UNIVERSITI PUTRA MALAYSIA

TOTAL PHENOLIC CONTENT OF SHOREA SP. EXTRACTS

NORHIDAYAH MUHAMAD

TOTAL PHENOLIC CONTENT OF *SHOREA* SP. EXTRACTS

By

NORHIDAYAH BINTI MUHAMAD

A Project Report Submitted in Partial Fulfillment of the Requirement for the Degree of Bachelor Science Bioindustry in the Faculty of Agriculture and Food Sciences Universiti Putra Malaysia Bintulu Sarawak Campus

2014

ABSTRACT

The aim of this study is to determine the total phenolic content in five selected *Shorea* species which are *Shorea ovata*, *Shorea macrophylla*, *Shorea dasyphylla*, *Shorea parvifolia* and *Shorea venulosa*. This experiment used successive extraction method for the extraction process using three solvent of different polarity (hexane, chloroform and methanol). Folin Ciocalteu reagent was used to estimate the total phenolic content in methanol, hexane and chloroform extracts of five selected *Shorea* sp. Gallic acid was used as a standard compound and the total phenol were expressed as mg/g gallic acid equivalent (standard curve equation : $Y = 1.550X + 0.036 = R^2 : 0.995$). The plants samples were collected at rehabilitated forest, Universiti Putra Malaysia Bintulu Sarawak Campus (UPMKB). The chloroform gives higher percentage yield in plant extracts followed by methanol extracts and hexane extracts. The total phenolic content of *S. macrophylla* extracts were ranged from 1.19 to 12.22 mg/g gallic acid equivalent (GAE), *S. ovata* ranged from 37 to 38.44 mg/g GAE, *S. dasyphylla* ranged from 0.11 to 5.05 mg/g GAE, *S. parvifolia* ranged 1.32 to 12.03 mg/g GAE and *S. venulosa* ranged from 0.82 to 6.75 mg/g GAE. *S. ovata* leaves extracts contained good amount of phenolic content and could be used as natural sources of antioxidant.

ABSTRAK

Tujuan kajian dijalankan adalah untuk menilai jumlah kandungan fenolik di dalam lima spesies *Shorea* terpilih iaitu *Shorea ovata*, *Shorea macrophylla*, *Shorea dasyphylla*, *Shorea parvifolia* dan *Shorea venulosa*. Kajian ini menggunakan ekstrak secara berperingkat untuk proses pengekstraktan menggunakan tiga jenis pelarut yang berbeza kepolaran (heksana, kloroform dan methanol). Reagen Folin Ciocalteu digunakan untuk menganggar jumlah kandungan fenolik di dalam ekstrak metanol, heksana dan kloroform bagi lima spesies *Shorea* yang dipilih. Asid galik digunakan sebagai kompoun seragam and jumlah kandungan fenolik diungkapkan sebagai asid galik equivalent (persamaan lekuk piawai : $Y = 1.550X + 0.036 = R^2$: 0.995). Sampel tumbuhan telah diambil di hutan simpan Universiti Putra Malaysia Kampus Bintulu Sarawak (UPMKB). Kloroform ekstrak mempunyai peratusan hasil yang paling banyak diikuti olek ekstrak methanol dan ekstrak heksana. Jumlah kandungan fenolik bagi ekstrak *S. macrophylla* berjulat daripada 1.19 hingga 12.22 mg/g GAE, *S. ovata* berjulat dari 37 hingga 38.44 mg/g GAE, *S. dasyphylla* berjulat dari 0.11 hingga 5.05 mg/g GAE, *S. parvifolia* berjulat dari 1.32 hingga 12.03 mg/g GAE dan *S. venulosa* berjulat dari 0.82 hingga 6.75 mg/g GAE. Ekstrak daun *S. ovata* mengandungi jumlah kandungan fenolik yang bagus dan boleh digunakan sebagai sumber antioksidan semulajadi.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisor Prof. Madya Dr. Yiu Pang Hung for his patience, guidance and advice which enabled me to complete this challenging project.

Sincere thanks to my co supervisor Dr. Ong Kian Huat who provide guidance background knowledge of *Shorea* sp. as well as thesis writing. Besides appreciation are also extended to Miss Phene Neoh, Miss Lee Hui Hong, Miss Weng and Mr. Wong for helpful suggestion and valuable guidance on equipment handling and trouble shooting.

Special thanks are extended to Mr. Muaish bin Sait for helping me in the sample collection and to Mr. Mohd Zulkipli bin Saali, lab assistant of Biotechnology and Microbiology lab for his help in the access to laboratory facilities.

Appreciation are also to all the lab members in the Department of Basic Sciences and Engineering for their companionship, opinions and also the joy we had together, I wish to thank my parents and family members and friend for their support.

