A COMPARATIVE STUDY ON THE ANTIOXIDANT ACTIVITY OF LEAF, FLOWER, STEM AND ROOT EXTRACTS OF *PEPEROMIA PELLUCIDA* (L.) HBK (PIPERACEAE)

by

MOK SIEW FANG

Dissertation submitted in partial fulfillment of the requirements for the degree of Bachelor of Health Science (Honours) (Nutrition)

June 2016

ACKNOWLEDGEMENT

I would like to take this opportunity to convey my sincerest thanks and appreciation to those who have supported and assisted me in accomplishing this study.

First of all, I would like to thank the course coordinator of Research Project in Nutrition, Prof. Wan Abdul Manan Wan Muda, for his guidance throughout the course.

Next, I also would like to express my deepest gratitude and appreciation to my supervisor, Dr. Hafzan Haji Yusoff, for her enlightening guidance, patience, and encouragement from the writing of research proposal to the presentation of research findings of my final year project.

Besides that, I would like to express my gratitude to all the Assistant Science Officers who work in the Science Laboratory Management Unit (UPMS) in School of Health Sciences, Universiti Sains Malaysia, especially Mr. Mohd Aminorddin Darus Mohamed Noor, Ms. Nor Azita Mohd. Nasir and Mr. Zulkhairi Othman, for their help in getting the chemical reagents and apparatus needed for my research project.

Moreover, not forget to extend my gratitude to all the laboratory assistants of the Nutrition Laboratory in School of Health Sciences, Universiti Sains Malaysia, for their guidance and supports in the completion of my research project.

Apart from that, I would like to express my heartfelt appreciation to Mr. Wan Mohd Sahnusi Wan Alias, the Medical Laboratory Technologist who responsible for the ultraviolet-visible spectrophotometer in the Analytical Laboratory. With his guidance, I managed to operate the machine well and completed my laboratory work smoothly.

Last but not least, I gratefully acknowledge statistical advice from Dr. Lim Poh Ying and Dr. Wan Amir Nizam Wan Ahmad.

KAJIAN PERBANDINGAN AKTIVITI ANTIOKSIDA ANTARA EKSTRAK DAUN, BUNGA, BATANG DAN AKAR *PEPEROMIA PELLUCIDA* (L.) HBK (PIPERACEAE)

ABSTRAK

Peperomia pellucida merupakan sejenis herba tahunan yang banyak terdapat di Malaysia tetapi belum dieksploitasi. Namun, penggunaan herba ini sebagai perubatan kaum dalam rawatan pelbagai jenis penyakit telah meliputi kawasan tropika dan subtropika di dunia. Kajian sebelum ini membuktikan bahawa *P. pellucida* memiliki sifat antioksida. Walau bagaimanapun, belum ada kajian antioksida yang dijalankan ke atas bahagian-bahagian yang berbeza daripada *P. pellucida*. Tujuan kajian ini adalah untuk menentukan dan membandingkan sifat-sifat antioksida antara daun, bunga, batang, dan akar *P. pellucida*, justeru menyumbangkan bukti saintifik tambahan terhadap potensinya. Setiap bahagian tumbuhan yang telah dikumpulkan dibasuh, dibeku-kering, dan dikisar menjadi serbuk halus sebelum diekstrak dengan menggunakan 70% etanol, 70% metanol dan air suling (1:10 berat/isipadu). Asai pemerangkapan radikal DPPH (2,2-difenil-1-picrylhydrazyl) telah digunakan untuk menentukan kapasiti antioksida. Hanya aktiviti antioksida secara sederhana [separuh kepekatan perencatan maksima (IC_{50}) >0.05 mg/ml] dikesan pada semua ekstrak *P. pellucida* dengan nilai purata IC_{50} dari 2.14 mg/ml (ekstrak etanol bunga) hingga 10.06 mg/ml (ekstrak metanol batang). Nilai purata IC_{50} bagi semua ekstrak *P. pellucida* berbeza secara signifikan daripada rujukan piawai, asid gallik (0.03 mg/ml) ($p < 0.05$). Secara umumnya, ekstrak daun *P. pellucida* menunjukkan aktiviti antioksida yang paling tinggi, diikuti dengan ekstrak bunga, akar, dan batang. Walau bagaimanapun, ekstrak etanol bunga (IC_{50} 2.14 mg/ml) menunjukkan aktiviti antioksida yang lebih tinggi daripada ekstrak etanol daun (IC_{50}

2.79 mg/ml) secara signifikan ($p < 0.05$). Dari segi pelarut pengekstrakan pula, ekstrak etanol *P. pellucida* secara umumnya menunjukkan aktiviti antioksida yang lebih tinggi berbanding dengan ekstrak metanol dan akues. Walau bagaimanapun, ekstrak metanol akar (IC_{50} 4.35 mg/ml) menunjukkan aktiviti antioksida yang lebih tinggi daripada ekstrak etanol akar (IC_{50} 6.15 mg/ml) secara signifikan ($p < 0.05$). Hasil kajian menunjukkan bahawa etanol merupakan pelarut pengekstrakan yang lebih baik berbanding dengan metanol dan air suling bagi daun, bunga dan batang *P. pellucida* untuk menunjukkan aktiviti antioksida yang paling tinggi manakala metanol merupakan pelarut pengekstrakan yang lebih baik bagi akar *P. pellucida* untuk menunjukkan aktiviti antioksidannya yang paling tinggi dalam asai DPPH.

