MORINGA OLEIFERA AS A POTENTIAL SOURCE OF LECITHIN

LEONG SIEW YIN

Report submitted in partial fulfillment
of the requirements for the award of Bachelor of Applied
Science (Honours) in Industrial Chemistry

Faculty of Industrial Sciences & Technology
UNIVERSITI MALAYSIA PAHANG

2012

ABSTRACT

Lecithin in strict scientific term refers to phosphatidylcholine. Lecithin which proves to provide sufficient amount of essential nutrients for the cells and biological functions of the body is also used in the food applications as food additives and stabilizers. The valuable properties of lecithin have created great demand and thus, attempt for new resources of lecithin from other plants has been done beside lecithin derived from soy beans. *Moringa oleifera* have been known for its rich sources of nutrition values and uses in curing much kind of diseases are found grown in tropical and humid climates areas. Fixed oils from the *M. oleifera* seeds are extracted using Soxhlet extraction with hexane solvent. Results showed that the solvent-extracted mature *M. oleifera* seeds have highly of 30% oil, the immature *M. oleifera* seeds have 25.0% oil, and the soybean seeds have 19.5% of oil. Compare to the cold maceration extracted method, the mature *M. oleifera* seeds have 25.4% of oil, the immature *M. oleifera* seeds have 20.2% of oil, and the soybean seeds have16.7% of oil. The percentage of total lecithin fraction (weight of pellet/weight of oil) showed that mature *M. oleifera* oil has 6.07%, immature *M. oleifera* has 4.96%, the soybean oil has 8.14% and the commercial soybean has 5.75% of phospholipids. The separation of individual phospholipid components of Moringa and soybean lecithin fraction were carried out using thin layer chromatography (TLC). The result showed Moringa lecithin contained 4-5 individual phospholipid and the presence of C=C group of fatty acid was found presence in Lane 1 and 2 of separated lecithin fraction of mature Moringa lecithin. Fatty acid compositions of Moringa seed oil, commercial soybean oil and Moringa lecithin (mature and immature) were determined using gas liquid chromatography (GLC). The result showed the relative percentage of fatty acid composition of Moringa oil (mature and immature) has higher stearic acid and oleic acid compare to the soybean oil as reported by a literature.

TRANSLATION OF ABSTRACT

Lecithin dalam istilah saintifik merujuk kepada phosphatidylcholine. Lecithin telah dibuktikan mempunyai jumlah nutrien yang mencukupi penting untuk sel-sel dan fungsi biologi badan juga digunakan dalam bidang aplikasi makanan sebagai bahan tambahan dan penstabil makanan. Kandungan zat lesitin yang berharga telah mendapat permintaan yang tinggi dan dengan ini,usaha untuk mencari sumber-sumber baru untuk mengantikan lesitin yang diperolehi dari kacang soya harus dijalankan. *Moringa oleifera* yang dikenali dengan sumber-sumber nutrisinya yang kaya telahdigunakan untuk menyembuhpelbagai jenis penyakit didapati tumbuh di kawasan iklim tropika dan lembap. Minyak dari biji benih *M. oleifera* diekstrak dengan menggunakan hexane sebagai pelarut dalam pengekstrakan Soxhlet dengan pelarut heksana. Keputusan menunjukkan bahawa pengestrakan soxhlet menggunakanpelarut hexane untuk biji *M. oleifera* yang matangmempunyai kandungan minyak sebanyak 30%, benih M. oleifera yang belum matang mempunyai kandungan minyak 25.0%, dan benih kacang soya mempunyai 19.5% kandungan minyak. Berbanding dengan pengestrakan mengunakan chloroform:methanol dalm nisbah 2:1, biji benih *M.oleifera* matang mempunyai kandungan minyak 25.4% biji benih *M.oleifera* yang belum matang mempunyai 20.2% minyak, dan benih kacang soya mempunyai kandungan minyak 16.7% .Jumlah peratusan kandungan phopholipid (berat pellet / berat minyak) bagi minyak *M. oleifera* yang matang ialah 6.07%, minyak *M. oleifera* yang belum matang ialah 4.96%, minyak kacang soya ialah 8.14% dan minyak kacang soya komersil ialah 5.75%. Pemisahan lecithin Moringa dan kcang soya kepada setiap individu komponen phospholipid dijalankan dengan menggunakan kromatografi lapisan nipis (TLC). Keputusan menunjukan bahawa lesitin Moringa mengandungi 4-5 individu komponen phospholipid dan Lane 1 dan 2 daripda pembahagian individu lecithin dari biji Moringa yang matang.menunjukkan kehadiran kumpulan asid lemak C = C. Penentuan komposisi asid lemak yang hadir daripada minyak biji Moringa, minyak kacang soya komersil dan lesitin Moringa (matang dan tidak matang) telah dianalysis dengan menggunakan gas kromatografi cecair (GLC). Keputusan menunjukkan peratusan komposisi relatif asid lemak daripada minyak Moringa (matang dan tidak matang) mempunyai kandungan asid stearik dan asid oleik yang tinggi berbanding dengan minyak kacang soya seperti yang dilaporkan dalam kesusasteraan.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