APPROVAL SHEET

I certify this this research project report entitled "Total Phenotic Content of Shorea Sp. Extracts" has been examinated and approved as a partial fulfillment of the requirement for the degree of Bachelor Science Bioindustry in the Faculty of Agriculture and Food Sciences, University Malaysia Bintulu Sarawak Campus.

_____

Prof. Madya Dr. Yiu Pang Hung
Faculty of Agriculture and Food Sciences
University Putra Malaysia Bintulu Sarawak Campus
(Supervisor)

_____

Prof. Dr. Japar Sidik bin Bujang
Dean
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia Bintulu Sarawak Campus

Date

TABLE OF CONTENTS

		Page
ABSTRACT		ii
ABSTRAK		iii
ACKNOWLEDGEMENTS		iv
APPROVAL SHEET		v
LIST OF TABLES		viii
CHAPTER		
1	INTRODUCTION	1
	1.1 Natural Product	1
	1.2 Potential of Plants in New Discovery of Medicine	2
	1.3 Present and Future of Plant Based Medicine in Market	3
	1.4 *Shorea* sp. in Malaysian Biodiversity	4
	1.5 Objective	6
2	LITERATURE REVIEW	7
	2.1 Biological Activities of *Shorea* sp.	7
	2.2 Antioxidant Activity of Plant	7
	2.2.1 Total Phenolic Content	8
	2.2.2 Total Phenolic Content of *Shorea* Plants	9
	2.2.3 Chemical Constituents	10
3	METHODOLOGY	12
	3.1 Plants Collection	12
	3.2 Plants Extraction	12
	3.2.1 Preparation	12
	3.2.2 Solvent Extraction	13
	3.3 Antioxidant Activity	13
	3.3.1 Total Phenolic Content (TPC)	13
4	RESULTS	15
	4.1 Effect of Different Solvent on the Percentage Yield of *Shorea* sp. Extracts	15
	4.2 Total Phenolic Content of the *Shorea* sp.	16

5	**DISCUSSION**	18
	5.1 Percentage Yield of the *Shorea* Plant Extracts	18
	5.2 Total Phenolic Content in *Shorea* sp. Extracts	20
6	**CONCLUSION**	22
	REFERENCES	23

LIST OF TABLES

Table		Page
4.1	Calibration of standard Gallic acid	16
4.2	Percentage yield of the plant extracts	17
4.3	Total phenolic content of the methanol, chloroform and hexane extract of *Shorea* sp.	18

CHAPTER 1

INTRODUCTION

1.1 Natural Product

A natural product is a chemical compound or substances produced by living organisms which is usually have pharmacological or biological activity for use in pharmaceutical drug discovery and drug design. The benefits of plants and plants extracts in the treatment and prevention of many leading health concern are historically well known and are becoming more widely studied and recognized. Natural products served humankind as the source of all drugs, and higher plants provided most of the therapeutic agents. Today, natural products and their derivatives still represent over 50% of all drugs in clinical used.

Natural products preparation has historically been the major source of pharmaceuticals agents. Analysis of FDA new drug approval from 1981 to 2002 reveals that natural products continued to play a vital role during that time, even if the industry had turned to other discovery strategies (Newman *et al*. 2003). Indeed, more than 90% of current therapeutic classes derive from a natural product prototype. Even today, roughly two thirds to three quarters of the world's populations relies upon medicinal plants for its primary pharmaceutical care. Those medicinal plants are either preparations of or natural products substances from plants that have potential utility as pharmaceutical agents (Balunas and Kinghorn

2005). Further evidence of the importance of natural products is based on the fact that most of the world 25 best selling pharmaceuticals in 1993 was the natural products and their derivatives (Balandrin and Lewis 1993).

1.2 Potential of Plant in New Discovery of Medicine

There is about more than 250 000 species of higher plants existing on this planet, and only a very small percentage of plants have been exhaustively studied for their potential value as source of drugs. Plants provide a large bank of rich, complex and highly varied structures which are unlikely to be synthesized in the laboratories. Plants have phytochemicals with various bioactivities. Currently, about 25% of the active component was identified from plants that are used as prescribed medicines (Gill *et al.* 2011).

Plants have always been a rich source of lead compounds. Lead compound is a compound that could potentially be converted to a new drug by optimizing its beneficial effects and minimizing its toxicity and side effects. Identifying lead compounds is the first step in the drug discovery process. Lead compounds that always present in the plant include alkaloids, morphine, cocaine, digitalis, tubocurarine, nicotine and muscarine. Many of these lead compounds are useful drugs. Clinically useful drugs which have been recently isolated from plants include the anticancer agent that is the paclitaxel from the yew tree and the antimalarial agent, artemisinin from *Artemisisa annua*.

Some examples of drugs from plants that served as a model for the next generation of drugs are as follow. Khellin from *Amni visnaga* (L.) Lamk was used as a bronchodilator in United States until it was shown to produce nausea and vomiting after prolonged use. In 1955, a group of chemist in England synthesis Khelinn analogs as potential bronchodilators with fewer side effects. This eventually led to the discovery of chromolyn used as sodium chromoglycate which stabilized cell membranes in the lungs to prevent the allergen induced release of the subtancess ultimately causing bronchoconstriction allergic in asthma patient. Further studies had led to the synthesis of amiodarone, a useful antianythmia agent (Sneader 1985).