A COMPARATIVE STUDY ON THE ANTIOXIDANT ACTIVITY OF LEAF, FLOWER, STEM AND ROOT EXTRACTS OF *PEPEROMIA PELLUCIDA* (L.) HBK (PIPERACEAE)

ABSTRACT

Peperomia pellucida is an underexploited annual herb abundantly available in Malaysia. It is widely used as ethnomedicines in treating various ailments throughout the tropical and subtropical regions of the world. Previous studies evidenced that *P. pellucida* possessed antioxidant properties. However, there is no antioxidant study on different parts of the plant was reported. The aim of this study is to determine and compare the antioxidant properties of leaf, flower, stem, and root of *P. pellucida*, hence providing additional scientific evidence of its potential. Each part of the plant collected was washed, freeze-dried, and ground into fine powder before extracted using 70% ethanol, 70% methanol and distilled water (1:10 w/v). DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was used to determine the antioxidant capacity. Only moderate [half maximal inhibitory concentration (IC_{50}) >0.05 mg/ml] antioxidant activities were detected in all *P. pellucida* extracts with mean IC_{50} ranging from 2.14 mg/ml (ethanol flower extract) to 10.06 mg/ml (methanol stem extract). The mean IC_{50} of all *P. pellucida* extracts is significantly different from that of standard reference, gallic acid (0.03 mg/ml) ($p<0.05$). Generally, *P. pellucida* leaf extracts showed the highest antioxidant activity, followed by flower, root, and stem extracts. However, ethanolic flower extract (IC_{50} 2.14 mg/ml) showed significantly higher antioxidant activity than ethanolic leaf extract (IC_{50} 2.79 mg/ml) ($p<0.05$). In terms of extraction solvent, ethanolic extracts of *P. pellucida* generally showed higher antioxidant activity than that of methanolic and aqueous extracts. However, methanolic root extract (IC_{50}

4.35 mg/ml) showed significantly higher antioxidant activity than ethanolic root extract (IC_{50} 6.15 mg/ml) ($p<0.05$). The results revealed that ethanol is a better extraction solvent than methanol and distilled water for leaf, flower and stem of *P. pellucida* to show their highest antioxidant activity while methanol is a better extraction solvent for *P. pellucida* root to show its highest antioxidant activity on DPPH assay.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATE

LIST OF ABBREVIATION

AFE	Aqueous flower extract
ALE	Aqueous leaf extract
ARE	Aqueous root extract
ASE	Aqueous stem extract
DPPH	2,2-diphenyl-1-picrylhydrazyl
EFE	Ethanolic flower extract
ELE	Ethanolic leaf extract
ERE	Ethanolic root extract
ESE	Ethanolic stem extract
GA	Gallic acid
IC_{50}	Half maximal inhibitory concentration
MFE	Methanolic flower extract
MLE	Methanolic leaf extract
MRE	Methanolic root extract
MSE	Methanolic stem extract
RSA	Radical scavenging activity

CHAPTER 1: INTRODUCTION

Figure 1.1: *Peperomia pellucida* (L.) Kunth

This study was carried out on a plant named *Peperomia pellucida* (Linnaeus) Kunth. This plant is an underexploited weed plant in Malaysia, a small annual herbaceous plant belonging to the Piperaceae family. It is native to tropical America and Asia (Akobundu & Agyakwa, 1998; Majumder, 2011), but currently distributed widely throughout tropical and subtropical regions on both hemisphere, mainly in Central and South America, West Africa, Southeast Asia and Australia (Rojas-Martínez, et al., 2013). It grows well in wet places, under the shades or within forests, rock crevices, bases of cliffs near sea level to 200 meter and on wooded rocky hillsides, up to 1100 meter altitude (Mosango, 2008). Currently, it has been a weed of cultivation widely grown in southern regions of China, such as Fujian, Guangdong, Guangxi, Hainan, and Yunnan (Flora of China, n.d.; Xu, et al., 2006).

Locally, *P. pellucida* is commonly known as *sirih cina*, *ketumpangan air*, *tumpang angin* or *cão hú jīao* (Ooi, Iqbal, & Ismail, 2012). In other parts of the world, it is variously known as silverbush, shiny bush, man-to-man, pepper elder, cow foot, rabbit ear or rat ear (North America), *kaca-kaca* or *surukan* (Indonesia), *pansit-pansitan* or *ulasimang bato* (Philippines), *pak-krasang* (Thailand), *càng cua* (Vietnam), *usuba sunakosho* (Japan), *pépéromie, herbe à couleuvre, cresson, salade soda, salade soldat* or *herbe à couresse* (France), *alumbre* or *erva-de-vidro* (Spain), *mashitandu chedi, pononoa, toyakandha* or *varshabhoo* (India), and *luchi pata* (Bangladesh) (Khan, Rahman, & Islam, 2010; Ooi, Iqbal, & Ismail, 2012). Furthermore, *P. pellucida* is also popularly known as "*coraçãozinho*" (little heart), "*língua de sapo*" (toad's tongue), "*erva-de-vidro*" (glass grass), and "*erva-de-jaboti*" (purpoise grass) in northeastern Brazil (de Fátima Arrigoni-Blank, et al., 2004; Mosango, 2008).

P. pellucida has long been used as a food item as well as a medicinal herb (Loc, Bach, Kim, & Yang, 2010; Egwuche, Odetola, & Erukainure, 2011). It can be eaten raw as salads or cooked as leafy vegetable or used as a condiment. It has also been used in variety ways to treat various ailments in many parts of the tropics. These days, it has been grown as an ornamental container plant by some people due to its ornamental-look foliage (Mosango, 2008).

In folk medicine, *P. pellucida* is used to prepare infusions and decoctions to treat various ailments including gastric ulcers as well as eye inflammation or conjunctivitis, flu, diarrhoea, colic, gout, arthritis, rheumatic pain, fatigue, trauma, convulsion, bleeding, skin wounds and skin related problems, such as acne, furuncles, and abscesses (de Fátima Arrigoni-Blank, et al., 2004; de Fátima Arrigoni-Blank, et al., 2002; Khan & Omoloso, 2002; Mitchell & Ahmad, 2006; Mutee, et al., 2010; Xu, et al., 2006).

1.1 Study Background

Oxygen is crucial for life. Oxidation reactions are used to produce energy. However, oxygen free radicals and other reactive oxygen species are produced within the cells as by-products through normal aerobic cellular metabolism as well as numerous physiological and biochemical processes. Free radicals are molecules with an unpaired electron in their outer orbit. The most common reported cellular free radicals are hydroxyl radicals (OH·), superoxide (O_2^{-}·) and nitric monoxide (NO·). Besides, free radicals also can be generated from other reactive species, such as hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^{-}$), and hypochlorous acid (HOCL) through various chemical reactions in many cases (Gilgun-Sherki, Melamed, & Offen, 2001).

