PHYTOCHEMICAL SCREENING, ANTIOXIDANT CAPACITY AND TOXICOLOGICAL STUDY OF MALAYSIAN *Peperomia pellucida* (L.) Kunth

AMIRAH BINTI SHAARI

DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE (BIOLOGY) MASTER BY RESEARCH

FACULTY OF SCIENCE AND MATHEMATICS
UNIVERSITI PENDIDIKAN SULTAN IDRIS

2019

UNIVERSITI PENDIDIKAN SULTAN IDRIS
SULTAN IDRIS EDUCATION UNIVERSITY

UPSI/IPS-3/BO 31
Pind.: 01 m/s:1/1

INSTITUT PENGAJIAN SISWAZAH /
INSTITUTE OF GRADUATE STUDIES

BORANG PENGESAHAN PENYERAHAN TESIS/DISERTASI/LAPORAN KERTAS PROJEK
DECLARATION OF THESIS/DISSERTATION/PROJECT PAPER FORM

Tajuk / *Title*: PHYTOCHEMICAL SCREENING, ANTIOXIDANT CAPACITY AND TOXICOLOGICAL STUDY OF MALAYSIAN *Peperomia pellucida* (L.) Kunth

No. Matrik /*Matric No.*: M20142002632

Saya / *I* : AMIRAH BINTI SHAARI

(Nama pelajar / *Student's Name*)

mengaku membenarkan Tesis/Disertasi/Laporan Kertas Projek (Kedoktoran/Sarjana)* ini disimpan di Universiti Pendidikan Sultan Idris (Perpustakaan Tuanku Bainun) dengan syarat-syarat kegunaan seperti berikut:-
acknowledged that Universiti Pendidikan Sultan Idris (Tuanku Bainun Library) reserves the right as follows:-

1. Tesis/Disertasi/Laporan Kertas Projek ini adalah hak milik UPSI.
   The thesis is the property of Universiti Pendidikan Sultan Idris
2. Perpustakaan Tuanku Bainun dibenarkan membuat salinan untuk tujuan rujukan dan penyelidikan.
   Tuanku Bainun Library has the right to make copies for the purpose of reference and research.
3. Perpustakaan dibenarkan membuat salinan Tesis/Disertasi ini sebagai bahan pertukaran antara Institusi Pengajian Tinggi.
   The Library has the right to make copies of the thesis for academic exchange.
4. Sila tandakan (√) bagi pilihan kategori di bawah */ Please tick (√) from the categories below:-*

☐	**SULIT/*CONFIDENTIAL***	Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub dalam Akta Rahsia Rasmi 1972. / *Contains confidential information under the Official Secret Act 1972*
☐	**TERHAD/*RESTRICTED***	Mengandungi maklumat terhad yang telah ditentukan oleh organisasi/badan di mana penyelidikan ini dijalankan. / *Contains restricted information as specified by the organization where research was done.*
☐	**TIDAK TERHAD / *OPEN ACCESS***	

(Tandatangan Pelajar/ Signature)

(Tandatangan Penyelia / *Signature of Supervisor*)
& (Nama & Cop Rasmi / *Name & Official Stamp*)

Tarikh: _____

Catatan: Jika Tesis/Disertasi ini **SULIT** @ **TERHAD**, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh laporan ini perlu dikelaskan sebagai **SULIT** dan **TERHAD**.

Notes: If the thesis is CONFIDENTAL or RESTRICTED, please attach with the letter from the related authority/organization mentioning the period of confidentiality and reasons for the said confidentiality or restriction.

