



UNIVERSITI PUTRA MALAYSIA

***DETERMINATION OF TOTAL PHENOLIC CONTENT, TOTAL
FLAVONOID CONTENT AND TOTAL ANTIOXIDANT ACTIVITY OF
TWO HYLOCEREUS FLOWERS EXTRACT***

HAFIYYAN NADHIRAH ZAINAL

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TWO *HYLOCEREUS* FLOWERS EXTRACT**

BY

HAFTYYAN NADHIRAH ZAINAL

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of Bachelor of Science (Nutrition and Community Health) from the Faculty
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ABSTRACT

DETERMINATION OF TOTAL PHENOLIC CONTENT, TOTAL FLAVONOID CONTENT AND TOTAL ANTIOXIDANT ACTIVITY OF TWO *HYLOCEREUS* FLOWERS EXTRACT

Hafiyyan Nadhirah Zainal

The flower of *Hylocereus undatus* or known locally as bawanghua is widely used in Traditional Chinese Medicine and is made into healthy soup to relieve cough, moisturize the lung and eliminating the phlegm. Previous study only include one species of *Hylocereus* flower which was *H.undatus* and the study reported that the antioxidant ability of *H.undatus* flower seems attributable to total phenolics which mainly total flavonoid. The objective of the study is to study total phenolic content (TPC), total flavonoid content (TFC) and the total antioxidant activity of two *Hylocereus* flower which are *H.undatus* and *H.polyrhizus* in 70% ethanol extract and aqueous extract. Determination of total phenolic content and total flavonoid content were done by Folin-ciocalteu method and aluminium chloride colourimetric method. The samples were analysed for antioxidant activity using ferric reducing antioxidant power (FRAP) and DPPH radical scavenging assay. Results showed that 70% ethanol extract obtained the highest value in TPC, TFC, FRAP value and have the lowest IC₅₀ value in DPPH radical scavenging activity. In comparison, between two sample which were the flower of *H.undatus* and *H.polyrhizus*, *H.undatus* flower contained higher TPC, TFC, FRAP and lowest IC₅₀ value in both ethanolic and aqueous extract. Analysis of variance showed significant difference at level $p < 0.05$ in TPC in ethanolic and aqueous extract *H.undatus* flower, significant difference in TFC in 70% ethanol extract of flower *H.polyrhizus* and *H.undatus*, and significant difference in antioxidant activity between ethanolic and aqueous extract of both flowers. Pearson correlation analysis showed that TPC has strong positive correlation with TFC, FRAP which indicate that as TPC value increases, TFC and FRAP also increases while TPC has strong negative correlation with IC₅₀ as the TPC value increase, IC₅₀ decreases which indicates stronger antioxidant activity. This showed that phenolic compounds and flavonoids may be the major contributors to the antioxidant activities in both *Hylocereus* flowers.

ABSTRAK

PENENTUAN JUMLAH KANDUNGAN FENOLIK, JUMLAH KANDUNGAN FLAVONOID DAN JUMLAH AKTIVITI ANTIOKSIDA DI DALAM DUA EKSTRAK BUNGA *HYLOCEREUS*

Hafiyyan Nadhirah Zainal

Bunga *Hylocereus undatus* atau dikenali tempatan sebagai bawanghwa digunakan secara meluas dalam Perubatan Tradisional Cina dan dijadikan sup yang sihat untuk mengurangkan batuk, melembapkan paru-paru dan menghilangkan kahak. Kajian terdahulu hanya satu spesies bunga *Hylocereus* digunakan iaitu *H.undatus* dan kajian melaporkan bahawa keupayaan antioksidan bunga *H.undatus* adalah disebabkan oleh jumlah fenolik yang kebanyakannya ialah dari jumlah flavonoid. Objektif kajian ini adalah untuk mengkaji jumlah kandungan fenolik (TPC), jumlah kandungan flavonoid (TFC) dan jumlah aktiviti antioksidan di dalam dua bunga *Hylocereus* iaitu *H.undatus* dan *H.polyrhizus* dalam ekstrak etanol 70% dan ekstrak air. Penentuan jumlah kandungan fenolik dan jumlah kandungan flavonoid telah dilakukan melalui kaedah Folin-ciocalteu dan kaedah kolorimetrik aluminium klorida. Sampel dianalisis untuk aktiviti antioksidan menggunakan kuasa antioksidan redaman ferit (FRAP) dan DPPH penghapusan radikal bebas. Keputusan menunjukkan bahawa ekstrak etanol 70% memperoleh nilai tertinggi dalam nilai TPC, TFC, FRAP dan mempunyai nilai IC₅₀ yang paling rendah dalam aktiviti penghapusan radikal DPPH. Sebagai perbandingan, antara dua sampel yang merupakan bunga iaitu *H.undatus* dan *H.polyrhizus*, bunga *H.undatus* mengandungi lebih tinggi TPC, TFC, FRAP dan nilai IC₅₀ yang terendah dalam kedua-dua etanol dan ekstrak air. Analisis varians menunjukkan perbezaan yang ketara pada tahap $p < 0.05$ dalam TPC dalam ekstrak etanol dan air bunga *H.undatus*, perbezaan ketara dalam TFC dalam ekstrak etanol 70% bunga *H.polyrhizus* dan *H.undatus*, dan perbezaan yang signifikan dalam aktiviti antioksidan antara etanol dan ekstrak air dalam kedua-dua bunga. Analisis korelasi Pearson menunjukkan bahawa TPC mempunyai korelasi positif yang kuat dengan TFC, FRAP yang menunjukkan bahawa semakin meningkat nilai TPC, TFC dan FRAP juga akan bertambah manakala TPC mempunyai korelasi negatif yang kuat dengan IC₅₀ kerana semakin meningkat nilai TPC, nilai IC₅₀ akan semakin menurun yang menunjukkan aktiviti antioksidan yang lebih kuat di dalam sampel. Ini menunjukkan bahawa sebatian fenolik dan flavonoid mungkin penyumbang utama kepada aktiviti antioksidan dalam kedua-dua bunga *Hylocereus*.

CHAPTER 1

INTRODUCTION

1.1 Background

Natural plant products are estimated to be used by more than 70% of the world population as herbal or food supplements or drugs and the top 50 drugs sold in European pharmacies are a plant-based product (Zhu, Huang, Tan, Sun & Whiteman, 2004). Western drugs used for treatment are widely accepted worldwide, but Asian communities are known for their culture to combine the use of herbal medicines with Western treatment due to their ancestor's experiences especially in Chinese communities. Traditional Chinese Medicine (TCM) is widely practised in Chinese communities for centuries and it is based on their fundamental theories, *yin-yang* and five elements, (*wu xing*) (Li & Cheng, 1987).

TCM includes Chinese herbal medicine, acupuncture and Chinese massage, (*tui na*), mind or body exercise and dietary therapy where Chinese herbal medicine and acupuncture the most commonly used. Chinese herbal medicine (*zhong yao*) is a part of Chinese traditional culture and medical practice which include the use of plants, minerals and animal parts in their medical practice. TCM is widely used in Asian countries including China to help cancer patient to improve their quality of life by relieving the symptoms and side effects from cancer treatment such as chemotherapy and radiotherapy and sometimes to treat cancer itself (Lao, Xu & Xu, 2012).

Reactive oxygen species (ROS) is a highly reactive compound produced by oxygen and some of the reactive species are free radicals which can result in cellular damage (Hajhashemi, Vaseghi, Pourfarzam & Abdollahi, 2010). Free radicals can damage genomic DNA and cancer is a disease of the genome. Antioxidants are molecules that can safely react with free radicals to neutralize or terminate the reaction to damage body cells. Almost all plant products contain natural antioxidants and intake of antioxidant has been associated to decrease the risk of cancer.

Fruits and vegetables are rich sources of natural antioxidants, minerals and vitamins. White flesh dragon fruit (*Hylocereus undatus* (Haw.) Britt. & Rose) and red flesh dragon fruit (*Hylocereus polyrhizus* (F.A.C WEBER) Britt. & Rose) are the fruits that have been studied to have a high antioxidant content. Dragon fruit which is in the family of Cactaceae and in genus *Hylocereus* is a type of climbing vine cactus species where the crop is widely distributed in Australia, China, Israel, Malaysia, Nicaragua, Taiwan and Vietnam. There are varieties of species of *Hylocereus* and there are about 18 tropical species in America (Gunasena, Pushpakumara & Kariyawasam, 2006).

In Malaysian market, the two most common species of *Hylocereus* are *Hylocereus undatus* which have white fruit pulp and red skin and *Hylocereus polyrhizus* which has red fruit pulp and red skin. The flower of dragon fruit is nocturnal where the flower opens at night and wilt during daytime and the flower is so huge and beautiful that it is nicknamed as “Queen of the Night” (Gunasena, Pushpakumara & Kariyawasam, 2006).

The flower of white flesh dragon fruit, *Hylocereus undatus* is an edible flower which is commonly used by people in provinces of China and they use the flower which is called “bawanghua” in Chinese as their medicinal food (Yi, Zhang, Li, Wang,

Ye, Zhao & Wang, 2012). The flower is usually sold either fresh or dried. *Bawanghua* is usually cooked with pork rib to relieve a cough, moisturize the lung and eliminating phlegm (Zhou, 2001). Besides using the flower in healthy soup, *bawanghua* is recently commercialized in China in form of healthy beverages (Xia, Zhong & Guo, 2004).

1.2 Problem statement

Modern society nowadays are more exposed to the health benefit of antioxidant thus the increasing trend in products that claimed to be a high antioxidant can be seen. Few studies have shown that antioxidant has a protective effect on our body cells from free radical that could result in the destruction of body cell which leads to various degenerative disease in humans. Fruits and vegetables are the common source of antioxidant as plants contain many derivatives such as carotenoid, phytoestrogen lignans, isoflavones, flavonoids, isothiocyanates, indoles and allium that contribute in preventing carcinogen from destroying body cells (Chan & Tomlinson, 2009). Due to increases in death caused by non-communicable diseases, a further research on alternative treatments for chronic diseases is needed.

Antioxidant is important in the food industry as antioxidant increases the shelf life of food through protecting the food from deterioration and become rancid due to the oxidation of lipids which decreases the nutritional value, become unsafe to eat and affect the food texture (Ngo, Wijesekara, Vo, Ta, Van & Kim, 2011). As antioxidant has been proved to improve the quality of food, the use of synthetic antioxidant is common in the food industry. However, the use of a synthetic antioxidant in the food industry has been restricted due to their toxic and carcinogenic effect and therefore there is an increasing trend on the usage of natural antioxidant (Barreira, Ferreira, Oliveira & Pereira, 2007).

The flower of white flesh dragon fruit, *Hylocereus undatus* which is called *bawanghua* is widely used in China as a medicinal food. In a previous study, it is reported that *bawanghua* contain six flavonoids (Yi, Wu, Wang & Ye, 2011). Flavonoid has an anti-inflammatory effect in a neurodegenerative disorder such as Alzheimer's disease (Spagnuolo, Moccia & Russo, 2017). There are also several studies on the *Hylocereus undatus* flower that found that the flower helps assist the therapy for atherosclerosis, diseases that related to lung such as tuberculosis and bronchitis (Liang, Li, & Wang, 1995; Zhonghua & Bencao, 2004). However, there is lack of data in the literature concerning the antioxidant properties of *Hylocereus* flower in Malaysia even in South East Asia. Other studies only focusing on the pulp, peel and seed of *Hylocereus* species and only a few studies on the flower. There is a study on the antioxidant activity, total phenolic and flavonoid content of *Hylocereus undatus* flower but the study only includes one species of *Hylocereus* flower (Li, Gao, Han, Lin, Hu & Chen, 2013).

Thus, this study aimed to determine the total phenolic content and total flavonoid content and the total antioxidant activity of two *Hylocereus* flower, which are *Hylocereus undatus* and *Hylocereus polyrhizus*.

1.3 Significance of the study

The medicinal plants are used to treat disease or alleviate symptoms, these Chinese medicinal plants also have been consumed by Chinese communities to ensure lifelong health. 'Pao' in Chinese means supplementation which is undifferentiated from the present meaning of functional food and medicinal plants under 'pao' category have been consumed by people in China as a wellbeing tonic or as a remedy to delay aging (Ko, Mak, Chiu, Poon, 2004; Wong, Li, Cheng & Chen, 2006). Although the flower of two species of *Hylocereus* is not under 'pao' category, it is hoped the data information obtained from this study may contribute a scientific evidence to prove the potential of dragon fruit flower as one of the sources of natural antioxidant and provide additional data for future researchers.

In Malaysia, Traditional Chinese Medicine is not widely practised due to lack of knowledge on the benefits of the medicinal plant. Thus, it is hoped that through the findings of this study, it will add to the body of knowledge of Malaysian on the benefit of medicinal plants. Moreover, the intake of natural antioxidant is more acceptable nowadays due to increasing awareness of the advantages of natural antioxidant derived from plants compared to synthetic antioxidants. The medicinal plant, *bawanghua* has been commercialized in China as healthy beverage thus it is hoped this study can help consumer on choosing natural food products rather than synthetic food products. Besides that, it is hoped that through this study it would promote the various use of dragon fruit including the flower part.

1.4 Objectives of the study

General objective

To study total phenolic content, total flavonoid content and the total antioxidant activity of *Hylocereus* flower which are *Hylocereus undatus* and *Hylocereus polyrhizus*.

Specific objectives

1. To determine and compare the total phenolic content and total flavonoid content of two *Hylocereus* flower in the aqueous and ethanolic extract.
2. To determine and compare the total antioxidant activity (DPPH free radical antioxidant activity and Ferric Reducing Antioxidant Power assay) in the aqueous and ethanolic extract.
3. To determine the correlation between total phenolic content, total flavonoid content and total antioxidant activity of two *Hylocereus* flower.