Oxygen radicals play very important role in origin of life and biological evolution, leaving beneficial effects on the organisms (McCord, 2000). They involved in various biochemical activities of cells, such as signal transduction, gene transcription and regulation of soluble guanylate cyclase activity. For examples, nitric oxide (NO) is an important signalling molecule that essentially regulates the relaxation and proliferation of vascular smooth muscle cells, vascular tone, leukocytes adhesion, platelets aggregation, hemodynamic, angiogenesis, and thrombosis (Zheng & Storz, 2000).

Apart from that, NO, O_2^{-}·, H_2O_2, and hypochlorite anion (^{-}OCl) are powerful oxidants used by leukocytes and other phagocyte to destroy bacteria, parasites and virus-infected cells to protect humans from infection. However, these oxygen-related free radicals and reactive species can cause oxidative damage and mutation to DNA as well as participate in the carcinogenic process if unchecked. Hence, plants and animals maintain complex systems of multiple types of antioxidants (Oloyede, Onocha, &

Olaniran, 2011). In-built antioxidant system of the body plays its pivotal role in prevention of any loss attributable to free radicals (Uttara, Singh, Zamboni, & Mahajan, 2009).

Nevertheless, humans are constantly exposed to free radicals created by electromagnetic radiation from the external environment in addition to those from cellular metabolisms, such as respiratory burst and enzyme reactions. Example of external or environmental free radicals include pollutants, cigarette smoke as well as natural resources, such as radon and cosmic radiation (Gilgun-Sherki, Melamed, & Offen, 2001).

Thus, overproduction or incorporation of free radicals from environment to living system, causing imbalanced defense mechanism of antioxidants due to inability of the body to counteract or detoxify the reactive intermediates, will lead to serious penalty of detrimental health effects. Toxicity of free radicals or oxidative stress (OS) can cause oxidative damage to biological macromolecules, such as lipids, proteins, and DNA (Betteridge, 2000). They contribute to proteins and DNA injury, inflammation, tissue damage and subsequent cellular apoptosis. These eventually lead to various non-communicable diseases such as cancer, atherosclerosis, cardiovascular diseases (CVDs), diabetics, rheumatoid arthritis, post-ischemic perfusion injury, chronic inflammation, stroke and septic shock, aging and other degenerative diseases in humans (Fang, Yang, & Wu, 2002; Temple, 2000).

Cancer is an umbrella term for a huge group of diseases that can affect any part of the body. Cell regeneration is important to replace worn out cells, heal injuries and help in growth and development. However, the genes, account for regeneration of cells, grow or multiply abnormally when there is DNA injury due to oxidative stress. The

cells grow into a lump or tumour, becoming cancer. Cancer can be benign or malignant tumours (National Cancer Society Malaysia, 2015). The rapid growth of abnormal cells beyond their usual boundaries can then invade adjacent parts of the body and spread to other organs. This process is known as metastasizing. Metastases are the major cause of death from cancer (World Health Organization, 2015).

Besides, oxidative stress provokes free radical attack on neural cells which eventually leads to neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease. This process plays a part in catastrophic role to neurodegeneration. Neural cells suffer functional or sensory loss due to imbalanced antioxidants defense mechanism and excess reactive oxygen species (ROS) exposure (Uttara, Singh, Zamboni, & Mahajan, 2009).

Antioxidants are compounds capable of removing of free radicals, scavenging ROS or their precursors, inhibiting the formation of ROS and binding metal ions needed for catalyzing ROS generation. Antioxidants can also terminate the chain reactions initiated by free radicals by removing the reactive intermediates and inhibit other oxidative reactions as well as having a wide scope to sequester metal ions. In many cases, it is concluded that antioxidants are able to modulate the pathophysiology of chronic inflammation up to some extent (Uttara, Singh, Zamboni, & Mahajan, 2009). However, the body cell can only synthesize a minority of these molecules (Gilgun-Sherki, Melamed, & Offen, 2001). Hence, diet is major source of antioxidants. This strongly suggests the protective role of antioxidants in our daily diet.

1.2 Problem Statement

Antioxidants are now being appraised as one of the persuasive therapeutic against those related diseases due to their capability in combating free radicals by neutralization and thus reducing the threats to biological macromolecules caused by oxidative process (Oloyede, Onocha, & Olaniran, 2011). There is currently an upsurge of interest in phytochemicals as potential new sources of natural antioxidants in foods and pharmaceutical preparations to replace synthetic antioxidants due to their potential health risks and toxicity (Katalinic, Milos, Kulisic, & Jukic, 2006). Public usually prefer to use natural treatments from food or natural compounds extracted from plant rather than synthetic alternative (Idu, et al., 2007). Therefore, plants, including medicinal herbs, are the major focus for isolation of antioxidant as the commercial source of antioxidants (Uttara, Singh, Zamboni, & Mahajan, 2009).

P. pellucida is a common annual herb abundantly available in Malaysia. It can be eaten raw as salads or cooked as vegetable. It is also used to prepare infusions and decoctions to treat some ailments. Few studies have been carried out to validate the medical properties of *P. pellucida. P. pellucida* is reported to exert hypotensive effects (Nwokocha, et al., 2012), possess analgesic activity as well as anti-inflammatory, anticancer, antibacterial, antimicrobial, and antioxidant properties (Aziba, Adedeji, Ekor, & Adeyemi, 2001; de Fátima Arrigoni-Blank, et al., 2004; Khan, Rahman, & Islam, 2010; Khan & Omoloso, 2002; Lee, Wee, Yong, & Syamsumir, 2011; Mutee, et al., 2010; Oloyede, Onocha, & Olaniran, 2011; Xu, et al., 2006; Akinnibosun, Akinnibosun, & German, 2008). However, there is lack of scientific data which compare the antioxidant capabilities among different parts of this plant. Hence, this study investigated the antioxidant activity in ethanol, methanol and aqueous extracts of *P. pellucida* leaf, flower, stem and root using DPPH radical scavenging assay.

