STUDIES ON THE ANTIOXIDANT PROPERTIES OF SELECTED MALAYSIAN "ULAMS"

by

NUR NADZIFAH BINTI CHE ALIAS

Dissertation submitted in partial fulfillment
of the requirements for the degree of
Bachelor of Health Sciences (Biomedicine)

March 2005

CERTIFICATE

This is to certify that the dissertation entitled "**Studies on Antioxidant Properties of Malaysian Ulams**" is the bona fide record of research work done by **Ms. Nur Nadzifah Binti Che Alias** during the period from **June, 2004 to March 2005,** under my / our supervision.

Signature of Supervisor:

Name and address of Supervisor:

Dr. Md. Rafiquzzaman
School of Health Sciences
Universiti Sains Malaysia

Date: 15.3.05

Signature of Supervisor:

PROFESSOR SYED MOHSIN SAHIL JAMALULLAIL
Timbalan Dekan
(Akademik & Pembangunan Pelajar)
Pusat Pengajian Sains Kesihatan
Universiti Sains Malaysia
Kampus Kesihatan

Name and address of Supervisor:

Prof. Dr. Syed Mohsin Syed Sahil Jamalullail
Deputy Dean of Student Affair and Academic
School of Health Sciences
University Science Malaysia

Date:

ACKNOWLEDGEMENTS

In The Name of Allah the Most Gracious and Merciful. *Selawat* and *salam* for our prophet, Muhammad S.A.W. I thank Allah the Lord for Him unceasingly granting me peace, joy, and faith, and guiding me throughout the research project. Without him, I could not do anything.

I would like to take the opportunity to thank and express sincere gratitude to my supervisors, Dr. Md. Rafiquzzaman and Professor Dr. Syed Mohsin Syed Sahil Jamalullail, School of Health Sciences, USM, who always made their valuable time available to me, taught and supervised me constantly in the course of my research project.

Not to forget, I would like to thank all scientific officers and technicians of the Laboratory Facilities Unit of the School of Health Sciences and Pharmacology Laboratory, School of Medical Sciences. Without their help, I would not be able to carry out this research work within the time period for the project.

I am grateful to Forest Research Institute of Malaysia (FRIM), Kepong, especially Dr. Vimala Subramaniam for her cooperation in various respects during my research work.

Lastly, I am very much thankful to my family members especially my parent, Che Alias Bin Teh and Masitah Binti Mustapha who constantly gave moral support and encouragement to me. Not forget to my friends especially Noor Shazilawati Mohd Rashid for her unfailing cooperation.

Nur Nadzifah Bt Che Alias

Date:

TABLE OF CONTENTS

LIST OF TABLE Page

LIST OF FIGURES Page

LIST OF CHART Page

ABSTRAK

Kajian terhadap kandungan aktiviti antioksidan dalam kalangan ulam yang terdapat di Kelantan, Malaysia telah dijalankan. Ulam-ulam yang telah dikaji adalah *Oroxylum indicum* (Beka), *Apium graveolens* (Sup/Sadri) dan *Vitex negundo* (Lemuni). Peratusan kembali bagi ekstrak ulam yang telah disediakan dalam lingkungan 2.47 hingga 2.54%. Ekstrak ulam dengan mengunakan methanol pada kepekatan 0.2 mg/mL diuji dengan ujian "autoxidation of lipid assay in a water-alcohol system", manakala 0.4 mg/mL untuk ujian DPPH radical scavenging dan 2.50 mg/mL menggunakan ujian xanthine/xanthine oxidase (X/XOD) superoxide scavenging test. 2, 6-di-tert-butylhydroxytoluene (BHT) merupakan antioksidan sintetik yang digunakan sebagai antioksidan piawai untuk ujian "autoxidation of lipid (ML) assay in a water-alcohol system" . Didapati *Oroxylum indicum* (Beka) dan *Vitex negundo* (Lemuni) mempunyai aktiviti antioksidan yang tinggi jika dibandingkan dengan BHT (92.9% dan 92.9% untuk ujian "xanthine/xanthine oxidase (X/XOD) superoxide scavenging" manakala 98.4% dan 98.6% untuk ujian DPPH radical scavenging. Turutan secara menurun bagi aktiviti antioksidan yang terdapat di dalam ulam untuk menghalang pengoksidaan lipid adalah *Vitex negundo* (Lemuni) > *Oroxylum indicum* (Beka) > *Apium graveolens* (Sup). Walaupun ketiga-tiga ujian menunjukkan nilai aktiviti antioksidan yang berlainan tetapi terdapat persamaan

di dalam turutan aktiviti antioksidan tersebut. Oleh itu, disarankan supaya penyelidikan seterusnya dijalankan terhadap ulam-ulaman yang terdapat di Malaysia terutamanya *Vitex negundo* (Lemuni). Kajian ini bertujuan menyediakan sesuatu produk yang boleh mengelakkan daripada penyakit berbahaya.

