UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF TOTAL ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC CONTENT AND TOTAL FLAVONOID CONTENT IN PEELS, SEEDS AND LEAVES OF Swietenia Macrophylla (TUNJUK LANGIT)

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BY

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A project submitted as a partial fulfillment of the requirement for the degree of Bachelor of Science (Nutrition and Community Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

ACKNOWLEDGMENT

First and foremost, I would like to thank ALLAH s.w.t. Most Gracious and Most Merciful for His Blessing and with that I can finish my study in B. Sc. Nutrition and Community Health. I also would like to take this opportunity to express my highest gratitude and sincere appreciation to my supervisor, Associate Professor Dr. Loh Su Peng for her invaluable guidance, encouragement, advice, support and his endless patience in providing me suggestions, advice and time in making this study a success.

I am also thankful to my co-supervisor, Dr. Siti Raihanah Shafie for her constructive comments and suggestions. Besides, I would also like to express my endless gratitude for tremendous help, support and contribution of the staff in the Department of Nutrition and Dietetics, especially to Mr. Syed Hasbullah, Mr. Syahidan and Mdm. Che Maznah for their precious time, technical advice and material provisions.

Appreciations are also to my dear parent and family members, a million thanks for their enormous amount of love, understanding, sacrifice, and continuous support that had make the task of completing this project possible. Lastly, I would like to thank and bless everyone in this universe for their support and contribution for the succession of this study.

May the Blessings of Allah will be upon all of you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BHT	Butylated hydroxytoluene
BHA	Butylated hydroxyanisole
DPPH	2-2-diphenyl-1-picrylhydrazyl
FTC	Ferric thiocynate
DW	Dry weight
GAE	Gallic acid equivalent
QE	Quercetin equivalent
HCl	Hydrochloric acid
SD	Standard deviation
UV	Ultraviolet
PG	Propyl gallate
HAT	Hydrogen atom transfer
ET	Electron transfer
ORAC	Oxygen radical absorbance capacity
TRAP	Total radical trapping antioxidant parameter
TEAC	Trolox equivalence antioxidant capacity
FRAP	Ferric reducing antioxidant potential assay
FCR	Folin–Ciocalteu reagent
TFC	Total flavonoid content
TPC	Total phenolic content

ABSTRACT

DETERMINATION OF TOTAL ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC CONTENT AND TOTAL FLAVONOID CONTENT IN PEELS, SEEDS AND LEAVES OF *Swietenia Macrophylla* (TUNJUK LANGIT)

CHE KU NUR SHALIANA BT CHE KU ANUAR

S. macrophylla is traditionally believed to impart health benefits among the local communities in Malaysia and other Asia countries. Many species have been used as medicinal plants to treat a variety of illnesses. In this study, the total antioxidant activity and total phenolic content in peels, seeds and leaves of *S. macrophylla* in aqueous and ethanol extracts was investigated. The total phenolic content was determined using a spectrophotometric technique, based on the Folin-Ciocalteu reagent. Ferric Reducing Antioxidant Power Assay (FRAP) was used for determination of total antioxidant activity. Results were expressed as mean values ± standard deviations. The results were analyzed statistically using One-way ANOVA analysis using Tukey test and Pearson correlations coefficient. In the present study; the highest percentage yield was demonstrated in aqueous and ethanol extracts of *S. macrophylla* leaves with percentage of yield of 29.2% and 22.4%, respectively followed by seeds and peels. For total phenolic content, *S. macrophylla* leaves for aqueous and ethanol extracts were found given better content of phenolic compound (16.85 mg GAE/g and 10.74 mg GAE/g sample) respectively compared to peels and seeds which was well-matched with the result previously since it has higher percentage yield. For antioxidant assay, the result shows that FRAP value for *S. macrophylla* leaves also gave higher values than seeds and peel in both ethanol and aqueous extracts with 111.65 and 94.99 mmol/g dried sample respectively. There was a significant difference at $p < 0.05$ exist between *S. macrophylla* leaves with seeds and peels. Results showed that both extracts possessed significant antioxidant activity. For the total flavonoid contents, between parts of the same solvent, leaves extracts contained significantly ($p < 0.05$) higher total flavonoid content than the peels and seeds extracts. The contents of phenolics and flavonoids exhibited good correlation with FRAP ($r=0.904$ and $r=0.987$). These results indicate that phenolic compounds are the major contributors to antioxidant activity. The results also provide justification for the use of the plant in folk medicine to treat various diseases.

ABSTRAK

PENENTUAN AKTIVITI ANTIOKSIDAN, JUMLAH KANDUNGAN FENOLIK DAN JUMLAH KANDUNGAN FLAVONOID DALAM KULIT, BIJI DAN DAUN *Swietenia Macrophylla* (TUNJUK LANGIT)

CHE KU NUR SHALIANA BT CHE KU ANUAR

S. macrophylla secara tradisional dipercayai memberi manfaat kesihatan di kalangan masyarakat tempatan di Malaysia dan negara-negara Asia yang lain. Banyak spesies telah digunakan sebagai tumbuhan ubat untuk merawat pelbagai penyakit. Dalam kajian ini, jumlah aktiviti antioksidan dan jumlah kandungan fenolik dalam kulit, biji dan daun *S. macrophylla* dalam ekstrak air dan etanol telah dikaji. Jumlah kandungan fenolik ditentukan dengan menggunakan teknik spektrofotometri, berdasarkan reagen *Folin-Ciocalteu*. *Ferric Reducing Antioxidant Power Assay* (FRAP) digunakan untuk penentuan jumlah aktiviti antioksidan. Hasilnya dinyatakan sebagai nilai *min* ± *standard deviations*. Hasilnya dianalisis secara statistik dengan menggunakan analisis *One-way ANOVA* menggunakan *Tukey test* dan *Pearson correlations*. Dalam kajian ini; hasil peratusan tertinggi ditunjukkan oleh daun *S. macrophylla* dalam ekstrak air dan etanol dengan peratusan 29.2% dan 22.4%, masing-masing diikuti oleh biji dan kulit. Untuk jumlah kandungan fenolik, daun *S.macrophylla* untuk ekstrak air dan etanol didapati lebih baik daripada kandungan sebatian fenolik (16.85 mg GAE / g dan 10.74 mg GAE / g sampel) masing-masing berbanding kulit dan biji yang sepadan dengan keputusan sebelumnya kerana ia mempunyai hasil peratusan yang lebih tinggi. Untuk ujian antioksidan, hasilnya menunjukkan nilai FRAP untuk daun *S. macrophylla* juga memberikan nilai yang lebih tinggi daripada biji dan kulit dalam kedua-dua ekstrak etanol dan air dengan 111.65 dan 94.99 mmol / g sampel . Terdapat perbezaan yang ketara pada $p < 0.05$ yang wujud di antara daun *S. macrophylla* dengan biji dan kulit. Keputusan ini menunjukkan bahawa kedua-dua ekstrak mempunyai aktiviti antioksidan yang penting. Untuk jumlah kandungan flavonoid antara bahagian pelarut yang sama, ekstrak daripada daun mengandungi kandungan flavonoid yang lebih tinggi ($p < 0.05$) lebih tinggi daripada ekstrak kulit dan biji. Kandungan fenolik dan flavonoid menunjukkan korelasi yang baik dengan FRAP ($r = 0.904$ dan $r = 0.987$). Keputusan ini menunjukkan bahawa sebatian fenolik merupakan penyumbang utama kepada aktiviti antioksidan. Hasilnya juga memberikan alasan untuk penggunaan tumbuhan dalam perubatan tradisional untuk merawat pelbagai penyakit.

CHAPTER 1

1.0 INTRODUCTION

1.1 Background

Antioxidants are substances that can prevent oxidative damage in the body cells. Imbalance free radicals caused their accumulation in the body which creates a phenomenon called as oxidative stress. Oxidative stress plays a major part in the development process of chronic and degenerative illness such as cancer, autoimmune disorders, arthritis, aging, cardiovascular and neurodegenerative diseases. Antioxidants play in role by neutralizes the excess of free radicals in the body to give the protection to cells against their toxic effects and also contribute to prevention of disease The body produced antioxidants to stop or limit the oxidative damage, either naturally generated in situ (endogenous antioxidants), or externally supplied through foods (exogenous antioxidants),(Pham-Huy, He & Pham-Huy, 2008).

S. macrophylla commonly known as West Indian mahogany, large-leaved mahogany, Honduras mahogany, jambolan or Java plum (Elevitch & Wilkinson, 2000) and locally known as Tunjuk Langit in Malaysia (Balijepalli, Suppaiah, Chin, Sagineedu, & Pichika, 2015). The genus belongs to the family *Meliaceae*. The

species of *S. macrophylla* are mainly found in indigenous to central and south America. The genus *S. macrophylla* is derived from the botanist and physician to Maria Theresa of Austria, Gerard von Swieten (1700-1772).

The specific name '*macrophylla*' refers to large leaves, which is derived from the Greek word "*makros*" meaning "large" and "*phyllon*" meaning "leaf" (Orwa et al., 2009). *S. macrophylla* is under category vulnerable in IUCN Red List of Threatened Species (World Conservation Monitoring Centre, 1998). The fruit of *S. macrophylla* is called "sky fruit" because its fruit seem to point upwards to the sky (Goh & Kadir, 2011). Besides, *S. macrophylla* also contain various chemical compounds. This plant mainly contains limonoids and other derivatives (Taylor & Taylor,1983). Active ingredients such as saponin, alkaloids and flavonoid that are known to be beneficial to health have been reported to be present in seed of *S. macrophylla.*

S. macrophylla is traditionally believed to impart health benefits among the local communities in Malaysia and other Asia countries. Many species have been used as medicinal plants to treat a variety of illnesses. In traditional medicine, *S. macrophylla* seeds have been used for the treatment of diabetes, hypertension and to relieve pain (Goh & Kadir,2011). The previous studies have reported that the seeds of *S. macrophylla* have antimicrobial activity (Durai, Balamuniappan, & Geetha, 2016), anti-inflammatory activity (Chen et al.,2015) and antitumor-promoting activities (Guevera., Apilado, Sakurai, Kozuka, & Tokunda, 1996). Furthermore, the *S. macrophylla* seeds are safe to consumed at a single dose of 2 g/kg bw or

equivalent to the human dose of 325 mg/kg bw. This study did not show any signs of toxicity in Sprague Dawley rats (Balijepalli et al., 2015).