1.3 Present and Future of Plant Based Medicine in Market

Obviously natural product will continue to be extremely important as sources of medicinal agents. In addition to the natural products which have been found direct medicinal application as drug entities, they also can serve as chemical models or templates for the design, synthesis and semi-synthesis of novel substances in order to treat the human diseases. Nowadays, there are new approaches to drugs discovery as for example the combinatorial chemistry and computer based molecular modeling design, none of them can replaced the important role play by the natural products in drug discovery and development.

Natural products have brighter future. Many significant advances in sciences and industry have been inspired by the pursuit of capturing the value of natural products. Treatment to the role natural products has played in the evolution of

organic synthesis. Efforts towards total synthesis of natural products have resulted in developments of new synthetic methods (Wilson and Danishefsky 2006), advances in the fields of medicinal chemistry, process chemistry and other aspect of the science of drug development. A number of advanced in capability and technology are becoming factor in advancing the natural products research and are directly or indirectly affects the development of natural products.

The most strong factor for the development of new natural products is the advancement in bioassay technology over the last several years (Littletonet *et al.* 2005). Currently, there is innovation of highly automated, very specific and selective bioassays. This is the factors that the natural products can be evaluated quickly and economically.

1.4 *Shorea* sp. in Malaysian Biodiversity

The dipterocarpaceae is a relatively large family of tropical plants consisting of 16 genera and approximately 600 species (Cronquist 1981). *Shorea* is the largest and most economically genus of the family of which 59 species are found in Peninsular Malaysia and at least 167 species in Borneo. This genus is widely distributed in the Southeast Asia Region, especially in Malaysia and Indonesia (Symington *et al.* 1974). In Peninsular Malaysia, *Shorea* is also known as Balau, Meranti Pa'ana, Meranti Damar Hitam and Meranti Merah. In this study, there are five selected *Shorea* sp. involved. They are *S. dasyphylla*, *S. venulosa*, *S. parvifolia*, *S. macrophylla*, and *S. ovata*.

The *Shorea* wood used for plants, building construction, furniture and plywood industry. Based on Wetphal and Batterman 2010, the resin in used for varnish glue, toreh fuel, medicine for diarrhoe, skin disease and dysentery. The people of South Asia utilized the *Shorea* plant resources for their various basic needs such as food, timber, fodder, medicine and raw materials. The *Shorea*'s timber is strong, elastic and durable and they are mainly used for construction, wimdow frames, planking, cart and carving. The wood used as firewood and to make charcoal.

The wood also used for planks, temporary constructions, packing case and furniture (Heyne 1987). Other than that, the leaves are lopped for fodder. They contain about 10% crude protein and the total digestible nutrients are 43%. The seeds can produce oil which usually used locally used locally as ointment oil. Then, after extraction of the oil, the cake is used to supplement cattle feed.

The resin of *Shorea* is used in indigenous medicine as an astringent and also used to treat diarrhea and dysentery (Shiva and Jantan 1998). Other than that, it also being used asan ingredient of ointment for skin diseases, ear troubles, toothaches, sore eyes, ulcers and wounds. Seeds of *Shorea* species especially under the red meranti groups produces illipe nute which are called "Engkabang". The The illipe fat extracted from the kernel is used in confectionary industry especially in the production of chocolate. Likewise, illipe fat can also be added to cosmatics such as lipticks.

1.5 Objective

The objective of this study is to screen the total phenolic content potential of Shorea

CHAPTER 2

LITERATURE REVIEW

1.1 Biological Activities of *Shorea* sp.

In natural product drug discovery, bioassay plays an important role. A bioassay will be applied to large amounts of initial sample to determine whether or not they have bioactivity of the desired type. This called the prescreening of biological activity. Through the screening process, bioassay will be used to select material for detailed individual study. Then bioassay will be used to guide fractionation of a crude material towards isolation of the pure bioactive compound in the monitoring process. For these purpose, bioassay test must be simple, rapid, reliable, reproducible, sensitive, meaningful and predictive.

2.2 Antioxidant Activity of Plant

Several plant extracts and chemical constituent have been found to show quite prominent antioxidant activity (Oseni and Akindahunsi 2011; Tripathi *et al*. 1996). According to Chu *et al*. (2002) the majority of the antioxidant activity may derived from the active substances such as flavonoid, isoflavone, flavones, anthocyanin, cetechin and iso-catechin.