ABSTRACT

The antioxidant activity of some edible Malaysian ulams found in Kelantan, Malaysia, was studied. The ulams studied were *Oroxylum indicum* (Beka), *Apium graveolens* (Sup/Sadri), and *Vitex negundo* (Lemuni). Percent recovery of the prepared ulam extracts was ranged from 2.47 to 2.54%. the methanolic extracts of the ulams at 0.2 mg/mL concentration level was tested using the autoxidation of lipid assay in a water-alcohol system, 0.40 mg/mL for DPPH radical scavenging assay and 2.50 g/mL for xanthine/xanthine oxidase (X/XOD) superoxide scavenging test. For the autoxidation of lipid (ML) assay in a water-alcohol system, 2, 6-di-tertbutylhydroxytoluene (BHT), a synthetic antioxidant, was used as standard antioxidant. It was found that *both Oroxylum indicum* (Beka) and *Vitex negundo* (Lemuni) possessed high antioxidant activity (92.9% and 92.9% for xanthine/xanthine oxidase superoxide scavenging test and 98.4% and 98.6% for DPPH radical scavenging assay, respectively). The decreasing order of antioxidant activity for inhibition of lipid autoxidation was observed as *Vitex negundo* (Lemuni) > *Oroxylum indicum* (Beka) > *Apium graveolens* (Sup/Sadri). Though all the test system did not yield the identical order of antioxidant activity but there was a similarity. However, irrespective of the type of the test carried out, the antioxidant activity of *Vitex negundo* (Lemuni) was found appreciably high. Therefore, it is worth to study further with

the ulams for the present study, especially with *Vitex negundo* (Lemuni) and the outcome of the study can be of importance in dietary control of diseases.

1. INTRODUCTION

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage (Buhler & Miranda, 2003). Thus is believed that antioxidants play a key role for longer and healthier life. Antioxidants promote good health by slowing down the aging process and delaying the onset of many chronic diseases. In some cases, antioxidants may even help to eliminate some diseases or actually reverse the damaging pathological processes that cause them (Lieskovan, 2004).

Scientists have identified different types of natural antioxidants and classified them into two different groups. Endogenous antioxidants are those that are produced within the body itself. This group includes glutathione peroxidase, catalase, and superoxide dismutase. Antioxidants that can't be produced within the body and must be obtained in the diet are called exogenous antioxidants. This group includes: vitamins C and E, β-carotene, lipoic acid, coenzyme Q10, grape seed extract, bioflavonoids, green tea polyphenols, and a great variety of other natural substances from herbs and other sources (Lieskovan, 2004).

Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many fruits and vegetables. (Luo, Basile, & Kennelly, 2002; Vinson *et al*,.1999). The best known antioxidant molecules are vitamin A, E, and β- carotene (Sies & Krinsky,

1995; Krinsky, 1998). A good antioxidant supplement should also contain trace minerals like selenium and zinc because these elements are required by the human body for the manufacture of several very important endogenous antioxidant (Lieskovan, 2004).

In aged people, the antioxidant defense mechanism becomes inefficient due to the reduction in enzyme and vitamin activity. As age increases, the production of antioxidant enzymes in the body is declined. And antioxidant enzymes become insufficient to scavenge and eliminate excess free radicals efficiently. Vitamin activity also drops due to inefficient absorption in old people. Thus repair mechanisms and turnover processes are slowed down (Vimala *et al.*, 2003).

Reactive oxygen species (ROS) have been implicated in initiating, accompanying or causing pathogenesis of many diseases (Facchinetti *et al.*, 1997; Prasad *et al.*, 1999; Delanty & Dichter, 2000). There is some evidence that abnormalities in lipid compounds may cause overproduction of ROS and, in turn, antioxidant enzyme activities and lipid peroxidation, and that these phenomena may be related to pathophysiology of major depression (Bilici *et al.*, 2001). Free radicals such as superoxide anion, hydrogen peroxide, hydroxyl radical, which cause lipid peroxidation can lead to cell death (Butterfield & Kanski, 2001). Transition metal alone as well as their chelates enhances lipid peroxidation process (Rafiquzzaman *et al.*, 1994; Miller, Buettner and Aust 1990; Arouma *et al.*, 1989; Rafiquzzaman, Komagoe & Tamagake 1995; Fukuzawa *et al.*, 2001). Hydrogen peroxide is thought to be the major precursor of highly reactive free radicals, and it has been reported to induce apoptosis in cells of the central nervous system (Candra *et al.*, 2001). Free radicals and lipid

peroxidation have been suggested as potentially important causative agents of aging and several human diseases (Zhou *et al.*, 1991).

There are two sources of free radical, the endogenous and exogenous sources. The endogenous source where the free radicals are produced inside the body during aerobic processes, such as metabolism, biochemical reactions in cells, detoxification in the liver and energy generation by mitochondria. Whereas the exogenous sources are coming by diets high in fats, saturated oils, barbecued meat, processed food products or stale food. A stressful life style, cigarette smoking and radiation also enhance free radical production (Vimala *et al.*, 2003).

Oxidative stress can damage many biological molecules. Proteins and DNA are significant targets of cellular injury. Another target of free radical attack in biological systems is the lipids of cell membranes (Halliwell *et al*,. 1992; Halliwell and Chirico, 1993). It also supported by Vimala *et al.*, (2003) that free radical cause lipid peroxidation which leads to aging symptoms and age-dependent condition. Excess free radical activity in the body is more common among omnivores than among vegetarians (Boyd, 1996).

Autoxidation of polyunsaturated lipids involves a free radical chain reaction that is most frequently initiated by exposure of lipids to light, ionizing radiation, metal ions and metalloprotein catalysts. It starts when the first hydroperoxides decompose to alkoxy and hydroperoxy radicals. These radicals abstract an H• from vulnerable sites in monoenoic and polyenoic (e.g. linoleic) fatty acid residues in the fats/oils, and foods containing fats or oils (Coultate, 1996). Biological organs contain many polyunsaturated fatty acids (PUFA), such as linoleic, linolenic and arachidonic acids, mainly in the form of esters with

phospholipids, triglycerides, or with cholesterol. These PUFA can undergo lipid peroxidation which can be interrupted by antioxidants by the donation of electrons (Vaya & Aviram, 2001)