^{o}E	East
^{o}N	North
>	more than
<	less than
%	percent
g	gram
m	meter
cm	centimeter
~	close to
mg	milligram
/	slash
^{0}C	degree celsius
±	plus minus
ml	milliliter
v/v	volume per volume
rpm	revolutions per minute
N	normality
mm	millimeter
µm	micrometer
µl	microlitre
w/w	weight per weight

LIST OF ABBREVIATIONS

PC	phosphatidylcholine
PE	phosphatidylethanolamine
PI	phosphatidylinositol
PA	phosphatidic acid
TLC	Thin Layer Chromatography
I	Iodine
O/W	oil-in-water
W/O	water- in- oil
W/O/W	water-in-oil-in-water
FAME	Fatty acid methyl ester
GC-FID	Gas chromatography Flame Ionization Detector
FTIR	Fourier Transform Infrared spectroscopy
C=C	unsaturated carbon bond
GLC	Gas Liquid Chromatography
IR	Infrared

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 OVERVIEW OF RESEARCH

The major phospholipids in lecithin are phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) where they have several characteristics in common, such as the presence of two fatty acids and the phosphoric group (Kuligowski et al., 2008). Lecithin, as a natural mixture, has moisturizing and natural emulsifier properties. It is generally non-irritating, non-sensitizing and safe to be used in leave-on products at concentrations up to 15%, the highest concentration tested in clinical irritation and sensitization studies (Fiume, 2001). Lecithin has importance in biological, pharmaceutical, and cosmetic applications (Weete et al., 1994), especially in the form of liposome. The liposomal form is used in cosmetics to increase active compound concentration in the deeper layers of the skin (Naglic et al., 1997).

Egg yolks (Maximiano et al., 2008) and soybean (Patil et al., 2009) has been used as lecithin sources. Currently, soybean oil is the primary and largest source of

commercial lecithin (Szuhaj et al.,1985) and are widely used as natural emulsifiers, wetting agents and baking improvers due to its high phospholipids content (Gunstone et al., 1994). The demand on lecithin has been increasing, in these recent years with numerous applications in dietetics, cosmetics and pharmaceuticals have been reported (Pardun, 1989). Lecithin can be found, together with soybean oil, at percentage concentration levels in a number of dietary products (Kuligowski et al., 2008).On the other hand, special characteristic of lecithin as naturally occurring phospholipids in skin and other biological membrane (Shono et al., 1979), causes highly demand application in cosmetic and refined grade lecithin (containing up to 99.7 g/100 g of phospholipids) is used for pharmaceutical applications and research (Ramadan , 2008).