Synthetic antioxidant are accepted as food additive, but the hazards and side effects are still in doubt. Synthetic vitamins are like any other synthetic molecules but because of their antioxidant nature, they are able to donate one electron, after which

they do not remain stable but are broken down in a metabolic process that yields hydrogen peroxide. Several synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have been extensively added to food and are commercially available but do not safe and their toxicity become problem (Ali and Nabavi 2007). Due to the problems, the research on the antioxidant activity of *Shorea* sp. done to determine the antioxidant in natural sources which is safer.

2.2.1 Total Phenolic Content

The antioxidant potential in the plant might due to the phenolic component present in the plant itself. The phenolic compounds prove the importance of antioxidant behavior and contribute significantly to the total activity of the medicinal and aromatic sample. Other than that total phenolic content of plants is a vital parameter for their antioxidant properties. The folin-ciocalteu has been used to measure total phenolic content in natural products.

There is increasing interest in the therapeutical potential of plants as antioxidant in reducing such free radical since it is belief that the antioxidant properties is the main contributory factor to the therapeutic benefit of many plants. Phenolic compound that mostly found in plant, have been reported to have several biological effects including antioxidant, antiapoptosis, anti aging, anti carcinogen and have highly considered for their important dietary roles as antioxidant and chemoprotective agents.

2.2.2 Total Phenolic Content of *Shorea* Plant

Based on the study done by Norizan and Wan (2012) found that antioxidant activities of these Malaysian dipterocarpacea can be used as potential sources of natural antiogidant. Among of all the *Shorea* extracts analyzed, *S. acuminata* showed the highest total phenolic content which is 2731.00 ± 0.09 mg GAE/100 g weight. This proved that *S. acuminata* contain high phenolic content and exhibited strong antioxidant potential. The result showed that all the *Shorea* sp. tested with TPC displayed high amount of phenolic with range of 2461-2731 mg/100 g GAE. Among the five *Shorea* tested, *S. acuminata* extracts displayed highest phenolic content. It then followed by *S. leprosula, S. resinosa, S. macroptera and S. bracteolata* have the lowest total phenolic content with 2434.08 ± 0.02mg/100 g which is considered very high. Most of the crude extracts of shorea gave total phenolic content range from 2461.54 ± 0.01 mg of GAE to 2731.00 ± 0.09 of GAE.

Shorea sp. plant extracts have antioxidant properties in which can bring benefits in prevention of human diseases. Antioxidant compound may function as free radical scavengers, complexing agents for pro-oxidants metals, as well as reducing agents and quenchers of singlet oxygen formation. Therefore, the importance of the search for natural antioxidants has greatly increased in recent years. A focus in plant derived polyphenols because of their potential antioxidant properties. Phenolic compounds exhibit considerable free-radicals scavenging activity, which is determined by their reactivity with other antioxidants and their metal chelating

properties as well as the stability of the resulting antioxidant derived radicals (Lee and Moss 2011).

The total phenolic contents could be regarded as an important indication of antioxidant properties of plants extracts (Wang *et al.* 2008). It is well known that plants contain many phenolic compounds which contain a hydroxyl group on an aromatic ring. These phenolic compounds interrupt oxidation chain by donating a hydrogen atom or chelating metals. So they act as reducing agents and antioxidants (Bursal and Ko Ksal 2010).

Free radicals are highly reactive and unstable compounds produced in the body during normal metabolic functions or introduced from the external environment such as pollution and cigarette smoke. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductant (Slater 1993). Human bodies are protected from oxidative damage of free radicals through some complex defense systems which are called antioxidants. Antioxidants works to maintain the oxidant at optimum level and to reduce free radical, stopping it from forming before it can disrupt living cells in our body

2.3.3 Chemical Constituents

Phytochemical studies of *Shorea* sp. showed that this genus is a source of Oligostilbenes such as resveratrol dimmers, trimers, tetramers, hexamers, heptamers and octamers (Ito 2011).

These compounds exhibited a variety of significant bioactivities including antibacterial (Nitta *et al*. 2002), anti fungal (Kusuma and Tachibana 2007; Bokel *et al*. 1988) and anti tumour (Jang *et al*. 1997).

There is a research conduct on chemotaxonomic of *Shorea* of South Asia, in this study flavonoid, aglycones and four flavonoid glycosidea (quercetin 3-glucoside, quercetin 3-rutinoside, kaempferol 3,5-glucoside and luteolin 7-glucoside) were successfully isolated from the leaves of *Shorea* using standard procedures after separation and purification by paper chromatography in several solvent systems. The isolated flavanoids can be used as chemotaxonomic markers and also as active ingredients of many medicines.

The pioneer study on flavonoid aglycone patterns of the species of *Shorea* was carried out by (Bate-Smith and Whitemore 1959). He had reported that flavonoid, aglycones allagic acis and some unidentified fluorescent compounds on 10 species of *Shorea* from Malaysia.