The initiation of lipid peroxidation also can be induced by HO• and metal ion-free radical (such as perferryl and ferryl) complexes. (Haliwell & Gutteridge, 1999). Peroxidase of lipid is implicated with numerous pathological states and disease condition (Kanner, German & Kinsella 1987; Monitti and Aust 1992; Miller, Buettner and Aust 1990; Fukuzawa *et al.*, 2001) like atherosclerosis, aging, cancer, etc. Therefore research interest in lipid peroxidation has intensified in recent years. Lipid peroxidation is known as a free radical chain reaction that takes place inside the human body, and produces many secondary products, such as alkanes, alcohols, acids and carbonyls. These secondary products are themselves highly reactive and they react with other biological components, such as protein, amino acids, amines, and DNA, leading to aging, mutagenesis and cancer (Vimala *et al.*, 2003). The overall mechanism of lipid oxidation consists of three phases, initiation the formation of free radicals, propagation cycles of free-radical chain reactions and termination, combination of radicals into non-radical products.

NTA (sodium nitrilotriacetic acid) is a synthetic chelating agent used in detergents (Bates and Sctabach, 1973). Unfortunately, iron-nitrilotriacetic acid can cause nephrotoxicity and renal cell carcinoma (Okada *et al.* 1987; Fukuzawa *et al.*, 2001).

According to International Agency for Research on Cancer (IARC) BHT has been used since 1947 as a common antioxidant in rubber and petroleum products and, more recently, in plastics. It has been used since 1949 as an

antioxidant in many fat-containing foods, in edible fats and oils and in cosmetics (Fulder, 1983). Although synthetic antioxidant such as butylhydroxytoluene (BHT) and butylhydroxyanisole (BHA) have been commonly used to prevent oxidation, but there is increasing demand for natural products that are free from chemical additives (Yepez *et al.*, 2001). Because synthetic antioxidants have been suspected to cause toxicity and promote negative health effect (Valentão *et al.*, 2002; Burlow, 1990; Branen, 1975; Namiki, 1990; Pokorny, 1991). Interest in employing antioxidants from natural sources to increase the shelf life of foods are considerably enhanced by consumer preference for natural ingredients and concerns about the toxic effects of synthetic antioxidants (Schwarz *et al.,* 2001)

"Ulams" are plants commonly consumed by Malaysians as raw salad or as lightly cooked vegetable dishes. "Ulams" are a good source of vitamins, and natural antioxidants which are recommended for prevention of diseases and general health-care purposes (Vimala *et al.*, 2003). Vimala and Adenan (1999) studied the antioxidant activity of some "ulams". But, yet there are "ulams" to be studied. Antioxidant activity of three "ulams" viz. *Oroxylum indicum* (Beka), *Apium graveolens* (Sadri) and *Vitex negundo* (Lemuni) were selected and have been evaluated in this study.

2. LITERATURE REVIEW

Antioxidant activity of selected plant species from the Canadian prairies are carried out by Amarowicz *et al.*, (2003). They used ethanolic extracts from the roots of wild licorice (*Glycyrrhiza lepidota*), narrow-leaved echinacea (*Echinacea angustifolia*), senega (*Polygala senega*), leaves of bearberry (*Arctostaphylos uva-ursi*) and aerial parts of two varieties of horsetail (*Equisetum* spp.) were prepared and evaluated for their free-radical scavenging capacity and their antioxidant activity, by a number of chemical assays. Assays employed included a β-carotene-linoleic acid (linoleate) model system, reducing power, scavenging effect on the DPPH• free radical and capacity to scavenge hydroxyl free radicals (HO•), by use of electron paramagnetic resonance (EPR) spectroscopy. The bearberry-leaf extract consistently exhibited the highest antioxidant activity based on the tests performed, and seems to be a promising source of natural antioxidants. The polyphenolic constituents appear to be responsible, at least in part, for the extract's radical-scavenging capacity. Research is progressing to characterize the antioxidant compounds in the bearberry-leaf extract and their mode of action in imparting antioxidant activity to various food systems.

Ng, Liu and Wang, (1999) studied on antioxidant activity of natural products from plants. A variety of flavonoids, lignans an alkaloid, a bis-benzyl, caumarins and terpenes isolated from Chinese herbs were tested for antioxidant activity and found to have the ability to inhibit lipid peroxidation in rat brain and kidney homogenates and rat erythrocyte hemolysis. The pro-oxidant activities of the aforementioned compounds were assessed by their effects on

bleomycin-induced DNA damage. The flavonoids baicalin and luteolin-7-glucuronide-6-methyl ester, the lignan 4-demethyldeoxypodopophyllotoxin, the alkaloid tetrahydropalmatine, the bis-benzyl erianin and the caumarin xanthotoxol exhibited potent antioxidative activity in lipid peroxidation assays. The flavonoid rutin and the terpene tanshinone I manifested potent antioxidative activity in the lipid peroxidation assay but no inhibitory activity in the hemolysis assay. The lignan deoxypodophyllotoxin, the flavonoid naringin and the caumarins columbianetin, bergapten and angelicin slightly inhibited lipid peroxidation in brain and kidney homogenous.

Yepez *et al.*, (2001) had studied on the extracted fractions of seeds of coriander (*Coriandrum sativum*) for their antioxidant activity. The antioxidant activity of the fractions was determined by measuring their ability to remove the free radicals present in a methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH). The extract exhibited a significant activity, comparable to that of commercial antioxidants.