The recognition that Moringa oil has value in cosmetics has increased interest in cultivating it for seed-oil (Foidl et al., 2001). Moringa seed contains 35–45% oil which is considered a great natural cosmetic emollient based on its tactile properties, almost total natural absence of color and odor, and high oleic acid concentration (>73%) and the low content of polyunsaturated fatty acids (<1%) gives to the oil remarkable oxidative stability (Lalas et al., 2002) and (Kleiman et al., 2008).

1.2 PROBLEM STATEMENT

Although soybean is the most studied source of lecithin, there is little or no research so far on lecithin of *Moringa oleifera* oil especially that of Malaysian Moringa. This research will focus on the fixed oil of *Moringa oleifera* which is of the potential use as emulsifier and cosmetic purposes.

1.3 OBJECTIVE OF STUDY

The objectives of studies are as follows:

a) To determine the percentage of oil content of *Moringa oleifera* and other seeds.

b) To determine the percentage of phospholipids from mature Moringa oil, immature Moringa oil, soybean oil and commercial soybean oil.

c) To separate the individual phospholipid components of Moringa and soybean lecithin fractions using thin layer chromatography (TLC).

d) To determine the fatty acid composition presence in Moringa seeds oil, commercial soybean oil, and Moringa lecithin.

CHAPTER 2

LITERATURE REVIEW

2.1 *Moringa oleifera*

Moringa oleifera is the best known of 13 species in genus Moringa which belong to the family of Moringaceae. These are *M. oleifera, M.arborea, M.borziana, M. concanensis, M. drouhardii, M. hildebrandtii, M. longituba, M. ovalifolia, M. peregrine, M. pygmaea, M. rivea, M. ruspoliana and M. stenopetala* (Mahmood et al., 2010). *M. oleifera* is known by its common name as drumstick tree, sajiwan, kelor, murungai kaai, saijhan and sajna. *M. oleifera* tree is native to sub-Himalayan of India, Pakistan, Bangladesh, and Afghanistan. The distribution of the drumstick tree is indigenous to the Himalayan foothills of South Asia from northeastern Pakistan (33 °N, 73 °E) to northern West Bengal State in India and northeastern Bangladesh where it is commonly found from sea level to 1,400 m on land or near riverbeds and streams (Ramachandran et al.,1980). Today it is widely cultivated in many locations of the tropics and being grown in the West, East and South Africa, tropical Asia, Latin America, the Caribbean, Florida and the Pacific Islands (Fahey, 2005).

2.1.1 Morphological Characteristic of *Moringa oleifera.*

M. oleifera is a shrub and fast growing tree of 2.5-10m in height and diameter of 20-40 cm at chest height, brittle branches, tripinnate leaves, and thick, corky, deeply fissured whitish bark. The fruits are three lobed pods and when the fruits mature, they turn brown. It takes approximately 3 months to mature after flowering (Palanisamy & Kumaresen, 1985). Each pod have10-50 seeds inside with 1.5-2.6 cm long (Vlahov et al., 2002). The tree grows with a short, straight stem and reaches a height of 1.5-2 m before it begins to branch up to maximum 3.0 m. The leaves are in the alternate or pinnately-compound and being spirally arranged, mostly grow at the branch tips. They are 20-70 cm long, grayish-downy when young, long petiole with 8-10 pairs of pinnae each bearing two pairs of opposite, elliptic or obovate leaflets and one at the apex, all 1-2 cm long, with glands at the bases of the petioles and pinnae (Morton, 1991). The seeds are in three-angled shape with papery wing and have an average weight ~0.3g with the kernel to hull ratio of 75:25 (Makkar &Becker, 1997). The Figure 2.1 showed the morphological characteristic of the pods and seeds of *M. oleifera.*