1.3 Research Objectives

1.3.1 General Objective

To compare the antioxidant activity among leaf, flower, stem and root of *P. pellucida*.

1.3.2 Specific Objectives

i. To determine percentage radical scavenging activity (% RSA) and half maximal inhibitory concentration (IC_{50}) of ethanol, methanol and aqueous extracts from different parts of *P. pellucida*.

ii. To compare the radical scavenging activity among leaf, flower, stem and root extracts of *P. pellucida*.

iii. To compare the radical scavenging activity among ethanol, methanol and aqueous extracts of *P. pellucida*.

1.4 Scopes of Study

Different parts of *P. pellucida* including leaf, flower, stem, and root were investigated separately. Ethanol, methanol, and distilled water were used individually as the extraction solvents to extract the potent antioxidant compounds from each part of the plant. Hence, the antioxidant capacities of potent compounds in each part of the plant were compared based on their respective antioxidant activities. The antioxidant activities were determined through their respective percentage radical scavenging activity (% RSA) and half maximal inhibitory concentrations (IC_{50}).

1.5 Research Questions

i. What are the percentage radical scavenging activity (% RSA) and half maximal inhibitory concentrations (IC_{50}) of ethanol, methanol and aqueous extracts from different parts of *P. pellucida*?

ii. Is there any difference in radical scavenging activity among leaf, flower, stem and root extracts of *P. pellucida*?

iii. Is there any difference in radical scavenging activity among ethanol, methanol and aqueous extracts of *P. pellucida*?

1.6 Research Hypotheses

i. Null hypothesis: There is no significant difference in radical scavenging activity among leaf, flower, stem and root extracts of *P. pellucida.*

Alternative hypothesis: There is a significant difference in radical scavenging activity among leaf, flower, stem and root extracts of *P. pellucida.*

ii. Null hypothesis: There is no significant difference in radical scavenging activity among ethanol, methanol and aqueous extracts of *P. pellucida.*

Alternative hypothesis: There is a significant difference in radical scavenging activity among ethanol, methanol and aqueous extracts of *P. pellucida.*

1.7 Significance of the Study

P. pellucida has been consumed for many years in many tropical and subtropical countries as food item or for its medicinal properties. Nevertheless, there are not many scientific studies on the antioxidant activity of *P. pellucida*. Despite the few studies evidenced that *P. pellucida* possess antioxidant activity, the amount of potent antioxidant compounds might vary in different parts of the plant. So far there was no antioxidant study on different parts of *P. pellucida* was found. Hence, this was the first study to look at the antioxidant capacity of *P. pellucida* in its different parts.

This study has provided scientific data regarding the antioxidant activity and thus estimated the antioxidant capacity of potent compounds in each part of the plant by using DPPH radical scavenging assay on different solvent extracts. The findings of this study have contributed additional scientific evidence which might be helpful in verifying or enhancing the use of this plant in treatment or prevention of some ailments more efficiently, in terms of the parts of the plant used in folk medicines as well as the extraction solvent used in commercial production of antioxidants.

CHAPTER 2: LITERATURE REVIEW

2.1 Physical Characteristics of *Peperomia pellucida*

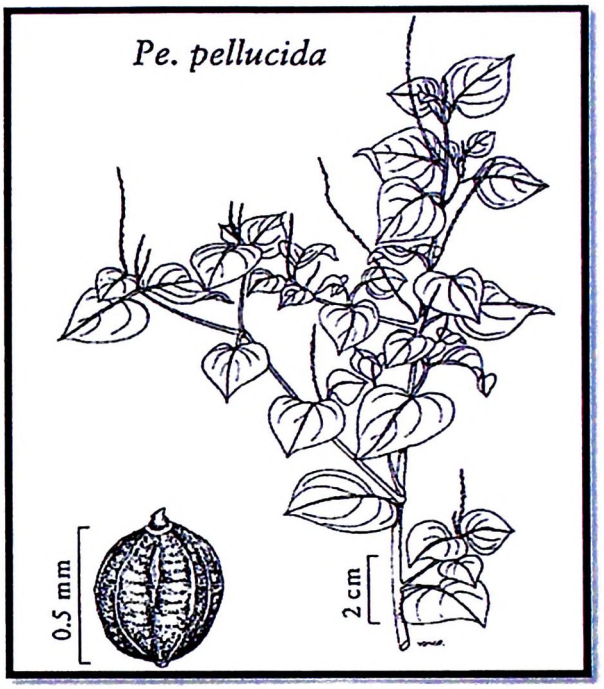

Figure 2.1: *Peperomia pellucida* (L.) Kunth structures

Note. From Flora of North America, n.d.

P. pellucida (L.) Kunth, with protologue of Humb., Bonpl. & Kunth, Nov. gen. sp. 1: 64 (1816) (Mosango, 2008), develops well during rainy season. It grows erect or ascending in clumps, usually about 15 to 45 cm long. It grows well in the loose and damp soils in shady areas throughout tropical and subtropical region of both hemispheres. This plant has shallow fibrous root system (Wagner, Herbst, & Sohmer, 1999). The stems are thread-like but angular trailing, with internodes usually 3 to 8 cm long. Those growing in rich habitats are usually fleshy and stout. They are translucent pale green, succulent, round, delicate, glabrous with shiny waxy surfaces. The elongated stems look like a vine with leaves rising 6 to 9 cm above the surface (Majumder, 2011).

The leaves of *P. pellucida* are rather thin, shrubby and fleshy. They are medium green on upper surface and whitish green on lower surface. The leaves are blunt, glabrous, shiny waxy and smooth on both surfaces. The blades are broadly-ovate, heart-shaped at the base, palmately 5-nerved to 7-nerved, apex acuminate, about 1.8 to 2.5cm long, 1.2 to 1.8cm wide with 0.5 to 2 cm long petioles alternate on the stalks at about 1 to 2 cm long (Akobundu & Agyakwa, 1998; Majumder, 2011). The foliage of the plant looks ornamental (Majumder, 2011).

