# KESAN PENURUNAN PARAS GLUKOSA-DARAH OLEH EKSTRAK BIJI *SWIETENIA MACROPHYLLA* DALAM TIKUS NORMAL DAN TIKUS DIABETIK TERARUH -STREPTOZOTOCIN

**oleh**

**MOHD AKMAL BIN HASHIM**

**Tesis yang diserahkan untuk**
**memenuhi keperluan bagi**
**Ijazah Sarjana Sains**

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# BLOOD-GLUCOSE LOWERING EFFECT OF *SWIETENIA MACROPHYLLA* SEED EXTRACTS IN NORMAL AND STREPTOZOTOCIN-INDUCED DIABETIC RATS

**by**

**MOHD AKMAL BIN HASHIM**

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In the Name of ALLAH

The Most Loving and the Most Merciful

**This thesis is dedicated to my parents and family for doing their best to educate and support me and to all my friends for their kindness in helping me to complete this study**

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*** indicate significant different between treated group and control (with insulin); $p<0.05$,

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| STZ | Streptozotocin |
| FBG | Fasting blood glucose |
| SAR | Structure activity relationship |
| DM | Diabetes mellitus |
| idDM | Insulin dependant diabetes mellitus |
| nidDM | Non-insulin dependant diabetes mellitus |
| IPGTT | Intraperitoneal glucose tolerance test |
| TZD | Thiazolidinedione |
| GIT | Gastrointestinal tract |
| GC-MS | Gas chromatography-mass spectrometer |
| LC-MS | Liquid chromatography-mass spectrometer |
| HPTLC | High performance Thin layer chromatography |
| TFA | Total flavonoid assay |
| OHA | Oral hypoglycaemic agent |
| TPA | Total phenolic assay |
| GAE | Gallic acid equivalent |
| QE | Quercetin equivalent |
| MW | Molecular weight |

**KESAN PENURUNAN PARAS GLUKOSA-DARAH OLEH EKSTRAK BIJI *SWIETENIA MACROPHYLLA* DALAM TIKUS NORMAL DAN TIKUS DIABETIK TERARUH -STREPTOZOTOCIN**

## ABSTRAK

Biji *S.macrophylla* telah digunapakai oleh orang yang terdahulu untuk merawat pelbagai jenis penyakit termasuk diabetes. Matlamat kajian ini adalah untuk menyiasat aktiviti antidiabetik ekstrak tumbuhan ini dan menentukan sebatian aktif yang mungkin menyumbang kepada aktiviti tersebut. Biji *S.macrophylla* yang telah hancur dan kering, diekstrak secara bersiri dengan Petroleum-eter, kloroform, metanol dan juga air melalui dua kaedah berbeza; pengekstrakan Soxhlet dan juga kaedah maserasi. Ekstrak-ekstrak yang diperolehi , diuji bagi aktiviti hipoglisemik ke atas tikus normal, perencatan kenaikan paras glukosa darah dalam ujian toleransi glukosa secara intraperitoneal (IPGTT) dan juga aktiviti antihiperglisemia ke atas tikus diabetik teraruh-streptozotocin.

Semua ekstrak daripada kaedah pengekstrakan Soxhlet didapati tidak mempunyai aktiviti antidiabetik. Manakala bagi ekstrak yang diperolehi melalui kaedah maserasi, didapati bahawa tiada ekstrak yang dapat menurunkan paras glukosa darah dalam ujian hipoglisemik ke atas tikus normal. Walaubagaimanapun, ekstrak petroleum-eter dan juga air yang diperolehi melalui kaedah maserasi, merencat dengan signifikan kenaikan paras glukosa darah dalam ujian IPGTT. Bagi tikus diabetik teraruh-streptozotocin, hanya ekstrak air berupaya menurunkan paras glukosa darah selepas 3, 5 dan 7 jam pemberian. Pemberian ekstrak petroleum-eter dan juga air secara oral sebanyak dua kali sehari, ke atas tikus diabetik menunjukkan hanya ekstrak air (1000mg/kg/sehari) dengan signifikan menurunkan paras glukosa darah puasa selepas

empat belas hari rawatan. Ekstrak air biji *S.macrophylla* kemudiannya difraksikan dengan kloroform, etil-asetat dan juga n-butanol.