ABSTRACT

This study performed to investigate the chemical compounds, toxicology, *in vitro* and *in vivo* antioxidant capacity of Malaysian *Peperomia pellucida* plant towards ICR mice. The qualitative chemical compound test was performed and the antioxidant capacity of *P. pellucida* plant extract was analyzed by 2,2-diphenyl-1-picrylhydrazyl (DPPH), *β*-carotene bleaching and oxygen radical absorbance capacity (ORAC) assays. The *in vivo* sub-acute toxicity study that used the best solvent from *in vitro* assay was done using ICR mice which were randomly divided. The different doses of 50 mg/kg, 150 mg/kg, 200 mg/kg, 500 mg/kg and 1000 mg/kg body weight of extract were administered daily for 21 days to the treated mice. The antioxidant enzymes in liver and kidney of mice were investigated using standard assays. *P. pellucida* only exhibited the presence of alkaloid, phenol and saponin. The ability to scavenge free radical in DPPH assay showed that methanol extract of Selangor (ground) sample depicted the highest value of 1108 ± 5.568 mg/ml compared to the other samples. In *β*-carotene bleaching assay, the ethyl acetate extract of Penang (ground) sample offered the highest percentage (86.111%) of scavenging activity from free radical compared to the other samples. In ORAC assay, methanol extract of Penang (ground) sample showed the highest value 397.691 µmol of Trolox equivalent/µl. Findings showed that ethyl acetate and methanol extracts exhibited a good solvent for antioxidant capacity of *P. pellucida*. However, in sub-acute toxicity study, ethyl acetate and methanol extract of higher doses lead to mortality in mice. Furthermore, the administration of ethyl acetate and methanol extract of *P. pellucida* did not affect the antioxidant enzymes in liver and kidney of mice. In conclusion, the antioxidant capacity of *P. pellucida* can only be seen by *in vitro* assay. Thus, *P. pellucida* could be a potential source of antioxidant substance.

PENYARINGAN FITOKIMIA, KAPASITI ANTIOKSIDA DAN KAJIAN TOKSIKOLOGI *Peperomia pellucida* (L.) Kunth DARI MALAYSIA

ABSTRAK

Kajian ini bertujuan mengkaji sebatian kimia, toksikologi serta kapasiti tumbuhan *Peperomia pellucida* dari Malaysia sebagai antioksida secara *in vitro* dan *in vivo* ke atas mencit ICR. Pengujian sebatian kimia secara kualitatif telah dijalankan dan kapasiti antioksida ekstrak tumbuhan *P. pellucida* dianalisa melalui kaedah 2,2-diphenyl-1-picrylhydrazyl (DPPH), ujian pelunturan *β*-karotena dan ujian kapasiti penyerapan radikal oksigen (ORAC). Kajian *in vivo* toksisiti sub-akut yang menggunakan pelarut terbaik daripada kajian *in vitro* telah dilakukan ke atas mencit ICR dimana mencit dibahagikan secara rawak. Dos ekstrak yang berbeza mengikut berat badan iaitu 50 mg/kg, 150 mg/kg, 200 mg/kg, 500 mg/kg dan 1000 mg/kg diberikan setiap hari selama 21 hari kepada mencit yang dirawat. Enzim antioksida dalam hati dan buah pinggang mencit dikenalpasti dengan menggunakan ujian standard. *P. pellucida* hanya menunjukkan kehadiran alkaloid, fenol dan saponin. Keupayaan menghalang radikal bebas melalui ujian DPPH mendapati bahawa sampel ekstrak metanol dari Selangor (tanah) menunjukkan nilai tertinggi iaitu 1108 ± 5.568 mg/ml berbanding sampel lain. Dalam ujian pelunturan *β*-karotena, ekstrak etil asetat sampel Pulau Pinang (tanah) menunjukkan peratusan tertinggi (86.111%) dalam menghalang aktiviti radikal bebas berbanding sampel lain. Dalam ujian ORAC, ekstrak metanol sampel Pulau Pinang (tanah) menunjukkan nilai tertinggi 397.691 μmol setara Trolox/μl. Dapatan menunjukkan ekstrak etil asetat dan metanol merupakan pelarut yang baik untuk mengkaji kapasiti antioksida di dalam *P. pellucida*. Walau bagaimanapun, melalui kajian ketoksikan sub-akut, ektrak etil asetat dan metanol pada dos yang tinggi menyebabkan kematian pada mencit. Tambahan pula, pemberian ekstrak etil asetat dan ekstrak metanol *P. pellucida* tidak menjejaskan enzim antioksidan dalam hati dan buah pinggang mencit. Kesimpulannya, kapasiti antioksida *P. pellucida* hanya dapat ditunjukkan melalui kajian *in vitro.* Oleh itu, *P. pellucida* berpotensi sebagai sumber bahan antioksida.