Vaya and Aviram, (2001), in their published paper discussed about the important role of dietary antioxidants in maintaining the integrity of living organisms. New data are being constantly gathered to show the role of oxidative stress and the involvement of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the pathogenesis of degenerative diseases. These diseases are associated with a disturbance in the necessary balance between oxidation and reduction status in blood and tissues, leading to oxidation of lipid, protein and nucleic acids. Such oxidative damage is accompanied by changes in macromolecules structure and function and by the manifestation of clinical disorders such as cardiovascular diseases and cancer.

The antioxidant properties of twenty medical herbs used in the traditional Mediterranean and Chinese medicine were studied by Schinell *et al.*, (2001). Extracts from *Forsythia suspensa*, *Helichrysum italicum*, *Scrophularia auriculata*, *Inula viscosa*, *Coptis chinensis*, *Poria cocos* and *Scutellaria baicalensis* had previously shown anti-inflammatory activity in different experimental models. Using free radical-generating systems *H. italicum*, *I. viscose* and *F. suspense* protected against enzymatic and non-enzymatic lipid peroxidation in model membranes and also showed scavenging property on the superoxide radical. All extracts were assayed at a concentration of 100 µg/mL. Most of the extracts were weak scavengers of the hydroxyl radical and *C. chinensis* and *P. cocos* exhibited the highest scavenging activity. Although *S. baicalensis* inhibited the lipid peroxidation in rat liver microsomes and red blood cells, the extract showed inhibitory actions on aminopyrine N-demethylase and xanthine oxidase activities as well as an pro-oxidant effect observed in the Fe^{3+} - EDTA - H_2O_2 system.

Einbond *et al.*, (2003) had studied on anthocyanin antioxidants from edible fruits, the edible fruits of 12 plants were extracted in methanol and subjected to solvent–solvent partitioning to yield three fractions, hexane, ethyl acetate, and aqueous. These fractions were then screened for antioxidant activity using the 1, 1-diphenyl-2-picrylhydrazyl assay. Nine of the semi-purified fractions exhibited high antioxidant capacity. Cyanidin-3-O-b-glucopyranoside, an anthocyanin antioxidant, was identified from semi-purified aqueous fractions of the tropical fruit star apple (*Chrysophyllum cainito*), Surinam cherry (*Eugenia unifora*), and jaboticaba (*Myrciaria caulifora*). Delphinidin-3-O-b-glucopyranoside was identified from *E. unifora*.

According to research conducted by Vimala and Adenan (1999), different type plant parts from 45 species of Malaysia tropical forest medicinal plants were screened for antioxidant activity against autoxidation of linoleic acid in a water-alcohol system. Antioxidative activities were assessed using ferric-thiocyanate method and some of the extracts were found to exhibit potent antioxidant activity relative to a synthetic antioxidant, i.e. 2, 6-di-tert-butyltoluene. The antioxidant activity observed in their study indicates that many of the local tropical medicinal plants posses free radical scavenging compounds that form a natural defense mechanism against activated oxygen damage in plants.

3. OBJECTIVE OF THE STUDY

Research is continually going on in search of an ideal natural antioxidant and its potential source. Natural antioxidants are capable of destroying free radicals in the body and thus help to keep the body's oxidative stress in balance. They are also thought to help in preventing degenerative diseases. The practice of eating 'ulams' (part of plants consumed as salad) by Malays is ancient. Though several studies have been performed on antioxidant properties of several Malaysian plants and 'ulams', the 'ulams' native to Kelantan (appendix 1) have not been given due attention. The objectives of the present study have been list below:

i) To identify the antioxidant activity of the methanolic extracts of:

a) Pucuk Beka/Bonglai *(Oroxylum indicum L)*.

b) Daun Sadri *(Apium graveolens)*.

c) Daun Lemuni *(Vitex negundo L)*.

ii) To standardize their antioxidant activity in relation to the standard antioxidant BHT.

iii) To evaluate their relative antioxidant activity.

4. MATERIAL AND METHODS

The following are the name of the instruments, chemicals and other materials used in this study:

Instrument:

TABLE 4.1 Instruments used throughout the studies.

No.	Instruction	Specification
1.	Grinder Macer	Memmert, Germany
2.	Rotary evaporator with Chiller cooling system	Heidolph, Germany
3.	Oven	Memmert, Germany
4.	UV-Visible Spectrophotometer	Jasco V-530 Model UV-Vis Spectrophotometer, Lambda 2S spectrophotometer
5.	Electronic balance	A & D Company Ltd., Japan
6.	Incubator	Binder, Model BD115

Chemicals:

TABLE 4.2 Chemicals used in the studies, with some relevant information.

No.	Chemical	M.W	Grade	Source
1.	Methyllinoleate (ML)	294.48	GR-grage	TCI, Japan
2.	Nitrilotriacetic acid (NTA)	191.14	ACS-grade	Sigma-Aldrich,USA
3.	Iron (III) chloride-6-hydrate	270.30	GR-grade	Bendosen Laboratory chemicals
4.	2,6-tert-butyl-hydroxytoluene (BHT)	220	GR-grade	TCI, Japan
5.	Glacial acetic acid	60	GR-grade	Merck, Germany
6.	Sodium hydroxide (NaOH)	40	LR-grade	Merck, Germany
7.	Methanol	32	GR-grade	Merck, Germany
8.	Distilled water (H_2O)	18		Prepared in PPSK lab
9.	Linoleic acid	280.4	99% LR-grade	Sigma-Aldrich, USA