CHAPTER 3

METHODOLOGY

3.1 Plants Collection

Fresh leaves of five selected *Shorea* sp. were collected at Phase 1 and Phase 4 rehabilitated forest, Universiti Putra Malaysia Bintulu Sarawak Campus (UPMKB). They were *S. dasyphylla* (03^0 12.646' N to 113^0 03.414'E), *S. venulosa* (03^0 12.741' N to 113^0 03.385'E), *S. parvifolia* (03^0 12.659' N to 113^0 03.396 E) of Phase 1 rehabilitated forest, UPMKB, *S. macrophylla* (03^0 12.787'N to 113^0 04.290' E) and *S. ovata* (03^0 12.780' N to 113^0 04.290') of Phase 4 rehabilitated forest, UPMKB.

3.2 Plant Extraction

3.2.1 Preparation

Fresh leaves were cleaned by mechanically technique to remove all the unwanted foreign material attached to the leaves. Then, they were air dried for 14 days at room temperature. The air-dried samples were ground into coarse powder form using laboratory blender and kept in airtight container at room temperature.

3.2.2 Solvent Extraction

Plant extract were extracted by using organic solvent in 1:10 (w/v) ratio of plant materials to solvent for maceration. Thirty grams of powdered sample were macerated in a conical flask with 300 ml of chloroform, methanol and hexane respectively on the orbital shaker for about 48 hours at 150 rpm. The mixture were

then filtered by using filter paper and the filtrate were then concentrated to a small volume to remove the organic solvent by using rotary evaporator. After that, the concentrated extract was air dried and kept in universal bottle wrapped with aluminium foil and parafilm before stored at 4^0C.

3.3 Antioxidant Activity

3.3.1 Total Phenolic Content (TPC)

The TPC content was determined spectrophotometrically according to the Folin Ciocalteu's method (Lachman *et al.* 1998) with slight modification. The crude extract (0.5 mL) were pipette into a 10 mL volumetric flask containing 0.5 mL of Folin Ciocalteu's reagent, The 0.01 g of crude extracts were appropriately diluted with 5 mL of methanol and 0.1 mL of the diluted sample was added into the Folin-Ciocalteu reagent. The flask was placed about 20 minutes in a dark place. Then 1.5 mL of a 20% Na_2CO_3 solution was added to the sample. The solution was immediately marked up with distilled water and mixed thoroughly by shaking the flask.

The Folin Ciacalteu's reagent were reduced to blue coloured molybdenum and tungsten oxides. The absorbance was measured at wavelength 760 nm using UV-visible spectrophotometer after incubation for two hours in the dark at room temperature. Total phenolic content determined as mg gallic acid equivalent (GAE)/100 g of sample using the formula C=cV/M where, C is the total content

compounds in mg/g GAE, c is the concentration of gallic acid (mg/mL) establish from the calibration curve, V is the volume of extracts and m is the weight of pure methanolic extracts.

CHAPTER 4

RESULTS

4.1 Effect of Different Solvent on the Percentage Yield of *Shorea* sp. Extracts

Table 4.1 showed the calibration of standard gallic acid. This was used as the standard in measuring the total phenol of the selected *Shorea* sp. Based on the Table 4.2, the extract of five different *Shorea* sp. were obtain after being soaked in three different solvent and undergo extraction process using rotary evaporator. Plant soaked in the methanol tends to have higher extract, followed by the plant soaked in the chloroform. The plant soaked in the hexane obtained the lowest extract. This finding proved that the different solvent affect the percentage yields of the plant extracts. Other than this, the selection of the solvent also depends on the cost, suitability and also the effect of solvent on health and environment. This yield also sometimes influence by the handling of rotary evaporator. Error while operating it can affect the yield gained.

Table 4.1 Calibration of standard gallic acid

Concentration (mg/mL)	Absorbance (760.00 nm)
0.000	0.013
0.031	0.088
0.063	0.129
0.125	0.209
0.250	0.426
0.500	0.895
1.000	1.547

Regression equation, R^2 : 0.99486 , Y = 1.5501 X + 0.036

Table 4.2 Percentage yield of the plant extracts

Species	Solvent	Initial weight(g)	Final weight (g)	Extraction yield (%)
S. venulosa	Chloroform	30.0	11.0	36.67
	Methanol	30.0	10.6	35.33
	Hexane	30.0	1.0	3.33
S. ovata	Chloroform	30.0	10.0	33.33
	Methanol	30.0	10.2	34.00
	Hexane	30.0	0.50	1.67
S. dasyphylla	Chloroform	30.0	5.30	17.67
	Methanol	30.0	5.90	19.67
	Hexane	30.0	1.50	5.00
S. parvifolia	Chloroform	30.0	2.20	7.33
	Methanol	30.0	6.50	21.67
	Hexane	30.0	0.70	2.33
S. macrophylla	Chloroform	30.0	0.80	2.67
	Methanol	30.0	7.30	24.33
	Hexane	30.0	2.60	8.67