CONTENTS

CHAPTER 2 LITERATURE REVIEW

CHAPTER 3 METHODOLOGY

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAPH	2, 2’ –azobis (2 amidinopropane) dihydrochloride
ANOVA	Analysis of Variance
BHA	Butylated Hydroxyabisole
BHT	Butylated Hydroxytoluene
BSA	Bovine Serum Albumin
CAT	Catalase
CDNB	1-chloro-2,4-dinitrobenzene
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDTA	Ethylenediamine Tetraacetic Acid
FL	Fluorescein
GPx	Glutathione Peroxidase
GR	Glutathione Reductase
GSSG	Oxidized Glutathione
GSH	Reduced Glutathione
GSt	Glutathone S-transferase
HAT	Hydrogen Electron Transfer
IC_{50}	Half Maximal Inhibitory Concentration
ICR	Strain of Albino Mice
LD_{50}	Median Lethal Dose
NADPH	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
OECD	The Organization for Economic Co-operation and Development

ORAC	Oxygen Radical Absorbance Capacity
ROS	Reactive Oxygen Species
SD	Standard Deviation
SET	Single Electron Transfer
SOD	Superoxide Dismutase
SPSS	*Statistical Package For The Social Science*
UV-Vis	Ultraviolet-Visible

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Introduction

This chapter concisely discussed about the research background, arising issues and the purpose of the study, the related research questions, the importance of this study and also the research limitations.

1.2 Research Background

The priority and concern towards having a healthy lifestyle has increased in recent years. Since ancient times, people have gained their knowledge in the use of plants as the important source of medicine (Mamedov, 2012). Medicinal plant is acceptable as a primary health care due to the minimal side effects and beneficial to the human body (Parekh, Jadeja, & Chanda, 2005). The popularity of plants in treating diseases leads to the investigation of medicinal plants by many researchers (Rafieian-Kopaei, 2012) whereby plant's bioactivities and the responsible phytochemicals were revealed (Johnson, Shringi, Patidar, Chalichem, & Javvadi, 2011; Amin, Jassal, & Tygi, 2013).

The chemical compounds in plants such as alkaloids, flavonoids, steroids, terpenoids, carotenoids, tannins and glycoside are the most important compounds (Amin et al., 2013) which are also responsible for the antioxidant activity (Husain & Kumar, 2012), antimicrobial activity, modulation of enzymes detoxification, response towards the immune system, decrease of platelet accumulation, modulation of hormone metabolism and as anticancer agent (Saxena, Saxena, Nema, Singh, & Gupta, 2013).

Basically, every part of a plant such as seed (Anwar, Qayyum, Hussain, & Iqbal, 2010), root (Pulak & NarayanaMurthy, 2011), bark (Jain, Bhatt, & Dhyani, 2012), flower (Nayak & Pereira 2006), fruit (Crişan, Cãlinescu, Zãlaru, & Moldovan, 2013) and leaf (Trifunschi & Ardelean, 2013) has its own medicinal values and has the ability to treat ailments (Zulazeze Sahri, Sharifalillah Nordin, & Haryani Harun, 2012). Generally, the

single or combination uses of the whole plant or only selected parts of plant and also combination with more than one plant are the common practices in the discovery of plant-derived drugs (Abas Hussin, 2001).

Antioxidant is a synthetic or natural substance which slows down the harmful effects of free radicals (Huang, Ou, & Prior, 2005). Medicinal plants consist of major classes of natural antioxidants such as polyphenols, carotenoids, glucosinolates and several vitamins which represent a good source of antioxidants (Abourashed, 2013). Antioxidant helps to delay or inhibits the formation of free radical which can form toxic wastes and dangerous to human body (Mahantesh, Gangawane, & Patil, 2012). In addition, free radical is a molecular species which is able to exist independently and contains an unpaired electron in an atomic orbital (Lobo, Patil, Phatak, & Chandra, 2010). In order to combat free radical, human body is struggling to obtain antioxidants whereby the consumption of medicinal plants is one of the alternative ways for antioxidants sources.

Malaysia is rich with varieties of medicinal plants species (A'attiyyah et al., 2017) and abundance of flora with 8300 species in Peninsular Malaysia and 12000 species were estimated in the areas of Sabah and Sarawak (Saw, Chua, Suhaida, Yong, & Hamidah, 2010). Many ethnics group in Malaysia such as Malays, Indians and Chinese consumed medicinal plants as well as Ayurvedic medicine, homeopathy and naturopathy (Ibrahim Jantan, 2006). Malaysian plants also become an interest and in high demand by public, not only due to the aromas and tastes, (Alsarhan, Sultana Al-Khatib, & Mohammed Rafiq

Abdul Kadir, 2014) but also due to the effectiveness as traditional medicine (Muhammad Shahzad Aslam & Muhammad Syarhabil Ahmad, 2016) to cure several diseases.