10.	Potassium thiocyanate	97.19	AR- garde	BDH, Chemical Ltd.
11.	Ferrous sulphate	278.01	GR-grade	Merck, Germany
12.	Phosphate buffer a) Sodium phosphate (monobasic) b) Sodium phosphate (dibasic)	 138.0 268.1	 ACS-grade ACS-grade	 Sigma-Aldrich, USA.
13.	Nitro Blue Tetrazolium (NBT)	817.6	TLC-grade	Sigma-Aldrich, USA.
14.	Tris[hydroxymethyl]-aminomethane hydrochloride (Tris (HCl))	157.6	Reagent-grade	Sigma-Aldrich, USA.
15.	Magnesium chloride	203.3	GR-grade	Sigma-Aldrich, USA.
16.	5-Bromo-4-chloro-3-indolyl phosphate	370.4	R&D-grade	Sigma-Aldrich, USA.
17.	Sodium carbonate	106.0	GR-grade	Sigma-Aldrich, USA.
18.	Ethylene diamine Tetraacetic acid (EDTA)	372.2	Sigma-grade	Sigma-Aldrich, USA.
19.	Xanthine		99-100%	Sigma-Aldrich, USA.
20.	Superoxide dismutase (SOD)			Sigma-Aldrich, USA.
21.	Xanthine oxidase (XOD)			Roche Diagnostics GmbH, Germany.
22.	N, N-dimethyl formamide	73.09	ACS-grade	Sigma-Aldrich, USA.
23.	1, 2-diphenyl-2-picrylhydrazyl (DPPH)	394.3		

Plant Material:

Methanolic extracts of the following ulams were prepared and used in the present study:

a. *Oroxylum indicum* (Beka)

b. *Apium graveolens* (Sadri/Sup)

c. *Vitex negundo* (Lemuni)

The names in *italic* are their scientific names, while those within the brackets are the vernacular names.

Other materials used:

- Test tube
- Test tube holder
- Conical flasks
- Volumetric flasks (25 mL, 50 mL, 100 mL)
- Glass pipette (5 mL)
- Micropipette
- Pasteur pipette
- Beakers
- Funnels
- Spatula
- Filter paper Whatman (110 mm Dia)
- Watch glass
- Parafilm foil
- Aluminium foil
- Tissue paper
- Disposable glove
- Cleaning agent