4.2 The Total Phenolic Content of the *Shorea* sp.

The total phenolic content of five different *Shorea* species is shown in the table 4.3. The mean for the Gallic acid quantification standard was 0.4716 ± 0.2114. Among the five different species of *Shorea*, the *S. ovata* showed highest total phenolic content in hexane (37.00 ± 0.25), chloroform (38.44 ± 0.24) and methanol (37.71 ± 0.01). This was followed by *S. macrophylla* with 12.22 ± 0.12, 1.19 ± 0.42 and 2.60 ± 0.01 in the chloroform, methanol and hexane extracts respectively. Meanwhile total phenolic content in the *S. dasyphylla* was 5.05 ± 0.06, 0.72 ± 0.03, and 0.11 ± 0.01 in chloroform, methanol and hexane extracts. For the *S. parvifolia,* the total phenolic content in chloroform (12.03 ± 0.21), methanol (1.32 ± 0.82) and hexane (2.77 ± 0.32). The total phenolic content in *S. venulosa* is 3.87 ± 0.03, 6.75 ± 0.03 and 0.82 ± 0.04 in chloroform, methanol and hexane extracts respectively.

Table 4.3 Total phenolic content of the methanol, chloroform and hexane extract of *Shorea* sp.

Species	Solvent	Phenolic Content (mg GAE/g of Extract)
S. macrophylla	Chloroform	12.22 ± 0.12
	Hexane	2.60 ± 0.01
	Methanol	1.19 ± 0.42
S. ovata	Chloroform	38.44 ± 0.24
	Hexane	37.00 ± 0.25
	Methanol	37.71 ± 0.01
S. dasyphylla	Chloroform	5.05 ± 0.06
	Hexane	0.11 ± 0.01
	Methanol	0.72 ± 0.03
S. parvifolia	Chloroform	12.03 ± 0.21
	Hexane	2.77 ± 0.32
	Methanol	1.32 ± 0.82
S. venulosa	Chloroform	3.87 ± 0.03
	Hexane	0.82 ± 0.04
	Methanol	6.75 ± 0.03

CHAPTER 5

DISCUSSION

5.1 Percentage Yield of the *Shorea* Plant Extracts

Chloroform, hexane and methanol extracts were prepared to examine the total phenolic content. The yield of extracts obtained from 30g of dry plant material was measured for each extract (Table 4.2). The highest yield of extract was obtained by using methanol as extraction solvent.

Solvent is a liquid act as the medium for a reaction. It function to dissolves the reactant (Ashenhurst 2012). In this experiment, three types of solvent were used. They are the hexane, chloroform and methane. All of the three solvents have different polarity. Polar solvent have large partial charges and contain bonds between atoms with very different electro negativity. Meanwhile, the non polar solvent contains bonds between atoms with similar electro negativities. This will lack partial charges. The absences of charge makes the molecules non polar.

The main function of solvents in a reaction step is solubilization. As the reaction media, solvents make solutes more reactive by breaking cohesive forces that hold crystalline and liquid solutes together. By selecting the solvents, manipulation of the reaction rate and in consequence product selectivity can be performed. The understanding about the properties of solvent and its chemical behavior is important.

In the plants extraction process, the rotary evaporator is used as tool. This is because it is the fastest, most efficient and most environmentally friendly way to remove a volatile solvent from a non-volatile sample. It is standard equipment in a modern chemistry laboratory. The rotary evaporator works by increasing the rate of evaporation of the solvent by reducing the pressure to lower the solvent boiling point, rotating the sample to increase the effective surface area and heating the solution.

Methanol is commonly used because it is relatively inexpensive, lots of compounds dissolve in it, relatively free of regulation compared to other chemical, and can easily evaporated (Silver 2014). Methanol is the best choices for the plant extraction. Methanol is an amphiphilic compound and helps to extracts all the various chemical groups from the plant material.

Broad range of solvents has been used for the extraction of bioactive compounds from plant materials. Studies have shown that the extraction yield is dependent on the solvent and the method of extraction. The extraction must allow complete extraction of the compounds of interest and it must avoid their chemical modification (Hayouni *et al.* 2007).

The extraction yields depend on solvents, time and temperature of extraction as well as the chemical nature of the sample. Under the same time and temperature

conditions, the solvent used the chemical property of the sample are the two most important factors (Shimada *et al.* 1992)

5.2 Total Phenolic Content in *Shorea* sp. Extracts

The amount of total phenol was determined using Folin – Ciocalteu reagent. Gallic acid was used as the standard reference and the total phenolic contents were expre sed as mg/g gallic acid equivalent in the equation of, $Y = 1.5501 X + 0.0363$ ($R^2 = 0.99486$) where Y was the absorbance at 760 nm and X was the total phenolic content in the different extract of *Shorea* sp. expressed in mg/gm.

