ANTIOXIDANT PROPERTIES OF WILD *NELUMBO NUCIFERA* (LOTUS PLANT) GROWN IN IPOH

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ABSTRACT

ANTIOXIDANT PROPERTIES OF WILD *Nelumbo nucifera* (LOTUS PLANT) GROWN IN IPOH

Wong Kah Wei Anthony

Wild *Nelumbo nucifera* (lotus plant) present in various Asia countries has been reported to contain numerous bioactive compounds leading to various pharmacological activities including antioxidant activity. The objectives of this study were to obtain the extracts of seed, receptacle, leaf and flower of wild lotus plant found in Ipoh and examine their antioxidant properties. Total phenolic content, total flavonoid content, total carotenoid content and DPPH radical scavenging activity of all four lotus ethanolic extracts were determined. Lotus seed extract showed significantly higher total phenolic content and total flavonoid content ($P < 0.05$) compared to the other three extracts. A significantly higher total carotenoid content ($P < 0.05$) was recorded by lotus leaf extract compared to the other three extracts. However, lotus receptacle extract was found to have the highest antioxidant activity using DPPH radical scavenging assay, evidenced by a significantly lower IC_{50} value ($P < 0.05$). This indicates that the receptacle of *Nelumbo nucifera* contains phytochemicals with a greater antioxidant activity although it had lower content of phenolic and flavonoid compounds ($P < 0.05$) compared to the seeds of the plant. Overall, the results from this study suggested that plant

organs of *Nelumbo nucifera* have a diverse distribution of phytochemicals contributing to variation in antioxidant activities. Therefore, there is a high potential for local *Nelumbo nucifera* to be established as a cost-effective source of dietary antioxidants for the functional food industry in Malaysia.

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DECLARATION

I hereby declare that the project report is based on my original work except for quotations and citations which have been duly acknowledge. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

WONG KAH WEI ANTHONY

APPROVAL SHEET

The project report entitled "**ANTIOXIDANT PROPERTIES OF WILD *Nelumbo nucifera* (LOTUS PLANT) GROWN IN IPOH**" was prepared by WONG KAH WEI ANTHONY and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Food Science at Universiti Tunku Abdul Rahman.

Approved by:

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Date: **30th August 2017**

PERMISSION SHEET

It is hereby certified that **WONG KAH WEI ANTHONY** (ID No: **14ADB07079**) has completed this final year project entitled "**ANTIOXIDANT PROPERTIES OF WILD *Nelumbo nucifera* (LOTUS PLANT) GROWN IN IPOH**" under the supervision of Dr. Lye Huey Shi from the Department of Agricultural and Food Science, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

WONG KAH WEI ANTHONY

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LIST OF ABBREVIATIONS

ABTS	2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid
$AlCl_3$	Aluminium chloride
ANOVA	Analysis of variance
BF	Butanol fraction
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CAT	Catalase
CCl_4	Carbon tetrachloride
DE	Dry extract
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
ECE	Ethanolic crude extract
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
HIV	Human immunodeficiency virus
HSV-1	Herpes simplex virus-1
HPLC	High performance liquid chromatography
IC_{50}	Extract concentrations which give 50% DPPH radical scavenging activity
LDL	Low density lipoprotein
Na_2CO_3	Sodium carbonate

$NaNO_2$	Sodium nitrite
NaOH	Sodium hydroxide
NMR	Nuclear magnetic resonance
PBMC	Peripheral blood mononuclear cell
PHA	Phytohemagglutinin
QE	Quercetin equivalent
QOG	Quercetin-3-*O*-β-D-glucopyranoside
$ONOO^-$	Peroxynitrite
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SPSS	Statistical Package for the Social Science
SRB	Sulforhodamin B
TBARS	Thiobarbituric acid reactive substances
TCC	Total carotenoid content
TFC	Total flavonoid content
TPC	Total phenolic content
Tukey's HSD	Tukey's Honestly Significant Difference
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

1.1 Background

Nowadays, several major life-threatening diseases such as cancer and cardiovascular diseases have been postulated to be associated with the condition known as oxidative stress that occurs in the human body (Dasgupta and Klein, 2014). During the period of escalated stress level, a high amount of reactive oxygen species (ROS) in the form of free radicals are produced inside the human body and this leads to numerous health complications (Krishnaiah, Sarbatly and Nithyanandam, 2010). In order to overcome the adverse effects linked to oxidative stress, the human body must have sufficient supply of both endogenous and exogenous antioxidants as the basis for the subsequent counteractive mechanisms. Hence, antioxidants are one of the core factors in preserving human health due to their primary function in removing free radicals and play a major role in disease prevention (Pham-Huy, He and Pham-Huy, 2008). In particular, antioxidants obtained or derived from natural sources are garnering tremendous attention as synthetic antioxidants used in the food industry have been suggested to be detrimental to human health (Krishnaiah, Sarbatly and Nithyanandam, 2010). One of the upcoming sources of natural antioxidants is the great variety of medicinal plants that can be found both in and outside of Malaysia (Sati, et al., 2010).

The World Health Organisation (WHO) estimated that around 80% of the world population utilise traditional medicine as their main source of health treatment which involves medicinal plant extracts which contain bioactive compounds (Krishnaiah, Sarbatly and Nithyanandam, 2010). One of these medicinal plants is *Nelumbo nucifera* which is commonly known as the Chinese lotus. It is a perennial plant that grows in an aquatic condition usually in lakes and has been reported to be documented in a well-known Chinese medicinal book in 400 years ago (Mehta, et al., 2013).

Due to certain similarities between both *Nelumbo nucifera* (sacred lotus) and *Nymphaea lotus* (waterlily), they are often misrecognised as the same plant. However, scientists have concluded that they are genetically different and are placed in different plant families (Biling and Biles, 2007). *Nelumbo nucifera* has been studied to have several pharmacological activities and among the prominent ones is its antioxidant activity due to the presence of a wide range of phytochemicals across different plant organs (Lim, 2016). In Malaysia, the rhizome of *Nelumbo nucifera* is regularly consumed as a vegetable dish and thus, there is a potential for other organs of the plant to be established as local sources of dietary antioxidants. Therefore, the wild *Nelumbo nucifera* that is present in Ipoh have been chosen to be the subject of this study.

1.2 Objectives

The objectives of this study were:

- To extract the phytochemicals from the seed, receptacle, leaf and flower of lotus plant using ethanol.
- To examine the total phenolic content (TPC), total flavonoid content (TFC) and total carotenoid content (TCC) of extracts obtained from seed, receptacle, leaf and flower of lotus plant.
- To determine the antioxidant activity of extracts obtained from seed, receptacle, leaf and flower of lotus plant.

CHAPTER 2

LITERATURE REVIEW

2.1 Antioxidants

2.1.1 Definition of Antioxidants

In general, antioxidants are defined as any type of molecules that are vital in maintaining tissue and structural integrity (Shekhar, Howlader and Kabir, 2017). Thus, various definitions are used or devised based on the different fields of study. In the field of health sciences, antioxidants are defined as substances that play a main role in minimising the risk of developing degenerative diseases due to excessive oxidative stress that is present in the human body. In the field of food science, antioxidants are defined as substances that are capable of controlling, inhibiting or retarding the oxidation process of foods or the deterioration in food quality aspects when these substances are made available or present in the food (Shahidi, 2015). Basically, antioxidants work or function by scavenging free radicals or stabilising reactive species that they come across with (Shekhar, Howlader and Kabir, 2017).

2.1.2 Classification of Food Antioxidants

Antioxidants that are added in or naturally-present in foods can be classified into various classes based on their mechanisms of functions. The first class is

known as the primary antioxidants. This specific group of antioxidants function by breaking the chain reaction of free radicals through donation of electrons or hydrogen molecules to free radicals and thereby converting them to highly-stable products (Madhavi, Deshpande and Salunkhe, 1996). Tocopherols and various phenolic compounds including flavonoids are examples of primary antioxidants (Maga and Tu, 1995). Next, secondary antioxidants, sometimes referred to as preventive antioxidants are a group of antioxidants which work by transforming or converting lipid peroxides into end products with high stability (Madhavi, Deshpande and Salunkhe, 1996). It has been reported that secondary antioxidants provide stability to lipids by slowing the rate of oxidation process through a variety of mechanisms (Akoh, 2017).

In addition, another unique class of antioxidants is classified as synergistic antioxidants. They have similar functions as primary antioxidants but are capable of regenerating and improving the stability of primary antioxidants that were used collectively (Madhavi, Deshpande and Salunkhe, 1996). This was supported by Hamdo, Khayata and Al-Assaf (2014), who have conducted a study on the synergetic properties between natural antioxidants and synthetic antioxidants. The authors found that different binary combinations of natural antioxidants and synthetic antioxidants exhibited various synergetic percentages, where a higher synergetic percentage was observed in the combination of tocopherol and ascorbyl palmitate. The authors suggested that this might be due to the donation of hydrogen by ascorbyl palmitate to regenerate the tocopherols that were used in the analysis. Considering the

synergistic effects provided by this class of antioxidants, smaller amounts or concentrations of primary antioxidants are needed when they are used together with synthetic antioxidants. Therefore, this can be used as one of the applicable methods to reduce production cost in the food industry (Shahidi, 2015).

2.1.3 Sources of Antioxidants

Most of the essential antioxidants are originated from a variety of natural sources such as grains, vegetables and fruits (Watson, Gerald and Preedy, 2011). Antioxidants are also available in various plant components such as barks, leaves, seeds and pods (Akoh and Min, 2008). Extracts of herbs and spices have also been studied for their antioxidant activities and used commercially as food antioxidants for decades (Akoh and Min, 2008). In addition, several antioxidants such as glutathione and several antioxidant enzymes can also be produced by the cells of human body (Watson, Gerald and Preedy, 2011). Other than the natural antioxidants, synthetic antioxidants are also manufactured for the use of consumers due to better antioxidant properties and low cost. However, there is a higher consumer demand for natural antioxidants due to the fact that they are common food components which can be safely incorporated into the human diet (Charles, 2013).

2.1.4 Natural Antioxidant Compounds

2.1.4.1 Vitamins and Minerals

Vitamins and minerals are also found to possess antioxidant properties other than functioning as nutrient sources (Bendich, Phillips and Tengerdy, 1990). According to Bendich, Phillips and Tengerdy (1990), vitamin C, vitamin E and beta-carotene are capable of functioning as antioxidants in the body. Traber and Atkinson (2007) found that different forms of vitamin E have different antioxidant potencies and the most potent form was α-tocopherol. On the other hand, selenium, manganese and zinc are minerals that can serve as antioxidants. These minerals can also act as cofactor to enhance the activities of several antioxidant enzymes (Woodrow, Colbert and Smith, 2011).

2.1.4.2 Simple Phenolics and Phenolic Acids

Simple phenolics are made up of substituted phenols whereby the position of substitution on the benzene ring can be *ortho*, *meta* or *para* (Vermerris and Nicholson, 2008). Simple phenolics are less available in most plants and a few examples that have been isolated are catechol, hydroquinone and pyrogallol (Harborne, 1984). Ozturk (2015) found that pyrogallol at concentration of 30 μg/mL was able to inhibit more than 75% of lipid peroxidation in an emulsion of linoleic acid. In the same study, pyrogallol showed positive results in several *in vitro* assays and this indicates that pyrogallol is an effective natural antioxidant.

On the other hand, phenolic acids are formed when there is a substituted-carboxyl group on the benzene ring of a phenol (Vermerris and Nicholson, 2008). Phenolic acids can be obtained from leaves of several plants species whereby they can be both alcohol-soluble and alcohol-insoluble (Harborne, 1984). A prominent example is gallic acid whereby it is available in most woody plants with high antioxidant activity (Badhani, Sharma and Kakkar, 2015). Madhavi, Deshpande and Salunkhe (1996) reported that phenolic acids obtained from leaf extracts, cottonseeds and oilseeds have been found to contain antioxidant properties.