Ujian selanjutnya menunjukkan bahawa fraksi n-butanol adalah yang paling poten dalam menurunkan paras glukosa darah puasa tikus diabetik teraruh-streptozotocin. Fraksi n-butanol didapati meningkatkan dengan signifikan penggunaan glukosa oleh sediaan otot abdomen terasing dan juga merencat penyerapan glukosa oleh kantung usus terbalik terasing. Ini mencadangkan bahawa kesan penurunan paras glukosa darah oleh fraksi n-butanol, setidak-tidaknya adalah melalui perencatan penyerapan glukosa pada saluran gastrousus dan juga meningkatkan penggunaan glukosa oleh otot. Analisa ekstrak petroleum-eter dengan menggunakan spektrometer jirim-kromatografi gas (GCMS) menunjukkan kehadiran fukosterol, β-sitosterol, asid linoleik dan juga γ-tokoferol yang mungkin bertanggungjawab ke atas aktiviti anti-hiperglisemik. Analisa ke atas fraksi n-butanol dengan menggunakan ujian saringan fitokimia dan juga spektrometer jirim-kromatografi cecair (LCMS) masing-masing telah menunjukkan fraksi tersebut mengandungi flavonoid, alkaloid dan juga saponin, dan flavonoid yang telah dikenalpasti adalah rutin dan isoquercitrin.

Kesimpulannya, kesan penurunan glukosa mungkin disumbangkan oleh sebatian tak berkutub yang merencat kenaikan paras glukosa darah ke atas tikus yang diberikan glukosa dan juga sebatian berkutub yang menurunkan paras glukosa darah ke atas tikus diabetik teraruh-streptozotocin. Penemuan-penemuan ini menyokong penggunaan tradisional *Swietenia macrophylla* untuk merawat diabetes

**BLOOD-GLUCOSE LOWERING EFFECT OF *SWIETENIA MACROPHYLLA* SEED EXTRACTS IN NORMAL AND STREPTOZOTOCIN-INDUCED DIABETIC RATS**

## ABSTRACT

*Swietenia macrophylla* (meliaceae) seeds have been used in folklore for treating various diseases including diabetes. The aim of the present work was to investigate the antidiabetic activity of the plant extracts and determine the active compound(s) that might contribute to the activity. Pulverized, dried powder of *S. macrophylla* seeds was extracted serially with petroleum ether, chloroform, methanol and water by two different methods; Soxhlet extraction and maceration methods. The extracts obtained were examined for hypoglycaemic activity in normal rats, inhibition of the rise of blood glucose level in intraperitoneal glucose tolerance test (IPGTT) and antihyperglycaemic activity in streptozotocin-induced diabetic rats.

It was found that all the extracts obtained from Soxhlet method did not possess antidiabetic activity. As for the extracts obtained from the maceration method, none of the extracts lowered the blood glucose levels in hypoglycaemic test in normal rats. However, petroleum-ether and aqueous extracts obtained by maceration method significantly inhibited the rise of blood glucose levels in IPGTT. In streptozotocin-induced diabetic rats, only aqueous extract at 3,5 and 7 hours after administration reduced the blood glucose level. Twice daily oral administration of aqueous and petroleum-ether extracts to diabetic rats showed that only aqueous extract (1000mg/kg/daily) significantly decreased the fasting blood glucose levels after 14 days

treatment. The aqueous extract of *S. macrophylla* seeds was then fractionated with chloroform, ethyl-acetate and n-butanol.