The inflorescence consists of slender spikes, 1 to 6cm long, arising from opposite side of leaf axils as well as stem terminals. The flowers are very small, numerous and bi-sexual. They are unnoticeable due to no petals, but well-spaced with only two stamens, with short filaments and oblong anthers, and a rounded-ovoid superior ovary of about 0.5 mm in diameter. The one-celled ovary is sessile with only one stigma. The bracts are rounded, up to 0.5 mm long. The fruits are minute, about 0.5 to 1 mm in diameter, blackish brown to orange colour, sticky, one-seeded berries attaching on the cord-like spikes with tiny dot-like seeds. The plant gives mustard-like odour when crushed (Akobundu & Agyakwa, 1998; de Fátima Arrigoni-Blank, et al., 2004; Majumder, 2011).

P. pellucida is short-lived, but its lifespan can exceed one year. The seed is set abundantly and dispersed by rain wash or more widely by human through contaminated soil. Seedlings emerge about 15 days after sowing and develop naturally during the rainy season. The growth is very fast in cultivation and the stems may reach a length of 60 cm in 100 days after transplantation. Inflorescences appear 4 to 6 weeks after transplantation (Mosango, 2008).

2.2 Ethnomedicinal Uses of *Peperomia pellucida*

P. pellucida is widely used as ethnomedicines in treating various ailments throughout the tropical and subtropical regions of the world. The way of using of *P. pellucida* for its pharmacology properties varies depending on the region. Some are eaten fresh or make into a solution of the fresh juice from stems and leaves or mashed and applied externally as warm poultice. Some use the decoction or infusion of the whole plant or certain parts of the plant, such as root or aerial part, solely or added with salt and palm oil before taken (Mosango, 2008; Muñoz, et al., 2000; Schmelzer & Gurib-Fakim, 2008).

This plant is considered refrigerant as its leaves are used in the treatment of headache, fever, sore throat, eczema, abdominal pains and convulsions (Khan, Rahman, & Islam, 2010). Besides, *P. pellucida* is also used to lower blood cholesterol level and treat common cold, cough, fatigue, wounds, colic, conjunctivitis, skin related problems, such as abscesses and abnormal complexion, kidney disorders, prostate problems, diabetes mellitus, cardiac arrhythmia, rheumatic pain, measles, small pox, male impotence, mental disorders, and breast cancer (Aziba, Adedeji, Ekor, & Adeyemi, 2001; Bayma, Arruda, Müller, Arruda, & Canto, 2000; Khan & Omoloso, 2002; Lans, 2006; Oloyede, Onocha, & Olaniran, 2011). In Malaysia, some local community claimed that decoction of the plant is useful in treating bone aches (Ong & Nordiana, 1999). According to Manila Medical Society, *P. pellucida* is useful in relieving rheumatoid arthritis joint pains. However, it may cause central nervous system (CNS) depression as the side effect as documented in Khan, Rahman and Islam (2008b) that *P. pellucida* leaves exert dose dependent depressant effects.

The pharmacological properties of *P. pellucida* are well documented and have been further affirmed by a number of scientific research reports.

Analgesic effect

Aziba *et al.* (2001) reported that the analgesic properties of *P. pellucida* seem to be related to its effect on the mechanism of prostaglandin synthesis, indicating the presence of an inflammatory pain process (Aziba, Adedeji, Ekor, & Adeyemi, 2001). This finding was supported by a study in 2004 using aqueous extract of the aerial parts of the plant. Using the same test, Aziba *et al.* (2001) obtained higher inhibition percentages (78.3%) by using methanolic extract of *P. pellucida* (210 mg/kg). These differences may due to the use of different extraction solvents. On the other hand, an analgesic effect was observed at concentrations of 100 and 200 mg/kg in the hot-plate test. The best dose was observed to be 100 mg/kg, indicating that the extract possesses an activity related to both inflammatory and non-inflammatory pain (de Fátima Arrigoni-Blank, et al., 2004).

Anti-inflammatory effect

Oral administration of the aqueous *P. pellucida* extract exhibited a dose-dependent anti-inflammatory activity at all concentrations tested in the carrageenin test. It inhibited edema starting from the first hour and during all phases of inflammation suggests that it probably inhibited different aspects and chemical mediators of inflammation (de Fátima Arrigoni-Blank, et al., 2004).

In 2010, Mutee *et al.* showed that 1000mg/kg of petroleum ether extract of *P. pellucida* significantly reduced carrageenan-induced hind paw edema compared with the control. However, there was no significant anti-inflammatory activity for chloroform and methanol extracts (Mutee, et al., 2010).

Anti-edematogenic activity

de Fátima Arrigoni-Blank *et al.* (2002) reported that there was variation in the potency of edema inhibition depending on the plant's phenophase. The *P. pellucida* aqueous extract showed significant anti-inflammatory activity during phenophases 1 and 2 of winter and spring. *P. pellucida* has a phenological cycle of approximately 100 days and it is recommended that the *P. pellucida* aqueous extract is used as an antiedematogenic only during phenophases 1 and 2 of winter and spring.

Anticancer property

Some compounds isolated from *P. pellucida* showed growth inhibitory effects on the three cancer cell lines, HL-60, MCF-7, and HeLa cell lines (Xu, et al., 2006). Furthermore, anticancer activity of *P. pellucida* leaf extract was determined through Colorimetric MTT (tetrazolium) assay against human breast adenocarcinoma (MCF-7) cell line by Lee *et al.* in 2011, indicated that *P. pellucida* leaf extract possessed anticancer activities with IC_{50} of 10.4±0.06 µg/ml. Findings from this study indicated that methanol extract of *P. pellucida* leaf possessed vast potential as medicinal drug especially in breast cancer treatment (Lee, Wee, Yong, & Syamsumir, 2011).