CHAPTER 2

LITERATURE REVIEW

2.1 Free radicals

Free radical can be defined as a molecule that has one or more unpaired electron in its outermost shell which are formed through the breakage of the chemical bond. They can act as oxidants and gains electron or as reductant and loss electron. Most of free radical are not stable and very reactive (Cheeseman & Slater, 1993). Free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated as part of physiological processes in normal metabolism (Valko et al., 2007). ROS which are radicals that have no oxygen are the byproducts formed in cells of aerobic organisms and can induce reaction which leads to cell damage (Rahman, 2007). When free radicals were present in food or in body cells, they can act readily with lipids, carbohydrates, protein and other molecules that are nearby which are harmful to human (Ozcan & Ogun, 2015).

Lipid peroxidation is one of the important mechanism and sequence of events of free radical where it causes damage to the cellular membrane. Lipid peroxidation caused damage to body cell through changing the net charge of the cells where it affects the osmotic pressure and causes cells to swell which lead to the death of the cell (Buettner, 1993). Few studies have shown that free radical and ROS has been associated with cardiovascular disease, inflammation, diabetes, cancer and

neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease (Halliwell, 1994).

The breakage of a chemical bond where each fragment keeps one electron, by breaking a bond to give another radical and redox reactions forms free radical. Free radicals, ROS have several types include hydroxyl, superoxide, hydrogen peroxide, peroxy, organic hydroperoxide, singlet oxygen and ozone. RNS includes nitric oxide, peroxynitrite, peroxynitrous acid and nitrogen dioxide (Devasagayam et al., 2004). All types of free radical capable to cause cellular damage by reacting with lipid membranes and other nearby molecules.

Free radicals can be formed through enzymatic and non-enzymatic reactions. Enzymatic reactions generating free radicals include those involved in the respiratory chain, the phagocytosis, the prostaglandin synthesis and the cytochrome P450 system (Halliwell & Gutteridge, 2007; Valko et al., 2004; Valko et al., 2007). For example, the superoxide anion radical is generated through several oxidation processes of cell systems such as NADPH oxidase, xanthine oxidase and peroxidases which when it formed, it generates various ROS and RNS. Free radicals also can be formed from non-enzymatic reactions of oxygen with organic compounds including a compound that is initiated by ionizing radiations. Besides that, free radical can be produced by enzymatic reaction through aerobic respiration in mitochondria (Pham-Huy, He, & Pham-Huy, 2008).

Reactive oxygen species or ROS are the oxygen-free radicals which are the main byproducts of free radical and were formed continuously in aerobic organisms. ROS can be generated endogenously and exogenously. ROS which is endogenously sourced include mitochondria, cytochrome P450 metabolism, peroxisomes and inflammatory cell activation. Besides that, neutrophils, eosinophils and macrophages

are ROS that is endogenously sourced. Macrophages that are activated increases the oxygen uptake which leads to the production of various type of ROS. ROS from the exogenous source is usually from xenobiotics, chlorinated compounds, environmental agents, a metal which are redox or non-redox and also from radiation and ions (Rahman, 2007).

Free radicals such as ROS and RNS plays two roles in the human body. It can be beneficial and deleterious to human. ROS and RNS have a beneficial effect on cellular signalling and immune defence in low or moderate levels (Losada-Barreiro & Bravo-Díaz, 2017). An overproduction of free radical which causes imbalance and have a deleterious effect as it can damage body cell and generate oxidative stress (Pham-Huy et al., 2008). Oxidative stress has been known to be the major cause of development of chronic and degenerative diseases. The deleterious effect of free radical is balanced with antioxidants that are present in the body and acts as defence system (Halliwell, 1996). However, the accumulation of free radical during life cycle can implicate health in long-term which causes ageing and degenerative diseases.

The biochemical and physiological damage caused by free radical through oxidative stress can be counteracted by antioxidants. Antioxidants system in human body protects the cells and organs from free radical damage by neutralizing and deactivating the free radical. Antioxidants play an important role to ensure the life-long health and maintaining the balance. However, under certain conditions, the antioxidant system in human body is not sufficient to counteract the damage by free radical. Natural plants such as fruits and vegetables are the most common source of food that is rich in antioxidants. Consumption of antioxidants from natural plants helps in neutralizing free radical and able to decrease the risk of degenerative diseases. Thus,

the increasing trend of consuming food that rich in antioxidants can be seen in the last decade (Alvarez-Parrilla et al., 2007).

2.2 Antioxidant

Antioxidants can be defined as molecules that neutralize the excess of free radicals in order to protect body cells against deleterious effect of free radical and contribute to preventing degenerative diseases (Pham-Huy et al., 2008). Antioxidants play important role in several mechanisms including reducing free radical activity, scavenging free radicals, potential complexing of pro-oxidant metals and quenching of singlet oxygen (Tachakittirungrod, Okonogi & Chowwanapoonpohn, 2006).

The human body has mechanisms to counteract oxidative stress by producing antioxidants, either naturally generated in situ (endogenous antioxidants), or externally supplied through foods (exogenous antioxidants). Endogenous antioxidants which are generated in body act as the first-line defence against free radical. However, the antioxidants are not enough thus human body mostly relies on antioxidant from food such as fruits and vegetables.

The protective effect at different stages of oxidation process can be used to classified antioxidant into primary and secondary antioxidants. Primary antioxidants stop or delay oxidation by releasing an electron to free radicals to make them more stable molecule while secondary antioxidant function in several mechanisms such as binding of metal ions, scavenging oxygen and converting hydroperoxides to non-radical species (Norshazila, Zahir, Suleiman, Aisyah, & Rahim, 2010).

The oxidation process is a chemical reaction that transfers electrons from a substance to an oxidizing agent and through this reaction, free radicals are produced.

The free radicals can initiate chain reaction process which could lead to cell damage and antioxidant plays its role to terminate these chain reactions. The reactions are stopped by antioxidant by removing the free radical intermediates and inhibit other oxidation reaction. Antioxidant molecules become oxidized when it destroys free radical thus, antioxidants are often reducing agents such as thiols, ascorbic acid and polyphenols (Sies, 1997).

Antioxidant molecules become oxidized when it destroys free radicals thus it is important that the antioxidant resources must be continuously restored. Antioxidant molecules may be effective against free radicals at one particular system, but the same antioxidant may not be effective in other systems. Besides that, an antioxidant in certain condition can act as a pro-oxidant where it generates toxic ROS or RNS (Young & Woodside, 2001).

Antioxidant also can be defined as molecules that possess radical scavenging capacity which have potential protective effect against deleterious effect of free radical. Oxidative stress alters cell membranes and other structures such as protein, lipids, lipoprotein and DNA. Oxidative stress can damage protein and lead to structural damage and affect the enzyme activity and oxidative stress on DNA can lead to mutation of cells and cause cancer. Oxidative stress which has a deleterious effect if not regulated properly, oxidative stress can cause degenerative and chronic disease as well as accelerate ageing process (Pham-Huy et al., 2008). Thus, these antioxidants are important in the prevention of degenerative diseases.

The antioxidant has been widely used in food industry to increase the shelf life of foodstuffs and the usage of synthetic antioxidant such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) is common food additives. Antioxidant

in food prevents the food from becoming rancid, maintain its colour, texture and nutritional value and prevent the formation of toxic compound (Carocho, Morales, & Ferreira, 2018). However, these synthetic antioxidants have shown a toxic effect on health compared to the usage of natural antioxidant and synthetic antioxidant also show low solubility and moderate antioxidant activity (Pourmorad, Hosseinimehr & Shahabimajd, 2006). Thus, the increasing demand for alternative and safe antioxidant from natural sources can be seen over the years.

2.3 Phenolic compound

The phenolic compound can be characterized by at least one aromatic ring (C_6) have one or more hydroxyl groups (Bravo, 1998). The group of compounds are usually referred to as polyphenols despite structural diversity. These polyphenols include simple phenols such as phenolic acids and derivatives and also complex structures such as flavonoids, flavones and anthocyanins (Alvarez-Parrilla et al., 2007).

Polyphenols are considered to be more important antioxidant compared to others due to a large array of reaction including free radical scavenging, metal chelation and enzyme modulation activities and inhibition of LDL oxidation (Rodrigo & Bosco, 2006). Phenolic compound is one of the most widely occurring groups of phytochemicals, are of considerable physiological and morphological importance in plants where the compound plays a role in growth and reproduction while providing protection from pathogen and predators (Balasundram, Sundram & Samman).

The shikimate pathways are the most important in the synthesis of plant phenolics (Strack, 1997). Phenolic compounds are secondary metabolites that are derivatives which are formed by condensation of addition reactions in plant pathways which are pentose phosphate, shikimate and phenylpropanoid (Randhir, Lin & Shetty,

2004). The phenolic compound can be classified as simple phenols and phenolic acids such as syringic acid, benzoic acid, gallic acid, chlorogenic acid and other associates and polyphenols which are classified into several groups including flavonoids, tannins and stilbenes.

Studies on phenolic compounds show that the compound has a beneficial effect on health as it is anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects (Balasundram, Sundram & Samman). The ability of a phenolic compound to scavenge free radicals, donate hydrogen atom or electron or chelate metal cation contribute to their antioxidant activity (Balasundram, Sundram & Samman).

Most of the health benefits of phenolic compounds are derived from high consumption of natural antioxidant such as fruits and vegetables which contain high antioxidant properties (Heim, Tagliaferro & Bobilya, 2002). Higher intake of natural antioxidant has been associated to lower the risk of degenerative diseases such as cancer, cardiovascular disease and diabetes (Cai, Luo, Sun & Corke, 2003). In the previous study which studies on 112 species of traditional Chinese plant shows that these medicinal plants have significantly stronger antioxidant activity and contain more phenolic compound compared to common fruits and vegetables (Cai, Luo, Sun & Corke, 2003).

An increasing interest in phenolic compounds can be seen among food and medical scientists since these compounds have a very strong *in vitro* and *in vivo* antioxidant as well as their abilities to scavenge free radicals, break radical chain reaction and chelate metals (Liu & Yao, 2006). All plant phenolic compounds have

the structural requirements of free radical scavengers and have potential to be a source of antioxidant (Jayathilakan, Sharma, Radhakrishna & Bawa, 2007).

2.4 Flavonoid

Among the naturally occurring phenolic compounds, flavonoid constitutes the largest group of plant phenolics (Harborne et al., 1999). Flavonoid is a plant pigment that is synthesized from phenylalanine and contributes to beautiful colours in flowering parts of a plant (Clifford & Cuppett, 2000). Flavonoid which is commonly found in fruits and vegetables comprises a large group of polyphenolic compounds that are characterized by a benzo- γ -pyrone structure (Yao et al., 2004).

Flavonoid cannot be synthesized by humans and animals because they are plant phytochemicals. Most of the flavonoid found in animals are originated from a plant that animal feed and not generated in situ (Cook & Samman, 1996). Flavonoid can be classified into 10 chemical groups including flavanones, flavanones, isoflavonoids, flavanols, anthocyanins and flavonols. Most of the flavonoid compound can be found in daily diet (Yao et al., 2004). Table 2.1 shows the main subgroups of flavonoids and its food sources. Flavonoid is a major colouring component of flowering plants and generally responsible for colour, taste, prevent lipid peroxidation and protection of vitamins and enzymes. Basically, any food that is plant-based which contain natural flavours and colourings may contain flavonoids (Huang, Osawa & Rosen, 1994).

Table 2.1: Main subgroups of flavonoids and food sources.

Subgroup	Food sources
Anthocyanins	Fruits and flowers
Flavanols	Apples, tea, beer, wine, fruit juice, black tea
Flavanones	Citrus fruits, cumin, oranges
Flavones	Herbs, cereal, fruits, parsley
Flavonols	Onion, cherries, apples, broccoli, kale, tomatoes
Isoflavones	Legumes such as soybean

(Sources: Yao et al., 2004)

Flavonoid which are from plants contain naturally occurring phenolic antioxidants and have been associated to have a health benefit to humanity. Flavonoid has the properties that are beneficial including anti-allergic, anti-inflammatory, anti-viral, anti-proliferative and anti-carcinogenic. Due to the beneficial effect of an antioxidant, flavonoid has been getting the attention of food and medical scientist in the prevention of degenerative diseases such as cancer and vascular diseases (Zand, Jenkins & Diamandis, 2002).

Flavonoid can scavenge free radicals and active oxygen species and due to this flavonoid has been known and labelled as “high level” natural antioxidants. Flavonoids have been reported to chelate iron and copper which may contribute to their antioxidant properties (Clifford & Cuppett, 2000). Previous in vitro and in vivo studies on flavonoids showed that flavonoid has antioxidant and antimutagenic activities and reduce the risk of cardiovascular disease and stroke (Aherne & Obrien, 2002; Duthie, Duthie & Kyle, 2000).

Consumption of natural antioxidant such as fruits and vegetables which contain flavonoid has been reported to be inversely associated with the incidence of vascular diseases as flavonoid reduce the rate of formation of oxidized LDL and inhibit the growth of plaque at artery which could lead to atherosclerosis (Naderi, Asgary, Sarraf-Zadegan & Shirvany, 2003). Flavonoid also acts as cancer chemopreventive agents as flavonoid can modify enzymes and bind carcinogens to DNA and prevents mutation of the cell which could lead to development cancerous cell (Siess et al., 1996). For example, green tea and black tea which is a significant source of antioxidants protect against cancer by causing cell cycle arrest and inducing apoptosis and inhibit tumour development (Ahmad, Feyes, Nieminen, Agarwal & Mukhtar, 1997; Nakamura, Harada, Kawase, Matsuda & Tomita, 1992).