1.2 Problem Statements

According to National Health and Morbidity Survey (2015), approximately 73% of total deaths in Malaysia are caused by Non-Communicable Diseases (NCDs). Non-communicable diseases are including cancer, diabetes, cardiovascular diseases and chronic respiratory diseases. Cardiovascular diseases including heart attacks and strokes are the main causes of death among Malaysian population. Previous study had been reported that the major antioxidant nutrients which are vitamin E, vitamin C, and beta-carotene play a beneficial role in prevention of several chronic diseases (Diplock et al., 1998).

In the next 10 years, the populations that estimate to die due to chronic diseases are approximately 388 millions of people (WHO, 2005). Chronic diseases will cause a greatest impacts on psychological functioning, physical and social functioning of patients (Hwu, 1995). In normal cellular function, production of free radical occurs continuously in all cells. However, excess production of free radical in body that originating from endogenous or exogenous sources may play role in many diseases (Young & Woodside 2001).

Currently, numerous plants are used for medicine purpose across the world because of their remedial properties. Ineffective of conventional medicine treatments, increase in self-health care and also desire for a healthy lifestyle also had increased the interest of the population to practice traditional medicine (Akerele, 1993).

Therefore, by investigating in depth the phytochemicals including the antioxidants in the plants of *S. macrophylla* can help to reduce the chronic disease in Malaysia.

Antioxidants can be found in foods naturally. They also can be extracted from natural resources or synthesized. In the past few years synthetic antioxidants have obtained growing attention in pharmacological and toxicological research. Synthetic antioxidants have been used as food additives in order to overcome the stability problems of frying oils and also as supplements and drugs (Akbarirad, Ardabili, Kazemeini, & Khaneghah, 2016). However, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) have been reported to have possible hazardous side effects that dangerous for human health (Bishi, Patil, Hota, Dagla, & Kumar, 2014). These compounds can cause health risks including cancer and carcinogenesis. Hence, natural antioxidants from plants products are being emphasize to replace synthetic antioxidants (Zhang et al., 2009).

Besides, although *S. macrophylla* have been practiced traditionally as medications since ancient times, their beneficial values have not yet been well-documented. There are limited studies have been carried out in Malaysia that investigate in depth the total antioxidant activity and also total phenolic content in this plant. In addition, too little attention has been paid especially on peels and seeds of *S. macrophylla* for their potential health benefit as natural antioxidant source. Many studies focused on antioxidant activity of extracts from leaves of *S. macrophylla* but not in peels and seeds. Previous study has compared the antioxidant activities of *S. macrophylla* leaves using different extracts which are methanol,

dichloromethane and n-hexane extracts as determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH.) radical scavenging assay and found the methanol extract to be the most active extract. Furthermore, the extracts were also found to contain some amount of total phenolic, total flavonoid contents and total tannin (Tan, Osman, Wong, Boey, & Ibrahim, 2009).

1.3 Significance of study

As the prevalence of chronic diseases is on rise, further research on alternative treatment for chronic disease such as medicinal plant need to be studied. According to World Health Organization (WHO), 80% of the populations in the world depend on the traditional medicine for their primary health care needs. Besides, there are increased interests in the use of natural antioxidants and plant extracts during the past few decades, due to the concerns about possible ill health effects caused by the use of synthetic antioxidants (Abramovic & Abram, 2006). Traditional medicines from plant sources also offer people to have access to safe and effective products through self-medication for the treatment and prevention of the diseases (Akerele, 1993).

Besides, previous study also shows that the natural antioxidants are safer and have better effects on human compared to synthetic antioxidants (Akbarirad et al., 2016). Hence, this study can provide one ways to expand resources and introduce *S. macrophylla* as alternative treatment of chronic diseases in Malaysia as well to replace the use of synthetic antioxidant. This may happen if the properties in this plant are well knows and verified. Besides, it also can provide new and useful information to the nutritionist and other agencies in promoting more this plant to help the community to become healthier.

In addition, nowadays public also become more conscious and aware about the importance to consume a healthy food for their health. However, there is still lack of scientific evidence that investigate in depth about the health benefit of this medicine plant. Hence, by examined the total antioxidant activity and total antioxidant contents in *S. macrophylla* would contribute to more scientific information of this species and probably provide a lead to the future development of new antioxidant drug. Moreover, this study also will increase the awareness and knowledge of the public regarding the important to consumed food from natural sources as well the health benefit of *S. macrophylla* as a source of natural antioxidant.

1.4 Objectives

1.4.1 General Objective

To study total antioxidant activity, total phenolic content and total flavonoid content in peel, seed and leaf of *S. macrophylla.*

1.4.2 Specific Objectives

- To determine and compare total antioxidant activity (Ferric Reducing Antioxidant Power Assay) in peels, seeds and leaves of *S. macrophylla* in aqueous and ethanol extracts.
- To determine and compare total antioxidant content (total phenolic content and total flavonoid content) in peels, seeds and leaves of *S. macrophylla* in aqueous and ethanol extracts.

- To determine the correlation between total antioxidant activity and total antioxidant content in peels, seeds and leaves of *S macrophylla* in aqueous and ethanol extracts.

1.5 Hypothesis

- There is significance difference in total antioxidant activity between peels, seeds and leaves of *S. macrophylla* in aqueous and ethanol extracts.
- There is significance difference in total antioxidant content between peels, seeds and leaves of *S. macrophylla* in aqueous and ethanol extracts.
- There is high correlation between total antioxidant activity and total antioxidant content between peels, seeds and leaves of *S. macrophylla* in aqueous and ethanol extracts.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Medicinal Plants

Ethno medicine is the oldest method of curing many types of diseases and infections. Medicine plants has a long history of use for the prevention and treatment of the various ailments. Besides, various part of plants has been used around the world to treat human diseases and infections (Durai et al., 2016). According to World Health Organization (WHO) (Goh &Kadir, 2011), 80% of the world's population using traditional medicine from plant source which continues to provide health coverage especially in the developing world. In Malaysia, more than RM4.5 billion annual sale contribute to country by alternative medicine industry (Mohamad, Ali, & Ahmad, 2010). Previous study also has reported that 80000 are medicinal plant from 2,500,000 plant species in earth which means that many plants have not been fully discovered yet for their properties and benefit in curing various of illness. In addition, since synthetic antioxidants have shown antagonistic effects on human health, there is need to develop antioxidant from natural origin that are much safer, less expensive and reliable (Lobo, Patil, Phatak & Chandra, 2010).

2.2 *S. macrophylla*

S. macrophylla is commonly known as "sky fruit" or "tunjuk langit" since the fruits seem to point upwards to the sky (Figure 2.1) (Goh & Kadir, 2011). Flowering and fruiting seasons happen distinctively as per graphical area. The *S. macrophylla* may produce fruits once a year. In addition, the trees will begin to produce fruit consistently when about 15 years old. The characteristic of *S. macrophylla* seeds which have a thin, taillike wing (Figure 2.2) caused them to rotate when they fall. They can dispersed by wind to 500 m from the tree (Orwa, Mutua, Kindt, R, & Anthony, 2009).

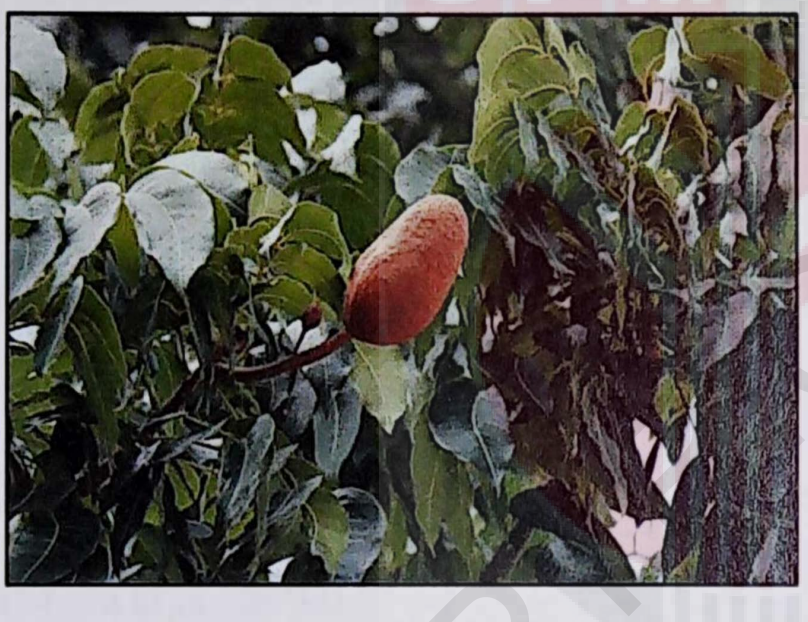

Figure 2.1: The S.*macrophylla*fruit that point upwards to the sky.