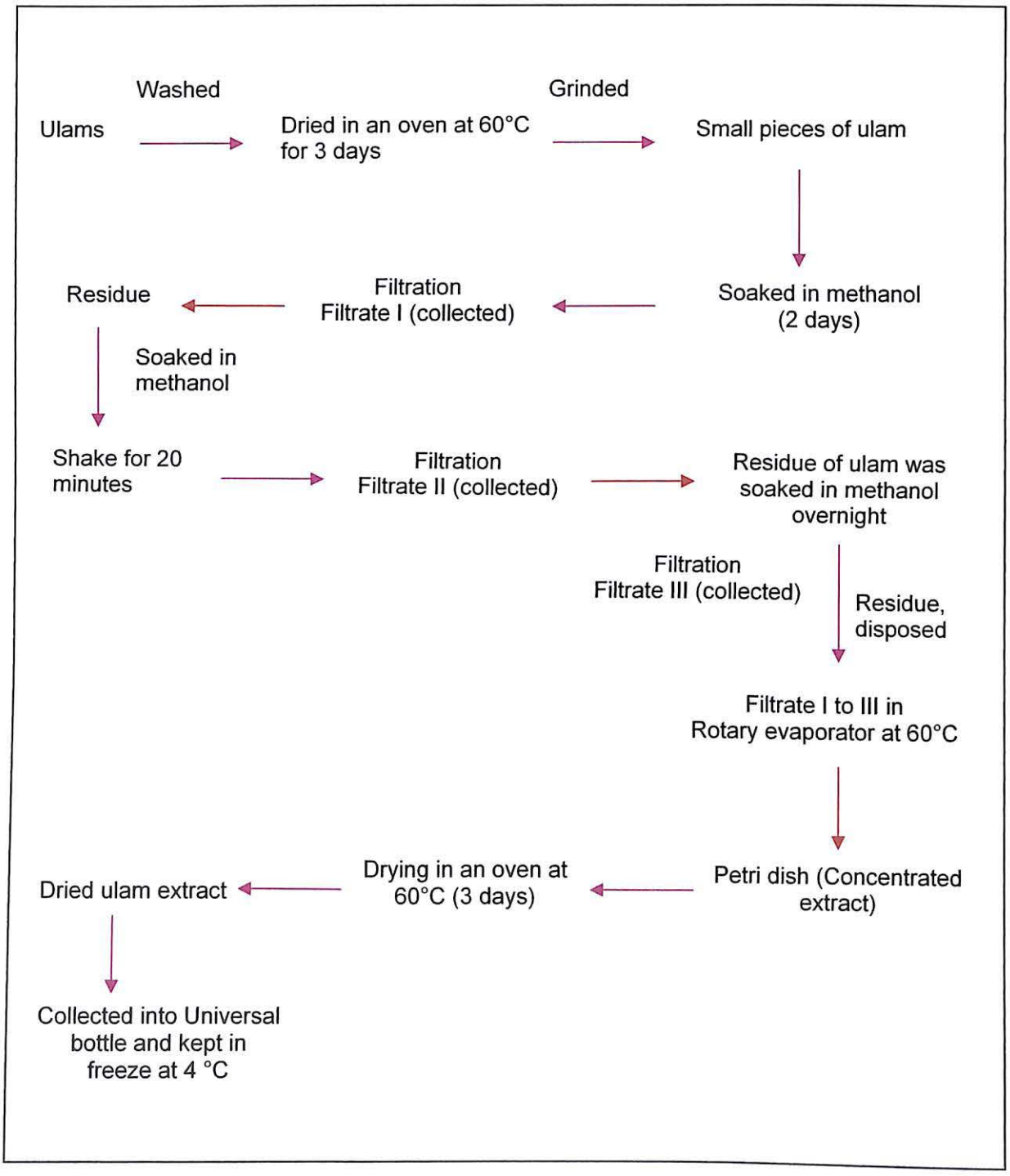
Collection of Ulams

Three 'ulams' namely *Oroxylum indicum* (Beka) (FIGURE 4.1), *Apium graveolens* (Sadri/Sup) (FIGURE 4.2), and *Vitex negundo* (Lemuni) (FIGURE 4.3) used in this study. *Oroxylum indicum* (Beka) and *Apium graveolens* (Sadri/Sup) were purchased from the local market of Pasar Jelawat, Bachok, Kelantan, Malaysia. Whereas the other ulam, *Vitex negundo* (Lemuni), was collected from the area of Pengkalan Chepa, Kota Bharu, Kelantan, Malaysia. Description about each 'ulam' has been included elsewhere in this dissertation.

Preparation of 'Ulams' Extract

After collection, the 'ulams' were washed with water and distilled water to remove dust and other dirt from them. All these 'ulams' were replaced into an oven for 3 days at not more than 60°C to prevent decomposition of any of the chemical components contained by the 'ulams'. The 'ulams' were grinded to small pieces using a grinder (FIGURE 4.5). The grinded 'ulams' were then extracted with methanol. In the extraction process, 150 gm of each 'ulam' was soaked in 300 mL of methanol for two days. After 2 days, each of the soaked 'ulam' was filtered with filter paper and the extract was collected in a beaker. 'Ulam' remained on the funnel, in each case, was soaked again in 150 mL of methanol separately and was shaken for 20 minutes. Then, the soaked 'ulam' was filtered with filter paper. Ulam residue was soaked again in 150 mL of methanol for overnight and filtered again. The collected extracts of each 'ulam' was then allowed to evaporate solvent under vacuum by a rotary evaporator (FIGURE 4.6) and at 60°C. Concentrated extracts were then taken into Petri dish and were dried in oven (FIGURE 4.7) at 60°C for 3 days. Finally the crude

extracts of ulams were placed into the universal bottles labeled from number 1 to 3 and were kept in refrigerator at 4°C for further use. The whole extraction process has been shown in the Flow Chart -4.1 illustrated below:



Flow Chart -4.1 Preparation of 'Ulam' Extract

Characteristic of *Oroxylum indicum* (Beka)

Taxonomy of *Oroxylum indicum*

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermotophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Rosopsida – eudicotyledons
Subclass	Lamiidae
Order	Lamiales
Family	Bignoniaceae
Genus	*Oroxylum Vent*
Species	*Oroxylum indicum*

TABLE 4.3 Taxonomy of *Oroxylum indicum*

Oroxylum indicum (L) Vent. also know as trumpet flower. It has bark light brown, fissured, and soft. It is a small tree about 7-12 m and leaves very large with two to tree pinnate with opposite pinnae. Fruits have very long capsule, straight, tapering both ends and flat, whereas, seeds are numerous and large with membranous cellophane-like wing. Methanolic extracts of the young leaves of *Oroxylum indicum* were prepared and used in the present study.