2.1.4.3 Polyphenols

At present, polyphenols are more appropriately defined as a class of antioxidants that encompass all phenolic compounds that have two or more hydroxyl groups in their aromatic ring structures (Watson, Preedy and Zibadi, 2014). Polyphenols are also part of the secondary metabolites synthesised by plants and function as antioxidants, major plant pigments and UV light screen that protects the plant (Daayf and Lattanzio, 2008). Examples include stilbenes, lignans, flavonoids and tannins (Daayf and Lattanzio, 2008). Compounds derived or originated from polyphenols are referred to as polyphenol-derived compounds (Watson, Preedy and Zibadi, 2014). Polyphenols are widely-distributed among plants such as fruits, berries and vegetables. The amount and phenol composition of fruits and vegetables are dependent on several variables like storage environment and geography. In addition, polyphenols are also found in beverages such as red wine, coffee and

tea (Vassallo, 2008). Seruga, Novak and Jakobek (2010) have analysed three types of red wines produced from different regions of Croatia and found that there was a high correlation between antioxidant activity and TPC of all the red wines analysed. This shows that the polyphenols present in red wine are able to function as antioxidants and this property is greatly enhanced when the polyphenols content are high. Similar observation was reported by Frei and Higdon (2003), where an improvement in the *ex vivo* oxidation resistance of lipoproteins in animals model of atherosclerosis was found upon the consumption of both green and black teas containing polyphenols.

2.1.4.4 Flavonoids

Flavonoids are a term used to represent a class of natural compounds that contains a C_6-C_3-C_6 carbon framework in its structure with the functionality of phenylbenzopyran (Grotewold, 2008). They are also part of the secondary metabolites synthesised by plants and are categorised as plant phenols (Atta-ur-Rahman, 2002). Based on the dissimilarities in their chemical structures, flavonoids can be further divided into six main classes known as flavonols, flavanones, flavanols, flavones, anthocyanins and isoflavones (Pandey and Rizvi, 2009). Their general chemical structures are shown in Figure 2.1. In addition, there are several classes such as chalcones and aurones which do not have the common basic skeleton of flavonoids but are categorised as flavonoids due to the fact that they are both chemically and biosynthetically similar (Yusuf, 2016).

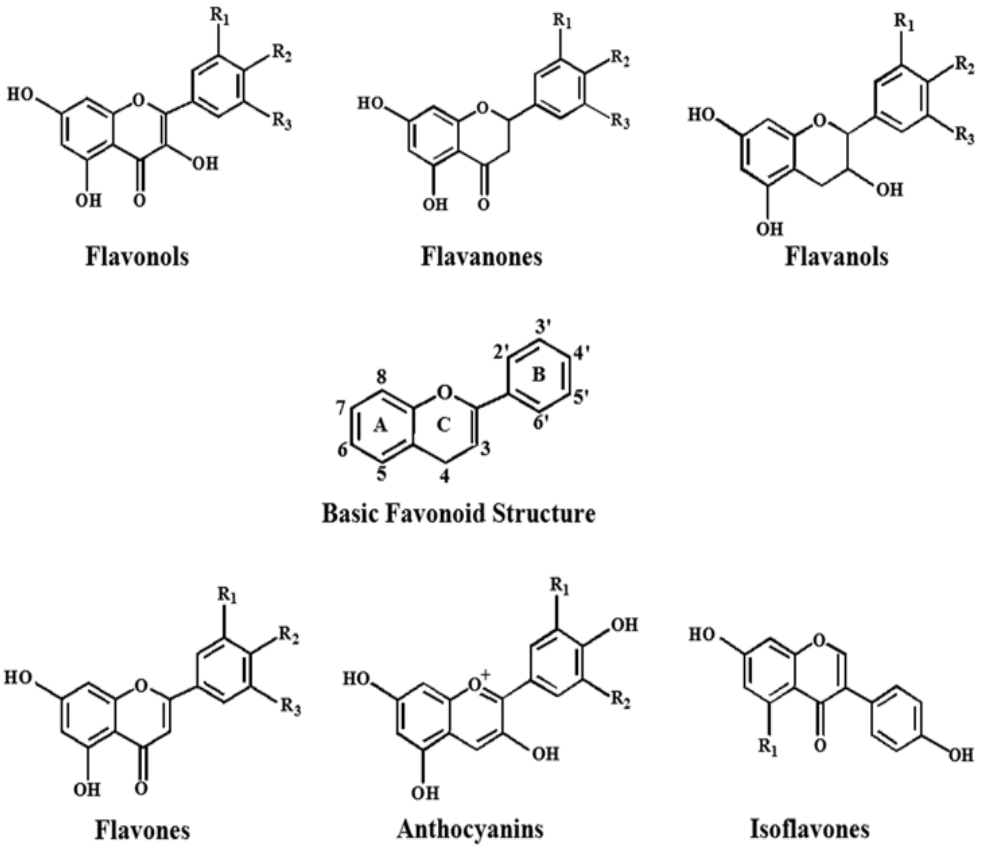


Figure 2.1: Chemical structures of various main classes of flavonoids (Pandey and Rizvi, 2009).

Natural sources of flavonoids are numerous and among the conventional ones are whole grains, spices, herbs, vegetables, fruits, teas and red wines. Meanwhile, flavonoids are also reported to be capable of acting as antioxidants that may aids in reducing cardiovascular diseases (DeBruyne, Pinna and Whitney, 2016). Yao, et al. (2004) found that flavonoids exhibited antioxidant as well as pro-oxidant activities in animal trials. The authors also noticed that most of the flavonoids show antioxidant properties in hydrophilic environment but only a handful are able to do so in a lipophilic environment. It has been suggested that the antioxidant activity of flavonoids is dependent on the arrangement of the functional groups in the physical structure. The configuration and total number of hydroxyl groups are the major influences on the antioxidant mechanisms of flavonoids (Kumar and Pandey, 2013). Other possible pharmaceutical functions such as antidiabetic, antiviral and anti-inflammatory of flavonoids have been studied and reported previously (Yao, et al., 2004).

2.1.4.5 Carotenoids

Carotenoids are lipid-soluble natural plant pigments. It is made up of a skeleton with 40 carbon atoms that presents itself in a variety of structures either hydroxylated or oxygenated and so on. There are two types of carotenoids; carotenes and xanthophylls. Carotenes are pure hydrocarbons while xanthophylls are derived from carotenes and oxygenated (Stange, 2016). However, there are also several carotenoids that contain less than 40 carbon

atoms in the whole structure. These carotenoids are known as diapocarotenoids (Krinsky, Mayne and Sies, 2004).

In addition, Tanumihardjo (2013) also reported that carotenoids contain certain functional properties as well as several benefits that contribute greatly to human's health. In the human body, several carotenoids play a few major roles such as serving as precursor for vitamin A, ensuring proper cell differentiation and essential for good vision (Stange, 2016). Stahl and Sies (2003) deduced that carotenoids are efficient antioxidants for plants that guard them from damage caused by oxidation. In addition, the authors also suggested that they might be a part of the antioxidant network and carry out tasks like scavenging radicals and protecting lipophilic components. Besides that, Sindhu, Preethi and Kuttan (2010) have analysed the antioxidant activity of lutein which is a type of carotenoid and found that it has the ability to scavenge superoxide and hydroxyl radicals on top of inhibiting lipid peroxidation in an *in vitro* manner. All these clearly portrayed the potential of carotenoids to be harnessed as an alternative source of antioxidants other than the common ones such as vitamin C and E.

2.1.5 Synthetic Antioxidants

Synthetic antioxidants are mostly synthesised for the stabilisation of polymers such as plastics and rubber. In the field of food manufacturing, only several synthetic antioxidants have been used as food additives due to rigid

toxicological testing procedures as well as strict regulations to safeguard the health and safety of potential consumers (Madhavi, Deshpande and Salunkhe, 1996). Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are common examples of synthetic antioxidants that are usually found in foods. However, the addition of synthetic antioxidants into foods are currently being debated due to recent studies indicating their carcinogenic effects on healthy human cells (Shebis, et al., 2013).

Several studies have been done on evaluating the antioxidant properties of synthetic antioxidants in recent years. Martinez, et al. (2012) studied the effect of rosemary extract and synthetic antioxidants on the oxidative stability of walnut oil. Results indicated that both natural and synthetic antioxidants were able to minimise lipid peroxidation significantly which subsequently increased the shelf-life of walnut oil up to six months of storage period. Moreover, Caleja, et al. (2016) also found that both natural and synthetic antioxidants were able to confer identical antioxidant activity without affecting the physical and nutritional qualities of the biscuits samples. Based on the findings of these studies, the antioxidant activities and properties of both natural and synthetic antioxidants are similar and thus, this indicates the potential of natural antioxidants to replace synthetic antioxidants in food applications in the near future due to the increasing consumer preference towards natural antioxidants.

2.1.6 Application of Antioxidants in the Food Manufacturing Industry

In the food industry, antioxidants have been applied or used in various food products as one of the main additives to preserve quality by minimising oxidation or oxidative damage. One of the primary functions of antioxidant as a food additive is to stabilise fats and oils. This is because fats from animal origin contain very low concentration of antioxidants in nature. Thus, both synthetic as well as natural antioxidants have been applied in this type of product due to their high stabilisation effect (Rahman, 2007). On the other hand, vegetable oils which naturally contain high amount of polyunsaturated fatty acids are harder to be stabilised due to their high susceptibility towards oxidation (Yun and Surh, 2012). However, several vegetable oils such as olive and sesame oils do contain high amount of natural antioxidant compounds such as tocopherol. Hence, they are less susceptible to oxidative damage compared to the other vegetable oils (Zeuthen and Bogh-Sorensen, 2003).

Several types of flavonoids such as quercetin have been used to minimise rancidification of fish oils and herbal extracts obtained from rosemary have also been reported to be very effective in preserving mackerel oil (Zeuthen and Bogh-Sorensen, 2003). Besides, antioxidants have been applied in food emulsions including water-in-oil and oil-in-water emulsions. For water-in-oil emulsion system, an example such as margarine whereby synthetic antioxidants are added to preserve the continuous oil phase of the emulsion (Pokorny, Yanishlieva and Gordon, 2001). Salad dressings such as

mayonnaise are made up of an oil-in-water emulsion system and natural antioxidants such as extracts of herbs and spices have been added to protect these products from oxidation besides conferring characteristic flavours to these products (Pokorny, Yanishlieva and Gordon, 2001).

2.1.7 Roles of Antioxidants in Human Health

2.1.7.1 Oxidative Stress in Human Body

Gelpi, Boveris and Poderoso (2016) defined oxidative stress as a condition inside the body whereby there is an imbalance between the amount of oxidants and antioxidants which disrupts the redox signalling or control and further leads to damage in the molecular level. Various metabolisms and the energy production process in humans can generate free radicals (Packer and Sies, 2008). Free radicals are generally defined as chemical molecules which have an unpaired electron located at the outermost electron shell. Thus, they are highly reactive compounds that can easily take part in redox reactions (Lobo, et al., 2010).

These free radicals are responsible for various health-related issues such as cell oxidative damage, aging and several degenerative diseases (Dasgupta and Klein, 2014). In a healthy human body, cells will start various antioxidant mechanisms to overcome the negative effects brought upon by these radicals which act as oxidants (Packer and Sies, 2008). One of the common mechanisms is by scavenging the free radicals through the action of

antioxidants present in human cells such as gluthathione (Lobo, et al., 2010). In addition, oxidative stress can exist in various forms such as nutritional oxidative stress or physiological oxidative stress (Gelpi, Boveris and Poderoso, 2016).

2.1.7.2 Reduce Risk of Cardiovascular Disease

Cardiovascular disease is a group of diseases that comprises of all the diseases that affects the heart and all types of blood vessels in the human body (DeFelice, 2005). For the past decade, there have been sufficient data from various clinical studies to show that there is some sort of correlation between oxidative stress and cardiovascular risk factors. Oxidative stress has been suggested to be induced by the increase in reactive oxygen species (ROS) produced in the vascular level (Landmesser and Drexler, 2006). In addition, oxidative stress can directly leads to the activation of genes that are associated with blood vessels inflammation. Besides that, oxidative stress also cause modifications in the signalling mechanism of arterial cells that is responsible for the proper functions of the arteries (Dasgupta and Klein, 2014).