In this study, the mean of total phenol content varied from 38.44 to 0.11. This showed that some of the selected *Shorea* sp. have high total phenolic content in the extracts. It is well known that there is strong relationship between total phenol content and antioxidant activity. This is due to the phenol posses strong scavenging ability for free radical due to their hydroxyl groups. Therefore, the phenol content of plants may directly contribute to their antioxidant activity (Wojdylo *et al.* 2007).

Shorea extract showed high amount of total phenolic content. This might due to the presence of the phenolics contents from secondary plants metabolites in every plants and plant productst. Plant with high total phenolic content may possed high antioxidant activity. Phenolic compounds presents in the plants acting as antioxidant or free radicals scavenger (Kahkonen and Rauha 1999) due to their hydroxyl groups which contribute directly to the antioxidative action.

Phenolic compounds are effective hydrogen donors, making them good antioxidants (Rice-Evan and Miller 1997). Phenolics presents in the *Shorea* extracts contain considerable amount of phenolic which had a good antioxidant potential.

The *S. ovata* extracts have the highest concentration of phenols and this means that the extracts perform highest antioxidant activity. Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore, the phenolic contents of plants may contribute directly to their antioxidant action.

It was previously reported that non phenolic antioxidant might also contribute to the antioxidant activity of plants extracts (Harish and Shivanandappa 2006). Thus, compounds other than phenolic might be responsible for the pronounced antioxidant activity observed with the plants extracts. Further investigation requires as compounds other than phenolic might also be responsible for the antioxidant activity.

Polyphenolics compounds are believed to have chemopreventive and suppressive activities against cancer cells by inhibition of metabolic enzymes involved in the activation of potential carcinogens or arresting the cell cycle. Further studies are therefore required to detect potential anticancer activities of the *Shorea* sp. extracts.

CHAPTER 6

CONCLUSION

The result obtained in the present study indicate that the *Shorea ovata* leaves extracts contain high amount of phenolic content and can be used as natural source of antioxidant that could have great importance as therapeutic agents in preventing or slowing the process of aging and age associated oxidative stress related degenerative diseases. Result of the study suggests the high value of the *S. ovata* species have potential for use in pharmacy and phytotheraphy. Based on this information, it could be concluded that this plant is natural sources of antioxidant substances of high importance.

It is noticed that the highest concentration of phenolic compounds in the extracts were obtained using solvent of high polarity which is the methanol. The methanolic extracts manifested greater power of extraction for phenolic compound in the *Shorea* sp. Further studies of this plant species should be directed to studies of its medicinal active components in order to prepare a natural pharmaceutical products of high value.

REFERENCES

Ali, M. and Nabavi, S. 2007. Antioxidant and free radical scavenging activity of *H. officinalis L. Var. angstifolius, V. odorata, B. hyrcana* and *C. speciosum*. *Journal of Pharmaceutical Science*, 23(1): 29-34.

Ashenhurst, J. 2012. Understanding the electron flow. *Organic Chemistry*, 23(4): 234-241.

Balandrin, M. and Lewis, J.A. 1993. Human Medicinal Agents from Plants. *ACS Symposium Series*, 34(4): 48 – 55.

Balunas, J. and Kinghorn, A. 2005. Drug discovery from medicinal plants. *Life Science*, 78(2): 431-441.

Bate-Smith, E.C, and Whitmore, T.C. 1959. *Chemistry and Taxonomy in the Dipterocarpaceae*. London: Nature.

Bokel, M., Diyasena, M.C., Gunatilaka, L., Kraus, W. and Sotheeswaran, S. 1988. Caliculatol, an antifungal resveratrol trimer from *Stemonoporus canaliculatus*. *Phytochemistry*, 27(3): 377-380.

Bursal, E. and Ko Ksal, E. 2010. A brief review on bioactive compounds in plants. Bioactive compounds in plants. *Food Chemistry,* 52(3): 1-22.

Chu, Y.F., Sun, J., Wu, X. and Liu, R.2002.Antioxidant and antiproliferative activities of common vegetables. *Journal Agricultural and Food Chemistry*, 50(3): 6910-6916.

Cronquist, A. 1981. *An Integrated System of Classification of Flowering Plants*. New York: Columbia University Press.

Gill, L., Suprava, S. and Goutama, G. 2011. Phytochemical investigation and in vitro antioxidant activity of an indigeneous medicinal plant *Alpinia nigra* B.L. *Asian Pacific Journal of Tropical Medicine*, 3(11): 871-876.

Harish, R. and Shivanandappa, T. 2006. Antioxidant activity and hypatoprotective potential of *Phyllanthus niruri. Food Chemistry*, 63(4): 446-448.

Hayouni, M., Manaf, A. and Moktar, H. 2007. The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of *Tunisian Quercus coccifera* L. *Food Chemistry*, 105(20): 1126– 1130.

Heyne, K. 1987. *Tumbuhan Berguna Indonesia*. Jakarta: Badan Litbang Kehutanan.