Peperomia pellucida (L.) is one of the well-known medicinal plant worldwide, belongs to the family of Piperaceae which is mainly distributed in the Central and South America, Africa, Southeast Asia and Australia (Rojas-Martínez et al., 2013). This plant is easily found in Malaysia especially in a moist habitat and is believed to confer antioxidant properties. *P. pellucida* functions well as alternative medicine and people have consumed this plant as salads or vegetables (Roslida & Noor Aini, 2009). The medicinal values of *P. pellucida* have been studied by scientists around the world. Several studies on *P. pellucida* focussed on the plant extract as immunostimulator (Lee, Sim, Wendy, & Zulhisyam, 2016), anticancer (Wei, Wee, Yong, & Syamsumir, 2011), antimicrobial (Wei et al., 2011; Habsah Mohamad et al., 2015), anti-inflammatory (Mutee et al., 2010), antibacterial (Igwe & Mgbemena, 2014), nutritional and mineral composition study (Ooi, Iqbal, & Maznah Ismail, 2012; Egwuche, Odetola, & Erukainure, 2011), toxicity studies (Khan, Rahman, & Islam, 2008b; Oloyede, Onocha, & Olaniran, 2011), antipyretic study (Khan, Rahman, & Islam, 2008a; Ali, Caballero, Maunting, Patricio, & Reyes, 2014), analgesic activity (Sheikh et al., 2013) and chemical compound studies (Ojo, Ajayi, & Owolabi, 2012). Due to its arrays of functions triggered interests among scientists and researchers to explore more on its medicinal values.

1.3 Problem Statement

Oxygen plays a vital role in human life. Oxygen is important for the chemical reaction and production of energy that occur inside the human cells (Wickens, 2001). Nevertheless, the activities of reactive oxygen species (ROS), which have an ability to give oxygen to other substances, could shift the oxygen into toxic and harmful molecules to human body (Husain & Kumar, 2012). The molecules such as superoxide, singlet oxygen, hydrogen peroxide, and hydroxyl radical are the examples of reactive oxygen species (ROS) which include both free radicals and non-free radicals (Poljsak, Suput, & Milisav, 2013). Meanwhile, free radical gives the meaning of any molecular species that are able to exist independently with an unpaired electron in the outer shell which is unstable and highly reactive (Lobo et al., 2010). According to Pham-Huy, He, and Pham-Huy (2008), free radicals are formed as the result of ATP production in the mitochondria when cells utilize oxygen in order to generate energy. The low concentration of free radical is important for the maturation process of cellular structures whilst high concentration will caused damaging effect in human cells (Pham-Huy et al., 2008). Other than metabolic process occurring in human body, the source of free radicals can be found in the ozone and from the exposure to X- rays, cigarette smoking, environmental pollution and chemicals from industries (Bagchi & Puri, 1998).

Oxidative stress is referring to the imbalance between the production and removal of reactive oxygen species (Gospodaryov & Lushchak, 2012). Human body is continuously exposed to oxidative stress and unhealthy environment every day in our life.

Oxidative stress is a factor that leads human to many diseases and exposes human to aging process (Breitenbach & Eckl, 2015; Urquiza-Martínez & Navarro, 2016) thus antioxidant is needed to slow down the process of aging (Sarma, Mallick, & Ghosh, 2010). In addition, human body needs more antioxidants to combat the excess free radicals and prevent oxidative damage occurring in target molecule (Mahantesh et al., 2012). Human body consists of mechanism that is important to scavenge the free radicals by producing antioxidants naturally in the body or can be obtained through diets and antioxidant supplements (Bhattacharya, 2015; Rani, 2017). The sophisticated of science and technologies developed numerous forms of natural antioxidants which are usually found in food and supplement intake (Chun et al., 2009). According to Mutee et al (2010), people choose to consume natural sources for example vegetables, fruits, herbs and spices rather than the synthetic forms of antioxidants. Natural antioxidant that is commonly found in food is vitamin C, vitamin A, vitamin E, anthocyanins, several polyphenols including flavonoids, coenzyme Q 10 and lycopene (Yadav et al., 2016).