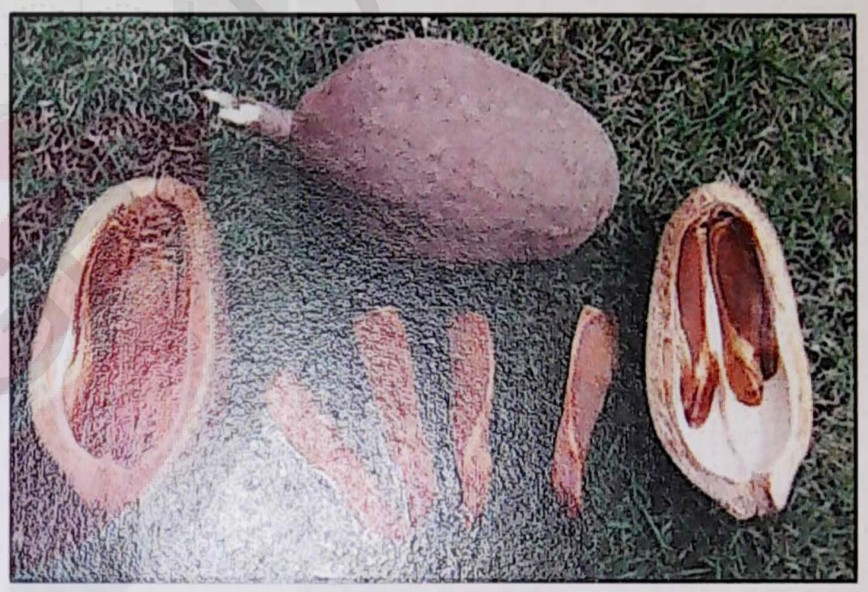

Figure 2.2: The fruit and seeds of *S. macrophylla*.

2.2.1 Taxonomy of *S. macrophylla*

The taxonomy of *S macrophylla* is as follows (World Conservation Monitoring Centre, 1998).

Kingdom	: Plantae
Phylum	:Tracheophyta
Class	: Magnoliopsida
Order	: Sapindales
Family	:Meliaceae

Scientific name : *Swietenia macrophylla*

Common Name(s):

English – Big-Leaf Mahogany, Brazilian Mahogany, Honduras Mahogany, Large-leaved Mahogany

French – Mahogani Grands Feuilles

Spanish – Caoba, Mara, Mogno

2.2.2 Habitat

S. macrophylla has a wide distribution in terms of altitude, precipitation, and soil types forests (Mejía, Buitrón, Pena-Claros, & Grogan, 2004). According to Brown, Jennings, and Clements (2003), *S. macrophylla* is commonly found in both moist and seasonally dry tropical rain forest and it seems to grow in a variety of soil conditions. Besides, it can procure greatest stature in rich, deep, well-drained riparian or seasonally wet soils, while additionally enduring dry conditions in open forests (Mejía et al., 2004).

For example, this plant can grow in three ecoregions: Amazon, pre-Andean Amazon, and the Chiquitano-Amazon transition in Peru, Bolivia and Brazil (Mejía et al., 2004). *S. macrophylla* have develops natively throughout the tropical districts of the Americas, specifically Central and South American nations including Bolivia, Mexico, west India, Malaysia, and southern China (Zorofchian Moghadamtousi, Hing Goh, Kei Chan, Shabab, & Abdul Kadir, 2013).

2.2.3 Plant Description

The plant of *S. macrophylla* is a lofty, evergreen large with an umbrella-shaped crown (Figure 2.3) (Krisnawati, Kallio, & Kanninen, 2011). The height normally around 30-40m while the girth was 3-4m with straight trunk and cylindrical bark. It can reach height up to 60 m and 9 m girth in favourable conditions (Orwa et al., 2009). While the *S. macrophylla* bark (Figure 2.4) are dark reddish brown, thick, deeply furrowed and have sweet scent (Eid, Elmarzugi, & El-Enshasy, 2013).

Figure 2.3: Five years old *S. macrophylla* trees.

Figure 2.4: Bark of 15 years old *S. macrophylla* tree.

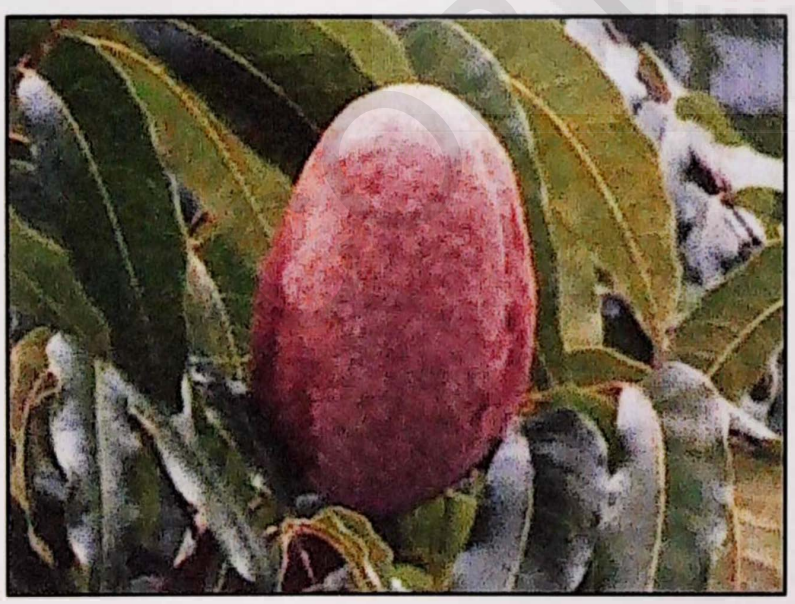

Figure 2.5: The brown of fruit of *S. macrophylla*.

Figure 2.6: *S. macrophylla* capsule showing the internal arrangement of winged seeds.

(Source: Krisnawati et al., 2011)

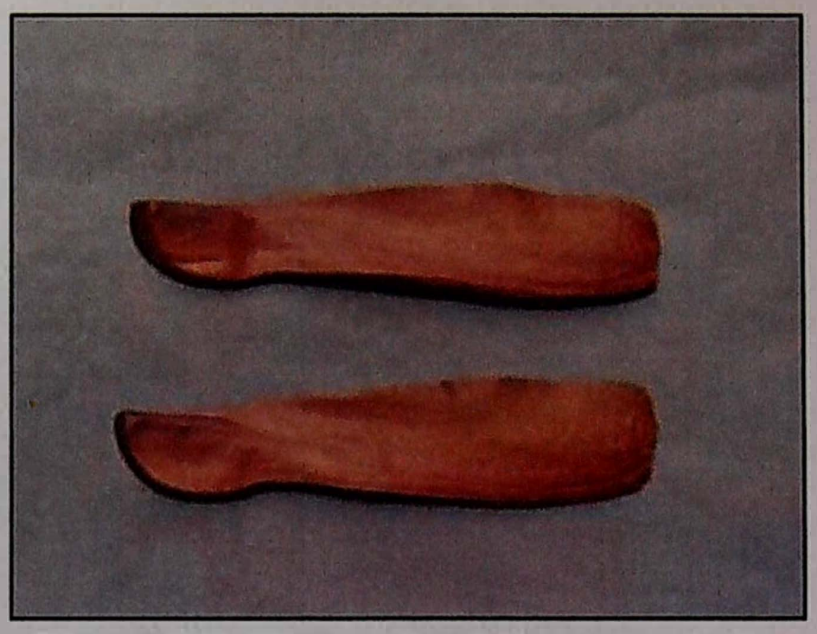

Figure 2.7: The seeds of *S. macrophylla*.

(Source: Krisnawati et al., 2011)

For the leaves, it is paripinnate, up to 60 cm long; leaflets 6-16, ovate, lanceolate, acuminate, slightly oblique, light green or reddish when young, dark green and shining when mature, up to 20 cm long (Orwa et al., 2009). It also has small flowers yellow-cream colored panicles and the *S. macrophylla* fruits are large woody capsules, ovoid, color light brown (Figure 1.5), 10–20 cm long which splits on 5 shares, with 10 to 14 winged seeds (Figure 2.6) (Eid et al., 2013). The *S. macrophylla* seeds are samaroid (Figure 2.7), bulky at their base, 7–12 cm long and 2–2.5 cm wide including the wing (Krisnawati et al., 2011).

Figure 2.8: *S. macrophylla* (1) tree, (2) flowering twig, (3) sectioned male flower, (4) sectioned female flower, (5) fruit, (6) seed.

(Source: PROSEA, 1886)

2.2.4 Phytochemical Compounds

S. macrophylla contain various bioactive compound. The major constituents in *S. macrophylla* are limonoids and their derivatives. Limonoids are derived from tetracyclic triterpenes similar with euphol (H-20b) or tirucallol (H-20α) by a series of oxidative changes. Oxidative changes occur at their side chain to a b-substituted furan ring which caused loss of four carbon atoms and also some molecular rearrangements (Moghadamtousi, Goh, Chan, Shabab, & Kadir, 2013).

S. macrophylla bark is found to have enylpropanoid-subtitutedcatechin which known as swietemacrophyllanin-catechin-8,7-7,2-epoxy-(methyl-4,5 dihydroxyphenylpropanoate). In addition, catechin and epicatechin are also isolated in *S. macrophylla* bark (Falah, Suzuki, & Katayama, 2008). While in the terminal shoots, senescent and mature leaves of *S. macrophylla* in form of fatty acids and

terpenoids, chemical constituents that have been identified were essential oil components such as γ-himachalene, germacrene D, germacrene A, cadina-1,4-diene, hexadecanoic acid and ethyl hexadecanoate (Soares, et al., 2003).