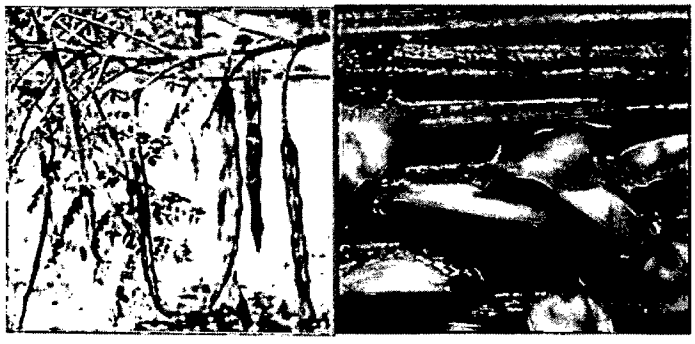

Figure 2.1: Morphological characteristic of the flowers, leaves, pods, and seeds of *M. oleifera.*

Source: Patel et al. (2010)

2.1.2 Uses of *Moringa oleifera* Parts.

Almost every part of the tree has its values. In Malaysia, the young pods are cut into small pieces and cooked as an herb curry using a minimum amount of spices. Almost all parts of the tree have been utilized for traditional medicine practices and the oil can be applied externally for skin diseases (Foidl et al., 2001). The leaves, flowers, fruits, and the roots of the tree can be used for many purposes. The leaves which are rich in vitamin A and C, are useful as respiratory ailments. The juice extracted from the leaves has strong antibacterial and anti-malarial properties and a paste of the leaves helps to promote healing of wounds (Gbeassor & Medjagni, 1990). The root of young trees and also the root bark are to cure inflammatory swellings and in the treatment of cholera (Lizzy et al., 1968). According to Fuglie (1999), there are many potential uses for Moringa which include the alley cropping (biomass production), animal forage (leaves and treated seed-cake), biogas (from leaves), domestic cleaning agent (crushed leaves), blue dye (wood), fencing (living trees), fertilizer (seed-cake), foliar nutrient (juice expressed from the leaves), green manure (from leaves), gum (from tree trunks), honey- and sugar cane juice-clarifier (powdered seeds), honey (flower nectar), ornamental plantings, biopesticide (soil incorporation of leaves to prevent seedling damping off) , pulp (wood), rope (bark), tannin for tanning hides (bark and gum), to flocculate contaminants and purify drinking water (powdered seeds). It has been used as lubricant in the machines, for salad preparation, manufacture of perfumes and hair care products (Gupta & Mazumder, 1999). Moringa oil is also used in cosmetic formulation due to its unique chemical, physical and tactile properties. It has very low iodine value of 65-68 which contributes to free spreading and elegant skin feel of this oil (Kleiman et al., 2008). The natural coagulant agents from *Moringa oleifera* seed extract has been found interesting as it has the ability to remove an ionic surfactant and is used in the water treatment (Heredia & Martin, 2009).

2.1.3 Composition of *M. oleifera* Seed Oil.

A search of the literature shows that the solvent-extracted oil from the *M. oleifera* seeds have 67.9% oleic acid compared to 70.0% in enzyme-extracted oil. The result showed highly unsaturated cause by presence of the high percentage of oleic acid. The other saturated fatty acids presence were palmitic (7.8% and 6.8%), stearic (7.6% and 6.5%), and behenic (6.2% and 5.8%) acids for solvent and enzyme-extracted oils, respectively (Abdulkarim et al.,2005). The result obtained showed that the seed contained 7.9% moisture, 38.3% crude protein, 30.8% crude oil,16.5% carbohydrate (by difference), 4.5% crude fiber,and 6.5% ash. The most abundance mineral element presence in *M. oleifera* seeds is magnesium which is 251.30±0.02 (mg/100). The other minerals presence are calcium (83.75±0.01), potassium (36.53±0.02), and sodium (22.50±0.01) (Makkar &Becker, 1997) and (Duke & Atchley,1984). Oil extraction by using Soxhlet method has the highest yield compared to extraction with a mixture chloroform:methanol (2:1). The oil extraction using soxhlet extractor showed 40% yield of oil while oil extracted using chloroform:methanol method yield 38.5% of oil *M. oleifera* oil (Abdulkarim et al.,2005). The mature seed of *M. oleifera* is rich in oil, containing between 22 and 40% crude oil (Ghazali et al.,2003).