Antimicrobial property

An antimicrobial study of the leaf extract revealed that the plant extract was able to inhibit the growth of 10 bacterial isolates at various concentrations (Lee, Wee, Yong, & Syamsumir, 2011). In the same year, *P. pellucida* was found to possess broad spectrum of antimicrobial activity on both Gram-positive and Gram-negative bacteria as well as the fungi used (Oloyede, Onocha, & Olaniran, 2011). Most of the microorganisms tested are different from those used by Lee *et al.* (2011).

The different fractions, namely crude methanol leaf extract, n-hexane, ethyl acetate, butanol and water fractions, examined inhibited the microorganisms growth in a concentration-dependent manner. Activity was generally observed at the higher concentrations of 25 to 200 mg/ml. Gentamicin (10mg/ml) and tioconazole (70%) were used as the positive control for bacteria and fungi respectively. Although the activities of the extracts were high, they are not as active as the reference compounds. The methanolic extract was active on *C. albicans* and *R. stolon* at 25 to 200 mg/ml which indicates a selective inhibition on fungi. The n–hexane fraction inhibited all the microbes at 50 to 200 mg/ml but showed mild activity on *R. stolon* and *P. notatum*. The ethyl acetate fraction exhibited a broad spectrum activity on the entire microorganisms at 50 to 200 mg/ml except on *E. coli*. The butanol fraction showed moderate activity for all tested microbes but was particularly active on *S. aureus* at 12.5 to 200 mg/ml. The aqueous fraction inhibited *P. aeruginosa, K. pneumonae, S. typhi, C. albicans* and *A. niger* moderately at 50 to 200 mg/ml. Overall, it was observed that the crude methanolic extract was low in activity compared to the other fractions while the hexane, ethyl acetate and butanol fractions at concentration of 50 to 200 mg/ml showed a broad spectrum antimicrobial activity on bacteria and the fungi used in this assay (Oloyede, Onocha, & Olaniran, 2011).

Apart from that, the antimicrobial properties of *P. pellucida* were also studied on Patuloside A regarding its *in vitro* antibacterial, antifungal and cytotoxic activities. Patuloside A (3-β-D-glucopyranosyloxy-1, 5, 6-trihydroxy-9H-xanthene-9-one) is a xanthone glycoside being isolated from *P. pellucida.* Disc diffusion technique was used to determine in vitro antibacterial and antifungal activities. Cytotoxicity was determined against brine shrimp nauplii. In addition, minimal inhibitory concentration (MIC) was determined using serial dilution technique to find out antibacterial potency. The

compound showed significant antibacterial activity against four Gram-positive bacteria (*Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus,* and *Streptococcus β-haemolyticus*) and six Gram-negative bacteria (*Escheichia coli, Shigella dysenteriae, Shigella sonnei, Shigella flexneri, Pseudomonus aeruginosa, Salmonella typhi*) but weak antifungal activities against *Aspergillus flavus* and *Candida albicans*. In cytotoxicity determination, LC_{50} of the Patuloside A against brine shrimp nauplii was 18.24 µg/ml (Khan, Rahman, & Islam, 2010).

In an investigation done by Khan and Omoloso (2002) on antibacterial activity of *P. pellucida*, the crude methanolic extract of aerial parts of *P. pellucida* was further extracted with petrol, dichloromethane, ethyl acetate and butanol. All the fractions exhibited very good level of broad spectrum antibacterial activity by using disc diffusion method on twelve Gram-positive bacteria and twelve Gram-negative bacteria. Fractionation is shown to enhance the activity as compared to the crude extract. The butanol fraction of *P. pellucida* was particularly good. However, no activity was observed against the protozoan, *Trichomonas vaginalis*, tested (Khan & Omoloso, 2002).

Antibacterial activity

Moreover, the antibacterial activity of *P. pellucida* was also investigated through aqueous and ethanolic leaf extracts on three gram-negative bacteria isolates, namely *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa* by Akinnibosun, Akinnibosun and German in 2008 using Agar-well diffusion method. The results showed that *E. coli* displayed the highest susceptibility in water extract, followed by *P. mirabilis* and least in *P. aeruginosa*. Conversely, the ethanolic extract showed the highest inhibition in *P. aeruginosa,* followed by *P. mirabilis* and the least was in *E. coli*.

The results revealed that water is the best extraction solvent for *P. pellucida* to show its highest inhibitory activity on *E. coli* while ethanol is better than water as the extraction solvent for of *P. pellucida* for it to show its highest inhibitory activity on *P. mirabilis* and *P. aeruginosa*. The results of this investigation supports the claims by local practitioners of ethnomedicine in the therapeutic efficacies of this herb as a potential source of antimicrobial agent against *E. coli*, *P. mirabilis* and *P. aeruginosa* and could be used in the management of nosocomial infection (Akinnibosun, Akinnibosun, & German, 2008).

Antifungal activity

Antifungal studies of *P. pellucida* reported its fungicidal activity against Helminthosporium oryzae by Singh (as cited in Ooi, Iqbal, & Ismail, 2012). In addition, chloroform extracts from dried leaves of *P. pellucida* have been shown to exhibit antifungal activity against *Trichophyton mentagrophytes* (Ragasa, Dumato, & Rideout, 1998). However, no activity was shown against the fungi tested in Khan and Omoloso (2002).

Anti-malarial activity

In a research done by Muñoz *et al.* (2000), *P. pellucida* extract of the whole plant inhibited 78% of parasite growth *in vivo* at 1000 mg/kg, displaying moderate anti-malarial activity. On top of that, the extract was also active *in vitro*, with an inhibition of 95% at 100µg/ml. In order to highlight the potential anti-malarial activity of the species, the *in vivo* results has been sorted with *in vitro* results, highlighting the extracts displaying good or moderate antiprotozoal activity. *P. pellucida* showed an *in vivo–in vitro* correlated activity (Muñoz, et al., 2000).