Further test showed that n-butanol fraction was the most potent in lowering the fasting blood glucose levels in streptozotocin-induced diabetic rats. The n-butanol fraction significantly increase the utilization of glucose by isolated abdominal muscles preparation and inhibited glucose absorption by isolated everted intestine. It may suggests that the blood glucose lowering effect of n-butanol fraction is by inhibition of glucose absorption in gastrointestinal tract and increasing utilization of glucose by muscles. Analysis of petroleum-ether extract by Gas-chromatography-mass spectrometer (GC-MS) showed the presence of fucosterol, β-sitosterol, linoleic acid and γ-tocopherol that might be responsible for the antihyperglycaemic activity. Analysis of n-butanol fraction, using phytochemical screening and LC-MS respectively indicated that the fraction contained flavonoids, alkaloids and saponins and the flavonoids that have been identified were rutin and isoquercitrin.

In conclusion, the glucose lowering effect might be contributed by the non polar compound(s) which inhibit the rise in blood glucose level in glucose loaded rats and polar compound(s) which reduced the blood glucose level in streptozotocin-induced diabetic rats. These findings support the traditional use of *Swietenia macrophylla* for treatment of diabetes.

# CHAPTER 1

# INTRODUCTION

## 1.0 **Pharmacognosy**

**Pharmacognosy** is the study of drugs of **natural origin**. It is one of the five major divisions of the pharmaceutical curriculum. In other words, pharmacognosy also means a knowledge of drugs or pharmaceuticals, that has been a part of healing arts and sciences, since mankind first began to treat illnesses (Varro *et al*., 1988). The ancient people, gathered herbs, animals, plants, and minerals and concocted them into plenty of ill-flavoured pungent mixtures, in order to treat diseases. The way of creating medication have progressed from an era of empiricism to the present age of specific therapeutic agent.

Recently, as a result of the intense concern with all aspects of ecology, there has been a renewed interest in so-called "natural" foods and drugs. The availability of an extremely wide variety of these products, ranging from the mint tea to ginseng chewing gum, has stimulated the scientific community to learn more about them. Nowadays, some natural compounds of drugs constituents have been partially replaced in commerce by synthetic compounds of identical chemical structure and therapeutic properties. For instance, natural camphor is obtained from the camphor tree by steam distillation, but in contrast, synthetic camphor was manufactured by either of two methods: by total synthesis from vinyl chloride and cyclopentadiene (a complete synthetic process) or semi synthesis from pine stumps (involve chemical modification of a natural product) illnesses ( Varro *et al*., 1988).

In future, the achievements of science and technology will be so great that, when brought to bear upon the mysteries of nature that have long puzzled us, those mysteries will yield their secrets amazingly. The plant and animal kingdoms will continue to serve mankind in the 21st century just as they have done since the dawn of history. Significant new drugs of natural origin and new methods of producing them will continue to be important parts for sustainability of human kinds.

## 1.1 **Herbs as an alternative medicine**

In recent years, medicinal herbs and their preparations have been increasingly considered in the treatment of illness, and at the the same time, Malaysia is a country that treasures its natural forests. These forests are the largest source of natural products which can be used for medicinal purpose. Since the early years, the Malays have been using plant products such as the leaves, seeds, roots, stems, flowers and fruit as ingredients in the preparation of traditional medicine. In the old days, plants were used primarily for treatment rather than cure, due to the tendency of practitioners of traditional medicine diagnosed diseases in a holistic manner. However, traditional medicine remains a popular method of treatment since it has been used in immemorial time until today.

Malaysia is a country with plenty of natural resources, rich with medicinal values. There are over 6000 species of tropical plants all over the country. Only in Peninsular Malaysia , there are 550 genera containing 1,300 species and most of these are medicinal plants good for the human body (*Muhammad & Mustafa*.,1994). Most drugs currently used as medicine today, are products of synthetic chemicals derived from natural chemicals. There are many types of traditional medicines that are widely used in

Malaysia for treating diabetes. It is a mixture of various plant parts. Some of the plants commonly used in Malaysia for treating diabetes are listed in Table 1. The composition of the herbs mixture varies in different places.