Studies of the physical and chemical characteristic of *Moringa oleifera* oil showed colour (red/yellow) is 0.20/3.14, refractive index is 1.457, density at 24^0C is 0.909 and the saponification value is 188.36 (Lalas & Tsaknis, 2002). The free fatty acid content of Moringa oil (0.20% of oleic acid) is much higher than virgin olive oil (0.11% of oleic acid) obtained by extraction using hexane. (Sengupta & Gupta, 1970). Moringa oil is characterized by a high content of oleic acid (71%) and belongs to the oleic acid oil category (Sonntag, 1982).

2.2 LECITHIN

Phospholipids occurs naturally in the cell membrane structures of plants and animal (Szuhaj, 1985). Phospholipid extracts are called lecithin and may contain many different types of phospholipids. Because of polarity of phospholipids, lecithin behaves as a surfactant. Commercial lecithin is mixtures of phospholipids which consists mainly of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidic acid (Schneider, 1989) is obtained from soybean during oil processing and is used as an emulsifier in many foods. With emulsifying, wetting, dispersing, and viscosity–modifying properties, lecithin has been widely applied in other industries. According to a report by (Scholfield & C.R, 1985), lecithins are used widely in food and beverages, cosmetics, industrial coatings and animal health and nutrition product. Although it has multiple uses, lecithin is most commonly used as an emulsifier, mainly for oil in water emulsion. The Figure 2.2 showed the structure of the phosphotidylcholine and the Figure 2.3 showed the structures of phospholipids.

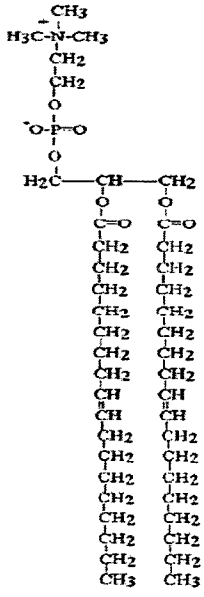

Figure 2.2: Structure of phosphotidylcholine.