Mosquitocidal activity

According to Mitchell (2006), *P. pellucida* may be used as an insect repellent since two out of five natural products isolated from the plant in Jamaica exhibited mosquitocidal activity.

Hypotensive effect

P. pellucida was studied for the possible mechanism of its hypotensive effect since it has been used as an antihypertensive remedy. The impact on cytochrome P450 (CYP) enzyme activity was also investigated. The mean arterial pressure and heart rate were recorded via cannulation of the carotid artery on anaesthetized, normotensive Sprague-Dawley rats following intravenous administration of *P. pellucida* aqueous extract (PPAE). Recordings of the contractile activity of the aortic rings to the extract of different concentration were done using standard organ bath techniques. Impact on CYP3A4 and CYP2D6 enzyme activities was investigated using human liver and heterologously expressed microsomes. The results suggest that the PPAE induces dose-dependent hypotensive, bradycardic and vasorelaxant effects via enhancement of endothelial Nitric oxide (NO)-dependent mechanisms. The data also suggested that the extract had a negative chronotropic effect. The impact on CYPs enzyme activities indicated unlikely adverse drug effect when the plant is consumed with other medications reliant on CYP3A4 metabolism (Nwokocha, et al., 2012).

Antipyretic effect

Antipyretic effects of petroleum ether and ethyl acetate soluble fractions of crude ethanol extract of *P. pellucida* leaves were also investigated. Intra-peritoneal administration of boiled milk at a dose of 0.5 ml/kg body weight in albino rabbit leads to pyrexia. Intra-peritoneal administration of the two fractions at a dose of 80 mg/kg

body weight significantly reduced the elevated body temperature of rabbit. The antipyretic effect has been compared with antipyretic effect of standard aspirin and the solvent used. It is shown that the antipyretic activity of petroleum ether fraction at 80 mg/kg body weight is almost similar to that of the standard aspirin group and is more active than the ethyl acetate fraction, hence supports the claims of traditional medicine practitioners as an antipyretic remedy (Khan, Rahman, & Islam, 2008a).

Gastroprotective effect

P. pellucida was reported with gastroprotective activity and dillapiole have been identified as the most active compounds in the gastroprotective activity of *P. pellucida* (Rojas-Martínez, et al., 2013). Isolation of this compound was done through a bioassay-guided fractionation by using an ethanol-induced gastric ulcer experimental rat model. The results were compared with the effect of carbenoxolone as the reference drug (Rojas-Martínez, et al., 2013).

A gastroprotective effect was observed when the hexane and dichloromethane extracts were tested, with the higher effect being obtained with the dichloromethane extract at 100 mg/kg. The effect elicited by dillapiole at 100 mg/kg was not attenuated by pretreatment with indomethacin, a prostaglandin synthesis blocker, NG-nitro-L-arginine methyl ester, a nitric oxide (NO) synthase inhibitor, and N-ethylmaleimide, a blocker of sulfhydryl groups. This suggests that the gastroprotective mechanism of action of dillapiole does not involve prostaglandins, NO or sulfhydryl groups. These results corroborate the use of *P. pellucida* to treat gastric ulcers in traditional medicine (Rojas-Martínez, et al., 2013). In addition, scientific studies on *P. pellucida* have also found anti-ulcerogenic and chemotherapeutic properties (Hamid, Zakaria, & Zuraini, 2007; Loc, Bach, Kim, & Yang, 2010).

Neuropharmacological effect

Neuropharmacological effects of *P. pellucida* leaves was investigated by (Khan, Rahman, & Islam, 2008b) due to the leaves are used by the local people in Lakshmipur district of Bangladesh in the treatment of excited mental disorder but its neuropharmacological effect has not yet been explored. Petroleum ether and ethyl acetate soluble fractions of crude ethanol extract of *P. pellucida* leaves were administered to mice intra-peritoneally and their effects on duration of diazepam-induced sleep, nikethamide-induced toxicity, light-dark test and force swimming test were examined. The results suggest that both fractions of the leaves extracts have dose dependent depressant effects. The duration of diazepam-induced sleep was extended by administration of these fractions. Nikethamide at high doses caused death of mice but administration of these fractions delayed the time that nikethamide caused the death of animals. In light-dark test and force swimming test, these fractions showed diazepam type effects (Khan, Rahman, & Islam, 2008b).

Toxicity test

Brine shrimp lethality tests were done on n-hexane, ethyl acetate, butanol and water fractions partitioned from crude methanol leaf extract of *P. pellucida.* The toxicity results revealed that the methanol, hexane and ethyl acetate fractions were toxic to brine shrimp larvae at different lethal concentrations (LC_{50}) while butanol and aqueous fractions were non-toxic due to having LC_{50} values greater than 1000 µg/ml (Oloyede, Onocha, & Olaniran, 2011).

The use of *P. pellucida* is reported to interfere with prostaglandin synthesis (Aziba, Adedeji, Ekor, & Adeyemi, 2001). Hence, pregnancy women or nursing mothers should avoid use due to lack of clinical data (Oloyede, Onocha, & Olaniran,

2011). The plant has a strong mustard-like odour and may cause asthma-like symptoms in patients with known hypersensitivity reactions to the plant species. Thus, patients with known hypersensitivity reactions to any of the components of the plant species should avoid its use (Oloyede, Onocha, & Olaniran, 2011).

Up to this point, there is no clinical data have been reported on human toxicity (Egwuche, Odetola, & Erukainure, 2011). Animals treated with 5g/kg of the aqueous extract of *P. pellucida* were observed and weighed daily for 14 days and showed no changes in behaviour or weight, which indicates low toxicity of the extract (de Fátima Arrigoni-Blank, et al., 2004).