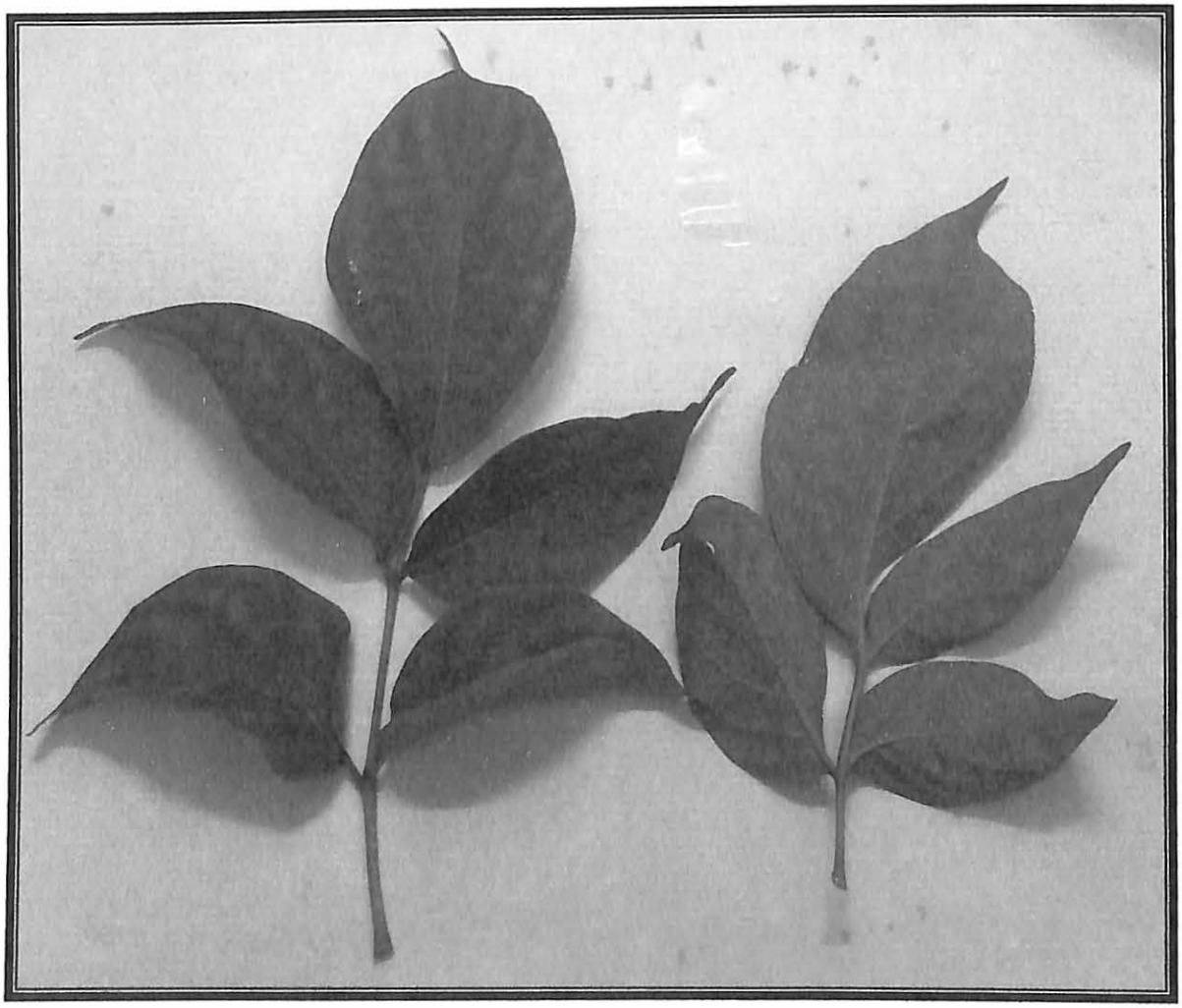


FIGURE 4.1 *Oroxylum indicum* (Beka)

Characteristic of *Apium graveolens* (Sup/Sadri)

Taxonomy of *Apium graveolens:*

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Rosidae
Order	Apiales
Family	Apiaceae – Carrot family
Genus	*Apium* L. – celery
Species	*Apium graveolens* L. – wild celery

TABLE 4.4 Taxonomy of *Apium graveolens*

Apium graveolens (L) are glabrous plants with small white flowers. Bracts are absent, whereas bracteoles present or absent. Fruit has glabrous, globose, or ovate-oblong, and somewhat compressed from the sides. *Apium graveolens* (L) has heavy aromatic smell and umbels short-peduncled. It has pinnate leaves with petioled 3-lobed leaflets. Methanolic extracts of the leaves of *Apium graveolens* were prepared and used in the present study.

FIGURE 4.2 *Apium graveolens* (Sup/Sadri)

Characteristic of *Vitex negundo* (Lemuni)

Taxonomy of *Vitex negundo:*

Kingdom	Plantae- Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Asteridae
Order	Lamiales
Family	Verbenaceae – Verbena family
Genus	*Vitex L.* – chastetree
Species	*Vitex negundo* L.

TABLE 4.5 Taxonomy of *Vitex negundo*

Vitex negundo is a large shrub or small tree to 5 m with quadrangular branchlets. Leaves are palmately compound and opposite while leaflets are 5 elliptic-ovate to lanceolate, entire, pinnately veined, and grayish-tomentose beneath. Flowers are perfect with lilac to lavender in loose, terminal and panicled clusters whereas, fruit has a small drupe and black-purplish color. Methanolic extracts of the young leaves of *Vitex negundo* were prepared and used in the present study.

FIGURE 4.3 *Vitex negundo* (Lemuni)