Several studies have been done to determine the effect of consuming natural antioxidants particularly polyphenols on minimising the risk of contracting cardiovascular disease and most of the studies so far have been found to be fruitful. This is because polyphenols have been reported to be effective in inhibiting the oxidation of low density lipoprotein (LDL) whereby this

scenario is the key factor that leads to atherosclerosis development (Pandey and Rizvi, 2009). Frankel, et al. (1993) conducted an *in vitro* study on antioxidant effect of phenolic substances originated from red wine. The authors found that these phenolic substances were able to inhibit the oxidation of human low density lipoprotein (LDL) and hence, the authors deduced that phenolic compounds present in red wine are potent antioxidants. It has also been suggested that catechins obtained from tea were able to hinder the cell proliferation of smooth muscles located in the arteries subsequently retarded the formation of atheromatous lesions (Pandey and Rizvi, 2009). In addition, pigments from tea such as flavonoids are capable of inhibiting the adhesion and aggregation of platelets besides increasing fibrinolysis (Yao, et al., 2004).

2.1.7.3 Prevention of Cancer

Cancer is defined as a condition whereby cells undergo uncontrolled proliferation which results in tumour formation due to mutations that have occurred within a single cell (Macdonald, Ford and Casson, 2004). The link between oxidative stress and cancer has long been established. One of the main reasons is the presence of ROS in significant amount during a state of oxidative stress. ROS are capable of attacking DNA of cells which may results in mutation of genes responsible for tumour-suppression (Dasgupta and Klein, 2014). In addition, oxidation of DNA by radical species has been reported to cause modifications or alterations in nucleotide base sequences (Herrera, Garcia-Bertrand and Salzano, 2016). This may further cause carcinogenesis

due to the mutagenic potential of affected nucleotide base pairing (Dasgupta and Klein, 2014).

Throughout recent years, numerous studies have been done on evaluating the role of natural antioxidants in cancer protection or prevention. Various types of polyphenols such as quercetin and lignans have been analysed and showed certain degree of cancer-protective effects in animal models although they have different action mechanisms (Pandey and Rizvi, 2009). Yao, et al. (2004) reported that flavonoids demonstrated ability to minimise carcinogenesis in animals models by having certain influence on the carcinogenic process stages of initiation and promotion. Several mechanisms of action of polyphenols in cancer protection have been identified, which are inhibition of cell proliferation, oxidation prevention and anti-inflammatory activity (Pandey and Rizvi, 2009). Teas are good sources of polyphenols and their role in cancer protection has been evaluated extensively. Green tea has been reported to be able to induce apoptosis and exhibits ability in preventing tumour initiation in various models of animal tumour while consumption of black tea helps in inhibiting the stage of tumour promotion (Yao, et al., 2004).

2.1.7.4 Minimises Risk of Diabetes

Diabetes mellitus is a group of various metabolic diseases and typically characterised by the presence of hyperglycemia and other distinctive health complications in the human body (Poretsky, 2010). In the past, multiple

studies on diabetic patients have shown that oxidative stress do play a vital role in causing systemic inflammation, impairment of pancreatic insulin secretion as well as impairment of glucose utilisation. In addition, oxidative stress also has been identified as a key factor in leading to increased mortality of diabetic patients (Dasgupta and Klein, 2014).

At present, there has been countless studies done on evaluating the anti-diabetic potential or role of natural antioxidants particularly polyphenols. Resveratrol, a well-known polyphenol found in red wine was reported to be able to delay the start of insulin resistance while ferulic acid, a polyphenol obtained from bran of maize plant was capable of reducing level of blood glucose combined with an elevation of plasma insulin content (Pandey and Rizvi, 2009). In 2002, a study on evaluating the insulin-enhancement activity of tea was done by Anderson and Polansky. The authors found that their samples of green and black tea were able to enhance insulin activity in an *in vitro* manner whereby the identified compounds that are responsible for this activity are reported to be epicatechin gallate and tannins, both are natural plant polyphenols. Besides, Pandey and Rizvi (2009) proclaimed the ability of quercetin to safeguard the alterations in diabetic patients brought upon by oxidative stress.

2.1.7.5 Other Diseases

Oxidative stress has also been established as one of the causative factors to several minor diseases which are not life-threatening such as age-related neurodegenerative diseases (Dasgupta and Klein, 2014). This is because neuronal cell membranes consist of high concentration of polyunsaturated fatty acids which are prone to oxidative damage by free radicals (Dasgupta and Klein, 2014). Natural antioxidants such as polyphenols have been studied to show beneficial effects in minimising and preventing certain Alzheimer's and Parkinson's diseases (Pandey and Rizvi, 2009). Rossi, et al. (2008) reported that polyphenols present in plants are better than vitamins in terms of their effectiveness as neuroprotective agents.

On the other hand, several studies on the anti-aging properties of natural antioxidants like polyphenols also have been done. It was suggested that the antioxidant and anti-inflammatory activity of polyphenols present in natural plant sources like fruits may play a role as effective anti-aging compounds (Pandey and Rizvi, 2009). Baxter (2008) reported that the administration of resveratrol on mice model had been found to prevent cell proliferation prior to irradiation by ultraviolet light thereby inhibiting photoaging. The author also suggested the topical usage of resveratrol is viable due to minimal risk and potentially wide range of benefits.

2.2 *Nelumbo nucifera*

2.2.1 Botany

2.2.1.1 Species

At present, there is only two species of lotus that have been identified and confirmed which are *Nelumbo nucifera* and *Nelumbo lutea* (Billing and Biles, 2007). Both of these species are categorised into the plant family known as Nelumbonaceae (Sridhar and Bhat, 2007). *Nelumbo nucifera* is widely distributed from the Caspian Sea to the Asia continent. *Nelumbo nucifera* has been found in various countries including China, Iran, Korea, Northen Australia as well as Malaysia (Lim, 2016). Due to its presence in a variety of countries, numerous local names have been developed to identify the *Nelumbo nucifera* and a few examples are sacred lotus, Chinese Lotus and Asian Lotus (Billing and Biles, 2007).

2.2.1.2 Cultivation of *Nelumbo nucifera*

In a worldwide-scale, thousands of cultivars of *Nelumbo nucifera* have been reported due to several reasons such as its function as a source of food and for beauty usage (Billing and Biles, 2007). In China, cultivation of *Nelumbo nucifera* is done in a massive scale. The province of Hubei is reported to have one of the largest area of cultivation comprising of 67,300 hectares of land in 2003 and the harvest output is reported to be in millions of tonnes (Liu, et al., 2006). In addition, Guo (2008) reported that plants of the genus *Nelumbo* are widely cultivated throughout India as it was considered as a sacred plant by

the Hindu religion. Classification of the countless cultivars of *Nelumbo nucifera* in China was done based on a ranking system developed for the difference in various morphological properties such as number of flower petals, petals colour, plant height and the shape of seed produced (Guo, 2008). Recently, scientists are looking into developing new cultivars which are hybrids with better tuber and flower production as well as having improved resistance towards pests and diseases (Billing and Biles, 2007).

2.2.1.3 Types of *Nelumbo nucifera*

In general, *Nelumbo nucifera* is also differentiated into various types other than based on the cultivation factor alone. Two of the most common parameters that have been used to categorise the types of *Nelumbo nucifera* are size and hardiness of the plant. The size of plant is divided into bowl, dwarf, medium and large. These divisions are based on several physical parameters of the plant (Billing and Biles, 2007). In the country of China, this plant which is locally named as Chinese lotus is categorised into three main types which are known as rhizome lotus, flower lotus and seed lotus. Rhizome lotus is known to have the tallest plant height and their rhizome is edible. Meanwhile, seed lotus contains the highest number of seed set compared to the other two types of lotus (Guo, 2008). For the type of flower lotus, it is known for having diverse flower types and shortest plant height (Guo, et al., 2007). In terms of plant hardiness, lotuses of the hardy type are known to only bloom during the summer season and their tuber size is larger. On the other hand, lotuses of the tropical type are found in tropical and subtropical climates

area and they are active bloomers compared to lotuses of the hardy type (Billing and Biles, 2007).

2.2.1.4 Plant Structure of *Nelumbo nucifera*

The lotus is known as an aquatic and perennial herb with both the root and rhizome component being submerged under muddy waters (Lim, 2016). As growth of the plant progresses during its growing season, the lotus will develop from its submerged network of root system. This root system is made up of numerous branches of horizontal stems that grow rapidly (Billing and Biles, 2007). The roots of the plant are grown from nodes located externally on the rhizome while the leaves are typically raised above the water or float on the water surface (Lim, 2016). Next, flower buds of lotus are also originated from the nodes and grow on a stem that extends to a height well above the water surface. The blooming of flowers will only occur in the suitable seasons (Billing and Biles, 2007). The structure of the plant that holds plenty of embedded seeds is known as the receptacle or seed pod and it achieves maturity when the petals of the flower start to fall off (Rosengarten, 2004). Lotus seeds are characterised with having yellowish-white dicotyledons and presence of a green embryo (Lim, 2016).

2.2.1.5 Life Cycle of *Nelumbo nucifera*

The life cycle of lotus typically starts when new growth occurred on tubers which are dormant in the spring season and is characterised with the

development of underground roots and formation of leaves. When the summer season approaches, the lotus will continue to extend its network of root system. In addition, it will also start to produce the stem that holds the flower bud at which it will bloom for three to four days when certain conditions are met (Billing and Biles, 2007). Once the flower petals start to fall, seeds will begin to develop in the receptacle if pollination do occurs whereby the receptacle will also experiences enlargement in size. Around the winter season, the seeds will achieve maturity and hardened while the receptacle will have woody and dry texture (Griffiths, 2009). When both of these parts are fully matured, the receptacle will position itself facing downwards towards the water and releases all the seeds in it (Billing and Biles, 2007). This marks the completion of a life cycle of a single plant of *Nelumbo nucifera.*

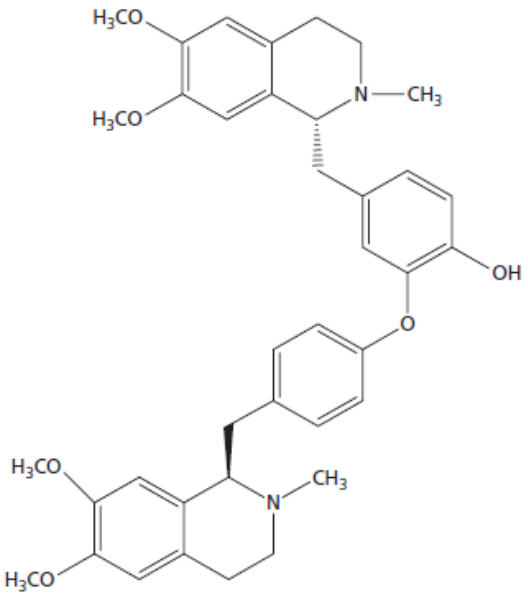
2.2.2 Nutrients and Phytochemicals Present in Seed

Several proximate analyses have been done in the United States on evaluating the nutrient content of lotus seeds. In 100 g of typical ripen and raw lotus seeds, there are 77 g of water, 4.13 g of protein, 0.53 g of total lipid, 1.07 g of ash and 17.28 g of carbohydrates contained in it. Out of all the unsaturated fatty acids that have been found available in lotus seeds, linoleic acid was the most abundant at 0.285 g per 100 g of lotus seeds (Lim, 2016). Chu, et al. (2012) reported that annexins, which are a type of proteins with various functions, were also found in lotus seeds.

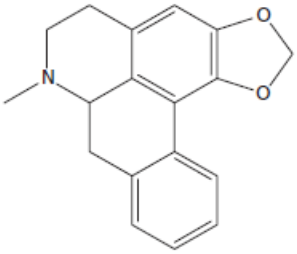
Countless natural compounds that are capable of functioning as antioxidants have been found in lotus seeds. Chen, et al. (2007) have successfully isolated and identified three alkaloids, liensine, isoliensinine and neferine from lotus seeds by using liquid chromatography analysis technique. Besides that, similar findings were reported by Liu, et al. (2009) where liensine, isoliensinine and neferine were successfully isolated and purified from crude extracts of lotus seed embryo up to 95 % purity and above. In addition, other alkaloids such as dauricine, roemerine and pronuciferine were reported as part of the major secondary metabolites that are available in lotus seeds and their chemical structures are shown in Figure 2.2 (Mehta, et al., 2013). Various flavonoids have been found present in lotus seeds coat and among of them are rutin, syringetin 3-*O*-glucoside and astragalin (Lim, 2016). Besides that, Youn, et al. (2010) had successfully isolated four different alkyl 4-hydroxybenzoates that are present in lotus seeds and their structures were analysed using NMR spectroscopy.