2.5 Dragon fruit

Dragon fruit was first introduced in Malaysia approximately more than ten years ago (Amat @ Halimi & Mohd Satar, 2007). Dragon fruit is known for their unique appearance and vibrant colour of their flesh. The most common species in Malaysia are white flesh dragon fruit (*Hylocereus undatus*) and red flesh dragon fruit (*Hylocereus polyrhizus*). Dragon fruit which belongs to a cactus family, Cactaceae can grow up to 20 ft high and climb using aerial roots on everything from trees to brick walls with ribbed stems, green and growing spinier with age (Halimoon & Hasan, 2010). The average weight of dragon fruit is around 350 g and this fruit grows better at climate that is dry with annual rainfall ranges from 20-50” per year (Nurliyana, Syed Zahir, Mustapha Suleiman, Aisyah, & Kamarul Rahim, 2010).

Dragon fruit is a popular crop in several countries such as Australia, China, Malaysia, Nicaragua, Taiwan and Vietnam and this fruit has become a major export in

Vietnam which led to a higher price in the market compared to Durian which is known as “King of Fruits”. The advantages of growing this crop are that once it is planted, it can grow up to 20 years and one plant of dragon fruit can produce up to four to six cycles of fruits per year (Gunasena, Pushpakumara & Kariyawasam, 2006).

Fruit maturity can be divided into physiological and commercial maturity where physiological maturity referred to the development stage of the plant starting from maximum growth to senescence stage while commercial maturity is the requirements need of the markets (Wills et al., 2007). Dragon fruit is considered to be ready for harvesting when they are fully expanded, and the skins become 85% red in colour.

The flower of dragon fruit is called “Queen of the Night” due to its nocturnal properties where it only blooms at night and starts to wilt during the day. The flowers bloom once every 15 days, around the 1st and 15th days of the lunar calendar. The flower of dragon fruit which is huge, beautiful and fragrant can grow up to 30 cm in diameter. The edible flower of white flesh dragon fruit (*H. undatus*) has been used in Chinese communities in healthy soups to remove phlegm, relieve a cough and moisturize the lung (Zhou, 2001).

The brilliant colour of red flesh dragon fruit is due to a pigment called betacyanins and the colour has led to many studies on the fruit to be used as colouring agents compared to white flesh dragon fruit which is lacking the pigment betacyanins (Wybraniec et al., 2001). The exotic features, attractive colours and the sweet taste of dragon fruit has led to several studies on the fruit. The red pigment of red flesh dragon fruit called betacyanins has been the interest of scientist and it is known that dragon fruit contains high antioxidant activity which is beneficial against free radical to

prevent the development of degenerative diseases. Previous studies on dragon fruit show that red flesh dragon fruit (*H. polyrhizus*) contain higher antioxidant activity compared to white flesh dragon fruit (*H. undatus*) as it is rich in an antioxidant-rich compound such as a flavonoid and phenolic compound (Park et al., 2008).

The peel of dragon fruit also contributes as a source of betacyanins compounds since they contain the same pigment as the pulp of the fruit. A study by Nurliyana, Syed Zahir, Mustapha Suleiman, Aisyah & Kamarul Rahim (2010), shows that the peels of red flesh and white flesh dragon fruit contain higher total phenolic content compared to the pulp of red flesh and white flesh dragon fruit. The higher total phenolic content in peels of dragon fruit compared to its pulp may be due to the presence of different kinds of antioxidant compounds. Besides that, a previous study confirmed the existence of organic acid and the peel carbohydrate components such as pectin, starch, cellulose and others (Jamilah et al., 2011).

Studies on the seed of dragon fruit revealed that there is major fatty acid present in the seed such as linoleic acid, palmitic acid and oleic acid (Chemah et al., 2010; Lim et al., 2010). The seed oil of red flesh dragon fruit and white flesh dragon fruit contain α -tocopherol and high amount of β -sitosterol (Lim et al., 2010).

Dragon fruit has been processed into several food products and been commercialized including beverages, jellies, jams and confectionery due to its attractive colour. The red colour of the fruit has been used as colouring agents and been a replacement for synthetic dyes. Besides the pulp, the flower also has been commercially advertised in China in form of health beverages (Xia, Zhong & Guo, 2004).

The fruit is believed to be a rich source of dietary fibres, minerals and antioxidants. The seed which contains α -tocopherol and high amount of β -sitosterol can be a good source of tocopherols and phytosterols. The red flesh dragon fruit (*H.polyrhizus*) contain higher antioxidant activities compared to white flesh dragon fruit which can be beneficial to human health as it has the ability to scavenge free radicals to prevent the development of degenerative disease and acts as chemopreventive agents.

2.6 Traditional Chinese Medicine

Traditional Chinese Medicine (TCM) which are originated in ancient China has been practised over thousands of years. In Chinese communities, the alternative treatment has been used alongside western treatment to relieve a disease or symptoms of a disease. In Singapore and Hong Kong, the use of TCM is significant as 54% of pregnant women in Hong Kong practised TCM mainly the usage of Chinese herbal medicine. TCM has become increasingly popular in western countries as it is safe, effective and free from side effects (Kam & Liew, 2002).

The medicinal herbs are classified based on the physical properties of the herb. The 'five elements' theory classified the herb based on taste whether it is sour, bitter, sweet, pungent or salty while the herb can also be classified using the properties of the herb whether it is cold, warm, heat or cold. For example, 'hot' symptoms will be treated with 'cold' herbs. The classification of the herb according to their chemical properties is a complicated process as there is no standardization of the classification system (Kam & Liew, 2002).

TCM uses the theories of *yin-yang* and "five elements" where the main goal of the treatment is to correct the imbalance. TCM includes many different practices

including acupuncture, Chinese herbal medicine, Chinese therapeutic massage, dietary therapy and tai chi. Several studies show that Chinese medicinal plants contain higher antioxidant content than common plants (Cai, Luo, Sun & Corke, 2004; Dragland, Senoo, Wake, Holte & Blomhoff, 2003). TCM which incorporate natural products from plants and plants contain naturally occurring antioxidant properties which can combat many degenerative diseases.

An in vivo study on antioxidant properties of herbal medicine *Salvia miltiorrhiza* Bunge which contain salvianolic acid B has shown that the herbal medicine able to treat atherosclerosis as it lowers the LDL cholesterol levels in a cholesterol-fed rabbit model (Wu, Hong, Lin, Wu & Shiao, 1998). Besides that, trilinolein which are abundant in plant *Panax pseudoginseng* has been used to treat circulatory disorder as it improves the erythrocyte deformation and inhibit platelet aggregation (Kam & Liew, 2002).

The Chinese herbal medicine is usually boiled in water and the extracts are used for consumption and it is been treated as a health tonic or as anti-ageing remedies. A herbal medicinal plant that is under “pro” category is consumed as supplementation to maintain overall health. The flavonoid, phenolic acid and tannins are the phenolic compounds that contribute the antioxidant activity in Chinese medicinal plants which have been associated to be anti-inflammatory, anti-carcinogenic and anti-atherosclerotic (Wong, Li, Cheng & Chen, 2005).

CHAPTER 3

MATERIALS AND METHODS

3.1 Reagents and chemicals

All chemicals used were purchased from Sigma Chemical Co. (St. Louis, USA) unless stated otherwise. All chemical reagents that will be used in this study are Analytical Reagent Grade. All glassware will be rinsed with distilled water before used.

3.2 Experimental Design

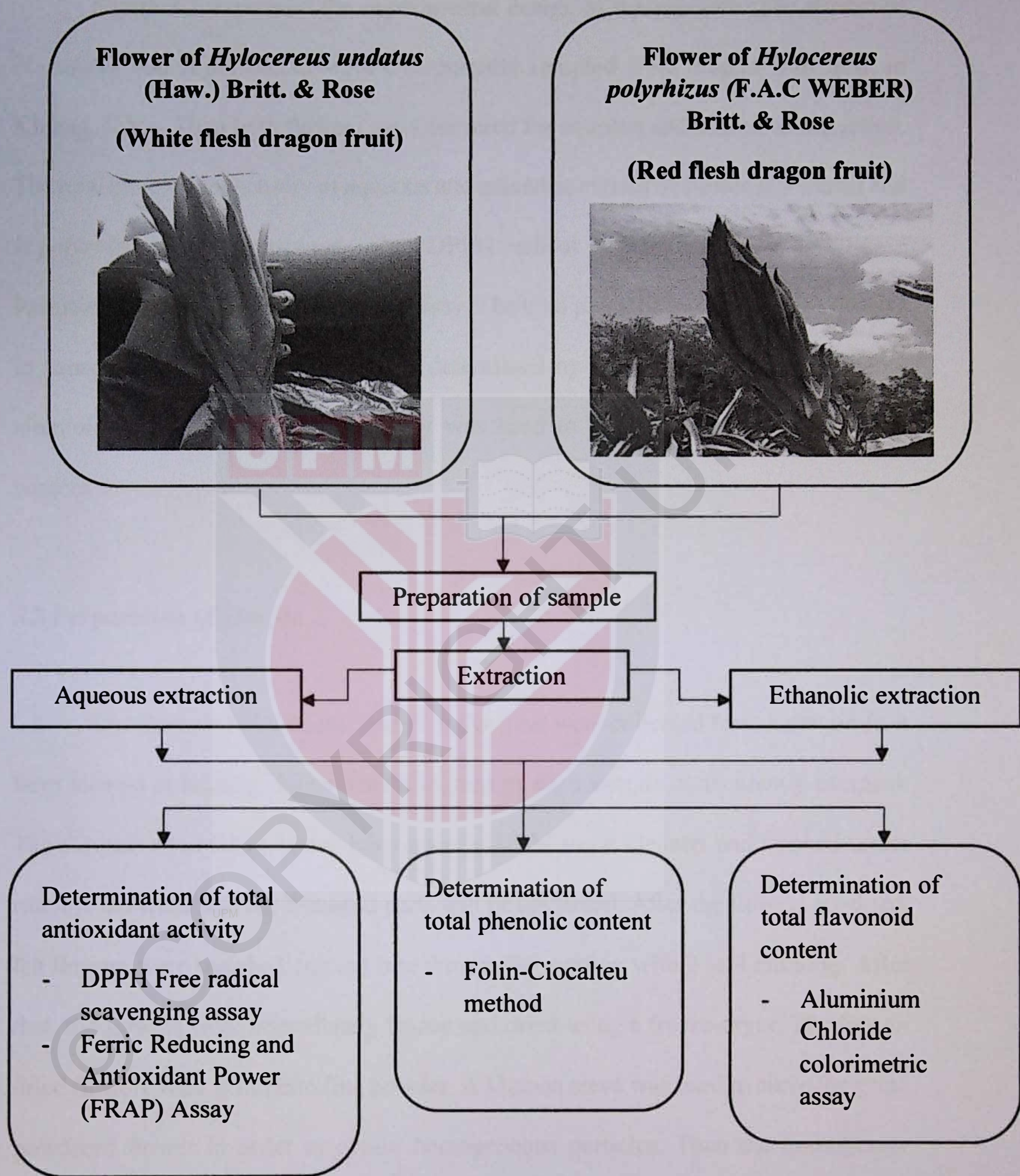


Figure 3.1: Schematic experimental design

Figure 3.1 explained the experimental design of the research. The flower of *H.undatus* and *H.polyrhizus* were conveniently sampled from dragon fruit farm in Kluang, Johor. Then both flowers were prepared for aqueous and ethanolic extraction. The total antioxidant activity of aqueous and ethanolic extract of flower *H.undatus* and *H.polyrhizus* were determined using DPPH radical scavenging assay and Ferric Reducing Antioxidant Power (FRAP) assay. The total phenolic content of both flowers in aqueous and ethanolic extracts was determined by Folin-Ciocalteu method while aluminium chloride colorimetric assay was used to determine the total flavonoid content.

3.3 Preparation of sample

The flower of *H. undatus* and *H. polyrhizus* were collected from a dragon fruit farm located at Kluang, Johor. One kilogram of each sample conveniently sampled. The flowers were taken to the laboratory in UPM to be cleaned and washed under running tap water and the damaged parts will be discarded. After the flowers air-dried, the flowers were weighed and cut into the smaller portion with 2 to 4 cm long. After that, the flowers were immediately frozen and dried using a freeze-dryer. The freeze-dried flowers were grind into fine powder. A kitchen sieve was used to sieve the grind powdered flower in order to obtain homogeneous particles. Then the homogenate flower powder was kept in an air-tight container and stored at -80°C prior to analysis.



Figure 3.2: The flower of *Hylocereus undatus*.



Figure 3.3: The flower of *Hylocereus polyrhizus*.



Figure 3.4: The flower is cut into small pieces flowers were before it is freeze dried.

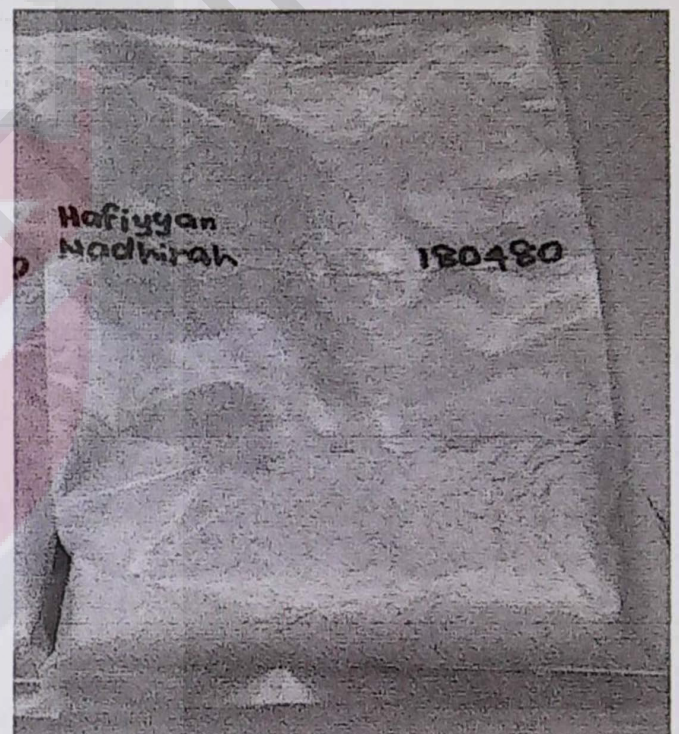


Figure 3.5: After the grind into powder.

3.4 Sample extraction method

3.4.1 Aqueous extraction

The aqueous extraction is according to the method of Amin and Tan (2002) with some modifications. Firstly, 4 grams of the free-dried samples was weighed and was extracted with 100 ml distilled water. Then, the mixture was stirred by using orbital shaker at 200 rpm for 2 hours at 50°C. After that, the mixture was poured into falcon tube and was centrifuged at 3000 rpm for 15 minutes at room temperature. Finally, the sediment was thrown while the supernatant was pipetted into a new tube wrap with aluminium foil and then was kept at 4°C for further analysis.

3.4.2 Ethanol extraction

The extract was prepared according to the procedure of Wen, Prasad, Bao & Amin (2010). The powdered sample was mixed with 70% aqueous ethanol in the ratio of 1 to 25 (g/v) in a conical flask. The mixture of the samples with the solvent was shaken for 2 h at 50°C using an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany). The extract was filtered using Whatman No. 1 filter paper to get the clear solution. The ethanol was removed under reduced pressure in a rotary evaporator at 40°C. The extraction yield was measured and expressed as a percentage (%). The filtered sample will be kept at -20°C for further analysis.

3.5 Determination of total phenolic content

The basic principle of the Folin-Ciocalteu assay is involving oxidation/reduction reaction based on the redox properties of antioxidant compounds that form a reaction with Folin-Ciocalteu reagent (Verzelloni, Tagliazucchi & Conte, 2007). The degree of colour change is correlated with the sample antioxidant concentrations. This assay involves electron transfer pathway that includes translocation of electrons from the antioxidant compounds to the molybdenum (Huang et al., 2005). The reduction of molybdenum will cause colour changes from yellow to blue.

However, there was some limitation of this assay as the colour measurement measured by Folin-Ciocalteu reagent is not specific phenolic compounds owing to its reaction to other reducing agents such as ascorbic acid, aromatic amines and sulphur dioxide (Huda-Faujan, Noriham, Norrakiah & Babji, 2007).

Total phenolic content was measured using Folin-Ciocalteu colorimetric method by Hajimahmoodi et al. (2008). The flower extracts of 200 μ L was mixed with 1.5 ml of 2.0N Folin-Ciocalteu reagent. The mixture was incubated for 5 min at room temperature. Then, the total phenolic was determined when 1.5 ml of sodium carbonate (60g/L) is added to the mixture and it was incubated for 90 minutes at room temperature. The absorbance was measured at 750 nm with UV-VIS spectrophotometer. Quantification was done with respect to the standard curve of gallic acid. All determinations will be performed in triplicate.

3.6 Determination of total flavonoid content

The total flavonoid content was determined using quantitative method by precipitating AlCl_3 in an alkalized medium where the precipitation acquires colour and as a consequence lends itself to the colorimetric assay (Luis, Domingues, Gil & Duarte, 2009). The principle involved in Aluminium chloride (AlCl_3) colorimetric method is that AlCl_3 react with C-4 keto groups, C-3 or C-5 hydroxyl group of flavones and flavonols then forms acid stable complexes (Bhaigyabati, Devi & Bag, 2014). An intense yellow fluorescence can be observed under UV filtered as a result of the flavonoid presence in the sample when reacted with the aluminium chloride (Alim et al., 2009).

Total flavonoid content was estimated by aluminium chloride method described by Chang et al. (2002). The sample extract of 2.0 ml was mixed with 0.1 ml of 10% aluminium chloride solution. Then, the mixture was vortexed for 10 seconds. The mixture was then added 0.1 ml of 0.1 mM potassium acetate solution. The mixture was incubated for 30 minutes in room temperature. The absorbance was measured at 415 nm after the mixture vortexed for 10 seconds. Quercetin at different concentrations was used as standard. The samples was analysed in triplicate.

3.7 DPPH radical scavenging activity

DPPH free radical scavenging assay is one of the most widely and commonly used method to measure the antioxidant activity of a sample as this method is simple and highly sensitive (Moon & Shibamoto, 2009). The concentration of active radical-scavenging materials is evaluated through chain-breaking mechanism. The formation of DPPH upon absorption of hydrogen from antioxidant can be seen when the purple colour of DPPH change to yellow (Moon & Shibamoto, 2009; Maisthisakul et al., 2005). The antioxidant effect of DPPH can easily be evaluated by observing the decreasing absorbance value at 517 nm (Liu & Yao, 2006).

Antioxidant present in the sample extract will reacts with the DPPH by destroying the DPPH radical by donating the electron to the DPPH free radical and it will be changed into inactive form (Prakash et al., 2006). The decolourisation of the purple colour of DPPH to yellow is parallel to the number of free radical electrons captured and the colour changes from purple to yellow indicate the free radicals have been neutralized by the antioxidant present in the sample and the decolourisation it is attributed by the hydrogen donating ability of the sample (Shimada, Fujikawa, Yahara & Nakamura, 1992). The colour changes can be monitored using spectrophotometer.

Free radical scavenging assay is not only specific to polyphenols but also to many interfering compounds which may react with the reagent (Prior, Wu & Schaich, 2005). According to Singleton and Rossi (1965), various phenolic compounds reacted differently in DPPH assay and this depends on the number of phenolic compounds they have. In addition, the responses of phenolics for antioxidant activity estimated by various methods also depend on their chemical structures (Zhao et al., 2007).

The ability of antioxidant in the samples to reduce DPPH radicals can be estimated and the efficiency of the sample in reducing the DPPH radical depends on the amount of antioxidants available in the sample. The negative control can be used to calculate the percentage of reduced DPPH radical by comparing to the absorbance (Prasad et al., 2013). The higher the percentage of DPPH radical scavenging activity, the better the antioxidant activity.

The IC_{50} of a compound is inversely related to its antioxidant capacity, as it expresses the amount of antioxidant required to decrease the DPPH concentration by 50%, which is obtained by interpolation from a linear regression analysis (Do et al., 2014). The lowest concentration of the extract needed to reduce 50% of the DPPH radical indicates the highest antioxidant activity.

Free radical scavenging is the mechanism use by antioxidant to inhibit lipid oxidation which are useful in protecting the food from spoiling and also important for the defence of living cells against oxidative damage (Wojdylo, Oszmianski & Czemerys, 2007; Barbaste et al., 2002). Thus, DPPH free radical scavenging assay is a preferable method to measure antioxidant activity of a sample as it is easy, rapid and sensitive enough to detect active phenolic compound at low concentration compared to other assay (Bondet, Brand-Williams & Berset, 1997; Do et al., 2014).

The antioxidant activity of the extract was measured with the DPPH method as described by Tang et al. (2002). DPPH solution was prepared freshly by dissolving 3.94 mg DPPH in 50 ml 70% ethanol (0.2 mM). The 0.2 ml flower extract with varying concentration (0.32-10 mg/ml) and 1.0 ml DPPH solution was mixed in a test tube. Then the test tube was kept at dark room with room temperature for 30 min. The

absorption was measured at 517 nm with a spectrophotometer. The percentage inhibition of radicals was calculated using the formula below:

$$\% \text{ inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100\%$$

where A_{control} is the absorbance of DPPH solution without sample and A_{sample} is the absorbance of the sample with DPPH solution. The half-maximal inhibitory concentration (IC_{50}) is the amount of antioxidant required to decrease the initial DPPH concentration by 50%. All tests were triplicated, and graphs were plotted using the average of three determinations. Ascorbic acid was used as standard for comparison.

3.8 Ferric Reducing and Antioxidant Power (FRAP) Assay

The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method employing an easily reduced oxidant, Fe(III). Reduction of a ferric tripyridyltriazine complex to ferrous-(2,4,6-tripyridyl-s-triazine) where the dye, 2,4,6-tripyridyl-s-triazine acts as an oxidant and has the ability to strongly bind to ferrous, Fe(II) ion forming navy blue colour and the absorbance measured can be used to determine the amount of iron reduced. The more the ferric, Fe(III) ion is reduced to ferrous ion by an antioxidant, the more intense the blue colour can be observed (Prasad et al., 2013).

The presence of compounds in the sample extracts possessed reducing properties as the compound in the sample breaking the free radical chain through donating a hydrogen atom (Abdullah et al., 2012). FRAP assay which is a simple assay that provides rapid, easy and reproducible results and it is also versatile as it can be used to both aqueous and organic solvent extracts of different plants (Wong, Li, Cheng &

Chen, 2005). However, the reducing capacity in FRAP assay may not reflect the antioxidant activity (Wojdylo, Oszmianski & Czemerys, 2007).

The total antioxidant activity of the flower was determined using ferric reducing and antioxidant power (FRAP) assay described by Benzie & Strain (1996). FRAP reagent was freshly prepared and mixed in the proportion of 10:1:1 (v:v:v) for A:B:C solutions, where A = 100 ml acetate buffer; B = 10 ml TPTZ solution; C = 10 ml ferric chloride solution. The test was run for blank by mixing 3 ml of FRAP reagent to 100 μ L of 70% ethanol. The absorbance was immediately measured at 593 nm (0 min) using a spectrophotometer. Then the 100 μ L of the sample was pipetted out and mixed with 3 ml of FRAP reagent. The mixture was vortexed then it is incubated for 4 minutes at 37°C. After the mixture is incubated and the absorbance was measured. The standards, ferrous sulphate was measured using the same procedure. All tests were triplicate, and graphs were plotted using the average of three determinations.

3.9 Data analysis

The data obtained were analysed by using Statistical Package for Social Sciences (SPSS version 22.0) and PRISM Graph Pad (version 7). Results that were obtained in triplicate were expressed as mean $\pm$ standard deviation (SD).

Pearson correlation coefficient was conducted in order to determine the relationship between total phenolic content with total flavonoid content and the antioxidant activity as well as total flavonoid content with total antioxidant activity in the sample extracts. Meanwhile, one-way ANOVA test was done to determine whether there are significant difference ($p < 0.05$) or no significant difference ($p < 0.05$) among the sample.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effect of extraction solvent on extraction yield

Phytochemical from plant can be obtained through many steps such as milling, grinding, homogenization and extraction. The most important step is extraction which recovers and isolates phytochemicals from plant materials. The chemical nature of phytochemicals, the method of extraction, the particle size of sample, the solvent used can affect the efficiency of extraction (Diem, Elisa, & Tran-nguyen, 2013). Efficient extraction method would enable high yield of extract and does minimal changes to the sample. Thus, it is important to select suitable extraction method and the solvent used according to the nature of the plant sample (Dhanani, Shah, Gajbhiye, & Kumar, 2017).

Different extraction solvents used in preparing the sample extract influenced the antioxidant activity and the yield of phenolic and flavonoid content in a sample (Sun & Ho, 2005). The solvent used in the extraction such as ethanol, methanol, water, acetone or the combination of these solvents become important factors that affect the

antioxidant activity in the extracts. Due to the complexities of chemical nature of solvents and the diversity in structure and composition of plant materials, it is hard to predict the different behaviours of material-solvent system. Besides that, there are no single solvent that are capable to extract all the antioxidants in a plant which have different polarity and solubility (Yang, Wang, Ke, Jiang & Ying, 2007).

The extraction yield depends on several factors such as the polarity, pH, temperature, extraction time and the composition of plant sample and become the most important parameters. In this work, the flowers of *Hylocereus* species which are *H. undatus* and *H. polyrhizus* were extracted using two different solvent namely water and 70% ethanol.

According to figure 4.1, the extraction yields were 22.97% for 70% ethanol extract *H. undatus* and 22.70% for 70% ethanol extract *H. polyrhizus*, 16.77% for water extract *H. polyrhizus* and 18.42% for water extract *H. undatus*. It can be seen that the extraction yield of aqueous ethanol was significantly higher than water extract which shows that with increasing polarity of the solvent used, the extraction yield will increase.

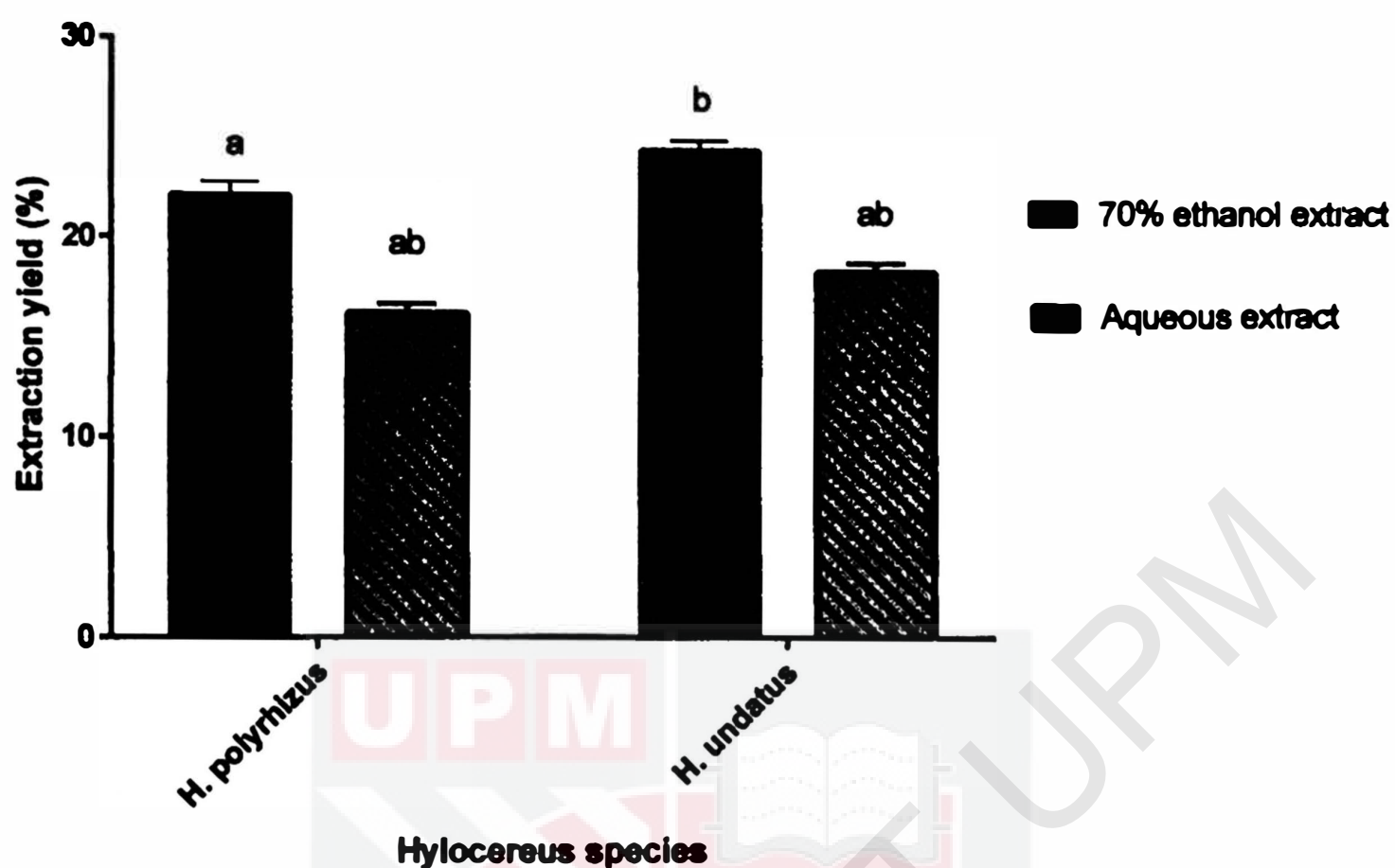


Figure 4.1: The extraction yield of *Hylocereus* flowers in 70% ethanol and aqueous extract

Values are expressed as mean $\pm$ standard deviation, SD (n=3). Mean value followed by different letter are significantly different at level of $p < 0.05$.

The higher extraction yield in aqueous ethanol compared to water extract can be explained by the combination of water and organic solvent facilitate the extraction chemicals that are soluble in both water and organic solvent which contribute to higher yield. The results are in agreement with extraction yields of some medicinal plants (Sultana, Anwar, & Ashraf, 2009; Wong, Li, Cheng, & Chen, 2006).

4.2 Total phenolic content (TPC)

In this experiment, when the flower extracts reacted with the Folin-Ciocalteu reagent and sodium carbonate it produces a blue-coloured solution which become the indicator to identify the presence of phenolic compounds in the flowers extracts. The blue colour of the solution become more concentrated as the phenolic content of the flower increases. The results in this study showed that flowers of *Hylocereus* in 70% ethanol extract contained the highest TPC compared to in aqueous extracts as the blue-colour solution in the ethanolic extract more concentrated.

Total phenolic content of both *Hylocereus* flowers were expressed in gallic acid equivalent by using a standard calibration curve generated from gallic acid that were prepared at several concentrations and it is commonly used since it is one of the major polyphenolic compound that occurs in plants (Lim, Lim & Tee, 2006). Different phenolic compounds such as ferulic acid or catechin can be used as standard depending on the nature of the studied sample. Gallic acid is preferable as it can be assumed as the key standard since it is widely used in many studies on antioxidant and it will be easier to be compared with other studies (Prasad et al., 2013).

In this study, gallic acid standard curve at concentration 0.5 to 6.5 mg/ml was used to determine the TPC in both flower extracts. From the standard curve, the equation obtained was $y=0.0483x - 0.014$ ($R^2=0.9901$) and this equation was used to determine the TPC of sample in unit of milligram Gallic Acid Equivalent per 100 gram of dry weight sample (mg GAE/ 100g dry weight).

4.2.1 Total phenolic content (TPC) in 70% ethanol extract and aqueous extract

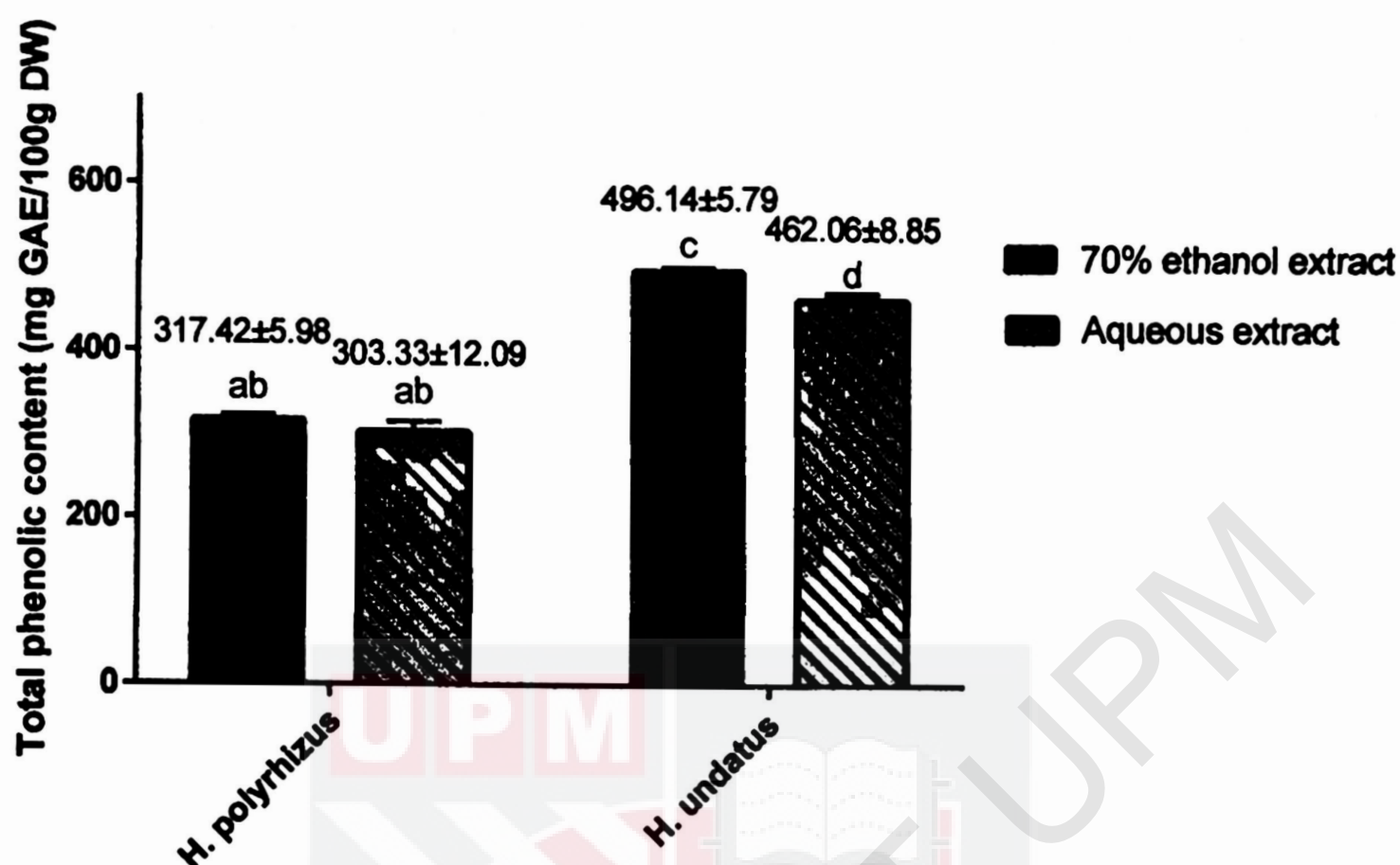


Figure 4.2 Total phenolic content of *Hylocereus* flowers in 70% ethanol and aqueous extract.

Values are expressed as mean $\pm$ standard deviation, SD (n=3). Results are expressed as gallic acid equivalents (GAE) / 100g dry weight. Mean value followed by different letter are significantly different at level of $p < 0.05$.

The figure 4.2 showed the differences of TPC between two types of sample extracts in 70% ethanol and aqueous extract for two *Hylocereus* flower extracts. For 70% ethanol extract, the flower of *H. undatus* contained higher phenolic content compared to *H. polyrhizus* flower. The phenolic content in *H. undatus* flower was 496.14 mg GAE/100g dry weight sample whereas for *H. polyrhizus*, it contained about 317.42 mg GAE/100g dry weight sample.

Previous study by Liu, Qiu, Ding & Yao (2007), reported that the ethanolic extract of 68 Chinese Herbal medicine ranged from 10 to 280 mg GAE/g dry weight

and another study by Ravipati et al. (2012) reported that 44 ethanolic extract of Traditional Chinese Medicine (TCM) plant contained TPC ranged from 80 to 340 mg GAE/100g dry weight. The TPC of *Hylocereus* flower in ethanolic extract were within the range of other medicinal plant however, the different levels of TPC reported in other study may due to the different sample used, procedures and standards used in the study to measure the total phenolic content.

From the ANOVA analysis of the flower of *H. polyrhizus* and *H. undatus* flower in 70% ethanol extract, there was significant difference between both sample at the level of $p < 0.05$.

For aqueous extract, figure 4.2 showed the differences of TPC between two types of sample extracts in 70% ethanol and aqueous extract for two *Hylocereus* flower extracts. For aqueous extract, the flower of *H. undatus* contained higher phenolic content compared to *H. polyrhizus* flower. The phenolic content in *H. undatus* flower was 462.06 mg GAE/100g dry weight sample whereas for *H. polyrhizus*, it contained about 303.33 mg GAE/100g dry weight sample.

According to Ravipati et al. (2012), the aqueous extract of 44 Traditional Chinese medicine plant contained TPC ranged from 40 to 14800 mg GAE/100g dry weight and another medicinal plant, TPC of aqueous extract of *Limnophila aromatica* was about 6.25 mg GAE/g dry weight (Do et al., 2012). As comparison, the TPC of aqueous extract of both *Hylocereus* flowers were higher than several local herbs that are widely available in Malaysia where the plants were also extracted by aqueous such as kesum, curry leaves, ulam raja, selom and pegaga (Huda-Faujan, Noriham, Norrakiah & Babji, 2007). Another study on selected lettuce which are commercially available in Malaysia reported that the TPC of the five varieties of lettuce ranged from

4.85 to 76.05 mg GAE/ 100g fresh weight (Gan & Azrina, 2016). Thus, it showed that the TPC of medicinal plant contained higher TPC than common herb and vegetables.

From the ANOVA analysis of the flower of *H. polyrhizus* and *H. undatus* flower in aqueous extract, there was significant difference between both sample at the level of $p < 0.05$.

According to the figure 4.2, 70% ethanol extract of both flower of *H. polyrhizus* and flower of *H. undatus* showed higher TPC which were 317.42 mg GAE/ 100g dry weight and 496.14 mg GAE/ 100 g dry weight respectively compared with aqueous which were 303.33 mg GAE/ 100 g dry weight and 462.06 mg GAE/ 100 g dry weight. These results were in agreement with a study on medicinal plant, *Tetrapleura tetraptera* which showed appreciable amounts of phytochemical in ethanolic extract than using solvents such as water by Gberikon, Adeoti and Aondoackaa (2015). Their finding was also supported by Sultana, Anwar and Ashraf (2009) where they reported that aqueous ethanol extract on selected medicinal plants were the most effective in extracting phenolic components from sample compared to pure ethanol and water.

Aqueous ethanol extract contained higher TPC and this could be explained by ethanol solutions that are mixed with water particularly those consisting of 40-80% of methanol or ethanol are more efficient to extract polyphenolic compound compared to pure water or alcohol (Maliarova, Mrazova, Havrlentova, & Sokol, 2015). and the combined use of water and organic solvent facilitate the extraction of phenolic compound in the sample extract (Do et al., 2014). Moreover, this result was supported by a study on selected Malaysian herb where they reported that ethanolic extract demonstrated significantly higher value of TPC compared to aqueous extract because compared to water, organic solvent like ethanol was more efficient in degrading the

polyphenol oxidases and cell thus more endocellular material can be extracted (Othman, Mukhtar, Ismail & Chang, 2014).

In this study, the flower of *H. undatus* which is a common edible flower in China called Bawanghua possessed higher TPC compared to flower of *H. polyrhizus*. As there is only one previous study on the flower of *Hylocereus* species and it only studies on flower of *H. undatus*, it is hard to compare the antioxidant content and activity of *H. polyrhizus*. Previous study on *H. undatus* flower was obtained from two different location, Shenjing flower and Qixing flower. The TPC of *H. undatus* flower in the study was higher than in this experiment Li, Gao, Han, Lin, Hu & Chen, 2013). This could be explained by the sample obtained in that study are grown specifically for medicinal purpose in China while in this experiment, the sample are obtained freshly from a dragon fruit farm in Kluang, Johor. Besides that, there are several other factors that may alter the content of antioxidant such as storage, processing and preparation conditions (Kaur & Kapoor, 2002).

Besides that, in previous study on the seed, pulp and peel of *H.undatus* and *H.polyrhizus*, the result shows that the TPC ranged from 11.79 to 62.95 mg GAE/100g fresh sample weight which was in order of *H.polyrhizus* seed > *H.undatus* seed > *H.polyrhizus* pulp > *H.polyrhizus* peel > *H.undatus* pulp > *H.undatus* peel (Adnan, 2012). As comparison, the flower of *H.undatus* and *H.polyrhizus* contained higher TPC than its other parts; seed, pulp and peel as other several studies have reported (Wu et al., 2006; Nurliyana et al., 2010; Khalili et al., 2011; Choo & Yong, 2011). This result was in line with another study that compare the phenolic content of the flower, leaf, skins and fruit of chestnut. The study reported that the skins, flowers and leaves contained higher TPC than the fruit of chestnut (Barreira, Ferreira, Oliveira & Pereira, 2007). Thus, from the results we can see that the other part of the plant such as flower

or leaf and medicinal plant may contain higher antioxidant content than the common fruits and vegetables.

4.3 Total flavonoid content (TFC)

Previous studies have reported that quercetin is the suitable reference for determination of total flavonoid content in plant sample extract. Thus, quercetin at concentration ranged from 0.01 to 0.10 mg/ml were used to make the calibration standard curve. From the standard curve, the equation obtained was $y = 6.5184x + 0.0244$ ($R^2 = 0.9917$) and the equation was used to determine the TFC of the sample in unit of milligram Quercetin Equivalent per 100 gram of dry weight sample (mg QE/100g dry weight).

4.3.1 Total flavonoid content (TFC) in 70% ethanol extract and aqueous extract

Figure 4.3 showed the differences of TFC between two types of sample extracts in 70% ethanol and aqueous extract for two *Hylocereus* flower extracts. For 70% ethanol extract, the flower of *H. undatus* contained higher flavonoid content compared to *H. polyrhizus* flower. The flavonoid content in *H. undatus* flower was 130.63 mg QE/100g dry weight sample whereas for *H. polyrhizus*, it contained about 119.30 mg QE/100g dry weight sample. From the ANOVA analysis of both *Hylocereus* flower in 70% ethanol extract, there are significant different between the samples at the level of $p < 0.05$.

For aqueous extract, figure 4.3 showed the differences of TFC between two types of sample extracts in 70% ethanol and aqueous extract for two *Hylocereus* flower extracts. For aqueous extract, the flower of *H. undatus* contained higher flavonoid content compared to *H. polyrhizus* flower. The flavonoid content in *H. undatus* flower

was 118,60 mg QE/100g dry weight sample whereas for *H. polyrhizus*, it contained about 107.30 mg QE/100g dry weight sample. From the ANOVA analysis of both *Hylocereus* flower in aqueous extract, there are no significant different between the samples.

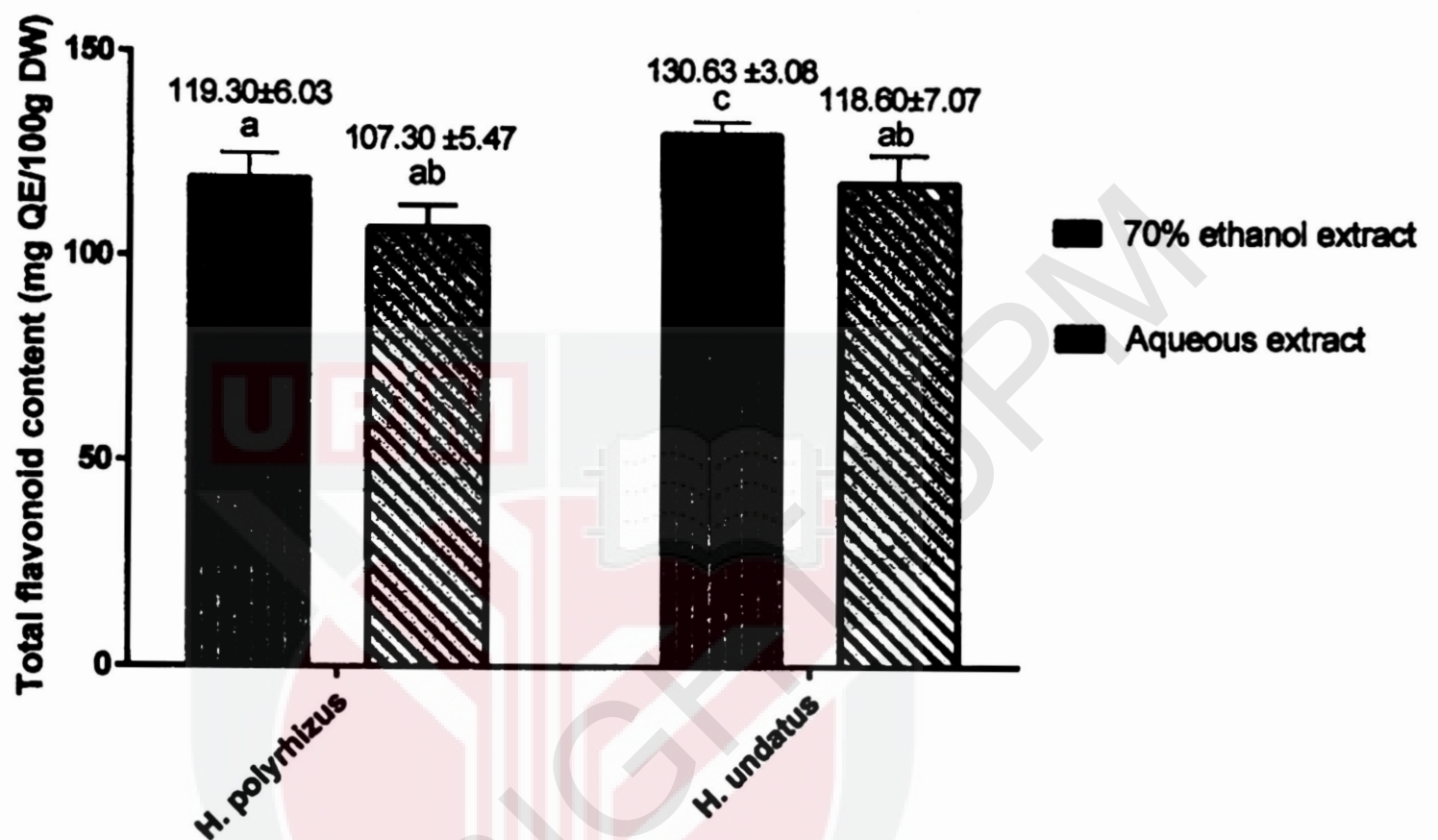


Figure 4.3: Total flavonoid content of *Hylocereus* flowers in 70% ethanol and aqueous extract.

Values are expressed as mean $\pm$ standard deviation, SD (n=3). Results are expressed as quercetin equivalents (QE) / 100g dry weight. Mean value followed by different letter are significantly different at level of $p < 0.05$.

In this experiment, the ratio of total flavonoid versus total phenolic for both aqueous and ethanolic extract of flower are high. The high ratios of total flavonoid versus total phenolics suggest that the antioxidant ability of both *Hylocereus* flower are mainly the effect of flavonoid. Previous study by Li, Gao, Han, Lin, Hu & Chen (2013) were in line with the data which reported that flavonoids were the main form of total phenolic which indicate that the antioxidant ability of *H. undatus* flower seems

mainly the effect of flavonoid as the high ratios of total flavonoids versus total phenolics.

4.4 Total antioxidant activity

The antioxidant activity of plants extracts vary with the assay methods used where each method has different mechanism to measure the antioxidant activity which could lead to different results although using the same sample (Sun & Ho, 2005; Yang, Wang, Ke, Jiang & Ying, 2007). Thus, using a single assay to measure the antioxidant activity of a sample may not be enough to represent and determine the antioxidant activity of the sample. In this study, two antioxidant activity assays were used to determine the antioxidant activity of *Hylocereus* flower in 70% ethanol and aqueous extract which were DPPH free radical scavenging assay and Ferric Reducing Antioxidant Power (FRAP).

4.4.1 DPPH free radical scavenging activity in 70% ethanol extracts and aqueous extract

Figure 4.4 shows the percentage of DPPH radical scavenging activity for the 70% ethanol extract and aqueous extract in different concentrations, expressed as the ratio of the sample absorbance decrease and the absorbance of DPPH solution in the absence of extract at 517 nm. According to figure 4.4, it showed that the DPPH free radical scavenging activity of the ethanolic and aqueous sample extracts increased with increasing concentration.

The extract obtained by 70% ethanol yielded the highest DPPH radical scavenging activity at concentration from 0.32 mg/ml to 10 mg/ml. The scavenging effect of the extracts range from 58.24% to 84.81%. The scavenging effect of ethanolic

and aqueous extract of both *Hylocereus* flower and standards on the DPPH radical decreased in the order of ascorbic acid > 70% ethanol extract *H.undatus* > 70% ethanol *H.polyrhizus* > aqueous extract *H.undatus* > aqueous extract *H.polyrhizus*. For 70% ethanol extract, at the highest concentration at 10 mg/ml, flower of *H.undatus* showed higher radical scavenging activity which was 84.81% compared to flower of *H.polyrhizus* which was 79.23%.

For aqueous extract, at the highest concentration at 10 mg/ml, flower of *H.undatus* showed higher radical scavenging activity which was 65.40% compared to flower of *H.polyrhizus* which was 58.24%. Ascorbic acid which was used as a standard showed the highest percentage compared to both samples. This result is in agreement with findings by Do et al. (2014) where they reported that their standard which was gallic acid are higher than the ethanolic and aqueous extracts of the *Hylocereus* flower. Although they used different standard from this experiment, the standard possessed higher antioxidant activity.

From the ANOVA analysis, there was significant different in inhibition concentration (IC_{50}) value between both sample at the level of $p < 0.05$ for both *Hylocereus* flower in 70% ethanol extract and aqueous extract. In both extract, *H.undatus* flower has lower IC_{50} value compared to flower of *H.polyrhizus* which indicate that *H.undatus* flower possessed stronger radical scavenging activity than *H.polyrhizus*.

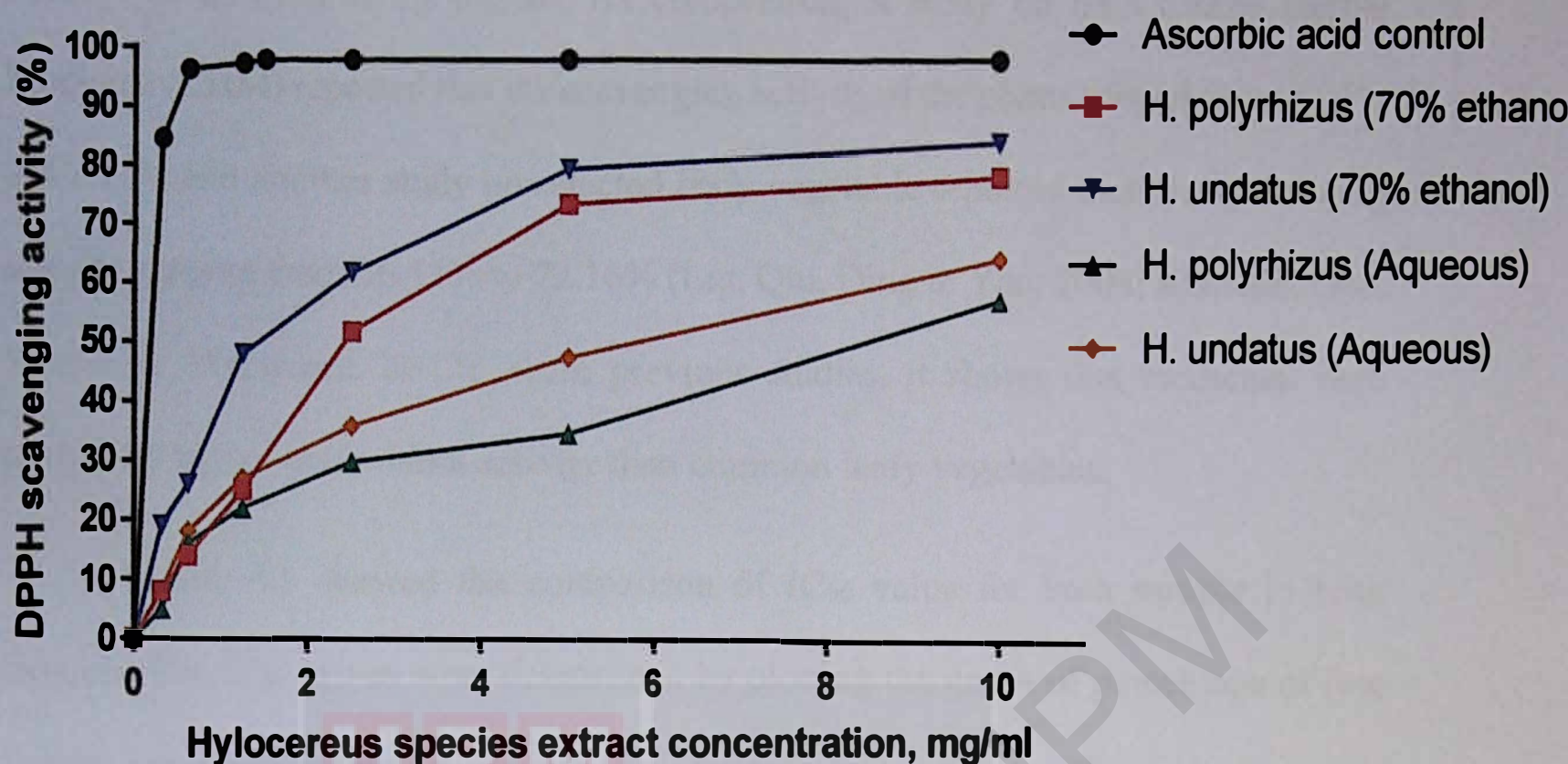


Figure 4.4: DPPH free radical scavenging activity in 70% ethanol extracts and aqueous extract

The concentration of 70% ethanol and aqueous extract was from 0.32 to 10 mg/ml. Values showed as mean $\pm$ standard deviation, SD (n=3). Ascorbic acid was used as standard.

In this experiment, the results showed that ethanolic extract of both *Hylocereus* flower possessed higher scavenging activity compared to aqueous extract. A similar trend was observed in study of DPPH radical scavenging activity on local medicinal plant misai kucing or *Orthosiphon stamineus* and bunga kantan (Chew et al., 2011; Wijekoon, Bhat & Karim, 2010). All extracts obtained by using aqueous organic solvent gave stronger radical scavenging capacity than water extract (Do et al., 2014).

Another study on selected Malaysian tropical fruit showed that aqueous organic solvent extract such as aqueous ethanol and methanol yielded the highest scavenging effect compared to pure alcohol or pure water (Allothman, Bhat & Karim, 2009). The scavenging activity of both *Hylocereus* flower in both extracts range from

58.24% to 84.81% at 10 mg/ml. As comparison, a study on 68 Chinese Herbal Medicine (CHM) reported that the scavenging activity of the plants ranged from 4.05% to 97.11% and another study on selected leafy vegetable reported that the scavenging activity ranging from 36.53% to 72.16% (Liu, Qiu, Ding & Yao, 2008; Khanam, Oba, Yanase & Murakami, 2012). From previous studies, it shows that medicinal herb possessed higher antioxidant activity than common leafy vegetables.

Figure 4.5 showed the comparison of IC_{50} value for both sample in both extracts. The IC_{50} values were determined by plotting the graph of percentage of free radical scavenging activity against the concentration extracts. IC_{50} values can be defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50% and lowest IC_{50} value means that it has the greater ability to limit the action of free radical DPPH. According to the figure 4.5, 70% ethanol extract was more efficient in scavenging free radical DPPH compared with aqueous extract through the determination of the IC_{50} value. Besides that, the flower of *H.undatus* showed lower IC_{50} value compared to flower of *H.polyrhizus* which indicate that the flower of *H.undatus* contain higher antioxidant activity than flower of *H.polyrhizus*.

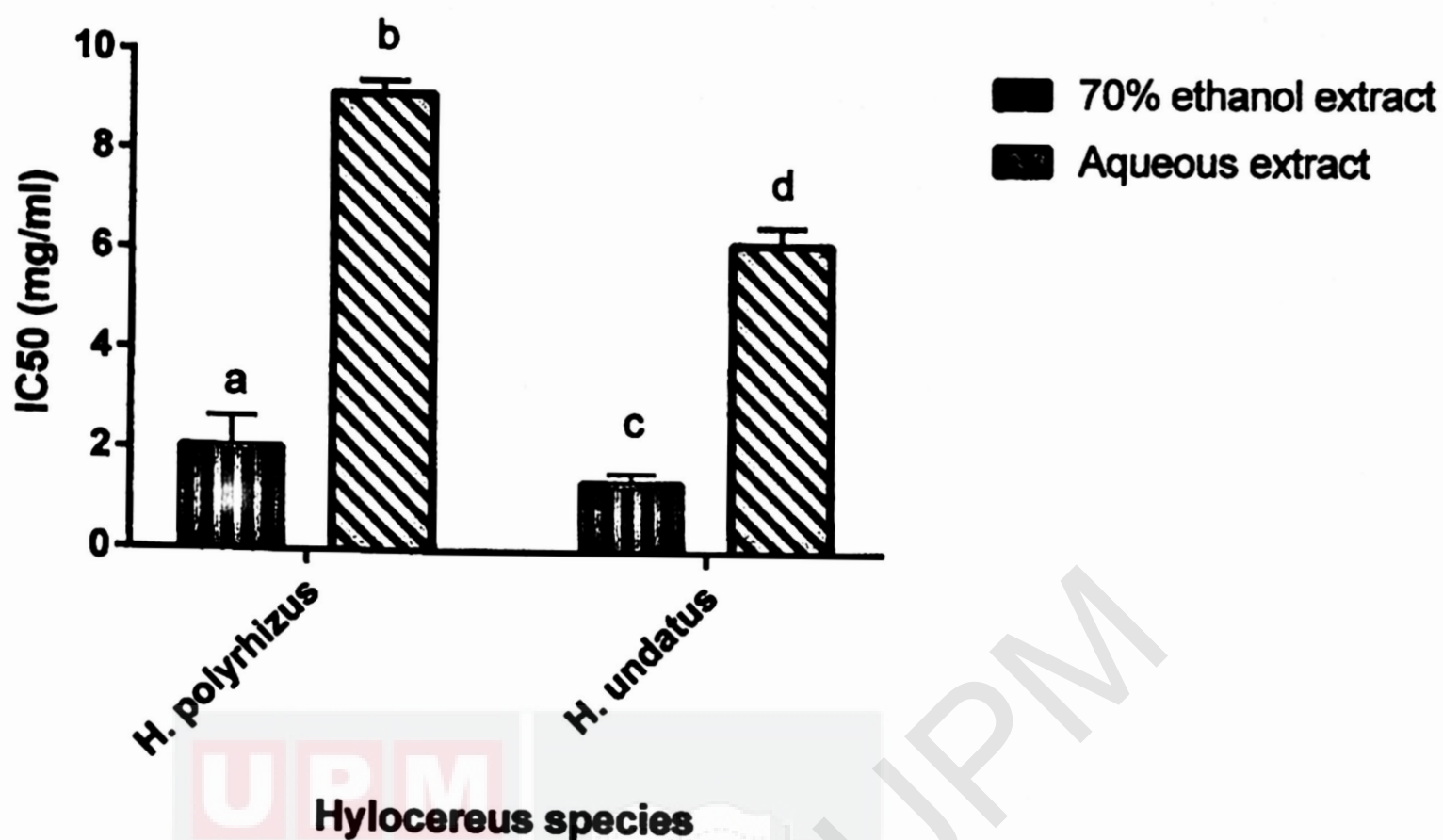


Figure 4.5: Free radical scavenging activity inhibition concentration (IC₅₀) for flower of *H.undatus* and *H.polyrhizus* in 70% ethanol and aqueous extract by using DPPH method.

Values showed as mean $\pm$ standard deviation, SD (n=3). Ascorbic acid was used as standard. Means with different letters were significantly different at the level of $p < 0.05$.

4.4.2 FRAP value in 70% ethanol extract and aqueous extract

In this study, ferrous sulphate standard curve at concentration 0.1 to 1.0 mM was used to determine the FRAP value in both flower extracts. From the standard curve, the equation obtained was $y = 0.4233x + 0.2555$ ($R^2 = 0.9953$) and this equation was used to determine the FRAP value of sample in unit of millimolar ferrous, Fe(II) per 100 grams dry weight sample (mM Fe(II)/ 100g dry weight).

Figure 4.6 shows the differences of FRAP value between two types of sample extracts in 70% ethanol and aqueous extract for two *Hylocereus* flower extracts. For 70% ethanol extract, the flower of *H. undatus* contained higher FRAP value compared to *H. polyrhizus* flower. The FRAP value in *H. undatus* flower was 114.61 mM Fe(II)/100g dry weight sample whereas for *H. polyrhizus*, it contained about 317.42 mM Fe(II)/100g dry weight sample.

Previous study by Wong, Li, Cheng and Chen (2005), they reported that the alcohol extract of 30 Chinese Herbal medicine ranged from 2.5 to 480 $\mu\text{mol Fe(II)/g}$ dry weight and another study by Liu et al. (2008) reported that 68 ethanolic extract of Chinese Herbal Medicine (CHM) plant contained FRAP value ranged from 0.89 to 157.67 mg RE/g dry weight.

From the ANOVA analysis of the flower of *H. polyrhizus* and *H. undatus* flower in 70% ethanol extract, there are significant different between both sample at the level of $p < 0.05$.

For aqueous extract, figure 4.6 showed the differences of FRAP value between two types of sample extracts in 70% ethanol and aqueous extract for two *Hylocereus* flower extracts. The flower of *H. undatus* contained higher FRAP value compared to *H. polyrhizus* flower. The FRAP value in *H. undatus* flower was 67.50 mM Fe(II)/100g dry weight sample whereas for *H. polyrhizus*, it contained about 50.50 mM Fe(II)/100g dry weight sample.

According to Wong et al. (2005), the aqueous extract of 30 Chinese Herbal medicine plant contained FRAP value ranged from 1.9 to 532 $\mu\text{mol Fe(II)/g}$ dry weight and another study reported that the FRAP value of aqueous extract of 32 selected herbs was ranged from 13.8 to 405 $\mu\text{M Fe(II)/100g}$ dry weight (Wojdylo, Oszmianski &

Czemerys, 2007). From the ANOVA analysis of the flower of *H. polyrhizus* and *H. undatus* flower in aqueous extract, there are significant different between both sample at the level of $p < 0.05$.

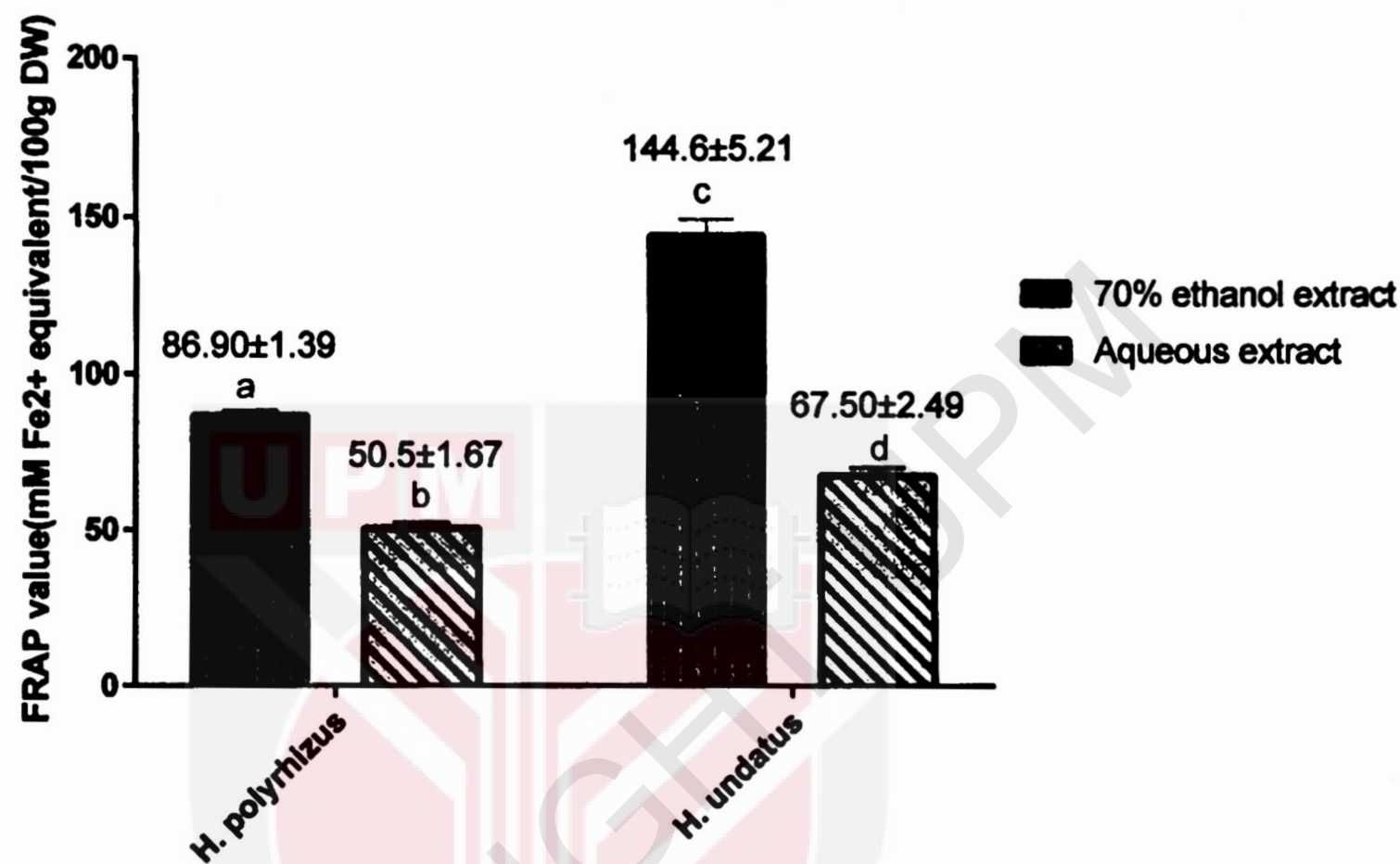


Figure 4.6: FRAP value of *Hylocereus* flowers in 70% ethanol and aqueous extract. Values are expressed as mean ± standard deviation, SD (n=3). Results are expressed as mM Fe(II) / 100g dry weight. Mean value followed by different letter are significantly different at level of $p < 0.05$.

According to the figure 4.6, the antioxidant activity in 70% ethanol extract showed stronger antioxidant activity compared to aqueous extract in both *Hylocereus* flower. Similar result can be seen from previous data on Iraqi medicinal plant where high value of antioxidant activity was found in ethanol extracts when compared to aqueous extract and another study by Cheung et al. (2003) also in line with the reported data.