In recent years, the interest towards the utilization of dietary supplement such as antioxidant supplement is high among public. The survey done by National Health and Nutrition Examination in 2005 showed that the results of supplement intake by South Korean adult was approximately 30% and increased to 45.96% in the fifth survey of South Korean adults with the age of 19 and older (Kim et al., 2016). Other than that, the consumption of antioxidant supplement in Korean adult of both sexes is found high especially in vitamin C (Kang et al., 2014). Meanwhile, in the United States, almost 50% of people consumed antioxidant supplements which is at least one tablet per day and it

was reported that the highest percentage of antioxidant supplement intake is from vitamin E (64%) and vitamin C (54%) (Chun et al., 2009). As reported by Kim et al. (2017), the use of antioxidant supplement was found to reduce the metabolic syndrome in Korean adults as compared to non-user of the antioxidant supplement. Meanwhile, the cancer survivors in Korean population showed that the consumption of dietary supplements such as multivitamins and minerals are higher among them (33.3%) as compared to the cancer-free individuals (22.1%) (Song et al, 2017). This was not surprising that highly demands in dietary supplement intake by public nowadays have led to the extensive growth of herbal product market (Ibrahim Jantan, 2006).

Despite the high consumption of supplements by public nowadays, there is still a relatively lack of awareness and knowledge among them regarding the correct way to consume the herbal products. Meanwhile, several issues have arisen in herbal market product and closely related to the safety of the product which provided less information especially in the toxicity of the products (Sharwan, Jain, Pandey, & Shukla, 2015). In addition, no specific data regarding the correct dosage, ingredients, possible implication, signs and symptoms, contraindications and pharmacology are stated with prolonged use (Ibrahim Jantan, 2004). According to Sharwan et al. (2015), inappropriate identification of herbals, wrong labelling of the plant material, herbal contamination with fungal toxins, microorganisms, pesticides and heavy metals, unethical herbal processing as well as insufficient standardization are the reasons for toxicity study. Therefore, more herbal investigation is needed to provide scientific data before any herbal products are marketed in the future.

Antioxidant can be natural or synthetic in which butylated hydroxyabisole (BHA), butylated hyroxytoluene (BHT), propyl gallate and tertbutylhydroquinone are the common synthetic antioxidants found in industries (Altemimi, Lakhssassi, Baharlouei, Watson, & Lightfoot, 2017). Other than that, alpha tocopherol (vitamin E) and ascorbic acid (vitamin C) are also categorized as common antioxidants which can be manufactured synthetically (Race, 2009). However, the prevalence of health problem is caused by the usage of synthetic antioxidant (Race, 2009). There are many issues regarding the safety of synthetic antioxidants and the use of these synthetic antioxidants is illegal in some countries (Thorat et al., 2013). The synthetic antioxidants need to be replaced with the natural sources (Bishi, Patil, Hota, Dagla, & Kumar, 2014**).** Some synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are unstable at elevated temperature and highly volatile (Anbudhasan, Surendraraj, Karkuzhali & Satishkumaran, 2014). As synthetic antioxidants give harmful effects to the human body, the alternative way of using medicinal plants is highly recommended as it is more effective and possesses high antioxidant property (Anbudhasan et al., 2014). Furthermore, it is clearly known that natural antioxidants are safer than synthetic antioxidants (Thorat et al., 2013). Further research for natural antioxidants is required for providing effective antioxidant agents that derived from the plant as antioxidant supplements. Therefore, plant-based supplement is one of the alternatives to obtain the antioxidant in a safer way as plants are the best reservoir for natural antioxidant (Mohiseni, 2017).

According to Edward (2017), plant-based supplements are produced from natural sources such as fruits, vegetables, flowers, leaves, barks, seeds, nuts, spices as well as other botanical ingredients which are better than synthetic vitamins or minerals. There are several plant-based antioxidant supplements which comes in various forms produced by industries such as those from the green tea extracts and powder which are one of the fast growing supplement in United States (Schneider & Segre, 2009), Mexico, Norway and Japan (Carlsen et al., 2010), proanthocyanidin extract from grape seed which is known in the countries such as United States, Japan, Korea and Australia (Yamakoshi, Saito, Kataoka, & Kikuchi, 2002), pomegranate juice (Johanningsmeier & Harris, 2011; Danesi & Ferguson, 2017), spices like oregano and rosemary (Yashin, Yashin, Xia, & Nemzer, 2017) and the antioxidant supplement from berries extract (Nile & Park, 2014).