In the *S. macrophylla* fruits is found to contain new phragmalin-type limonoid namely 6-O-acetyl-30-demethylswietephragmin together with 16 known compounds. Furthermore, the other compounds such as 6-O-Acetyl-30 demethylswietephragmin, 3,6-O,O-diacetylswietenolide, 3-O-tigloylswietenolide, 3-O-tigloyl- 6-O-acetylswietenolide, swietemahonin, and 6-O-acetylswietemahonin are also isolated and identified from its fruit (Chen et al., 2010).

Previous studies also have been reported that *S. macrophylla* seeds contain limonoids or also known as tetranortriterpenoids which including swietenine, swietenolide (a bitter compound), 8,30-epoxy-swietenine acetate (Falah et al., 2008;Chan, Tang, & Toh, 1976), swietenolide diacetate, augustineolide and 3β,6-dihydroxydihydrocarapin (Chan et al., 1976; Mootoo et al.,1999). Besides, fatty acids and terpenoids are also isolated from the seeds including palmitic acid, stearic acid, arachidic acid, oleic acid, linoleic acid and linolenic acid (Falah et al., 2008;Chan et al., 1976).

2.2.5 Uses of *S. macrophylla*

According to World Wildlife Fund, (2018) *S. macrophylla* has been a prized timber product since 1500s. The woods have been sought, traded and used for the manufacture of furniture, cabinet making, and plywood (veneer). S. *macrophylla* is valued for its rich reddish color, durability, beauty and good characteristics. Besides,

the woods also have been used for flooring, panelling, automobile bodies, interior trim of boats, mouldings and other wood products (Figure 2.9). It also has technical qualities that make it suitable for making range of musical instruments (Krisnawati et al., 2011).

Figure 2.9: Items made from *S. macrophylla* timber: (a) door pane, (b) cupboard, (c) flooring and (d) urn.

(Source: Krisnawati et al., 2011)

Furthermore, *S. macrophylla* has been widely used in the common folk communities in Asia and many other countries because of the benefits that have in each part of this plant whether as a medicine or other purposes. *S. macrophylla* has been used to treat various ailments based on its antimicrobial, antimutagenic, antioxidant effects, anticancer, anti-inflammatory, antidiabetic activities and antitumor (Moghadamtousi et al., 2013). In Malaysia, the *S. macrophylla* seeds are eaten by chewing or pounded and then swallowing which used traditionally to treat high blood pressure, diabetes, and also to relieve pain (Chan et al.,1976; Goh & Kadir, 2011).

Besides, the seeds are also used to reduce the diarrhea. For example, in East Midnapore, West Bengal, India, the local healers believed that the *S. macrophylla* seeds can help them for curing diarrhea (Maiti, Dewanjee, & Mandal, 2007).

S. macrophylla seeds also used for the treatment diabetes mellitus as well hypertension in India (Hashim et al., 2013). The decoction of the crushed seeds of this plant are used to treat wounds and also for skin ailments. *S. macrophylla* also is used as an abortion medicine for Amazonian Bolivian ethnic group while in Indonesia, it has been used for the treatment of hypertension, diabetes and malaria as a folk medicine (Bourdy et al., 2000; Moghadamtousi et al., 2013).

2.3 Antioxidants

Antioxidants are substances that can inhibit or delay free oxidation process in body. Free radicals and oxidants can be either harmful or helpful to the body. This is because both play a dual role as both can becomes toxic and beneficial compounds (Pham-Huy et. al., 2008). In addition, antioxidants are the stable molecule which donates an electron to a free radical and neutralizes it. Thus, accordingly diminishing its ability to harm the body cell. The substances that can prevent and reduce the oxidative damage including through scavenging free radicals and reactive oxygen species (ROS), acting as reducing agents, chelating metal ions, or by quenching secondary products of lipid oxidation (Bishi et al., 2014).

There are distinctive attributes to categorize the antioxidants. The first attribute is depending on the function which are primary and secondary antioxidants. Primary antioxidants donating hydrogen for the formation of more stable radicals to prevent propagation (Bishi, Patil, Hota, Dagla, & Kumar, 2014). The compounds include phenolics, antioxidant minerals, antioxidant vitamins and phytochemicals including β-carotene, flavonoids, lycopene, catechins, carotenoids, diterpene of, black pepper, thyme, garlic, cumin and their derivatives. Secondary antioxidants are

the phenolic compounds that carry out the function of capturing free radicals and ceasing the chain reactions. Antioxidants of this group are mainly Butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and propyl gallate (PG). The second attribute is depending on enzymatic and non-enzymatic antioxidants (Figure 2.10) (Moharram & Youssef, 2014).

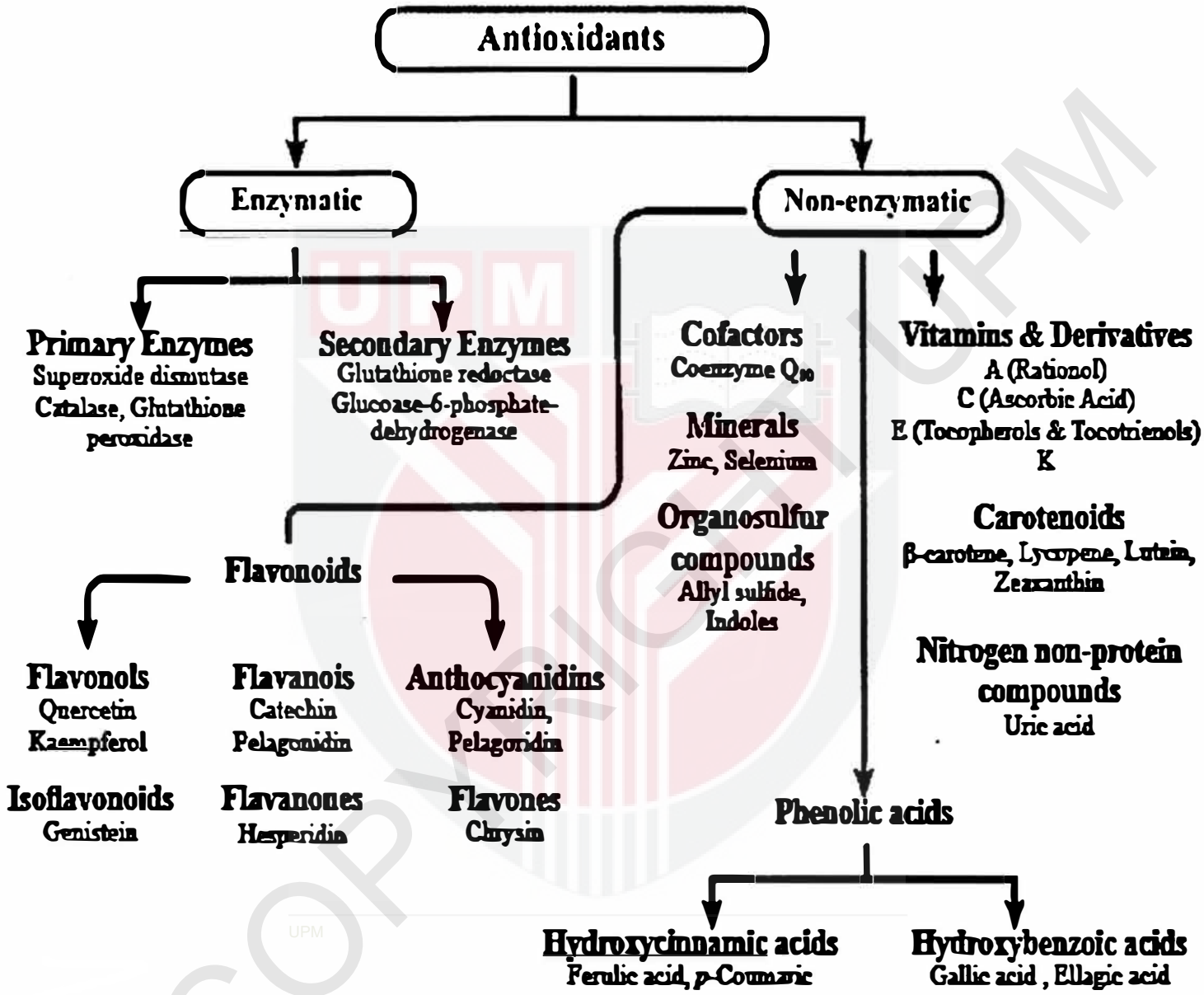

Figure 2.10: Classification of antioxidants.

(Source: Carocho & Ferreira, 2013)

2.3.1 Antioxidant Compounds

Antioxidant can be classified into two groups which are natural and synthetic antioxidants. Natural antioxidant is antioxidant that comes from natural sources such as Vitamin C, Vitamin E, and various polyphenol including carotenoids and

flavonoids. Fruits and vegetables are the main source of antioxidants that believed to be rich in antioxidants contents. Besides, studies also show that fruits and vegetables can lower the cancer risk in human. Sufficient supply antioxidants from dietary sources such as fruits and vegetables intake can give protection to our body against the harmful effects of oxidative stress (Sun & Ho, 2005).

Synthetic antioxidants have been widely uses as antioxidants in food industry, therapeutic and cosmetics industry. However, synthetic antioxidants such as butylated hydroxyl toluene (BHT) and synthetic butylated hydroxyanisole (BHA) can causes negative health effects. This is due to some physical properties of BHT and BHA such as their high volatility and instability at elevated temperature can give negative effects to consumer. Previous studies also have shown that some of the synthetic antioxidant can caused cancer and other diseases due to its ability that can intercept free radicals. Thus, the search for effective, nontoxic natural compounds with antioxidative activity has been intensified in recent years and have shifted the attention of manufacturers from synthetic to natural antioxidants (Lobo et al., 2010).

2.4 Antioxidant Capacity Assay

According to Shahidi and Zhong, (2007), antioxidant capacity is defined as the potential of the antioxidant to prevent oxidation. The antioxidant capacity assays can generally be categorized into two types depending upon the reactions involved: assays based on hydrogen atom transfer (HAT) reactions and assays based on electron transfer (ET). For the HAT-based assays, the antioxidant and substrate compete for thermally generated peroxyl radicals through the decomposition of azo compounds. These assays include inhibition of induced low-density lipoprotein

autoxidation, oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP), and crocin bleaching assays (Huang, Boxin, & Prior, 2005).

The capacity of an antioxidant in the reduction of an oxidant is measure for ET-based assays, which the color will changes when reduced. Sample's antioxidant concentrations influence the degree of color change. ET-based assays include the total phenols assay by Folin–Ciocalteu reagent (FCR), Trolox equivalence antioxidant capacity (TEAC), ferric ion reducing antioxidant power (FRAP), "total antioxidant potential" assay using a Cu(II) complex as an oxidant, and DPPH (Huang et al., 2005).

2.4.1 Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric reducing antioxidant power (FRAP) assay had been used by Benzie and Strain to estimate antioxidant potential of plasma (Benzie & Strain, 1996). FRAP assay relies on the efficiency of antioxidant from sample to donate an electron. Yellow ferric tripyridyltriazine complex (Fe(III)-TPTZ) will reduce to blue ferrous complex (Fe(II)-TPTZ). The binding of Fe^{2+} to the ligand creates a very intense navy-blue color. The reduced form (blue colour) is measured spectrophotometrically at 593nm. The absorbance can be measured to test the amount of iron reduced and can be correlated with the amount of antioxidants. Trolox or ascorbic acid can be used as references (Pellegrini et al., 2003; Gil, Tomas-Barberan, Hess-Pierce, & Kader, 2002).

2.4.2 2,2-diphenyl-1picrylhydrazyl (DPPH) Assay

DPPH assay was developed by Blois (1958) with the perspective to measure the antioxidant activity in a like manner by using a stable free radical DPPH(2,2-diphenyl-1picrylhydrazyl). Besides, this assay is based on the measurement of the scavenging capacity of antioxidants towards it. By receiving a hydrogen atom from antioxidants to the corresponding hydrazine, the odd electron of nitrogen atom in DPPH will be reduced (Figure 2.11) (Contreras-Guzman & Srong, 1982). In addition, DPPH is a commercially available radical and the method is simple and rapid for determination of antioxidant activity of sample based on reduction of DPPH radical (Huang et al., 2005).

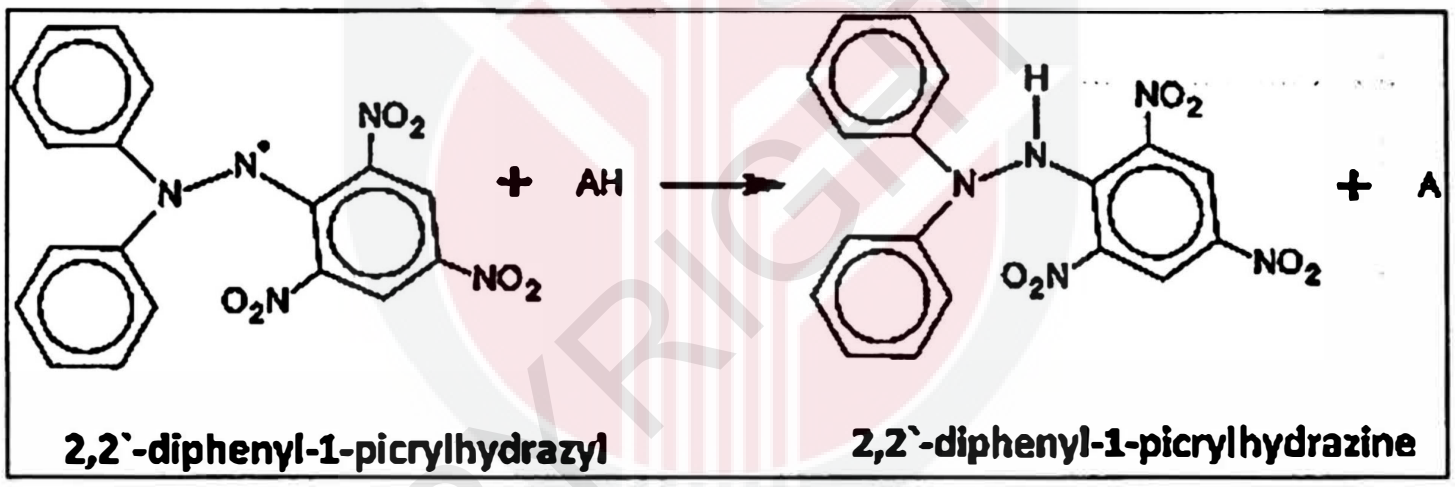

Figure 2.11: Conversion of radical DDPH to DPPH molecule from antioxidant.

(Source: Pyrzynska & Pe, 2013)

Furthermore, there is delocalization of the spare electron on the whole molecule makes DPPH(2,2-diphenyl-1picrylhydrazyl) to become a stable free radical. Therefore, DPPH does not dimerize, as occurs with most free radicals. Besides, DPPH radical is also soluble in organic reagents and purple in colour giving maximum UV-visible absorption at 515-520. It produces pale yellow hydrazine after reduced. When DPPH reacts with a hydrogen donor, the reduced (molecular) form (DPPH) is generated, go along with the disappearance of the purple colour. Therefore, the efficiency of the samples in reducing the DPPH radical depends on the

amount of antioxidant available. For this method, Trolox can be used as standard antioxidant. The maximum absorbance reading obtained at 517nm is normally expressed as percentage of inhibition of DPPH chromogen (Thaipong et al., 2006; Pisoschi, Cheregi, & Danet, 2009).

2.5 Phenolic Compound

Phenolic can be divided into two groups which are simple phenols and polyphenols based on the number of phenol subunit. Simple phenol contains one aromatic ring while polyphenol contain two or more aromatic rings (Marinova, Ribarova, & Atanassova, 2005). Besides, simple phenols contain phenolic acids or phenol. Phenolic acids are hydroxylated derivatives of cinnamic and benzoic acids (Figure 2.12). Polyphenol have abilities to donate hydrogen ion to make free radicals become stable. Hence, polyphenol can scavenge free radicals in human body. Redox properties of phenolic components such as phenolic acids and polyphenols can play an important role in absorbing and neutralizing free radicals (Arts & Hollman, 2005).

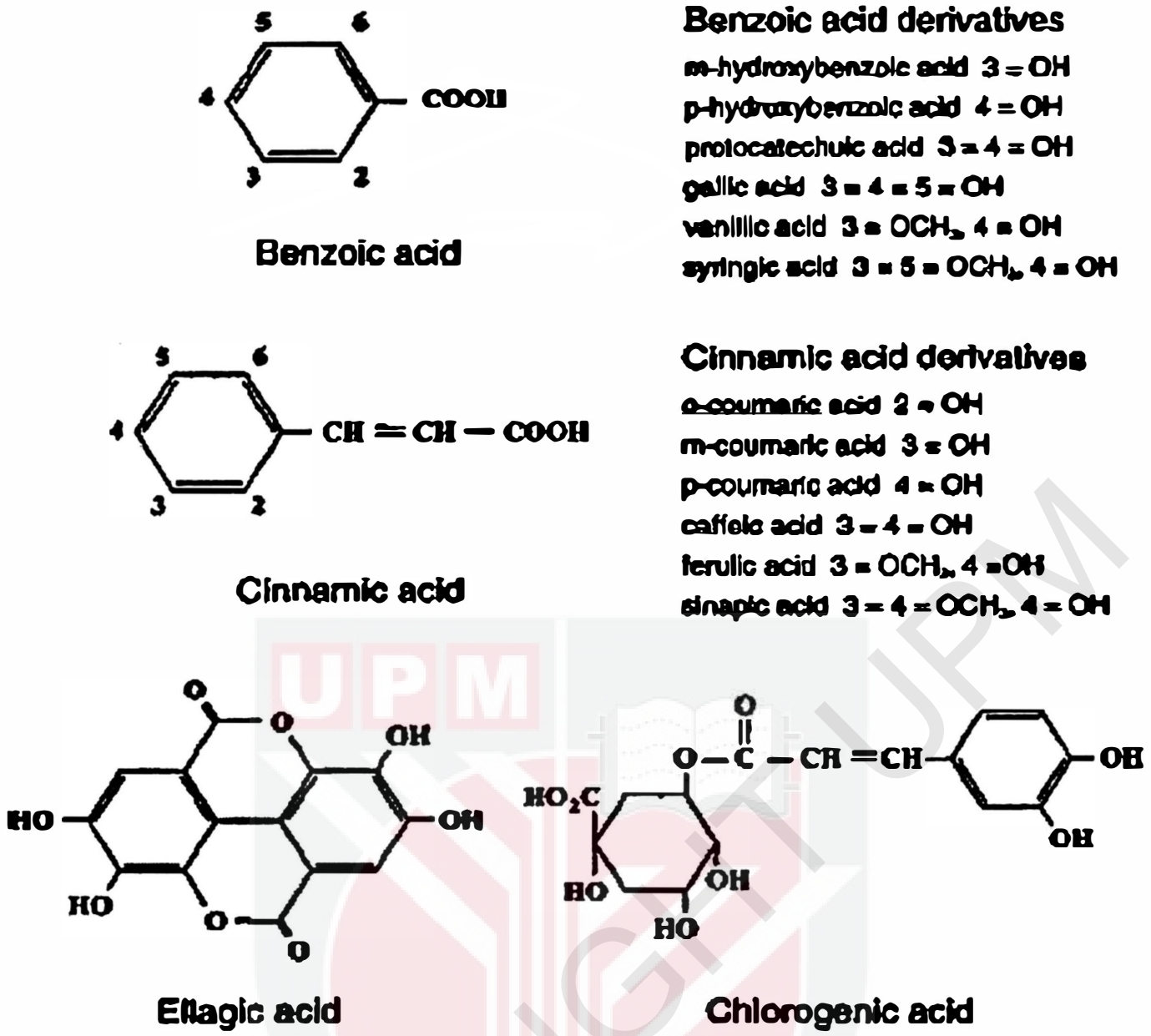

Figure 2.12: Chemical structures of phenolic acid

(Source: Mattila & Kumpulainen., 2011)

Total phenolic assay is electron transfer pathway involving Folin- Ciocalteu reagent. This assay is simple, easy to perform and the results are reproducible. Total phenolic assay has been used in accessing phenolic antioxidants or phenolic content of food sample. Besides, it also includes translocation of electrons from antioxidant compounds to the molybdenum. The reduction of molybdenum will cause color changing from yellow to blue that can be monitored through spectrophotometer at 750-765nm. Gallic acid can be used as standard and the degree of color change is associated with the antioxidant concentration of sample (Huang et al., 2005).

2.6 Flavonoids

Flavonoids are a class of secondary plant phenolics which are present in most plants that can characterized by benzo-y-pyrone structure (Miller,1996; Subramaniam, Stacey, & Yu, 2007). In the human diet, they are most concentrated in green tea, grapes (red wine), cocoa (chocolate), apple, ginkgo biloba, curcuma, soybean, berries, onion and broccoli. For example, green tea contains a rich source of flavonoids, especially flavonols (catechins) and quercetin (Pham-Huy, He, & Pham-Huy, 2008). Furthermore, over 4000 flavonoids have been discovered and classified into flavanols, flavanones, flavones, isoflavones, catechins, anthocyanins, proanthocyanidins based on chemical structure (Miller,1996). Flavonoids that present in foods are primarily as glycosides and polymers that are degraded to variable extents in the digestive tract.

Flavonoids have many beneficial effects on human health. Previous study have been reported that flavonoids can prevent or delay a number of chronic and degenerative diseases including cancer, arthritis, aging, cardiovascular diseases, Alzheimer's disease, memory loss, cataract, stroke, infection, and inflammation (Hanneken, Lin, Johnson, & Maher, 2006). This is due to flavonoids have capability to scavenge free radicals directly by hydrogen atom donation as well ability to inhibit lipid peroxidation. Hence, flavonoids can protect the body against the harmful effects of reactive oxygen species (Procházková et al., 2011). Radicals are made inactive according to the following equation, where $R^{\bullet}$ is a free radical and $Fl\text{-}O^{\bullet}$ is a flavonoid phenoxyl radical (Figure 2.13).

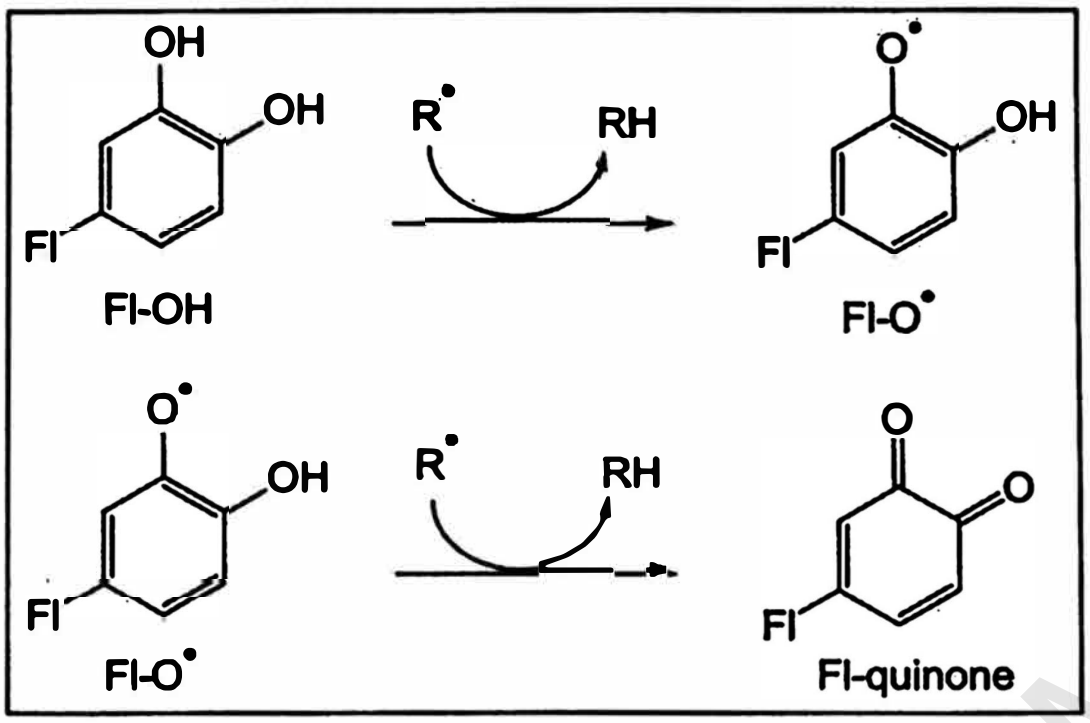

Figure 2.13: Scavenging of reactive oxygen species (R•) by flavonoid. The free radical Fl-O• may react with a second radical, acquiring a stable quinone structure.

(Source: Procházková et al., 2011)

CHAPTER 3

3.0 METHODOLOGY

3.1 Sample

The sampling method was convenient sampling. The fruits and leaves of *S. macrophylla* were collected from the area of Besut, Terengganu, Malaysia in December 2017.

3.2 Chemicals

Sodium phosphate buffer (pH 6.6), 1% potassium ferricyanide, 10% trichloroacetic acid (w/v), 0.1% of ferric chloride, Butylated hydroxytoluene (BHT), Folin-Ciocalteu reagent, gallic acid, quercetin, sodium nitrite ($NaNO_2$), and 10% aluminium chloride ($AlCl_3$) were all purchased from Sigma- Aldrich Co. (St. Louis, MO, USA).

3.3 Study Design

Figure 3.1 shows the summary of the flow chart of this present study. Aqueous and ethanol extracts of peels, seeds and leaves of *S. macrophylla* were studied for their total antioxidant activity, total phenolic content and total flavonoid content.

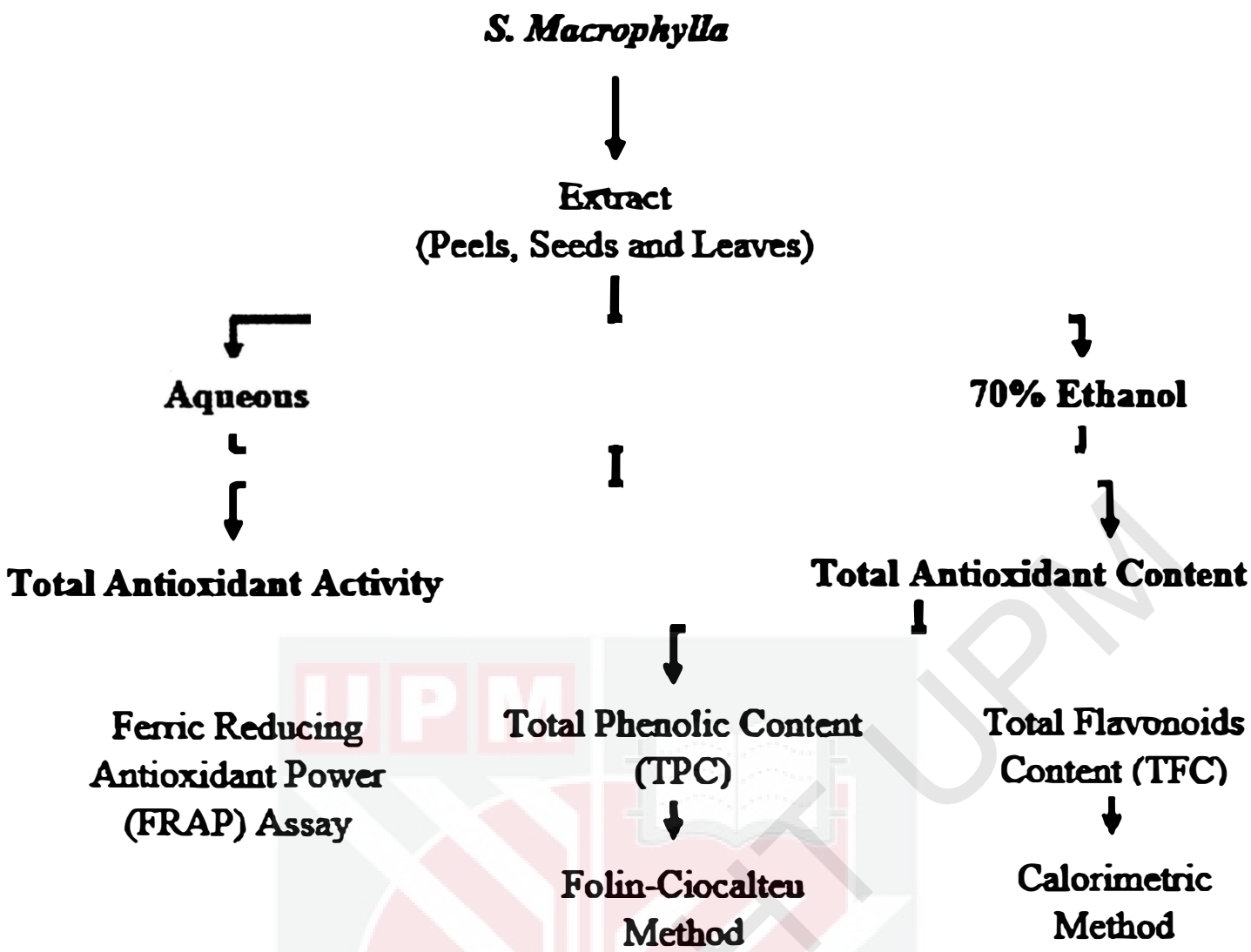

Figure 3.1: Study design of sample preparation of *S. macrophylla*.

3.4 Methods

3.4.1 Sample Preparation

The *S. macrophylla* samples were send to laboratory of Faculty of Medicine and Health Sciences in Universiti Putra Malaysia for analysis. Firstly, the samples were cleaned and thoroughly washed under running tap water. Secondly, the parts were separated into leaves, seeds, and peels. The inedible parts of the samples were removed. Then, the samples were weighed and freeze dried. After that, the dried samples were grounded into a fine particle size using a dry grinder. The dried samples were powdered using electrical blender. Finally, the samples were weighted and kept in an airtight container. The samples were stored in a freezer at -20 °C for further extraction.

3.4.2 Sample Extraction

3.4.2.1 Ethanol Extraction

For the 70% ethanol extraction, 125.0 ml of distilled water was added into 5.0 g of dried sample for extraction. Then, the mixture was shaking continuously for 2 hours at 50 °C using a temperature controlled orbital shaker. After that, the extract was filtered using Whatman No. 1 filter paper. Then, the extract was concentrate using rotary evaporator and dried using freeze dry. The filtrate was keep at -20 °C until further analysis. This procedure adapted from the method Wen et al., (2010) with slight modifications.

3.4.2.2 Aqueous Extraction

For the aqueous extraction, 125.0 ml of distilled water was added into 5.0 g of dried sample for extraction. Then, the mixture was shaking continuously for 2 hours at 50 °C using a temperature controlled orbital shaker. After that, the extract was filtered using Whatman No. 1 filter paper. Then, the extract was dried using freeze dry. The filtrate was keep at -20 °C until further analysis. This procedure adapted from the method Wen et al., (2010) with slight modifications.

3.4.3 Antioxidant Assay

3.4.3.1 Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric reducing scavenging activity was determined according to the procedure described by Yen, Duhand and Chaung (2000). Various concentrations of samples (100-400 μg/ml) were mixed with 2.5ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. then 2.5ml of 10% trichloroacetic acid (w/v) were added, 5 ml of

above solution was mixed with 5ml of distilled water and 1ml of 0.1% of ferric chloride. The absorbance was measured spectrophotometrically at 700 nm. Butylated hydroxytoluene (BHT) was used as standard. The measurements were run triplicate and results average.

3.4.4 Determination of Total Phenolic Content

The total phenolic content of each sample of *S. macrophylla* was determined by using Folin-Ciocalteu method as described by Singleton and Rossi, (1965). Briefly, 0.2 mL of the sample extract was mixed with 1.5 mL of Folin-Ciocalteu reagent was added and the mixture was then allowed to stand at room temperature for 5 min. After that 1.5 mL of sodium carbonate solution was added to the mixture and stirred. After 90 min at room temperature the absorbance was measured at 750 nm using a UV/Vis 1601 spectrophotometer (Shimadzu, Kyoto, Japan).

In this study, calibration curve was plotted with four different concentrations of gallic acid as standard ranging from 0.025 to 0.1 mg/mL versus their absorbance values. The linear equation from the calibration curve of gallic acid was then used to quantify the total phenolic content in samples. Total phenolic content for each sample were calculated from mean of triplicates and expressed as miligram (mg) gallic acid equivalent (GAE) per g dry weight (DW). Total Phenolic Content (TPC) of sample was calculated using formula:

$$\text{Total Phenolic Content (TPC)} = \frac{\text{TPC per ml sample x Dilution factor x Total sample volume used}}{\text{Sample weight}}$$

3.4.5 Determination of Total Flavonoid Content

The total flavonoid content was determined by $AlCl_3$ using the colorimetric method as describe by Bera, Chatterjee, and Ghosh (2015) with quercetin as the standard. The reaction mixture was prepared using 1 mL of extract, 4 mL of distilled water, and 0.3 mL of $NaNO_2$ and after 5 minutes, 3 mL of 10% $AlCl_3$ was added to the reaction mixture. After 5 minutes incubation, the absorbance was measured at 510 nm. The result was expressed in terms of mg quercetin equivalents (QE)/g of extract.

3.6 Statistical Analysis

Statistical analysis was performed using SPSS (Version 22.0 software). Results were expressed as mean values ± standard deviations. The results were analyzed statistically using One-way ANOVA analysis using Tukey test and Pearson correlations coefficient were used to determine whether there were correlations between total phenolic content and antioxidant activity in peel, seed and leaf of *S. macrophylla*. The level of significance of this study based on $p<0.05$. All experiments were performed triplicate.

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 Percentage Yield

Selection of an extraction solvent was one of the preliminary parameter before an extraction started.The extraction solvents that was commonly used were ethanol, methanol, hexane and acetone whilst inorganic extraction solvents included water (Wang et al., 2008). Aqueous and ethanol were the most commonly solvents used in plant extraction because of their low toxicity and safer to be used as compared to acetone, methanol and other organic solvent (Do et al., 2014). The amount of extraction yields was depended on the solvent and method of extraction.

The result in Table 4.1 shows the weight and the percentage yield for *S. macrophylla* in aqueous and ethanol extracts. From the Table 4.1, the highest yield of extracts was demonstrated in aqueous and ethanol extracts of *S. macrophylla* leaves with percentage of yield of 29.2% and 22.4%, respectively. The lowest extraction yields were obtained in peels of aqueous extracts (2.0%) and ethanol extracts (0.6%). of *S. macrophylla* Results found that the yields of aqueous extracts were varied in the range between 2.0% and 29.2% while the ethanol extracts yields were in the range between 0.6% and 22.4%.

Table 4.1: The weight and percentage yield of extract from the samples of *S. macrophylla*.

Samples	Extract	Weight (g)
Peels	Dried and ground material	5.00
	Aqueous extract	0.10 (2%)
	Ethanol extract	0.03 (0.6%)
Seeds	Dried and ground material	5.00
	Aqueous extract	0.71 (14.2%)
	Ethanol extract	0.74(14.8%)
Leaves	Dried and ground material	5.00
	Aqueous extract	1.46 (29.2%)
	Ethanol extract	1.12(22.4%)

For all the samples, the aqueous extracts give the most abundant yield (2% for peels and 29.2% for leaves) compared to ethanol extracts (0.6% for peels and 22.4% for leaves). The results for the yields are comparable with those reported in the literature using different extraction solvents. Chigayo, Mojapelo, Mnyakeni-Moleele, and Misihairabgwi, (2016) reported that percentage yield of *Kirkiawilmsii (K.wilmsii)* tubers for water extraction higher compared to ethanol extraction 20.9% and 7.3% respectively.

However, the ethanol extract of *S. macrophylla* seeds gives the higher yield which was 14.8% compared in aqueous extract 14.2%. This was due to bioactive compound that present *in S. macrophylla* seeds contained more non-polar because most of the yield extract from seeds contained fatty acid mostly compared to leaves and peels of *S. macrophylla* (Suliman et al., 2013).The different pattern was found in

the study reported by Tabart et al. (2009), water was a good extraction medium in extracting the corn polyphenols instead of using 70% ethanol. According to Wang et al., (2008), the extraction efficiency can be improved by selecting the optimum solvent-to-water ratio, which therefore can increase the extraction yield.

4.2 Total Phenolic Content

Table 4.2 shows the total phenolic content (TPC) of aqueous and ethanol extracts determined using Folin-Ciocalteu. In this study, TPC in *S. macrophylla* leaves for aqueous and ethanol extracts were found given better content of phenolic compound (16.85 mg GAE/g and 10.74 mg GAE/g sample) respectively compared to peels and seeds which was well-matched with the result previously since it has higher percentage yield.

Meanwhile, *S. macrophylla* seeds had lower phenolic content 0.56 mg GAE/g sample for aqueous extract and 0.95 mg GAE/g sample in the ethanol extract. It was also obtained that *S. macrophylla* leaves and seeds in ethanol extracts contained higher phenolic compounds compared with aqueous extract. The result was comparable to what was reported by Othman, Mukhtar, Ismail, and Chang, (2014) where TPC of *Melicope Lunuankeda* (locally known as tenggek burung) and *Polygonum minus* (locally known as kesum) were higher in ethanol extracts than in aqueous extract.

Table 4.2: Total phenolic content (TPC) of the peels, seeds and leaves of *S. macrophylla*.

Extraction Solvent	Total Phenolic Content (TPC) (mg GAE/g sample)
Aqueous	
Peels	6.61±0.07[a]
Seeds	0.56±0.03[a]
Leaves	10.74±0.08[b*]
Ethanol	
Peels	3.04±0.21[b]
Seeds	0.95±0.10[a]
Leaves	16.85±1.00[a*]

Results are expressed as mean ± standard deviation, SD (n=3). Values with different letter in the same column are significant ($p < 0.05$) between the parts of the same solvents and * indicates significant difference ($p < 0.05$) between the extraction solvents of the same parts.