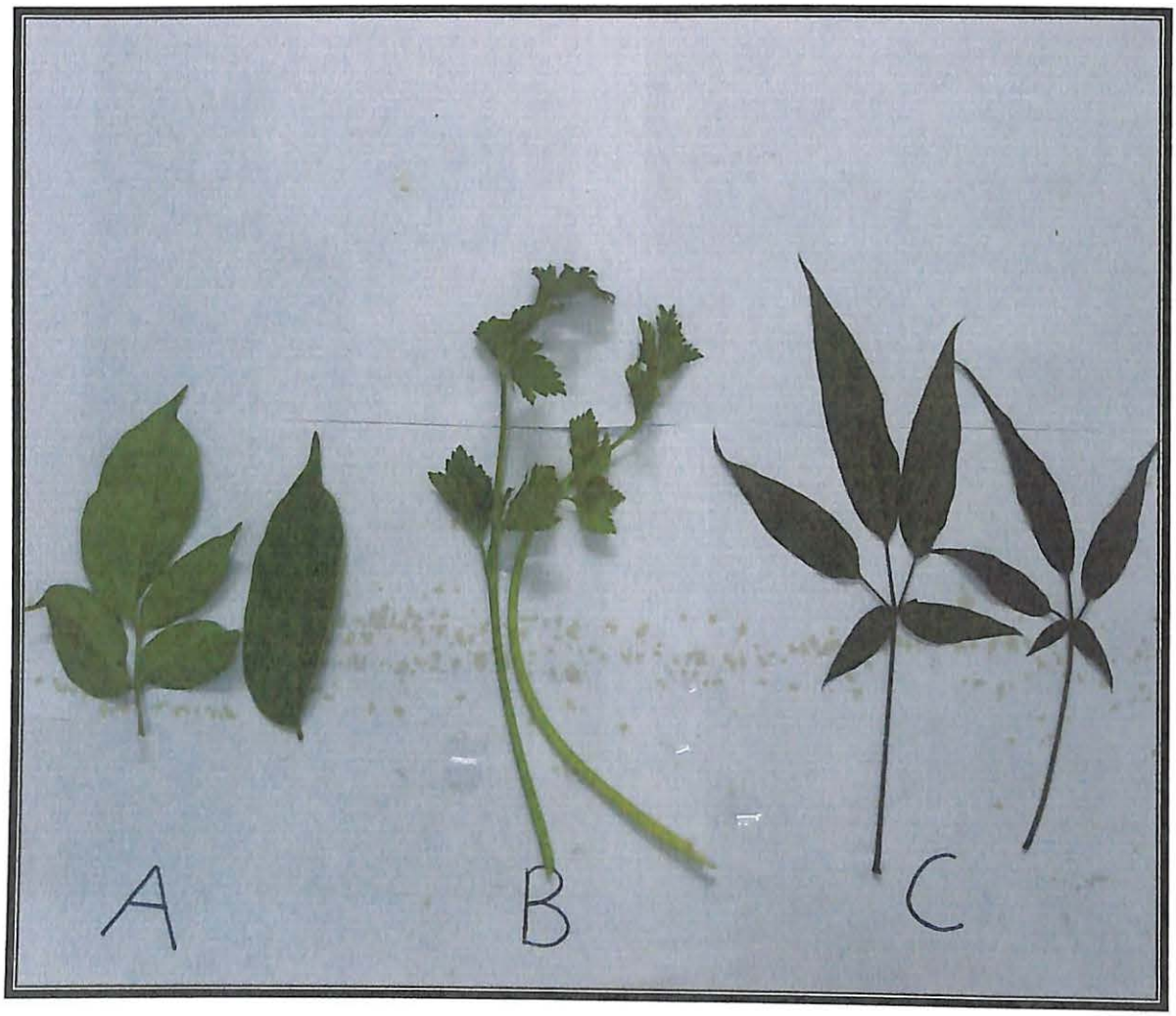


FIGURE 4.4 Picture of Ulams (in the single figure for comparison) studied.

A. *Oroxylum indicum* (Beka)

B. *Apium graveolens* (Sup/Sadri)

C. *Vitex negundo* (Lemuni)

FIGURE 4.5 Grinder

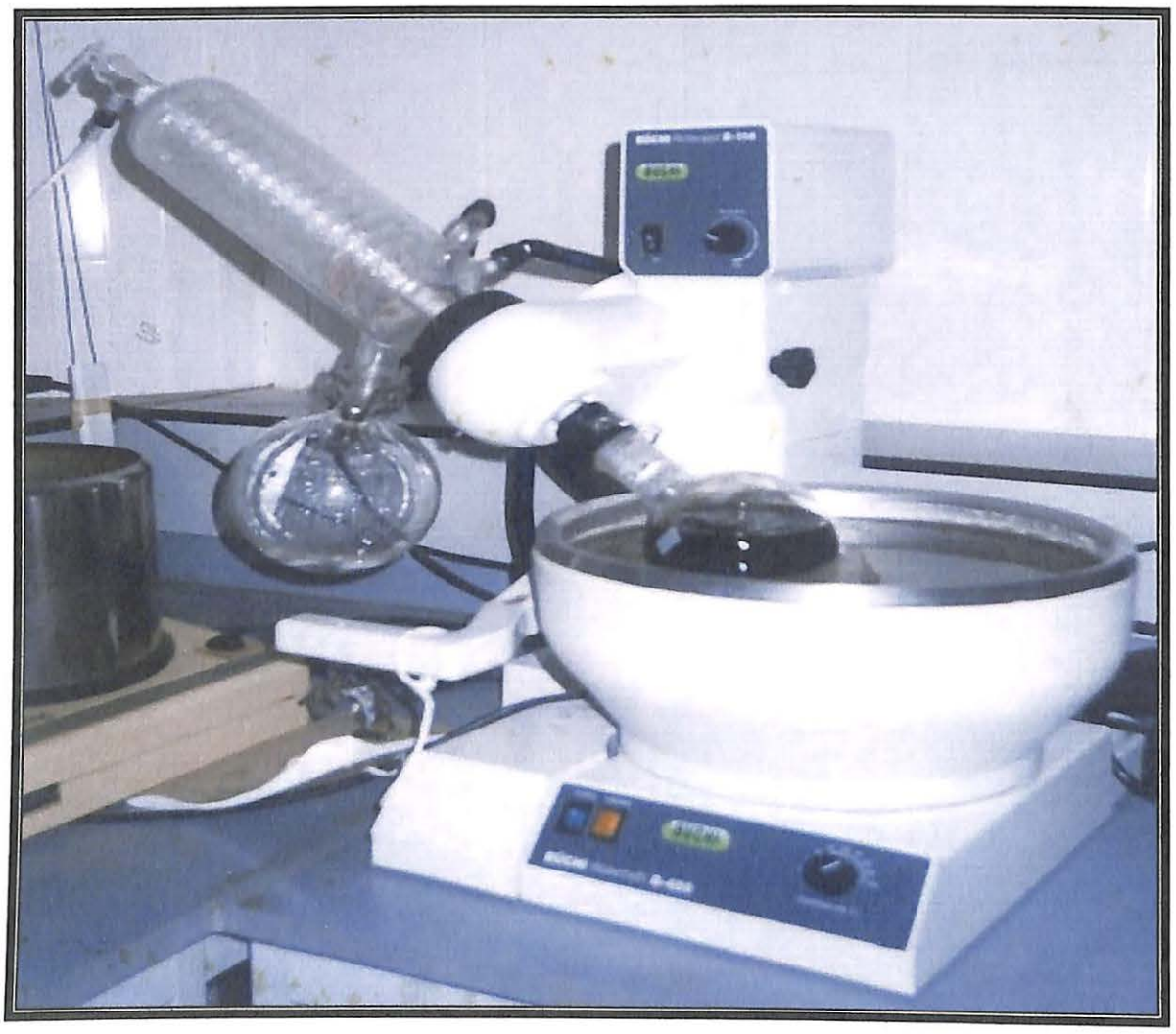


FIGURE 4.6 Rotary Evaporator