Peperomia pellucida is one of the known medicinal plants that have high potential to be a plant-based antioxidant supplement. The utilization of *P. pellucida* in herbal product could be an alternative way to replace the synthetic antioxidant which is harmful to human body. This plant is numerous, easily grown and found arounds us especially in moist area. The presence of chemical compounds in *P. pellucida* such as the phenolic compounds (Anokwuru, Anyasor, Ajibaye, Fakoya, & Okebugwu, 2011), flavonoids (Shalaby & Shanab, 2013; Rao, Muhammad Abdulrrazak, & Khamsah Suryati Mohd, 2016), alkaloids, tannins and saponins (Benhammou, Ghambaza, Benabdelkader, Atik-Bekkara, & Kadifkova, 2013) are responsible for the antioxidant properties of this plant and this finding has become the main reason for further antioxidant investigation of this plant.

Previous studies showed the presence of alkaloids, tannins, saponin, flavonoids (Ojo et al., 2012), steroids, triterpenoids, carbohydrate (Majumder & Arun, 2011), resins, phenols (Oloyede et al.,2011), cardenolides (Egwuche et al., 2011) and cardiac glycoside in *P. pellucida* leaves (Mensah, Ihenyen, & Okhiure, 2013). Meanwhile, the root and the stem of *P. pellucida* revealed six compounds which are carbohydrates, alkaloids, tannins, flavonoids, steroids and triterpenoids (Majumder, 2012). In addition, many studies have been done on the antioxidant activities of this plant (Mutee et al., 2010; Wei et al., 2011; Oloyede et al., 2011; Hamzah, Odetola, Erukainure & Oyagbemi, 2012; Pappachen & Chacko, 2013; Phongtongpasuk & Poadang, 2014; Habsah et al., 2015) and this clearly shown that this plant has potential to exhibit antioxidant properties and could be effective as antioxidant supplements.

However, previous studies on *P. pellucida* are still limited and require more research as the whole plant is being used traditionally on a major scale (Ooi et al., 2012). As reported by Majumder (2011), research has been made for pharmacognostical study of *P. pellucida* stem as well as its phytochemical as there is no systematic data has been discovered yet. In India, only a few scientific data have been found in the pharmacological, chemical and biological activities of *P. pellucida* (Majumder, Abraham & Satya, 2011). Despite the wide research on *P. pellucida,* there is still no nutritional and mineral profile report of Malaysian *P. pellucida* L. has been claimed that are indigenous to Malaysia (Ooi et al., 2012). To date, very little studies were done in Malaysia especially on the toxicology and antioxidant activity of this plant that caused limitation of references regarding *P. pellucida*. Meanwhile, there was no data documented on *β*-

carotene antioxidant assay and oxygen radical absorbance capacity (ORAC) in Malaysian *P. pellucida.* Previous studies on antioxidant capacity of *P. pellucida* commonly use 1, 1-diphenyl-2-picrylhydrazyl (DPPH) as the main assay (Mutee et al., 2010; Wei et al., 2011; Oloyede et al., 2011; Phongtongpasuk & Poadang, 2014; Habsah Mohamad et al., 2015).

The knowledge on the healing qualities of *P. pellucida* is very limited among local communities. This knowledge gap in people knowledge on medicinal plants still exist despite numerous studies on this area which have been done around the world by botanists, scientists, healers, herbalists, companies as well as other plant specialist (Kathe, 2006). The knowledge on medicinal plants is found unorganized and scattered in journals, websites, books and portals and to date, no centralized system found in Malaysia to reorganize the data on herbal plants (Zulazeze Sahri et al., 2012). To solve all the problems above, more studies on *P. pellucida* is recommended to provide more documented data of this plant. Therefore, the main purpose of this study is to investigate the phytochemical constituents and antioxidant capacity of *P. pellucida* plant from two different locations in Malaysia.

1.4 Research Objectives

The objectives that had been lined for the research are:

1. To determine the chemical compounds of *Peperomia pellucida* (L.) collected from two different locations which are Selangor and Penang area.
2. To investigate the antioxidant capacities of *Peperomia pellucida* (L.) collected from two different locations which are Selangor and Penang area by *in vitro* antioxidant study.
3. To study the effects of sub-acute toxicity in ICR mice on their body weight, relative organ weight and mortality rate after being administered with *Peperomia pellucida* extract.
4. To evaluate the antioxidant enzymes capacity of *Peperomia pellucida* (L.) in the liver and kidney of ICR mice.