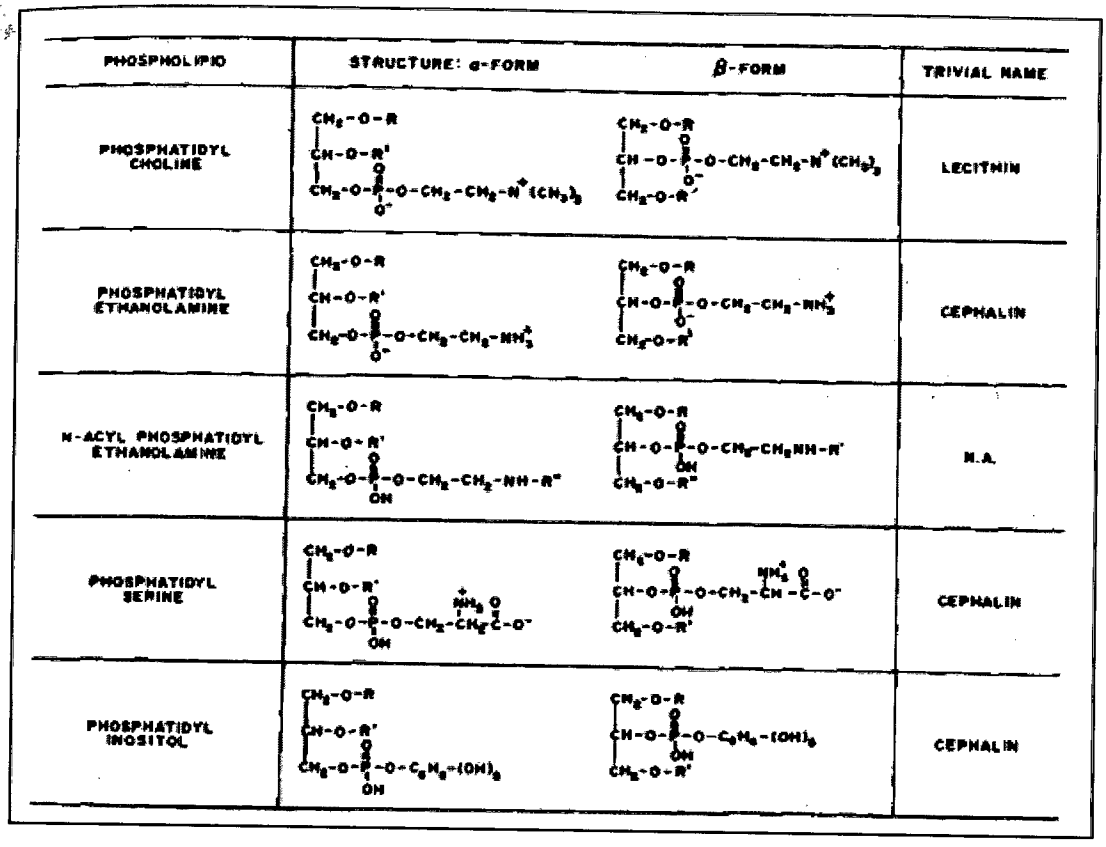

Figure 2.3: Structures of phospholipids. R, R'= various fatty acids: cephalin currently refers to phosphatidylethanolamine, NA= not available

Source: Cherry et al. (1981).

Lecithin is widely used in the food, feed and pharmaceutical industry due to its surface activity, high nutritional value and high consumer demand (Minifie, 1982). It is well known that lecithin, a mixture of various phospholipids, can act as a penetration enhancer for topically applied substances and is able to facilitate the transport of molecules into cell (Katoet al., 1987). Lecithin as assumed influenced the properties of both primary and the multiple emulsion. This connected with the

amphiphilic properties of lecithin, which commonly used as an emulsifier for water and oil emulsions (Kato et al, 1987).

The consumer's demand for natural products gives lecithin an advantage in today's cosmetic surfactant market. Early uses of lecithin in cosmetic included the addition of egg yolk to skin and hair preparations, but soybean lecithin can be found in a variety of products (Baker, 1989). It has been reported that lecithin is used in cosmetics enhances spreading and absorption as well as moisturizing efficiency (Szuhaj, 1989). Many cosmetic products are oil-in –water (o/w) or water in oil(w/o) emulsions, which are usually stabilized by petrochemical emulsifiers. However, some synthetic surfactant cause eye or skin irritations (Conry, 1980). Lecithin has not been found irritating and is an effective emulsifier and recognized as GRAS that is Generally Recognized as Safe, (Weete et al., 1994).Therefore, it has the potential to replace synthetic emulsifier in stabilizing cosmetic emulsion. Lecithin studies have focused on the emulsification properties in o/w or w/o emulsion (Johanssan et al., 1995) and on the effects of phospholipid composition and the fatty acid composition on those properties (List, 1989).

2.2.1 Emulsifying properties

An emulsion may be single for example, oil-in-water (O/W) and water-in-oil (W/O) emulsions, or it may be an emulsion of an emulsion such as in water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O) emulsions or it is also termed as multiple or double emulsions. The food and feed industries mostly use soybean lecithin, which is a complex mixture of mainly phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidic acid (PA), and may contain relatively large amounts of triglycerides. They are widely used as emulsifiers to control rheological properties of dispersions and fat crystallization