For leaves, ethanol extract contained significantly ($p < 0.05$) higher TPC than aqueous extract. For seeds, there was no significant difference between ethanol and aqueous extracts. For peels, aqueous extract contained significantly ($p < 0.05$) higher TPC than ethanol extract. Between parts of the same solvent, leaves extracts contained significantly ($p < 0.05$) higher TPC than the peels and seeds extracts. From the results of TPC, it was clear that ethanol extracts exhibited a significantly higher TPC compared with aqueous extracts for most samples. This was because ethanol as

an organic solvent was able to denature polyphenol oxidases and was more efficient in degrading cell wall, thus able to extract more endocellular materials compared to water (Prior et al., 2005).

4.3 Total Flavonoids Content

The total flavonoid content (TFC) in peels, seeds and leaves of *S. macrophylla*were shown in Figure 4.3. The highest amount of flavonoid content was found in leaves for both ethanol and aqueous extracts (262.72 mg (QE)/g dry wt. and 206.61 mg QE/g dry wt.) respectively. The lowest flavonoid content was found in seeds of *S. macrophylla* 16.61 mg (QE)/g dry wt. and 8.83mg (QE)/g dry wt. respectively for ethanol and aqueous extracts.

Table 4.3: Total flavonoid content (TFC) of the peels, seeds and leaves of *S. macrophylla*.

Extraction Solvent	Total flavonoid content (TFC)
Aqueous	
Peels	185.5±13.02[a]
Seeds	8.83±6.67[a]
Leaves	206.61±5.36[b*]
Ethanol	
Peels	41.06±10.59[b]
Seeds	16.61±4.19[a]
Leaves	262.72±1.92[a*]

Results are expressed as mg (QE)/g DW and mean ± standard deviation, SD (n=3). Values with different letter in the same column are significant ($p < 0.05$) between the parts of the same solvents and * indicates significant difference ($p < 0.05$) between the extraction solvents of the same parts.

. Besides, the total flavonoid content for ethanol extract was higher compared to aqueous extract for leaves and seeds of *S. macrophylla* with 262.72 QE/g dry wt. and 16.61 QE/g dry wt. respectively. Previous study has been reported that green leafy vegetables and medicinal plants exhibited higher levels of flavonoid contents. Polyphenolic compounds such as flavonoids were widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (Molehin & Adefegha, 2013).

Between parts of the same solvent, leaves extracts contained significantly ($p < 0.05$) higher total flavonoid content than the peels and seeds extracts. Flavonoid was one of the phenolic components. Hence theoretically, values of phenolic content should be more than flavonoid content during quantification of phenolic and flavonoids. However, the result for total flavonoid content for this study was higher than total phenolic content due to different standard used for expressing the results which mg gallic acid equivalent for total phenolic content and mg quercetin for total flavonoid content. The content of phenolic and flavonoid follow the same trends which leaves was the highest followed by peels and seeds for aqueous and ethanol extract. Furthermore, all phenolics could not be estimated by single extraction or by any single method due to complexity of compounds.

4.4 Ferric Reducing Antioxidant Power (FRAP) Assay

Table 4.4 shows the FRAP values of peels, seeds and leaves of *S. macrophylla*. The leaves extract of *S. macrophylla* contained the highest total antioxidant capacity in both ethanol and aqueous extracts with 111.65 and 94.99 mmol/g dried sample respectively. The lowest total antioxidant capacity for both extracts can be detected in *S. macrophylla* seeds extracts with 0.50 and 3.32mmol/g dried sample for ethanol and aqueous extracts respectively. *S. macrophylla* leaves gave higher FRAP values than seeds and peels.

Table 4.4: Ferric reducing antioxidant power (FRAP) assay of the peels, seeds and leaves of *S. macrophylla*.

Extraction Solvent	Ferric reducing antioxidant power (FRAP)
Aqueous	
Peels	$91.03 \pm 0.24^{a*}$
Seeds	$3.32 \pm 0.49^{a*}$
Leaves	$94.99 \pm 0.25^{b*}$
Ethanol	
Peels	$1.34 \pm 0.24^{b*}$
Seeds	$0.50 \pm 0.25^{b*}$
Leaves	$111.65 \pm 0.00^{a*}$

Values are expressed as mmol FeSO4 equivalents per g sample DW and mean ± standard deviation, SD (n=3). Values with different letter in the same column are significant ($p < 0.05$) between the parts of the same solvents and * indicates significant difference ($p < 0.05$) between the extraction solvents of the same parts.

For ethanol extract, *S. macrophylla* leaves has FRAP value of 111.65mmol/g dried samples followed by peels with 1.34mmol/g dried samples and seeds with 0.50 mmol/g dried samples. There was a significant difference at $p < 0.05$ exist between *S. macrophylla* leaves with seeds and peels. For aqueous extract, *S. macrophylla* leaves also had the highest FRAP value of 94.99 mmol/g dried sample, followed peels with 91.03 mmol/g dried sample and seeds with 3.32 mmol/g dried sample. The finding of this present study complied with the research study reported by Ao et al (2008) that found out the high antioxidant capacity from leaves extract compared to bark and fruits extracts.

The results showed that FRAP values were higher in ethanol extract samples compared to aqueous extract for leaves and peels of *S. macrophylla*. This showed that ethanol extract was more efficient in extracting antioxidants in plant materials compared to water. However, the total antioxidant capacity for the *S. macrophylla* seeds was highest in aqueous extract compared to ethanol extract with 3.32 and 0.50mmol/g dried sample respectively. The result indicates that different parts of plants have different total antioxidant capacity.

The extracts that exhibit the high antioxidant showed that more ferrous tripridyltriazine complexes that produce during the reaction. Ferrous tripridyltriazine complexes were produced as product from the reaction in which samples had the ability to reduce from Fe^{3+} to Fe^{2+}. The greater Fe^{3+} reduced to Fe^{2+}, the higher total antioxidant activity will be observed.

4.5 Correlation Between Antioxidant Activity and Antioxidant Content

Table 4.5 shows the correlation between antioxidant activity and antioxidant content of the peels, seeds and leaves of *S. macrophylla*. Based on table 4.5, the contents of phenolics and flavonoids exhibited good correlation with FRAP (r=0.904 and r=0.987).The strong positive correlation (r = 0.93) between TPC and TFC with FRAP which was similar to the findings for gold mohar flower, leaves and bark extracts (Habir et al, 2011).A study by Piluzza and Bullitta, (2011) also showed linear correlations between TPC and antioxidant activity in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area.

Besides, Fernandes de Oliveira et al., (2012)also stated that the amount of TPC will affect the antioxidant capacity. In the study, four species of the *Malvaceae* family were used as sample to determine the TPC and FRAP assay to determine the antioxidant capacity. The results showed that there was a strong correlation between total polyphenol contents and antioxidant activity of the crude extract of *Sidastrummicranthum* and *Wissadulaperiplocifolia* where high TPC gives high antioxidant capacity.

Table 4.5: Correlation between the total antioxidant activity and total antioxidant content in peels, seeds and leaves of *S. macrophylla*.

		FRAP
TPC	r value	0.904**
TFC	r value	0.987**

**Correlation is significant at $p < 0.01$

Furthermore, Maisuthisakul et al. (2008) also revealed that plants with high antioxidant activities also have high total phenolic and flavonoid content. These was in agreement with our study that plants extracts which have high antioxidant activities possess high antioxidative compounds such as polyphenol, phenolic acid and flavonoid This suggests that phenolic compounds in plant extracts might have acted as powerful radical scavenging and reducing agents.

CHAPTER 5

5.0 CONCLUSIONS AND FUTURE RECOMMENDATIONS

5.1 Conclusions

Ethanol extracts of leaves and peels of *S macrophylla* parts showed highest antioxidant activity as compared to the aqueous extracts. Among the ethanol extracts of the different parts of *S. macrophylla*, the extracts of leaves signified best activities and can be recommended as a prophylactic agents against oxidative stress and infectious diseases. For the total phenolic content and total flavonoids contents, leaves also the highest compared to peels and seeds for both extracts. This shows that aqueous and ethanol extracts of *S. macrophylla* leaves contain total phenolic and flavonoids compounds and were capable of inhibiting, quenching free radicals to end the free radical chain reaction and acting as reducing agents.

The antioxidant activity of *S. macrophylla* and antioxidant content has very strong correlations between total phenolic content and ferric reducing antioxidant activity (FRAP) r= 0.904 and also total flavonoid content and FRAP r=0.987. These results indicate that phenolic compounds are the major contributors to antioxidant activity. Furthermore, various solvent having different polarities were employed for the extraction antioxidant components present in *S. macrophylla*. The present study

confirms the different in extraction efficiency of various solvent which affects the total antioxidant activity and total antioxidant content in peels, seeds and leaves of *S. macrophylla*. From the results of this study, it was suggested that solvents effect should be taken into account while addressing the antioxidant potential of any sample. The results of the antioxidative assays in the current study also suggest that *S. macrophylla* of ethanol extract can be good substitutes for the synthetic antioxidants, which are suspected for their negative impact on the health of human and animals.

5.2 Recommendations

Based on the result of present study, leaves of *S. macrophylla* contain highest total antioxidant activity and total antioxidant content for both aqueous and ethanol extracts compared to peels and seeds. Hence, further research need to be done on leaves to know more depth other possible health benefit in this *S. macrophylla* leaves. Besides, since, it contains considerable amount of antioxidant it should be recommended as a good source of health-promoting bioactive compounds compared to synthetic antioxidants.

Moreover, further researcher was suggested to use the same standard to compare the values between total phenolic content and total flavonoid content to have better comparison. From the result the lowest extraction yields were obtained in peels of aqueous extracts (2.0%) and ethanol extracts (0.6%). of *S. macrophylla.* Hence, further study can use other extraction method to have better and higher yield. In addition, different method of antioxidant assay can be used for the future study.in order to know which one is much better and efficient.

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