1.5 Research Questions

1. What are the chemical compounds of Malaysian *Peperomia pellucida* (L.) collected from two different locations in Selangor and Penang?
2. What are the antioxidant capacities of Malaysian *Peperomia pellucida* (L.) collected from two different locations in Selangor and Penang?
3. What are the effects of *Peperomia pellucida* extract on the body weight, relative organ weight and mortality rate of ICR mice?

4. What are the antioxidant enzymes capacities of *Peperomia pellucida* (L.) found in liver and kidney of ICR mice?

1.6 Significance of Research

Plants play a significant role in maintaining human health where the effectiveness of medicinal plants in the treatment of disease and illness are undeniable. The study on the active compounds of medicinal plants helps in having a better understanding on how plant's healing properties work. The present investigation provides systematic data on phytochemical and antioxidant properties of *P. pellucida.* However, only little investigations were done by local researchers especially using this species. Therefore, the more herbal investigations are needed in order to discover the potential of this plant as a new plant-based drug. This study can help to gain people's knowledge on this plant regarding the effectiveness.

Peperomia pellucida has been discovered around the world in which this plant was extremely used in pharmaceutical and food industry. However, the study on this plant is still limited especially in the toxicology and antioxidant activity of this plant. People nowadays have increased their concern to have a healthy lifestyle by consuming more plant-based product. Hence, this is a good signs to study this plant in depth as *P. pellucida* could highly potential to be an antioxidant supplement. Although plant-based products have been discovered as the effective remedies, consumers should take more

alert on the safety and quality of the product. It is a bit worrying when some irresponsible manufactures are processing their herbs-based products without considering the safety and quality of products before it is marketed. With the presence of toxicity study on this plant, Malaysia can produce a better quality of herbal products. In addition, the safety of the herbal product can be increased with the correct dosage, ingredients, side effects and any information related to the usage of the herbal product. Public also will have a greater knowledge and increase the awareness towards the safety of any herbal products they consumed every day.

Malaysia is abundant with medicinal plants which can be found in the Tropical Rain Forest. Although many herbal plants can be found around us, local communities are still lack in knowledge on the healing potential of these plants. Therefore, the findings from previous researchers regarding *P. pellucida* will help people to discover the variety of medicinal plants exist around them. The goal for this research is to trigger people's interest in finding more natural antioxidants from their surroundings and provide opportunity to public to have a healthy lifestyle. The beneficial scientific findings on *P. pellucida* will increase the demand for this plant by the public which simultaneously may lead to the extensive growth of herbal market product in Malaysia.

1.7 Research Limitations

This study is focusses on the phytochemical screening, toxicity study and antioxidant study of *P. pellucida* whole plant collected from two sampling locations in Penang and Selangor. The chemical compounds in plant are responsible for the plant's bioactivities and each of medicinal plants exhibits different bioactivities. So, the findings for the phytochemical study cannot be generalized to all medicinal plants and to all areas in Malaysia. The study done used different solvents for extraction. The choice of solvent is important in the isolation of chemical compounds in which the different solvents used will isolate the different chemical compounds. Moreover, this study focussed on the whole plant. The isolation of chemical compound differs in each part of the plant. Therefore, the results of chemical compounds obtained in the present study cannot be generalised with certain parts of plant although with the same species.

In vitro antioxidant assay is an assay which is done outside living organisms and in a controlled environment. There are several *in vitro* antioxidant assays that are needed to determine the antioxidant activity of the plant. However, the present results between several *in vitro* antioxidant assay done cannot be compared with each other. The mechanism of reaction for each assay is different so the comparison between several *in vitro* antioxidant assays cannot be done. In addition, the result of *in vitro* antioxidant assay and *in vivo* antioxidant assay also cannot be compared each other due to the different environment of study. Contradict to *in vitro* antioxidant assay, *in vivo* antioxidant assay is an assay which is done inside living organisms. Meanwhile, the

findings for sub-acute toxicity study cannot be generalised to all toxicity study. Acute, sub-acute and chronic toxicity study gives different results due to the different treatment given to the mice. Moreover, different experimental period will show different effect at the end of the experimental study.