(Smith, 1995). An emulsifier is a substance with a hydrophobic and a hydrophilic part which has the capacity to form W/O or O/W emulsions by reduction of interfacial tension (Nieuwenhuyzen, 1976).It is known that hydrophobic lecithin with reduced phosphatidylcholine (PC) content can act as an emulsifier in W/O emulsions (Johannson & Bergenstahl, 1995) and to produced multiple W/O/W emulsions with lecithin as the emulsifier in the primary W/O emulsion (Akhtar et al.,2001).A study from Weete et al., (1994) reported improved emulsifying properties for W/O emulsions when lecithin was thermalized.Having a small and uniform droplets are desirable to reduce the rate of creaming during the first stage of emulsion breakdown (Sagitani,1981).

2.2.2 Hydratable lecithin and non –hydatable lecithin.

Some lecithin compounds do not separated into the aqueous phase during degumming and they are referred as non-hydratable phospholipids (Liu,K., 1997). The extent to which a phosphatide present in the crude oil is removed during water degumming depends on its hydrophilicity. Phosphatidylinositol (PI) has five free hydroxyl groups on the inositol moiety that make PI strongly hydrophilic. PI will be hydrated during the water degumming and the PI content of properly water-degummed oil is negligible. Similarly, the positive charge of the trimethylamino group in phosphatidylcholine makes this phosphatide hydrophilic. This hydrophilicity does not depend on the pH of the water used and when the phosphate group in the PC is dissociated with a negative charge and does not form an internal salt with the quaternary amino group for steric reasons. Consequently, the positive quaternary amino group remains isolated at all pH values and causes PC to be hydrophilic at all pH values (Dijkstra,2011). Cationic surfactants are of limited use as emulsifiers (Venable, 1985). They are mostly used as antistatic agents or as fabric softeners, and they have great potential in cosmetology and food manufacture (Ackerman, 1983)

CHAPTER 3

METHODOLOGY

This research was conducted to determine the percentage of oil extracted from *M. oleifera* (mature and immature) and soybean seeds using soxhlet apparatus and cold maceration. The main purpose of the research is to determine the phospholipid content and the fatty acid composition from the fixed oil of *M. oleifera* (mature and immature).

Laboratory chemicals equipments. Standard laboratory equipment was used for soxhlet extraction, weighing balance, drying oven, centrifugation and etc. All solvents and chemicals used were is analytical grade. Pre-coated TLC plates were obtained from Merck.

3.1 COLLECTION OF PLANT MATERIALS.

M. oleifera (mature and immature) were collected from Taman Taiping, Perak and the soybean seeds are bought from the market in Carrefour East Coast Mall Kuantan, Pahang (Appendix A1).

3.2 FIXED OIL EXTRACTION FROM SEEDS.

3.2.1 Soxhlet Extraction

Dried seeds of *Moringa oleifera* (mature and immature) seeds are ground into fine powder using mortar and pestle. 4 g of ground *M. oleifera* seeds (mature and immature) were placed into filter paper and extracted using hexane solvent in soxhlet extractor (Appendix A2) for 4 hours and 30 minutes. The oil is then recovered by evaporating of the solvent using rotary evaporator (Appendix A3). The amount of oil extracted is expressed in percentage of total weight of sample used during extraction. The same procedure was used to extract soybean seeds.

3.2.2 Cold Macerations

10g of dried *M. oleifera* seeds (mature and immature) are ground into fine powder using mortar and pastel (Appendix A4). Then, it was mixed with solvent chloroform: methanol with ratio 2:1 at room temperature and placed into a 500ml volumetric flask with a closed vessel (Appendix A5). It is allowed to stand for a 7 days shaking occasionally. Then, it is filtered and let to evaporate till concentrated oil is obtained (Appendix A6). This procedure is repeated using soybean seeds.

3.2.3 Marc Recovery

The solid residue (marc) is extracted using solvent chloroform: methanol ratio 2:1 (Appendix A7). After 4 days, it is then filtrated and let it evaporated to obtain a concentrated remain residues.