2.2.3 Phytochemicals Present in Receptacle

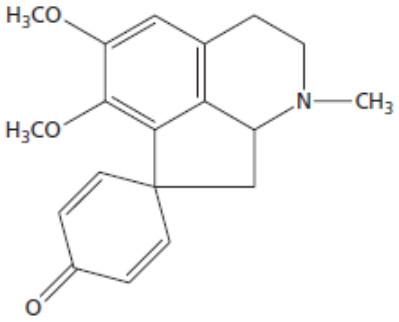
In recent years, various studies have been done on isolating and identifying the phytochemicals that are present in lotus receptacle which holds the seeds of the plant. In 2005, Ling, Xie and Yang had successfully recovered, characterised and analysed the antioxidant activity of procyanidins present in receptacle of *Nelumbo nucifera* Gaertn. Based on their results of mass spectroscopy, they deduced that their sample extracts contain large concentration of dimers followed by monomers and tetramers of procyanidins.



Dauricine



Roemerine



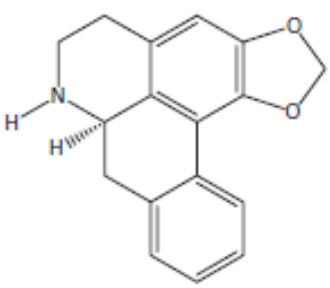
Pronuciferine

Figure 2.2: Chemical structures of dauricine, roemerine and pronuciferine found in seeds of *Nelumbo nucifera* (Mehta, et al., 2013)

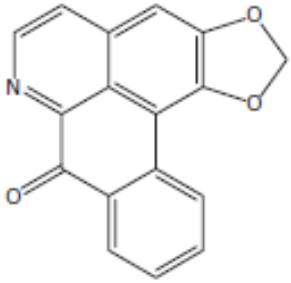
In addition, the authors mentioned that the base units of the identified procyanidins are catechin and epicatechin. Thus, this indicates the presence of natural polyphenols in lotus seedpod thereby signifiying the potential of lotus seedpod to be serve as a source of antioxidants. In a separate study, five different flavonol glycosides were successfully isolated and determined in lotus receptacle and among of them are the antioxidants hyperoside and isoquercitrin (Wu, et al. 2013). Hu, et al. (2005) analysed the nutritional composition as well as antioxidant activity of receptacle of lotus and they were able to acquire and identify the presence of a dietary flavonoid, quercetin-3-*O*-β-D-glucopyranoside (QOG) from their samples.

2.2.4 Phytochemicals Present in Leaf

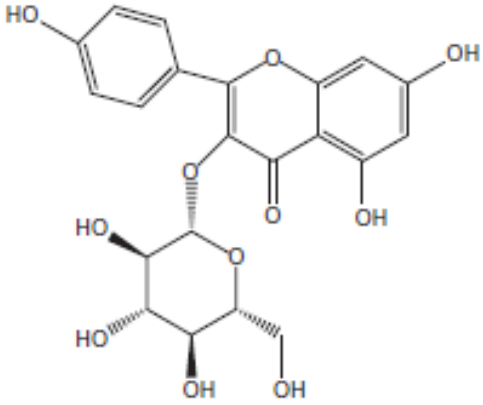
The gigantic leaves of *Nelumbo nucifera* have been studied for the past decades on determining its potential as a source of natural antioxidants. Various alkaloids were found to be available in leaves of the plant and among of them are (–)-caaverine, (–)-nuciferine, *N*-Nornuciferine and roemerine (Lim, 2016). Liu, et al. (2014) had successfully isolated and identified a total of 15 phytochemical compounds from lotus leaves and among of them were lysicamine, (−)-nuciferine and (−)-asimilobine that are suggested as potential natural antioxidants. In addition, Mehta, et al. (2013) reported that phytochemicals like anonaine, liriodenine and astragalin have been found in lotus leaves and their chemical structures are shown in Figure 2.3.



Anonaine



Liriodenine



Astragalin

Figure 2.3: Chemical structures of anonaine, liriodenine and astragalin found in leaves of *Nelumbo nucifera* (Mehta, et al., 2013).

Flavonoids which are potent natural antioxidant compounds have also been found in lotus leaves across different studies done in the past. Flavonoids such as astragalin, rutin and quercetin have been successfully recovered from ethanolic extract of lotus leaves (Ohkoshi, et al., 2007). Similar findings were also reported by Goo, Choi and Na (2009) where six different forms of the flavonoid quercetin have been acquired from methanolic extract of lotus leaves. Phenolic compounds available in leaves of *Nelumbo nucifera* are consisted mainly of gallic acid, quercetin and rutin (Lim, 2016). In addition, Chen, et al. (2012a) successfully extracted 13 different flavonoids from lotus leaves. Five out of all the flavonoids obtained were claimed to be present in lotus leaves for the first time by using an optimised extraction and HPLC analytical method. Therefore, all these studies accentuate the diverse content of bioactive compounds present in leaves of *Nelumbo nucifera*.

2.2.5 Phytochemicals Present in Flower

Lotus flowers are available in different colours and this has attracted the interest of the scientific community on examining the phytochemicals content of this plant structure. Jung, et al. (2003) had recovered seven known flavonoids from stamens of lotus flowers including a few variants of the flavonoid kaempferol. In addition, anthocyanins are also reported to be present in petals of lotus flowers (Yang, et al., 2009). In an alternative study, Deng, et al. (2013) did an identification and quantification study on the flavonoids composition of lotus flower petals obtained from 108 cultivars.

Based on their results, the authors had successfully found a total of 19 different flavonoids available in their samples belonging to the classes of anthocyanins, flavonols and flavones by utilising the HPLC analysis technique. Similar findings were also reported by Chen, et al. (2013) where five variants of anthocyanins and 20 other flavonoid compounds were isolated from extracts of lotus petals originated from 12 different genotypes. One of the reported anthocyanins was cyanidin 3-*O*-glucoside. The stamens and petals component of the lotus flower are comprised of almost identical range of flavonoids such as rutin, astragalin, and myricetin 3-*O*-galactoside (Lim, 2016). Besides that, carotenoids in the form of beta-carotene were identified to be present in four varieties of *Nelumbo nucifera* Gaertn flowers obtained from two different provinces in the country of Thailand (Phonkot, Wangsomnuk and Aromdee, 2010). Hence, all these studies clearly depict the potential of lotus flower to be established as a flavonoids source.

2.2.6 Consumption of Various Parts of *Nelumbo nucifera*

The sacred lotus or *Nelumbo nucifera* has been utilised as a food ingredient and incorporated into various recipes by the Asian communities (Lim, 2016). The food preparations or consumption of the different parts of the lotus plant are shown in Table 2.1.

Table 2.1: The food preparation and consumption of various lotus plant structures.

Lotus plant structures	Food preparation/Consumption	References
Seed	Used as an ingredient to make the paste of premium mooncakes by Chinese bakeries.	(Hu, 2005)
	Lotus seeds are added into soups, variety of food dishes and Chinese desserts.	(Lim, 2016)
	Lotus seeds are eaten raw, roasted or ground into powder form.	(Sridhar and Bhat, 2007)
Receptacle	Non-edible.	(Zheng, et al., 2012)
Leaf	Used as a packaging material to wrap and cook rice in Korea.	(McWilliams, 2013)
	Utilised as the main ingredient for several herbal beverages in China.	(Lim, 2016)
Flower	Utilised as a flavouring agent in tea-making.	(Sridhar and Bhat, 2007)
	Dried petals of lotus blossoms are added into popular Chinese dishes.	(Lim, 2016)

2.2.7 Potential Health Benefits of Consuming *Nelumbo nucifera*

2.2.7.1 Anti-Cancer Activity

Multiple studies have been done in the past to have a better understanding on the anti-cancer potential of *Nelumbo nucifera.* Liu, et al. (2004) evaluated the ability of extracts derived from *Nelumbo nucifera* in suppressing the cell proliferation process of human peripheral blood mononuclear cells (PBMC) in an *in vitro* manner. The authors found that ethanolic extracts of *Nelumbo nucifera* were capable of inhibiting the proliferation of PBMC activated by phytohemagglutinin (PHA). Hence, this indicates that the presence of phytochemicals in lotus possess certain anticancer activity. Moreover, Kim, et al. (2009) found that luteolin and maslinic acid isolated from *Nelumbo nucifera* leaves exhibited substantial cytotoxicity against a few human cancer cells such as ovarian and colon cancer cells by utilising the Sulforhodamin B (SRB) bioassay.

Neferine, an alkaloid present in embryo of lotus seed was found to inhibit lung cancer cells, A549 obtained from humans by stimulating the process of apoptosis in a dose-dependent approach (Poornima, Weng and Padma, 2014). In a separate study, a similar anticancer activity of neferine obtained from *Nelumbo nucifera* was reported by Yoon, et al. (2013). The authors reported that neferine showed cytotoxicity towards Hep3B cells of hepatocellular carcinoma, a form of liver cancer but not towards healthy liver cells of humans. In addition, flavonoids present in lotus leaf extracts like quercetin were found to be capable of minimising both the volume and weight of tumour

in mice which were inoculated with breast cancer cells of human labelled as MCF-7 cells in advance (Yang, et al., 2011).

2.2.7.2 Anti-Diabetic Activity

Several studies have been done to determine the anti-diabetic activity of *Nelumbo nucifera* in the past several years. The alkaloid nuciferine obtained from *Nelumbo nucifera* has been found to be capable of inducing the secretion of insulin in islets cells and its primary action mechanism involves the closing of K-ATP channels (Nguyen, et al., 2012). Similar anti-diabetic activity results were also reported in an isolated study done by Huang, et al. (2011). The authors reported that extracts of *Nelumbo nucifera* were capable of enhancing the secretion of insulin in β-cells particularly the bioactive compound, catechin. The authors also suggested that extracts of *Nelumbo nucifera* containing catechin have potential to be used as controller of hyperglycaemia based on their ability to function as insulin secretagogues. Other than nuciferine and catechin, Pan, et al. (2009) examined the effect of introducing neferine isolated from lotus seed to insulin-resistant rats and they found that neferine showed a similar effectiveness as rosiglitazone in raising the sensitivity of their rat models toward insulin. In other words, neferine has been found to confer substantial degree of anti-insulin resistance to live samples. In addition, Sakuljaitrong, et al. (2013) examined both hypoglycaemic and hypolipidemic effects of lotus flower extracts on diabetic rats. Based on their findings, the introduction of flower extracts at a dosage of 250 mg/kg were

able to cause considerable reduction in fasting blood glucose level besides lowering cholesterol levels in diabetic rats.

2.2.7.3 Anti-Viral Activity

At present, the anti-viral activity of compounds present in *Nelumbo nucifera* extracts have been analysed across multiple studies. In 2012, Knipping, Garssen and Land evaluated the inhibition of rotavirus infection of several plant species including *Nelumbo nucifera* Gaertn. They found that the fruit extracts of lotus exhibited notable anti-viral activity with a significant ($P < 0.05$) 50% inhibitory concentration of less than 300 μg/mL. In addition, the authors also reported that lotus fruit extracts showed synergistic effects with extracts of a few other plants such as *Glycyrrhiza glabra* L in their anti-viral activities and hence, they proposed the potential of lotus extracts to be used in treating rotavirus-induced diarrhoea.

Furthermore, several compounds have been obtained from lotus leaves and all these compounds showed anti-HIV activities. The concentrations of each compound that inhibited viral replication by 50% were reported to be lower than 0.8 μg/mL (Kashiwada, et al., 2005). Among of those analysed compounds were (−)-1(S)-norcoclaurine, nuciferine and liensinine. In addition, lotus seed extracts obtained using ethanol as a solvent were reported to show high efficacy in inhibiting the replication of herpes simplex virus-1 (HSV-1). Extracts of *Nelumbo nucifera* seeds at a dosage of 50 μg/mL was

able to suppress the replication of HSV-1 in HeLa cells up to 85.9% besides capable of reducing the synthesis and transcription of infected cell proteins in HeLa cells treated with the extracts beforehand (Mukherjee, et al., 2009).

2.2.7.4 Anti-Obesity Activity

In the past decade, a number of studies have been done on evaluating the anti-obesity potential of phytochemicals present in *Nelumbo nucifera*. Velusami, Agarwal and Mookambeswaran (2013) have examined the anti-obesity activity of both methanol and aqueous extracts of lotus flower petals. Their results showed that both types of extracts were able to suppress lipid storage in adipocytes besides enhancing the process of lipolysis in an *in vitro* model. Moreover, the authors reported that methanol extracts have better overall anti-obesity activity compared to aqueous extracts. Thus, this indicates organic extracts of the plant are more potent in their pharmacological activity compared to their aqueous counterparts. Next, the administration of lotus leaf extracts was capable of hindering the increase of body weight, adipose tissue weight and rise in triacylglycerol levels in the liver of obesity induced-mice (Ono, et al., 2006). Based on their findings, the author suggested that leaf extracts of lotus can be utilised as a suppressor of obesity.

In addition, the introduction of *Nelumbo nucifera* leaf extracts in rats fed with a diet of high fat content has been found to cause a remarkable decrease in total lipids present in liver tissues (Lim, 2016). You, et al., (2014) investigated

the anti-obesity activity of ethanol extracts of lotus seeds in cultured human adipocytes. Their results showed that the treatment of human adipocytes with lotus seed extracts prevented the occurrence of lipid accumulation besides minimising the expression of the glucose transporter known as GLUT4 present in the adipocytes. Besides, Ahn, et al. (2013) had conducted a study on evaluating the anti-obesity effect of various chemical compounds isolated from leaves of *Nelumbo nucifera*. The authors reported that *cis-N*-feruloyltyramine, an alkaloid found in lotus leaf exhibited a significant ($P < 0.05$) inhibition against the pancreatic lipase enzyme while the alkaloids (6R,6aR)-roemerine-Nβ-oxide and liriodenine were able to strongly restrain the cell differentiation process of adipocytes.

2.3 Antioxidant Properties of *Nelumbo nucifera*

2.3.1 Seed

Numerous studies have been done to evaluate the antioxidant activity and properties of seeds of *Nelumbo nucifera* due to its profound status as a medicinal plant. In 2004, Yen, Duh and Su examined the effects of lotus seed extracts on DNA damage that occurred in lymphocytes obtained from the human body. They found that their sample extracts were able to suppress the DNA damage that was induced in human lymphocytes by hydrogen peroxide in advance. Besides that, the extracts also depicted formidable antioxidant activity and chelating ability on ferrous ions. Thus, the authors suggested that the powerful antioxidant activity of lotus seeds extracts is strongly-linked to

its ability to scavenge radicals, thereby preventing DNA damage and as well as its high content of phenolic compounds.

In a different study of Rai, et al. (2005), the authors investigated the antioxidant activity of seeds extracts of *Nelumbo nucifera* in both *in vivo* and *in vitro* models. Their results were shown in terms of concentration of sample required to inhibit 50% of nitric oxide radical (IC_{50}). Based on their results, the seeds extracts of lotus showed a better antioxidant activity in the nitric oxide radical inhibition assay with an IC_{50} value of 84.86 µg/mL when compared to the standard used which was rutin with an IC_{50} value of 152.17 µg/mL. In addition, their *in vivo* studies showed that the introduction of lotus seeds extracts to Wistar rats leads to a rise in levels of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD). This scenario was coupled with the decrease in thiobarbituric acid reactive substances (TBARS) level in the kidney and liver of the rats. The rats were then intoxicated with CCl_4 after four days. However, it was reported that rats which were not introduced with lotus seeds extracts prior to CCl_4 treatment have been found to have lower levels of both CAT and SOD in their kidneys and livers besides having higher level of TBARS. Hence, this indicates that compounds present in lotus seeds are able to enhance the activity of antioxidant enzymes in addition to having potent antioxidant activity. Therefore, *in vivo* and *in vitro* studies should be done on local species of *Nelumbo nucifera* to evaluate its antioxidant properties and compare it with those species found in other countries.

2.3.2 Receptacle

Recently, the structure of the lotus plant which holds the seeds known as the receptacle has been gaining tremendous attention due to various reports on its high content of phytochemicals including procyanidins and flavonoids (Lim, 2016). Wu, et al. (2012) had evaluated the antioxidant activity of extracts obtained from receptacle of *Nelumbo nucifera* purchased in Fujian, China. The authors used several organic solvents including butanol, ethanol and petroleum ether for the extraction process and analysed the antioxidant activity of all these fractions individually. Their results showed that both the ethanol crude extract (ECE) and butanol fraction (BF) have higher radical scavenging activity compared to the standard used, BHT in the ABTS assay. Based on their findings, the concentration of ECE, BF and BHT which gave 50% scavenging activity on the ABTS radicals (IC_{50}) were reported to be 73.6 μg/mL, 48.4 μg/mL and 157.9 μg/mL, respectively.

A similar study was also done by Zheng, et al. (2012), the authors investigated the antioxidant activity of lotus receptacles harvested from 11 different Chinese cultivars. They found that all their 11 samples have stronger ability to scavenge superoxide radicals compared to the standard used, rutin in the superoxide anion scavenging assay. Furthermore, it was also reported that two different cultivars receptacle extracts labeled as L-8 and L-11 have the highest antioxidant activities and even surpassed BHT which was used as the standard as evidenced by their reported lower IC_{50} values in the ABTS assay. Hence, the authors suggested that the receptacle of *Nelumbo nucifera* has the potential

to be established as an economical antioxidants source. Subsequently, there is a potential for the receptacle of local *Nelumbo nucifera* to be developed as an antioxidants source for the nutraceutical industry in Malaysia although it is not edible.

2.3.3 Leaf

Lotus leaf has been well-established as a source of various phytochemicals including antioxidant compounds and thus, a number of studies have been done to investigate its antioxidant properties. Huang, et al. (2009) had assessed the antioxidant activity of lotus leaf extracts in both *in vivo* and *in vitro* methods. Based on their *in vitro* studies, extracts of lotus leaf has similar scavenging ability as ascorbic acid in the ABTS assay evidenced by their similar IC_{50} values. In addition, the *in vivo* works done by the authors showed that the administration of lotus leaf extracts into rats can lead to reduction of TBARS level while at the same time increases both levels of CAT and SOD in the kidney and liver of the rats. Liu, et al. (2014) had successfully isolated and studied the antioxidant activity of 15 compounds present in leaves of *Nelumbo nucifera* which comprised of alkaloids, carotenoids and steroids. Their results showed that all 15 compounds exhibited positive antioxidant activities in at least one type of assays being conducted which include DPPH, ABTS, ferrous ion chelating and reducing power assays. Thus, this indicates lotus leaf contains multiple active substances that have notable antioxidant activity.

Besides that, Zhu, et al. (2015) had extracted a total of 14 different flavonoids from lotus leaves and examined their antioxidant properties in a collective way using various assays. Based on their studies, the flavonoids fraction labelled collectively as Fraction II was found to have a better radical scavenging activity and a lower IC_{50} value compared to butylated hydroxytoluene (BHT) which was used as the standard in the ABTS assay. In addition, it was also reported that this fraction of flavonoids has a slightly lower reducing power compared to the Trolox standard used in the FRAP assay. In short, all these studies highlight the prospective of the gigantic lotus leaf to be utilised as a source of antioxidants.

2.3.4 Flower

Both petals and stamens of *Nelumbo nucifera* flower have also been analysed for their antioxidant potential in a number of studies done in the past. Organic extracts of lotus flowers obtained using ethanol have been found to have a better antioxidant activity than ascorbic acid in an *in vitro* study (Lim, 2016). In 2008, Phonkot, Wangsomnuk and Aromdee had successfully identified the DNA fingerprints of the stamens of four different *Nelumbo nucifera* flowers obtained from different regions across Thailand and evaluated their antioxidant activities. Their findings showed that all four variants of stamen extracts exhibited positive antioxidant activities in the DPPH radical scavenging assay. Besides, Jung, et al. (2003) examined the antioxidant activity of methanolic extracts of Korean lotus stamens and found that their samples showed significant ($P < 0.05$) antioxidant activities by scavenging

peroxynitrites ($ONOO^-$). In addition, the authors also had isolated seven variants of flavonoids from their samples and analysed their antioxidant activity individually. Based on their results, several identified compounds such as kaempferol and kaempferol 3-*O*-β-D- galactopyranoside exhibited notable $ONOO^-$ scavenging activity. However, the antioxidant properties of *Nelumbo nucifera* found in Malaysia particularly on the flower of the plant has not been studied extensively. Therefore, the antioxidant property of local *Nelumbo nucifera* flower is an interesting research subject that can be looked into.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Design

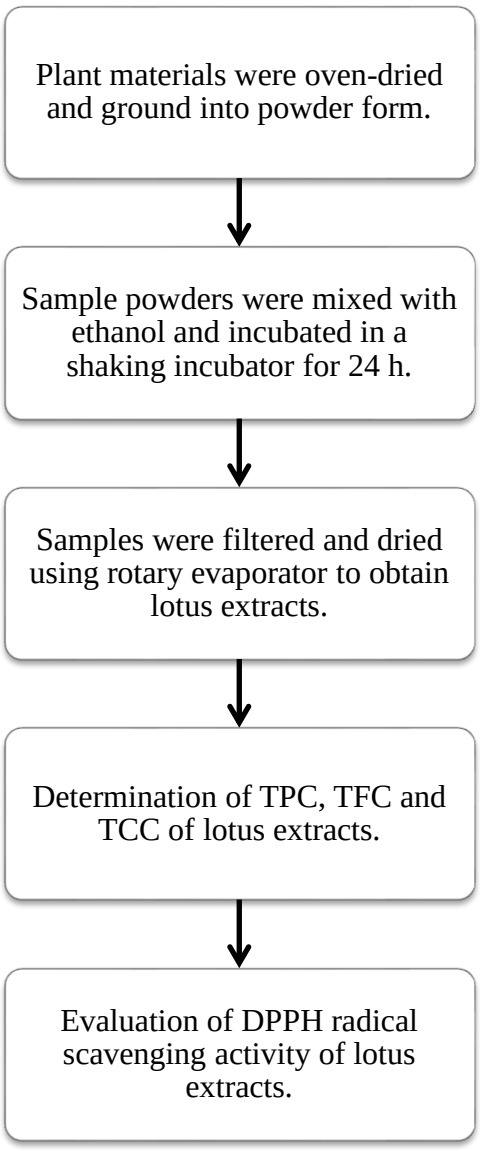


Figure 3.1: Experimental design of this research study

3.2 Sample Preparation and Extraction

Seeds, receptacles, leaves and flowers of *Nelumbo nucifera* were purchased from Buntong wet market in Ipoh, Perak, Malaysia. All plant materials obtained were dried using an oven (Binder, Tuttlingen, Germany) for two days to obtain a constant weight. Then, the plant materials were ground into powder form using a grinder (IKA, USA) and were stored separately in media bottles (Wu, et al., 2011). Sample powders were mixed with 95% ethanol (Medigene, Malaysia) at a ratio of 1:10 in a conical flask and wrapped with aluminium foil. The samples were shaken at 100 rpm and 30°C for 24 h using a shaking incubator (Infors HT, Switzerland). The samples were then filtered using Whatman filter paper and the filtrates obtained were dried at 40°C using a rotary evaporator (Büchi, Switzerland) (Leong, Tan and Chang, 2012). The dried extracts obtained were transferred into their respective 50 mL centrifuge tubes and stored at -20°C in a laboratory refrigerator (Liebherr, Germany) for further analysis. In this research study, flower extract of *Nelumbo nucifera* was made up of petal and stamen extracts only and were analysed collectively as an entity termed flower extract.

3.3 Determination of Total Phenolic Content

3.3.1 Total Phenolic Content of Lotus Extracts

The TPC of all lotus extracts were examined according to Chai and Wong (2012) with minor modification. All lotus extracts were prepared in concentration of 1.0 mg/mL by dissolving 10 mg of lotus extracts in 10 mL of 95% ethanol (Medigene). Then, all lotus extracts were further diluted to the concentration of 0.1 mg/mL. Next, 0.2 mL of each lotus extracts were mixed with 0.8 mL of distilled water and 0.1 mL of Folin-Ciocalteu's phenol reagent (Merck, Darmstadt, Germany) and incubated in the dark for 3 min. Then, 0.3 mL of 20% (w/v) sodium carbonate (Merck, Darmstadt, Germany) was added into each lotus extracts and the mixtures were incubated for 2 h. Then, the absorbance of each mixture was read at 765 nm using a spectrophotometer (BMG Labtech, Ortenberg, Germany). Ethanol (Medigene) was used as the blank. The absorbance of each lotus extracts were recorded after subtracting it with the absorbance of the blank. TPC of each lotus extracts were expressed in terms of mg of gallic acid equivalent per g of dry extract (mg GAE/g DE).

3.3.2 Gallic Acid Standard Curve Preparation

Gallic standard curve was prepared according to Chai and Wong (2012) with minor modification. A stock solution of 500 mg/L concentration of gallic acid (R&M Chemicals, United Kingdom) was prepared by dissolving 5 mg of gallic acid in 10 mL of 95% ethanol (Medigene). Then, the stock solution was diluted to obtain gallic acid solution of 100 mg/L. Subsequently, serial

dilutions were carried out further to prepare 80 mg/L, 60 mg/L, 40 mg/L and 20 mg/L concentrations of gallic acid. Next, the TPC of each gallic acid concentrations was determined using the procedures as described previously in 3.3.1. Then, the gallic acid standard curve was prepared by plotting absorbance versus concentrations of gallic acid in mg/L.

3.4 Determination of Total Flavonoid Content

3.4.1 Total Flavonoid Content of Lotus Extracts

The TFC of all lotus extracts was examined according to a method described by Chai and Wong (2012). All lotus extracts were prepared in concentration of 1.0 mg/mL by dissolving 10 mg of lotus extracts in 10 mL of 95% ethanol (Medigene). Then, extracts of lotus seed, receptacle and leaf were further diluted to 0.5 mg/mL. Next, 0.2 mL of each lotus extracts were mixed with 0.15 mL of 5% (w/v) sodium nitrite (Bendosen, Malaysia) and incubated for 6 min in the dark. Then, 0.15 mL of 10% (w/v) aluminium chloride (Friendemann Schmidt, Parkwood, Western Australia) was added to the mixtures and incubated for another 6 min in the dark. After that, 0.8 mL of 10% (w/v) sodium hydroxide (Merck, Darmstadt, Germany) was added to the mixtures and incubated for 15 min in the dark. The absorbance of each lotus extracts were measured at 510 nm using a spectrophotometer (BMG Labtech). Ethanol (Medigene) was used as the blank.

To deal with background absorbance, each extracts measurements were accompanied with their respective control reactions prepared simultaneously. For these control reactions, addition of 0.15 mL of 10% (w/v) aluminium chloride (Friendemann Schmidt) was replaced with 0.15 mL of ethanol (Medigene) while all the other steps remained unchanged. The absorbance of each lotus extracts were recorded after subtracting it with the absorbance of the blank and the absorbance of each respective control reactions. TFC of each lotus extracts were expressed in terms of mg of quercetin equivalent per g of dry extract (mg QE/g DE).

3.4.2 Quercetin Standard Curve Preparation

Queretin standard curve was prepared according to Chai and Wong (2012). A stock solution of 1000 mg/L concentration of quercetin (Sigma-Aldrich, Darmstadt, Germany) was prepared by dissolving 10 mg of quercetin in 10 mL of 95% ethanol (Medigene). Then, the stock solution was diluted to obtain quercetin solution of 500 mg/L. Subsequently, serial dilutions were carried out further to prepare 400 mg/L, 300 mg/L, 200 mg/L and 100 mg/L concentrations of quercetin. Next, the TFC of each quercetin concentrations was determined using the procedures as described previously in 3.4.1. Then, the quercetin standard curve was prepared by plotting absorbance versus concentrations of quercetin in mg/L.

3.5 Determination of Total Carotenoid Content

The TCC of all lotus extracts were examined using a spectrophotometric assay (Lichtenthaler and Buschmann, 2001; Maadane, et al., 2015). All lotus extracts were prepared in concentrations of 1.0 mg/mL by dissolving 10 mg of extracts in 10 mL of 95% ethanol (Medigene). Then, the absorbance of all lotus extracts was measured at 470 nm, 648 nm and 664 nm using a spectrophotometer (BMG Labtech). Ethanol (Medigene) was used as the blank. The absorbance of each lotus extracts were recorded after subtracting it with the absorbance of the blank. The TCC of each lotus extracts was determined by using the formula shown below as described by Lichtenthaler and Buschmann (2001). TCC of all lotus extracts were then expressed in terms of mg/g dry extract (mg/g DE).

Chlorophyll a content (µg/mL) = (13.36 × Absorbance at 664 nm) – (5.19 × Absorbance at 648 nm)

Chlorophyll b content (µg/mL) = (27.43 × Absorbance at 648 nm) – (8.12 × Absorbance at 664 nm)

Total carotenoid content (µg/mL) = [(1000 × Absorbance at 470 nm) – (2.13 × Chlorophyll a content) – (97.64 × Chlorophyll b content)]/209

3.6 Determination of DPPH Radical Scavenging Activity

The antioxidant activity of all lotus extracts was examined by using DPPH radical scavenging activity assay as described by Leong, Tan and Chang (2012) with slight modification. At first, lotus extracts and ascorbic acid (HmbG Chemicals, Malaysia) were prepared in concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/mL in 95% ethanol (Medigene). Then, 0.3 mM of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Darmstadt, Germany) was prepared by dissolving 5.9 mg of DPPH in 50 mL of methanol (QReC, New Zealand). Next, 0.1 mL of each lotus extract concentration was mixed with 2.9 mL of DPPH solution vigorously. In order to prepare the control, 0.1 mL of methanol was mixed with 2.9 mL of DPPH solution vigorously.

In this assay, ascorbic acid was used as the positive control and for comparison with lotus extracts. All the lotus extracts, ascorbic acid and the control were incubated for 30 min in the dark. Then, the absorbance of all lotus extracts, ascorbic acid and the control were measured at 517 nm. Methanol was used as the blank. The absorbance of each lotus extracts, ascorbic acid and the control were recorded after subtracting it with the absorbance of the blank. The DPPH radical scavenging of all lotus extracts and ascorbic acid were calculated using the equation shown below:

DPPH radical scavenging activity = $[(A_C - A_S) / A_C] \times 100\%$

In the equation above, A_C represents the absorbance of the control and A_S represents the absorbance of sample. The DPPH radical scavenging activity of all lotus extracts and ascorbic were then expressed in terms of IC_{50}. IC_{50} represents the extract concentrations which give 50% DPPH radical scavenging activity and was determined using a non-linear regression model (Nikolova, Evstatieva and Nguyen, 2011).

3.7 Statistical Analysis

In this study, all measurements were carried out in triplicates. Data obtained were tabulated and expressed as means ± standard deviations. IBM Statistical Package for the Social Science (SPSS) version software version 20.0 was used to carry out one-way analysis of variance (ANOVA) and Tukey's HSD multiple comparison test was used to assess the significant differences between means ($P < 0.05$) of lotus extracts.

CHAPTER 4

RESULTS

4.1 Determination of Total Phenolic Content of Seed, Receptacle, Leaf and Flower of *Nelumbo nucifera*

The TPC for different parts of *Nelumbo nucifera* and the standard curve of gallic acid are shown in Table 4.1 and Figure 4.1, respectively. The TPC found in all of the extracts was varied and ranged from 414.10 to 737.18 mg GAE/g dry extract. A higher TPC was observed for seed extract, followed by receptacle, leaf and flower ($P < 0.05$). The phenolic content of seed extract was found to be higher compared to the receptacle, leaf and flower extracts by 15%, 59% and 78%, respectively.

Table 4.1: Total phenolic content of seed, receptacle, leaf and flower extracts of *Nelumbo nucifera*.

Sample extract	Total phenolic content (mg gallic acid equivalent/g dry extract)
Seed	737.18 ± 6.83^{A}
Receptacle	642.74 ± 16.98^{B}
Leaf	464.96 ± 8.37^{C}
Flower	414.10 ± 10.35^{D}

Results are represented as mean ± standard deviation obtained from values of triplicates from two separate runs (n = 6).

$^{A-D}$ Means with different superscripts (uppercase letters) indicate significant differences at $\alpha = 0.05$.

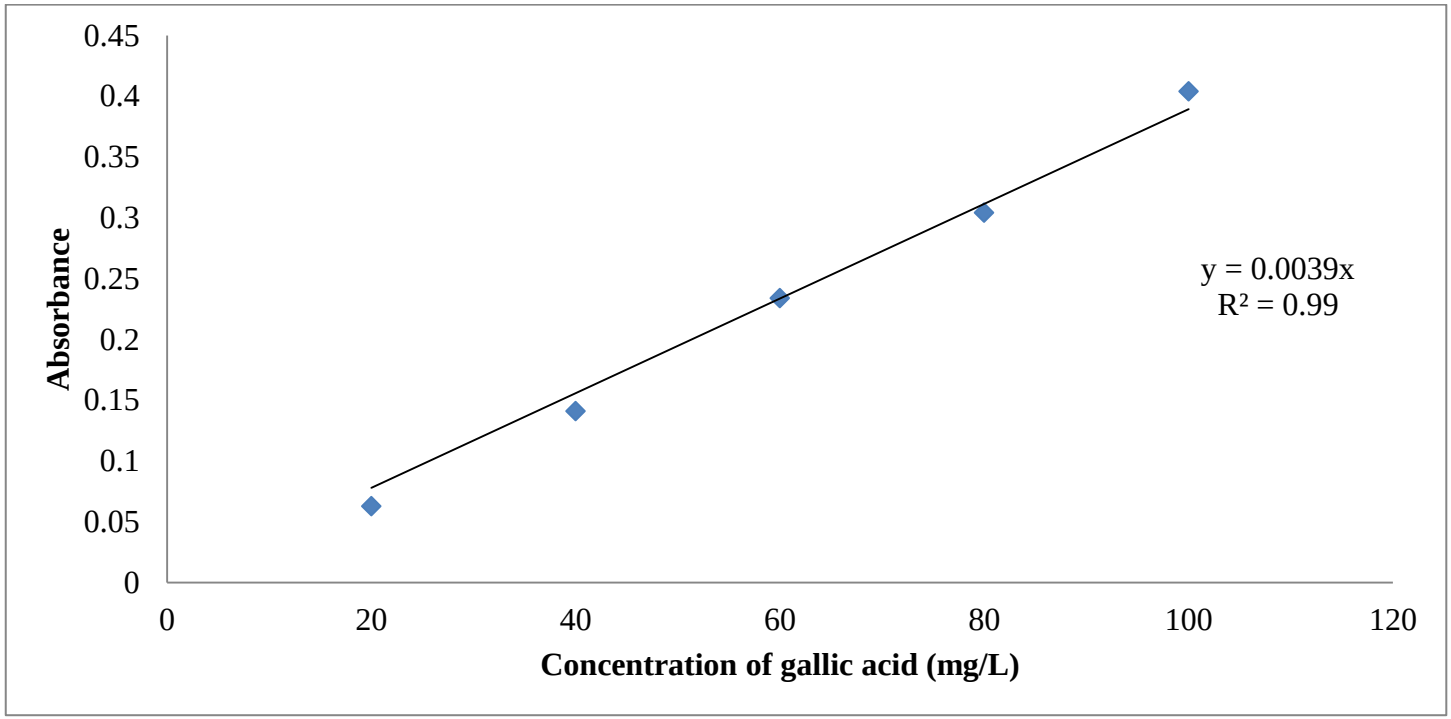


Figure 4.1: Standard curve of gallic acid for the determination of total phenolic content.

4.2 Determination of Total Flavonoid Content of Seed, Receptacle, Leaf and Flower of *Nelumbo nucifera*

The TFC of the extracts is shown in Table 4.2 while the standard curve of quercetin is shown in Figure 4.2. The TFC of all the extracts was in the range from 287.50 mg QE/g dry extract to 926.67 mg QE/g dry extract. Among all of the extracts studied, extract obtained from seed showed a higher ($P < 0.05$) concentration of flavonoid compared to the extracts of receptacle, leaf and flower. The TFC of seed extract was found to be higher compared to the receptacle, leaf and flower extracts by 22%, 49% and 222%, respectively.

Table 4.2: Total flavonoid content of seed, receptacle, leaf and flower extracts of *Nelumbo nucifera*.

Sample extract	Total flavonoid content (mg quercetin equivalent/g dry extract)
Seed	926.67 ± 42.97^{A}
Receptacle	760.83 ± 24.38^{B}
Leaf	622.50 ± 56.01^{C}
Flower	287.50 ± 14.83^{D}

Results are represented as mean ± standard deviation obtained from values of triplicates from two separate runs (n = 6).

$^{A-D}$ Means with different superscripts (uppercase letters) indicate significant differences at $\alpha = 0.05$.

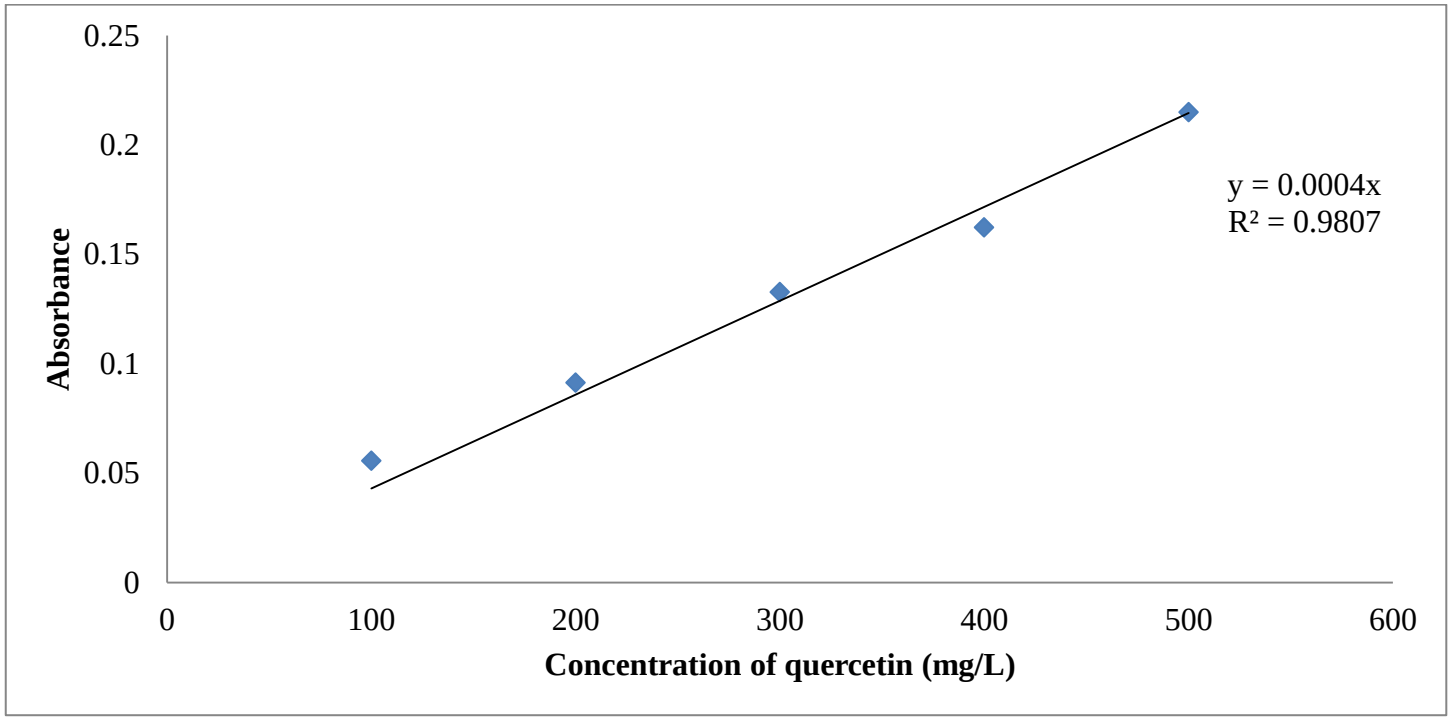


Figure 4.2: Standard curve of quercetin for the determination of total flavonoid content.

4.3 Determination of Total Carotenoid Content of Seed, Receptacle, Leaf and Flower of *Nelumbo nucifera*

Table 4.3 shows the TCC of *Nelumbo nucifera* seed, receptacle, leaf and flower. The TCC of the extracts was in the range of 0.17 mg/g dry extract to 2.40 mg/g dry extract. A higher ($P < 0.05$) concentration of carotenoid was found in leaf extract of *Nelumbo nucifera*, while a lower ($P < 0.05$) concentration of carotenoid was detected in seed and receptacle extracts compared to the other extracts studied. The TCC of leaf extract was higher than the extracts of seed, receptacle and flower by 1,312%, 515% and 229%, respectively.

Table 4.3: Total carotenoid content of seed, receptacle, leaf and flower extracts of *Nelumbo nucifera.*

Sample extract	Total carotenoid content (mg/g dry extract)
Seed	0.17 ± 0.05^{C}
Receptacle	0.39 ± 0.08^{C}
Leaf	2.40 ± 0.26^{A}
Flower	0.73 ± 0.04^{B}

Results are represented as mean ± standard deviation obtained from values of triplicates from two separate runs (n = 6).

$^{A-C}$ Means with different superscripts (uppercase letters) indicate significant differences at $\alpha = 0.05$.

4.4 DPPH Radical Scavenging Activity of *Nelumbo nucifera*

The DPPH radical scavenging activity of all extracts is shown in terms of IC_{50} in Table 4.4. The DPPH radical scavenging activity of all extracts against different extract concentrations was shown in Figure 4.3. Among the lotus extracts that were analysed, extract of receptacle showed a significant ($P < 0.05$) lower IC_{50} value than the other extracts. In contrast, a higher ($P < 0.05$) IC_{50} value was recorded by the flower extract of *Nelumbo nucifera*. This indicates a lower radical scavenging activity of the flower extract. The antioxidant activity of all extracts can be summarised in the following order of flower < leaf < seed < receptacle < ascorbic acid ($P < 0.05$).

Table 4.4: Antioxidant activity of seed, receptacle, leaf and flower extracts of *Nelumbo nucifera* based on DPPH radical scavenging assay.

Sample	IC_{50} of DPPH radical scavenging activity (mg/mL)
Seed	0.59 ± 0.02^{C}
Receptacle	0.35 ± 0.02^{B}
Leaf	0.69 ± 0.01^{D}
Flower	1.20 ± 0.02^{E}
Ascorbic acid	0.14 ± 0.02^{A}

Results are represented as mean ± standard deviation obtained from values of triplicates from two separate runs (n = 6).

$^{A-E}$ Means with different superscripts (uppercase letters) indicate significant differences at $\alpha = 0.05$.

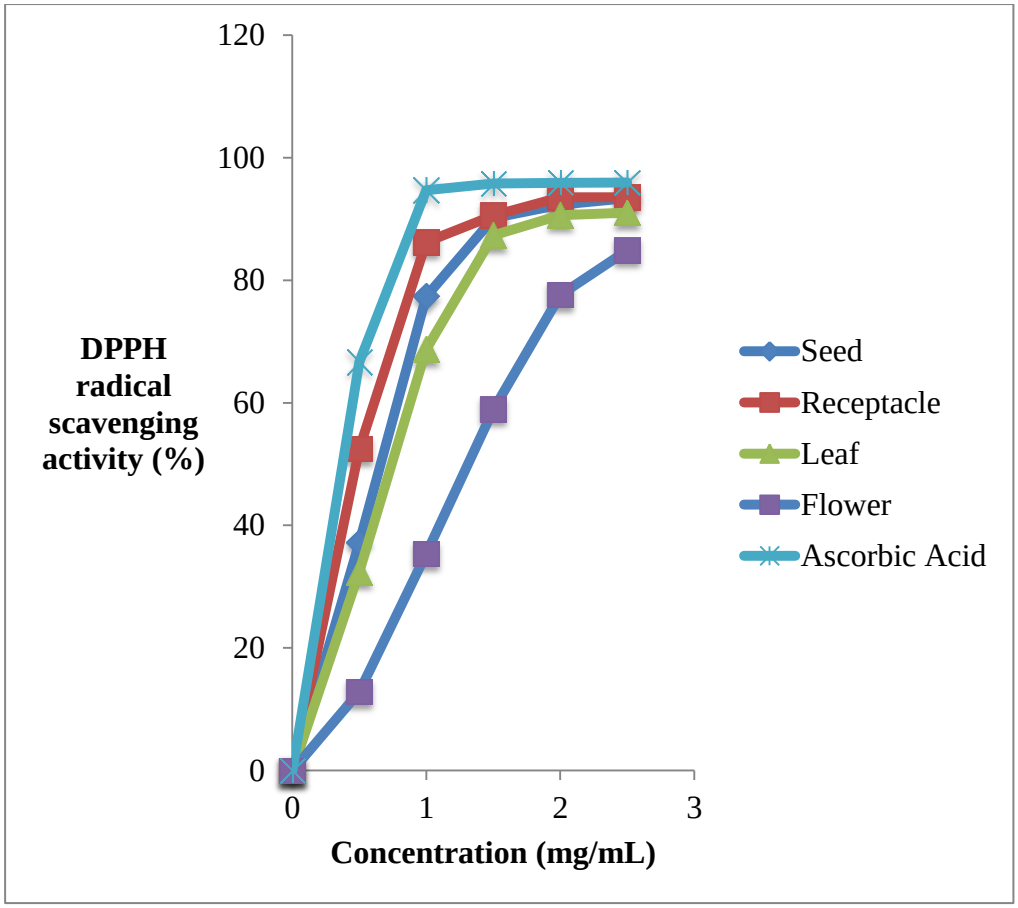


Figure 4.3: DPPH radical scavenging activity of various *Nelumbo nucifera* extracts with ascorbic acid used as comparison.

CHAPTER 5

DISCUSSION

5.1 Total Phenolic Content of Seed, Receptacle, Leaf and Flower of *Nelumbo nucifera*

Based on the findings of this study shown in Table 4.1, seed extract of *Nelumbo nucifera* had the highest TPC among the four extracts that were analysed. A similar finding was reported by Leong, Tan and Chang in 2012. The authors found that the seed extract of *Nelumbo nucifera* obtained in Kampar, Perak had the highest TPC compared to the extracts of other parts of the same plant that were analysed concurrently.

Next, by comparing among the three other *Nelumbo nucifera* extracts which were receptacle, leaf and flower, their TPC followed the order of receptacle > leaf > flower. Similar results were reported in a study done by Wu, et al. (2011). The authors examined the TPC of 10 different extracts of *Nelumbo nucifera* obtained from the Fujian province of China and their results showed receptacle extract had a higher TPC than leaf extracts. In addition, their results also showed that the TPC of stamen extracts and flower extracts were almost identical to each other whereby both of these extracts had lower TPC compared to leaf and receptacle extracts. However, extracts of seed, seed epicarp and embryo were reported to have lower TPC than the receptacle extract in the same study done by Wu, et al. (2011). Moreover, a higher TPC

was also reported in lotus seedpod compared to lotus seeds found in Tamil Nadu, India (Ruvanthika, Manikandan and Lalitha, 2017). These results were not comparable with the results obtained in this study whereby seed extract was found to contain a higher TPC than the receptacle extract.

Hence, this indicates that there is disparity in terms of phenolic contents between lotus seeds harvested in Fujian, Tamil Nadu, and those harvested in Ipoh. This phenomenon may be due to their different habitats of growth which leads to a different pattern of metabolites development (Saboonchian, Jamei and Sarghein, 2014; Liu, et al., 2015a). In addition, this may also suggest that the lotus found in Ipoh might be of a distinctive cultivar than those found in Fujian and Tamil Nadu. Both of these statements are supported by a study done in 2012. Zheng, et al. (2012) examined the TPC of 11 different *Nelumbo nucifera* receptacle extracts originated from three different provinces in China and their results showed there is a large variation of TPC among all the 11 extracts although they were originated from the same parts of the plant. The authors deduced that this may be attributed to the different ecological conditions and cultivars of *Nelumbo nucifera* present in those provinces which are Hunan, Zhejiang and Fujian.

5.2 Total Flavonoid Content of Seed, Receptacle, Leaf and Flower of *Nelumbo nucifera*

Presently, various parts of *Nelumbo nucifera* including seeds, receptacle, leaf and flower have been examined and reported to possess numerous flavonoid compounds (Lim, 2016). Based on the results shown in Table 4.2, seed extract of *Nelumbo nucifera* recorded the highest flavonoid content among the four extracts that were analysed in this study, followed by receptacle extract, leaf extract and flower extract. However, this is in contrast with the findings reported by Wu, et al. (2011). The authors found that receptacle extract had the highest TFC among the 10 lotus extracts that were analysed, including extracts of seed, seed epicarp and embryo. In addition, a similar finding was reported by Ruvanthika, Manikandan and Lalitha (2017) whereby lotus seedpod harvested from Tamil Nadu, India was found to have a higher content of flavonoids compared to seeds of the plant. It was suggested that the disparity in content of bioactive compounds such as flavonoids in *Nelumbo nucifera* may be due to different environments of growth and climate factors (Huang, et al., 2010). In addition, elevated climate temperatures have been found to decrease the flavonoids content in grapes that are cultivated for wine-making (Cloudhary, et al., 2015). Hence, this indicates the difference in climate conditions between Malaysia and other countries may cause the plant organs of *Nelumbo nucifera* to have dissimilar flavonoids content.

Meanwhile, Chen, et al. (2012b) reported that both mature and young lotus leaves have higher flavonoid content than lotus petals and stamens. Hence, this was in accordance with the results obtained in this study whereby lotus leaf extract had higher TFC than lotus flower extract.

In addition, Liu, et al. (2015b) found that the content of the flavonoids hyperoside and isoquercitrin increased in lotus seeds across three different ripening stages. In a separate study, Zhu, Liu and Guo (2016) reported that the prominent secondary metabolites that are available in lotus seeds were made up of flavonoid and alkaloid compounds. The authors also proclaimed that a total of 30 different flavonoids such as rutin, quercetin and hyperoside have been successfully isolated or obtained from lotus seeds. Furthermore, the authors also mentioned that during the tissue development period of the lotus plant, the flavonoids content in lotus seed plumules continued to rise while the flavonoids content in lotus seedpod remained unchanged. Hence, this phenomenon may serve as one of the factors that contributes to the higher TFC found in lotus seeds compared to lotus receptacle as reported in this research study.

Based on the results obtained in this study, the TFC of all samples has been observed to follow the order of flower < leaf < receptacle < seed. This identical pattern was also found in the determination of TPC as shown in Table 4.1 whereby a higher TPC was recorded by *Nelumbo nucifera* seed extract, followed by receptacle extract, leaf extract and flower extract. This

scenario was in accordance with a study done by Wu, et al. (2011). The authors found that there was a great correlation between the TPC and TFC of all their lotus extracts. Thus, it was postulated that the major composition of phenolic compounds found in lotus extracts were flavonoids (Wu, et al., 2011). Hence, this suggests that flavonoids are the major constituents of phenolic compounds present in the plant structures of *Nelumbo nucifera* grown in Ipoh.

5.3 Total Carotenoid Content of Seed, Receptacle, Leaf and Flower of *Nelumbo nucifera*

In plants, carotenoids are well-known as the vital compounds that play a key role in photosynthesis as well as have been reported to possess antioxidant activity (Ohmiya, 2011). Based on Table 4.3, the highest TCC was recorded by leaf extract of *Nelumbo nucifera* compared to the other extracts that were analysed concurrently. This was supported by a study done by Miller, Watling and Robinson in 2009. The authors claimed that the amount of chlorophyll and carotenoids were higher in lotus leaves compared to lotus receptacle although both of these plant structures have photosynthetic activity as evidenced by their similar content of ribulose-1,5-bisphosphate carboxylase (Rubisco). Rubisco is a vital enzyme that must be present in the first stage of the Calvin cycle and is responsible for the carbon-fixation reaction (Russel, Hertz and McMillan, 2017). Meanwhile, Zeb and Mehmood (2004) stated that carotenoids present in leaves of plant are usually masked by the green-chlorophyll pigments. A similar statement was also reported by Baranski,

Baranska and Schulz (2005) whereby they claimed that the carotenoids content is usually high in leaves of numerous plants but there is a possibility of them being covered by other plant pigments such as anthocyanins. Furthermore, the authors also found that a high amount of carotenoids was complemented with high anthocyanins level in their Raman-spectroscopy analysis of plant tissues including leaf tissues.

Hence, this may explain why lotus leaf extract had higher TCC compared to the others although it does not have the appearance that indicates the presence of carotenoids compounds. Moreover, the carotenoids pigments found abundant in plants are synthesised in the chloroplasts of plant cells (Tanaka, Sasaki and Ohmiya, 2008). Thus, a higher TCC in lotus leaves was observed compared to the other extracts that were examined. This was due to the presence of chloroplasts in the leaf cells.

On the other hand, a lower TCC was found in seed extract. In the process of photosynthesis, carotenoids have two major functions which are to take part in absorption of sunlight and confer protection to plants against photo-oxidative damage (Ruiz-Sola and Rodriquez-Concepcion, 2012). Hence, this may be one of the factors that cause the carotenoids content present in lotus seed extract to be lower when compared to lotus leaf extract as it is not a major photosynthetic organ of the plant.

5.4 DPPH Radical Scavenging Activity

The DPPH radical scavenging assay has been utilised extensively to determine the antioxidant activity of numerous substances by evaluating their ability in scavenging radicals (Zheng, et al., 2012). In this study, all the lotus extracts exhibited positive DPPH radicals scavenging activity and the most potent antioxidant activity was recorded by receptacle extract with the lowest IC_{50} value as shown in Table 4.4. Similar findings were observed in a study done by Wu, et al. (2011). The authors evaluated the antioxidant activity of 10 different lotus extracts using DPPH radical scavenging assay and the receptacle extract was found to have the most significant ($P < 0.05$) DPPH radicals scavenging activity compared to the other extracts. In addition, they also suggested that the antioxidant potency of lotus extracts were attributed to their TPC as a strong correlation has been determined between these two parameters.

Although seed extract had a higher TPC than receptacle extract, it had a slightly weaker DPPH radicals scavenging activity compared to receptacle extract based on the results of this study. This may be due to both of these plant structures having different types or structures of phenolic compounds present particularly flavonoids. In general, phenolic compounds of different structures will differ in their antioxidant activity due to dissimilarities in ability to donate electrons and the stability of the resonance structure formed (Maisuthisakul and Gordon, 2009). Thus, this indicates the receptacle of *Nelumbo nucifera* contains phytochemicals with greater antioxidant activity

compared to the seeds of the plant although it had a lower content of phenolic and flavonoid compounds.

In a separate study, Wu, et al. (2012) examined the antioxidant activity of lotus receptacle extracts and found that the ethanolic crude extract had a greater scavenging activity than butylated hydroxyl toluene (BHT) which was used as a control. Besides that, Ling, Xie and Yang (2005) claimed that procyanidins, a class of flavonoids isolated from lotus receptacle have better antioxidant activity than BHT in preventing lipid peroxidation at the same concentrations. Hence, this indicates that lotus receptacle extract has substantial antioxidant activity which was in tandem with the results obtained in this research study.

5.5 Future Recommendations

In this present study, the content of phenolic compounds, flavonoids and carotenoids have been examined in seed, receptacle, leaf and flower of wild *Nelumbo nucifera* found in Ipoh. However, the results of this study did not indicate what categories or types of phenolic compounds, flavonoids and carotenoids are present in all the four plant organs. Hence, for future studies, chemical analysis technique such as high performance liquid chromatography (HPLC) should be used to characterise the phenolic compounds, flavonoids and carotenoids that can be obtained from various structures of local *Nelumbo nucifera*. Besides that, other novel antioxidant assays like ABTS radical scavenging assay should be carried out to provide a comprehensive evaluation on the antioxidant activities of *Nelumbo nucifera*. Next, the extraction yield of each plant organs should be calculated in future studies to enable the quantification of extracts that can be obtained from each respective plant organs. Consequently, this information can be used as one of the basis to determine the viability of this plant to be established as a local source of natural antioxidants. Last but not least, the petals and stamens of the lotus flower should be analysed individually in the future as both of these compounds may have distinctive content of phytochemicals as suggested by other studies.

CHAPTER 6

CONCLUSION

In this study, there was a great variation in the distribution of phenolic compounds and flavonoids between the four plant structures that were analysed. Lotus seed extract was found to have a significant ($P < 0.05$) higher TPC and TFC compared to the other extracts. This may be attributed to factors such as climate and growth conditions which might lead to different patterns of metabolites development in plant. Besides that, the distribution of phenolic and flavonoid compounds were found to have a similar order whereby seed extract had the highest content followed by extracts of receptacle, leaf and flower. This suggests that the phenolic compounds present in *Nelumbo nucifera* were mainly made up of flavonoid compounds. In addition, the highest TCC was found in lotus leaf extract although it does not have an appearance which indicates the presence of carotenoids. This indicates the possibility of the carotenoids present in lotus leaf to be covered by other plant pigments such as chlorophylls.

Lotus receptacle extract was found to have the most potent antioxidant activity although it had lower contents of phenolic and flavonoid than lotus seed extract. Consequently, this implies the phytochemicals present in lotus receptacle particularly flavonoids were structurally-different than those found in lotus seed leading to better antioxidant activity. Overall, the results from

this study suggested that the plant organs of *Nelumbo nucifera* have a diverse distribution of phytochemicals contributing to variation in antioxidant activities. At present, the lotus plant has been incorporated into various dishes and consumed by the local communities in Malaysia. Therefore, there is a high potential for local *Nelumbo nucifera* to be established as a cost-effective source of dietary antioxidants for the functional food industry in Malaysia.

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APPENDIX A

STATISTICAL ANALYSIS

Table A1: Statistical analysis of Total Phenolic Content (TPC) of *Nelumbo nucifera* seed, receptacle, leaf and flower

Tukey HSD

Sample	N	Subset for alpha = 0.05			
		1	2	3	4
Flower	6	414.1026			
Leaf	6		464.9573		
Receptacle	6			642.7350	
Seed	6				737.1795
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table A2: Statistical analysis of Total Flavonoid Content (TFC) of *Nelumbo nucifera* seed, receptacle, leaf and flower

Tukey HSD

Sample	N	Subset for alpha = 0.05			
		1	2	3	4
Flower	6	287.5000			
Leaf	6		622.5000		
Receptacle	6			760.8333	
Seed	6				926.6667
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table A3: Statistical analysis of Total Carotenoid Content (TCC) of *Nelumbo nucifera* seed, receptacle, leaf and flower

Tukey HSD

Sample	N	Subset for alpha = 0.05		
		1	2	3
Seed	6	.1707		
Receptacle	6	.3940		
Flower	6		.7281	
Leaf	6			2.3952
Sig.		.054	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table A4: Statistical analysis of IC_{50} of *Nelumbo nucifera* seed, receptacle, leaf and flower in DPPH radical scavenging assay

Tukey HSD

Sample	N	Subset for alpha = 0.05				
		1	2	3	4	5
Ascorbic acid	6	.1354				
Receptacle	6		.3473			
Seed	6			.5890		
Leaf	6				.6918	
Flower	6					1.1977
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.