Antioxidant activity

Other than that, antioxidant activity of *P. pellucida* was first reported by Mutee *et al.* in 2010. The antioxidant effect was evaluated *in vitro* by determining the free radical scavenging activity of petroleum ether, chloroform and methanol extracts of *P. pellucida* whole plant by using DPPH radical scavenging assay. Butylated hydroxytoluene (BHT) was used as the standard reference agent. Half maximal inhibitory concentration (IC_{50}) less than 50 µg/ml is considered high antioxidant capacity while IC_{50} more than 50 µg/ml is considered moderate antioxidant capacity (Omisore, et al., 2005). The methanol extract showed a strong and dose-response free radical scavenging activity. Petroleum ether and chloroform showed lower free radical scavenging activity compared to methanol extract. The results from this study suggested that this plant is a good natural source for antioxidant therapy (Mutee, et al., 2010).

On the contrary, antioxidant activity of the methanol leaf extract of *P. pellucida* characterized by Lee, Wee, Yong and Syamsumir (2011) using the same method, α, α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging method, was found to inhibit

only 30% of DPPH free radical at the concentration of 0.625 mg/ml. The reported moderate antioxidant activity of *P. pellucida* was contrary to the finding of Mutee *et al.*, in which methanol extract of *P. pellucida* whole plant was reported to exhibit high antioxidant activity with IC_{50} 0.083 mg/ml (Mutee, et al., 2010). Discrepancy of results may due to difference in the extraction method applied. Finely ground plant sample would probably yield more potent plant extract (Lee, Wee, Yong, & Syamsumir, 2011). It may also due to different parts of the plant being used in the extraction. Mutee *et al.* used the whole plant for extraction while Lee *et al.* only used the leaf. Hence, high antioxidant activity may due to active compounds extracted from other parts of *P. pellucida* other than the leaf. Investigation on the antioxidant activity of different parts, such as leaf, stem or root, may reveal different level of antioxidant activity in each part.

Another study focus on antioxidant activity of *P. pellucida* was conducted by Oloyede, Onocha and Olaniran (2011) in Nigeria. The antioxidant activity of *P. pellucida* was determined by three methods, namely scavenging effect on 2, 2-diphenyl-1-picryhydrazyl radical (DPPH), hydroxyl radical generated by hydrogen peroxide and peroxide oxidation by ferric thiocyanate method. Three reference standards are used in the assay, which are butylated hydroxyl anisole (BHA), ascorbic acid and α- tocopherol. The results revealed that the fractions have good antioxidant activity when compared with antioxidant reference standards. Among those three methods, the extracts were most active in the hydroxyl radical method giving a percentage inhibition of over 98% scavenging activity (Oloyede, Onocha, & Olaniran, 2011).

For DPPH radical scavenging assay, all the fractions tested have better antioxidant activity than the reference standards used and inhibition increased with decrease in concentration (Oloyede, Onocha, & Olaniran, 2011). This observation is

contrary to the previous two studies done on antioxidant activity of *P. pellucida*, which showed a dose-respondent effect.

In hydrogen peroxide assay, all the fractions showed better scavenging activities when compared to the standards. The percentage inhibition was between 92 and 99% at all the concentrations used. Activity was found to be better particularly with the n-hexane fraction. *P. pellucida* therefore is a source of antioxidant compounds especially in scavenging the highly reactive hydroxyl radicals which are known to cause oxidative damage to biological macromolecules (Oloyede, Onocha, & Olaniran, 2011).

The Ferric thiocyanate method (FTC) method was used to determine the amount of peroxide, which oxidized ferrous chloride $FeCl_2$) to a reddish ferric chloride ($FeCl_3$) pigment. All those fractions, hexane, ethyl acetate, butanol, crude methanol and aqueous extracts showed moderate antioxidant activities at various concentrations in a dose-dependent manner (Oloyede, Onocha, & Olaniran, 2011).

The high antioxidant activity of the plant at low concentration indicates that it could be very useful for the treatment of ailments resulting from oxidative stress such as Parkinson's disease, Alzheimer's disease, cancer, cardiovascular disorders, bacterial and viral infections and inflammation, coronary heart disease and stroke. This result confirms the use of *P. pellucida* as an antioxidant agent and further corroborates the ethnomedicinal uses of the plant (Oloyede, Onocha, & Olaniran, 2011).

However, usage of such toxic chemical compounds at high doses should be properly monitored despite their medicinal benefits in the therapy of some ailments involving cell or tumour growth due to the brine shrimp lethality tests revealed that extracts of some solvent are considered toxic if their LC_{50} (concentration at 50% of lethality) is higher than 1000 µg/ml (Oloyede, Onocha, & Olaniran, 2011).

2.3 Antioxidant Studies on Different Parts of Plants

According to Jahan *et al.*, there was a different in DPPH radical scavenging activity between broccoli flower and broccoli stem (Jahan, et al., 2010). Besides, Khan *et al.* have also shown that methanolic extracts from different parts of Tut plant exhibited different level of antioxidant activity (Khan M. A., et al., 2013). In addition, higher antioxidant property was exhibited by the *Dorstenia barteri* twig extract when compared with the leaf extract (Omisore, et al., 2005). Other than that, a comparative assay for the different plant parts of *Cassia auriculata* showed that the leaves exhibit higher radical scavenging activity than the stem and fruit (Gaikwad, Kamble, Devare, Deshpande, & Salvekar Jyoti, 2011). These studies revealed that different parts of a plant may have their relative difference in the presence or distribution of the potent antioxidant components. However, there was so far no antioxidant study on different parts of *P. pellucida* has been found. Thus, this study investigated the antioxidant activity of extracts from different parts of this plant.

2.4 Selection of Freeze-Drying Method for Sample Preparation

The effects of different drying methods studied by Ibrahim, Mat, Lim and Ahmad showed that different drying treatments resulted in significant differences in the antioxidant properties as well as the phenolic and flavonoids contents of the plant extracts studied. Freeze-dried leaf extracts exhibited significantly higher radical scavenging activity compared to that of oven-dried extracts (Ibrahim, Mat, Lim, & Ahmad, 2013). Moreover, the effect of thermal treatment on antioxidant activity and phenolic content studied by Ismail, Marjan and Foong in 2004 indicated that the antioxidant activities of selected vegetables were significantly decreased after thermal