Ito, T. 2011. Structures of oligostilbenoids in dipterocarpaceaeous plants and their biological activities. *Yakugaku zasshi. Journal of Pharmaceutical Society of Japan*, 13(4): 93-100.

Jang, M., Cai, L., Udeani, G.O., Slowing, K.V., Thomas, C.F., Beecher, C.W., Fong, H.H.S., Farnsworth, N.R., Kinghorn, A.D., Mehta, R.G., Moon, R.C. and Pezzuto, J.M. 1997. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science*. 275(13): 218-220.

Kahkonen, M.P. and Rauha, J. 1999. Antioxidant activity of plant extracts containing phenolic compounds. *Journal Agricultural and Food Chemistry*, 47(23): 3954-3962.

Kusuma, I.W. and Tachibana, S. 2007. Antifungal compounds isolated from tropical and temperate woods. *ACS Symposium Series*, 95(4): 377-390.

Lachman, J., Hosnedl, V., Pivec, V. and Orsak, M. 1998 . Sample preparation methods for the determination of the antioxidative capacity of apple juices. *Food Chemistry*, 84(17): 435–438.

Lee, M.K. and Moss, S.J . 2011. Herbal medicines and preoperative care. *The Journal of the American Medical Association*, 286(5): 208-216.

Littletonet, Palfreyman and J orgensen. 2005. *In Vivo Analysis of Membrane Fusion. In: Encyclopedia of Life Sciences*. Chichester: J ohnWiley and Sons, Ltd.

Newman, J .,Gordon, M. and Kenneth, M. 2003. Natural products as sources of new drugs over the period 1981-2002. *Journal of Natural Products*, 66(2): 1022-1037.

Nitta, T., Arai, T., Takamatsu, H., Inatom, Y., Murata, H., Iinuma, M., Tanaka, T., Ito, T., Asai, F. and Watabe, K. 2002. Antibacterial activity of extracts prepared from tropical and subtropical plants on methicillin-resistant *Staphylococcus aureus*. *Journal of Health Science*, 48(4): 273-276.

Norizan, A. and Wan, Z. 2012. Antioxidant and antimicrobial studies on selected species of *Dipterocarpaceae* from Uitm forest reserve, J engka,Pahang. *Journal of Natural Products*, 12(2): 36-42.

Oseni, O.A. and Akindahunsi, A.A. 2011. Some phytochemical properties and effect of fermentation on the seed of *Jatropa curcas* L. *Journal of Food Technology*, 6(3): 158-165.

Rice-Evan, C.A. and Miler, N. 1997. Antioxidant property of phenolic compounds. *Trends in Plant Science*, 2(1): 152-159.

Shimada, K. Fujikawa, Nakamura, T. 1992. Evaluation of the antioxidant and antibacterial properties of various solvents extracts of *Annona squamosa* L. leaves. *Journal of Agricultural and Food Chemistry*, 40(12): 945–948.

Shiva, M.P., Jantan, I. 1998. Non-Timber Forest Product from Dipterocarps. *In*: Appanah, S. and Turnbull, M.J. eds. *A Review of Dipterocarps: Taxonomy, Ecology and Silviculture*. Jakarta: Center for International Forestry Research.

Silver, J. 2014. Solvent and purification review. *Bioorganic and Medicinal Chemistry*, 15(2): 6882–6892.

Slater, K. 1993. Extracellular superoxise dismutase in biology and medicine. *Free Radical Biology Medicine*, 35(12): 236–256.

Sneader, W. 1985. *Drug Discovery: The Evolution of Modern Medicines by Walter*. New York: Wiley and Sons Incorporated.

Symington, N., Hakim, E.H., Makmur, L., Sakai, K. and Shimizu, K. 1974. *Foresters Manuals of Dipterocarps*. Kuala Lumpur: Universiti Malaya.

Tripathi, Y.B., Chaurasia, S., Tripathi, E., Upadhyav, A. and Dubey, G.P. 1996. Bacopa monniera Linn. As an antioxidant: Mechanism of action. *Indian Journal of Experimental Biology*, 34(12): 523-526.

Wang, H., Gao, X.D., Zhou, G.G., Cai, L. and Yao, W.B. 2008. In vitro an in vivo antioxidant activity of aqueous extract from *Choerospondias axillaris* fruit. *Food Chemistry*, 106(24): 888-895.

Westphal, P.and Battermann, M. 2010. Shampoos and hair conditioners containing fine wood extracts. *African Journal of Pharmacy and Pharmacology*, 32(12): 26-27.

Wilson, K. and Danishefsky, J. 2006. Small molecule natural products in the discover of therapeutic agents: The synthesis connection. *Journal of Organic Chemistry*, 71(22): 8329-8351.

Wojdylo, A., Oszmianski, J. and Czemerys, R. 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, 105(34): 940-949.

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled ''Total Phenolic Content of *Shorea* sp. Extracts" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus and published in the form approved by the Faculty.

_____

Norhidayah Bt. Muhamad
Date: