample | Yield of Methanol Extract (%) | Yield of Chloroform Extract (%) |
|---|---|---|
| **Foliage** | 44.44 | 22.22 |

### *3.2 Antioxidant Acitivity of Hylocereus Undatus Foliage*

It is important to determine the reductive capacity of *Hylocereus undatus* extracts to indicate the amount of their antioxidants. The method used to determine the antioxidant activity in this study was ferric reducing antioxidant power assay (FRAP) designed by Benzie & Strain (1998). The results of reducing power can be seen in Table 2 and Figure 1.

The reducing capacity of certain compound may serve as an indicator of its potential antioxidant activity (Meir et al., 1995). Same as free radical scavenging, reductive capabilities also increase as the increase in its concentration. FRAP assay has taken an advantage of an electron transfer reaction in which a ferric salt is used as an antioxidant (Benzie & Strain, 1998). The reducing power ability of $Fe^{3+}$ was carried out by varying the concentration of the sample extract. Based on the graph in Figure 1, the value of absorbance increases as the concentration of the sample extract increases which represent the increase in reducing power. The assay in this study has changed the yellow colour of the test solution to various shades of dark green as these colours depend on the reducing power of antioxidant samples. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

**Table 2:** Absorbance Measurement of BHT/BHA, Methanol and Chloroform Extract

| Concentration (ppm) | Volume of Sample (mL) | Absorbance Measurement (at 700 nm) | | |
|---|---|---|---|---|
| | | BHT/BHA (1:1) | Methanol Extract | Chloroform Extract |
| **200** | 3.6 | 1.428 | 0.622 | 0.243 |
| **400** | 7.1 | 2.023 | 0.930 | 0.272 |
| **600** | 10.7 | 2.204 | 1.210 | 0.435 |
| **800** | 14.3 | 2.449 | 1.285 | 0.450 |
| **1000** | 17.9 | 2.464 | 1.455 | 0.507 |

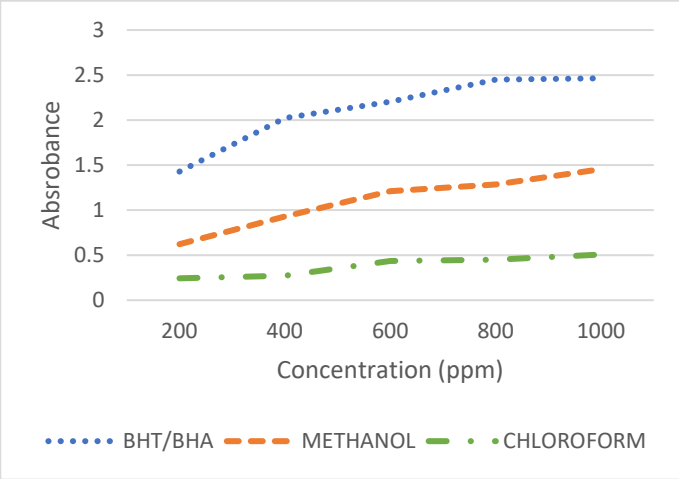


**Figure 1:** Absorbance Measurement of BHT/BHA and Methanol and Chloroform Extract of *Hylocereus Undatus Foliage*

As shown in Figure 1, BHT/BHA with the ratio one to one had the highest absorbance reading compared to methanol extract followed with chloroform extract. The reducing power of BHT/BHA, methanol extract and chloroform extract increased when the concentration of the sample extract increased. The absorbance of 200 ppm of BHT/BHA has no significant difference with 800 ppm of methanol extract, while 200 ppm of methanol extract has no significant difference with 600 ppm of chloroform extract. This can be concluded that the reducing power of 200 ppm BHT/BHA can be compared with 800 ppm of methanol extract, and the reducing power of 200 ppm methanol extract can be compared with 600 ppm of chloroform extract in reducing ferric ions to ferrous ions ($Fe^{3+}$ to $Fe^{2+}$). Table 3 shows the antioxidant activity in methanol extract and chloroform extract while Table 4 shows the comparison of antioxidant activity in this study with the previous studies on *Hylocereus undatus* (Som et al., 2019). From the results, it shows that the antioxidant activity in this study is comparable to the previous studies.

**Table 3:** Antioxidant in Methanol Extract and Chloroform Extract

| Concentration (ppm) | Antioxidant (%) | |
|---|---|---|
| | Methanol Extract | Chloroform Extract |
| **200** | 43.56 | 17.02 |
| **400** | 45.97 | 13.45 |
| **600** | 54.90 | 19.74 |
| **800** | 52.47 | 18.37 |
| **1000** | 59.05 | 20.58 |

**Table 4:** comparison of antioxidant activity in this study with the previous studies on *Hylocereus undatus.*

| Type of Solvent | Method Used | Antioxidant Activity (%) | References |
|---|---|---|---|
| **Methanol (Foliage)** | FRAP | 59.05 | This study |
| | DPPH | 88.81 | (Som et al., 2019) |
| **Methanol (Peels)** | FRAP | N/A | - |
| | DPPH | 97.42 | (Som et al., 2019) |
| **Chloroform (Foliage)** | FRAP | 20.58 | This study |
| | DPPH | 38.30 | (Som et al., 2019) |
| **Chloroform (Peels)** | FRAP | N/A | - |
| | DPPH | 18.71 | (Som et al., 2019) |
| | DPPH | 38.30 | (Som et al., 2019) |
| **Chloroform (Peels)** | FRAP | N/A | - |
| | DPPH | 18.71 | (Som et al., 2019) |

## 4.0 Conclusion

This study was conducted to analyze the antioxidant activity in *Hylocereus undatus* or white dragon fruit foliage by using two different solvents for extraction namely; methanol and chloroform. In determining antioxidant activity, the powder of *Hylocereus undatus* foliage being extracted first and the yield of sample extraction shows that methanol extraction is higher than chloroform extraction. This is because methanol solvent can extract both polar and non-polar compounds while chloroform solvent can only extract non-polar compound. Then, Ferric Reducing Antioxidant Power (FRAP) assay was used to determine the antioxidant activity. In conclusion, the antioxidant activity obtained using FRAP assay (59.05%) and (20.58%) to comparable to the DPPH

(88.81% and 38.30%) using methanol and chloroform solvent, respectively.

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