4.5 Pearson correlation coefficient between total phenolic content and total flavonoid content, DPPH free radical scavenging assay and FRAP in 70% ethanol extract and aqueous extract

Table 4.1: Correlation between total phenolic content and total flavonoid content, DPPH free radical scavenging assay and FRAP

Total Phenolic content		Pearson correlation		
		TFC	IC50	FRAP
Aqueous extract	r value	0.994**	-0.996**	0.969**
	p value	0.000	0.000	0.001
70% ethanol extract	r value	0.951**	-0.888	0.996**
	p value	0.004	0.018	0.000

**Correlation is significant at $p < 0.01$

4.5.1 Relationship between total phenolic content (TPC) and total flavonoid content (TFC)

In this study, 70% ethanol extract and aqueous extract both shows a very strong and significant ($p<0.01$) correlation with TFC ($r= 0.951$, $p= 0.004$; $r= 0.994$, $p= 0.000$). Positive relationship between TPC and TFC in 70% ethanol and aqueous extract means that as the TPC value increases, the TFC value also increases which indicate that flavonoid was important phenolic group that present in sample in ethanolic and aqueous extract. This result was in agreement with previous study where they reported that the flower of *H.undatus* has high ratios of total flavonoid versus total phenolic which indicate that the antioxidant ability of *H. undatus* flower seems mainly the effect of flavonoid (Li, Gao, Han, Lin, Hu & Chen, 2013).

The result was supported by the study done by Alothman, Bhat and Karim (2009) on several tropical fruits from Malaysia and study by Maisuthisakul, Suttajit and Pongsawatmanit (2007) also showed positive correlation between TPC and TFC in some Thai medicinal plant. Recent study by Do et al. (2014) on medicinal herb *Limnophila aromatica* also showed positive relationship between TPC and TFC.

The results found in this study was in contrast with the results by Maisuthisakul, Suttajit and Pongsawatmanit (2007) where they reported that extracts that possessed high phenolic content did not always have higher flavonoid content which may due to the different plant extracts contain different levels of TFC as a proportion to TPC. Besides that, there were several studies which reported there is no correlations exist or weak correlation between total phenolic content and total flavonoid content (Ghasemi et al., 2009; Ebrahimzadeh et al., 2009).

4.5.2 Relationship between total phenolic content (TPC) and IC₅₀ value

In this study, 70% ethanol extract and aqueous extract both showed a very strong correlation with IC₅₀ ($r = -0.888$, $p = 0.018$; $r = -0.996$, $p = 0.000$). For 70% ethanol extract the correlation between TPC and IC₅₀ value was significant at level $p < 0.01$. Negative relationship between TPC and IC₅₀ in 70% ethanol and aqueous extract means that as the TPC value increases, it causes the IC₅₀ of DPPH scavenging activity to decrease which lower IC₅₀ value indicate increasing in antioxidant activities.

The results are in agreement with a study on selected seeds of Malaysian tropical fruit where they reported a strong negative correlation between TPC and IC₅₀ ($r^2 = -0.9849$). The negative correlation between TPC and IC₅₀ proves that the samples with highest phenolic contents showed lower IC₅₀ value which indicate that phenolic compound are more likely contribute to the antioxidant activity in both ethanolic and

aqueous extracts (Barreira et al., 2008). The results were also supported by more recent study on medicinal plant from Indonesia which found negative and strong correlation between TPC and IC₅₀ (Fidrianny, Budiana & Ruslan, 2015).

The results found in this research was in contrast with a study on *L. aromatica* extract where they found that total phenolic contents were directly proportional to their antioxidant activity (Do et al., 2014). Study by Liu, Lin, Wang, Chen and Yang (2009) also reported that there was a positive correlation between TPC and IC₅₀ ($r^2 = 0.834$) and the antioxidant activity IC₅₀ values of DPPH radical scavenging activity correlated with its contents of phenols which also supported by a study by Marimuthu, Wu, Chang and Chang (2008). However, a study by Eberhardt, Liu, Smith and Lee (2001) found that there was no correlation between TPC and IC₅₀ which indicate that phenolic compound does not play significant role in the antioxidant activity of the sample.

4.5.3 Relationship between total phenolic content (TPC) and FRAP value

In this study, 70% ethanol extract and aqueous extract both shows a very strong and significant ($p < 0.01$) correlation with FRAP ($r = 0.996$, $p = 0.000$; $r = 0.969$, $p = 0.001$). Positive relationship between TPC and FRAP in 70% ethanol and aqueous extract means that antioxidant components in these *Hylocereus* flowers could reduce oxidants and scavenge free radical and proves that phenolic compound had a major contribution to antioxidant activity. Besides that, positive correlation can be used as an important indicator for antioxidant activity of the sample which may preliminarily screen edible medical plant and use as natural sources of antioxidant functional foods.

This result was in agreement with study by Gardner et al. (2000) on fruit juices and study by Abdullah et al. (2012) on traditional Chinese medicine, wax gourd. Positive correlation showed that the samples exhibited high reducing power on ferric

ions and redox potential of phenolic compound plays an important role in determining antioxidant capacity (Abdullah et al., 2012). Similar finding was found by Nuri et al. (2010) on the antioxidant activity of vegetables from Indonesia who reported high and positive correlation between total phenolic content and FRAP ($r^2 = 0.850$). Since total phenolic content have good correlation with total antioxidant activity, this indicate that phenolic compounds may be the major contributor to total antioxidant activity of the sample.

Previous study on 68 Chinese Herbal medicinal plants were in line with the result as they reported that there was significant linear correlation ($r^2 = 0.9467$) between TPC and FRAP values. Another study on 30 Chinese medicinal plant also show positive correlation between TPC and FRAP ($r^2 = 0.7917$) (Wong, Li, Cheng & Chen, 2005). The result in this study and several previous studies shows that phenolic compounds plays important role and contribute significantly to the antioxidant activity in different Chinese medicinal plants (Cai et al., 2004; Tang et al., 2004).

4.6 Pearson correlation coefficient between total flavonoid content and DPPH free radical scavenging assay and FRAP in 70% ethanol extract and aqueous extract

Table 4.2: Correlation between total flavonoid content and DPPH free radical scavenging assay and FRAP

Total Flavonoid content		Pearson correlation	
		IC ₅₀	FRAP
Aqueous extract	r value	-0.858	0.847
	p value	0.095	0.103
70% ethanol extract	r value	-0.957**	0.934**
	p value	0.003	0.006

**Correlation is significant at p < 0.01

4.6.1 Relationship between total flavonoid content (TFC) and IC₅₀ value

In this study, 70% ethanol extract and aqueous extract both shows a strong correlation with IC₅₀ (r= -0.957, p= 0.003; r= -0.858, p= 0.095). For 70% ethanol extract the correlation between TFC and IC₅₀ value was significant at level p<0.01. Negative relationship between TFC and IC₅₀ in 70% ethanol and aqueous extract means that as the TFC value increases, it causes the IC₅₀ of DPPH scavenging activity to decrease which lower IC₅₀ value and it showed that flavonoid compounds in the extracts influence the antioxidant activity and increase the antioxidant activity.

The results are in line with previous study by Li, Gao, Han, Lin, Hu & Chen (2013) which reported that flavonoids are the main form of total phenolics meaning that the antioxidant ability of *H. undatus* flower seems mainly the effect of flavonoid.

Strong and negative correlation between TFC and IC₅₀ of DPPH scavenging activities can be seen from previous study by Fitriansyah, Aulifa, Febriani & Sapitri (2018) on Indonesian medicinal plant, *Phyllanthus emblica*.

4.6.2 Relationship between total flavonoid content (TFC) and FRAP value

In this study, 70% ethanol extract and aqueous extract both shows a very strong and significant ($p < 0.01$) correlation with FRAP ($r = 0.934$, $p = 0.006$; $r = 0.847$, $p = 0.103$). Positive relationship between TFC and FRAP in 70% ethanol and aqueous extract means that antioxidant components in these *Hylocereus* flowers could reduce oxidants and scavenge free radical and with increasing TFC it showed that flavonoid compounds are the main contributor of strong antioxidant potential in the flower extract.

The result was in agreement with study on traditional balsamic vinegar where the total flavonoid content is highly correlated with the antioxidant properties ($r^2 = 0.988$). Previous study by Nurain et al. (2013) on aromatic Malaysian herb showed strong correlation between TFC and FRAP assay. Several other studies also find that phenolic compounds including flavonoid are good source of natural antioxidant and found strong correlation between TFC and FRAP (Othman et al., 2007; Faujan et al., 2007 & Verzelloni et al., 2007).

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study had been conducted to determine the antioxidant activity in the flower of *Hylocereus undatus* and *Hylocereus polyrhizus* in 70% ethanol extract and aqueous extract. The antioxidant activity was determined by using two method which were DPPH free radical scavenging assay and Ferric ion Reducing Antioxidant Power (FRAP). Folin-ciocalteu assay was conducted to determine the total phenolic content of the sample in both extract while aluminium chloride colorimetric assay was carried out to determine the total flavonoid content.

From the results obtained in this study, 70% ethanol extract showed higher total phenolic content (TPC) compared to aqueous extract for both *Hylocereus* flower. For the comparison between sample, the flower of *H.undatus* possessed higher TPC compared to flower of *H.polyrhizus*. TPC of the sample was in order of flower *H.undatus* in 70% ethanol > flower *H.undatus* in aqueous extract > flower *H.polyrhizus* in 70% ethanol extract > flower *H.polyrhizus* in aqueous extract.

For total flavonoid content (TFC), the flower of *H.undatus* possessed higher TFC compared to flower of *H.polyrhizus*. Based on the result obtained, TFC of the sample was in order of flower *H.undatus* in 70% ethanol > flower *H.polyrhizus* in 70% ethanol extract > flower *H.undatus* in aqueous extract > flower *H.polyrhizus* in aqueous extract.

From the results of IC₅₀ value by DPPH assay, 70% ethanol extract showed the lowest value as been compared to aqueous extract. Since IC₅₀ values indicated the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%, thus, the 70% ethanol extract which have lowest IC₅₀ value means that the ethanolic extract have greater ability to limit the action of free radical DPPH compared to the ability in the aqueous extract. The order of the IC₅₀ values from the lowest to the highest was as following: flower *H.undatus* in 70% ethanol > flower *H.polyrhizus* in 70% ethanol extract > flower *H.undatus* in aqueous extract > flower *H.polyrhizus* in aqueous extract.

For Ferric Reducing Antioxidant Power (FRAP), the flower of *H.undatus* possessed higher FRAP value compared to flower of *H.polyrhizus*. Based on the result obtained, FRAP value of the sample was in order of flower *H.undatus* in 70% ethanol > flower *H.polyrhizus* in 70% ethanol extract > flower *H.undatus* in aqueous extract > flower *H.polyrhizus* in aqueous extract.

From the result of Pearson correlation test, TPC in 70% ethanol and aqueous extract both shows very strong positive and significant ($p < 0.01$) correlation with TFC ($r = 0.951$, $p = 0.004$; $r = 0.951$, $p = 0.004$) and TPC in both extract also shows positive and significant ($p < 0.01$) correlation with FRAP ($r = 0.996$, $p = 0.000$; $r = 0.969$, $p = 0.001$) which indicate that as TPC value increases, TFC and FRAP also increases.

For the correlation between total phenolic content (TPC) and IC₅₀ value, in this study, 70% ethanol extract and aqueous extract both shows a very strong correlation with IC₅₀ ($r = -0.888$, $p = 0.018$; $r = -0.996$, $p = 0.000$). For 70% ethanol extract the correlation between TPC and IC₅₀ value was significant at level $p < 0.01$. Negative relationship between TPC and IC₅₀ in 70% ethanol and aqueous extract means that as the TPC value increases, it causes the IC₅₀ of DPPH scavenging activity to decrease which lower IC₅₀ value indicate increasing in antioxidant activities. The same result can be seen for the correlation between total flavonoid content (TFC) and IC₅₀ value where both extract shows a very strong correlation with IC₅₀ where in 70% ethanol extract ($r = -0.957$, $p = 0.003$) while in aqueous extract ($r = -0.858$, $p = 0.095$).

Finally, the correlation between TFC and FRAP value were as follow where in 70% ethanol extract and aqueous extract both shows a very strong and significant ($p < 0.01$) correlation with FRAP ($r = 0.934$, $p = 0.006$; $r = 0.847$, $p = 0.103$). Positive relationship between TFC and FRAP in 70% ethanol and aqueous extract means that flavonoid compounds are the main contributor of strong antioxidant potential in the flower extract.

As a conclusion, binary solvent (ethanol and water) systems are more efficient than mono-solvent systems (pure water) in the extraction of phenolic and flavonoid compounds as well as in the determination of antioxidant activity using DPPH free radical scavenging activity and Ferric Reducing Antioxidant Power (FRAP). The flowers of *H. undatus* and *H. polyrhizus* have potential to be natural sources of antioxidant as it exhibits antioxidant activity from antioxidant test.

5.2 Recommendations

As different extraction solvents like methanol, acetone, chloroform, diethyl ether or hexane may have different effect on the antioxidant activity of the sample, other extraction solvents can be used to find the effect of solvent on extraction efficiency and the most effective solvent used to extract antioxidant content can be determined.

Besides that, study on the effect of extraction temperature to antioxidant content and antioxidant activity of *Hylocereus* flowers can be conducted to know the effect of temperature on antioxidant content of sample. Moreover, different antioxidant activity tests such as oxygen radical absorbance capacity (ORAC), cupric ion reducing antioxidant capacity (CUPRAC), Beta-carotene bleaching assay and others can be used to see the most suitable assay to measure the antioxidant content of sample. Finally, future study to focus on determination of other phenolics compounds such as anthocyanins or ascorbic acid can be conducted to add to the body of knowledge.

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