| **Local name** | **Scientific name** | **Family** | **Part Used** |
|---|---|---|---|
| Mengkudu | *Morinda citrifolia L* | Rubiaceae | Leaves |
| Lidah buaya | *Aloe vera L.* | Liliaceae | Leaves |
| Kunyit | *Curcuma Domestica Val.* | Zingiberceae | Rhizome |
| Kacang soya | *Glycine max [L.] Merr.* | Papilionaceae (Leguminoceae). | Seeds |
| Jambu batu | *Psidium guava L* | Myrtaceae | Fruit |
| Bawang merah | *Allium cepa L.* | Liliaceae | The entire parts |

Table 1.1 : Some of the herbs commonly used in Malaysia for treating diabetes.
Sources : (http://jamu-herbal.com, accessed on 2 July 2009)

1.2 **Compounds from herbs with antidiabetic activity**

Anti-diabetic secondary metabolites

Diabetes mellitus is a debilitating and often life-threatening disorder with increasing incidence throughout the world (WHO, 1985). Literature surveys show that more than **400 plant** species were reported to have **anti-diabetic** activity, and most of the anti-diabetic natural products were, so far, isolated from plants (Mukherjee, 1981; Rai, 1995).

| **SECONDARY METABOLITES** | Description |
|---|---|
| Flavonoids | Originate from a large group of polyphenolic compounds, commonly in plants.There are the pigments responsible for the shades of yellow, orange and red in flowering plants. Many are endowed with biological activities, such as anti-diabetic, anti-inflammatory,antiallergic, antischemic, antiplatelet and anti-tumoral activities. (Catherine and Lester, 2003) |
| Alkaloids | An organic nitrogenous compounds with a limited distribution in nature. May occur in various parts of the plants: in seeds, fruits, leaves, in underground stems, roots, rhizomes and barks. The pharmacologic actions varies widely : from anti-diabetic to analgesics and narcotics whereas others are central stimulants. (Varro *et al*.,1988). |

| | |
|---|---|
| Diterpenes | Naturally occurring organic compounds that comprise two monoterpene molecules; found in some essential oils; reported to have antibacterial, anti-diabetic, antiviral, antifungal, anti-inflammatory and expectorant properties ( Jonas., 2005) |
| Triterpenoids | Terpenoid derivatives of triterpene molecules. They may have useful activities including immunostimulation, anti-tumour-promoting activity, antiinflammatory and anti-cancer properties . ( Joseph *et al*., 2002) |
| Sesquiterpene lactones | It is another member of the terpene group; comprises three units of isoprene. Molecular formula is $C_{15}H_{24}O_6$. Reported to be useful as an antiseptic, antibacterial, or anti-inflammatory, anti-diabetic and as a calming agent by aromatherapists. ( Jonas., 2005). |
| Saponins | A group of glycosides that is widely distributed in the higher plants. It form colloidal solutions in water that foam upon shaking. They have a bitter and acrid taste . Saponins have been reported to beneficial to control blood cholesterol levels, bone health, cancer, diabetic and building up of the immune system. (Varro *et al*., 1988). |

| | |
|---|---|
| Anthocyanins | Water-soluble, terrestrial plant pigments that can be classified chemically as both flavonoid and phenolic. Act as a colouring agents for fruit juices and wine. These pigments are linked to an amazingly broad range of health benefits such as treating diabetes, hypertension, vision disorders, microbial infections and also diarrhea. ( Jonas., 2005) |
| Lignans | A type of polyphenolic compound that occurs naturally in some plants. Sources that are particularly rich in lignin, in order of highest to lowest concentration are, flax, sesame, sunflower, and pumpkin seeds. This substance has an excellent anti-oxidant properties and belief to help in preventing the development of diabetes and arteriosclerosis. (Iqbal ahmad *et al*., 2006) |
| Tannins | A naturally occurring polyphenolic compound with antioxidant and anticarcinogenic effects. *In vitro*/animal studies indicate it may have health benefits in treating **diabetes** (Liu X *et al*., 2005) |

Table 1.2 : Some of the secondary metabolites that have been reported to have anti-diabetic properties.

## 1.3 **Approaches to anti-diabetic screening of *S. macrophylla* seeds extract.**

### 1.3.1 Extraction of *S. macrophylla* seeds.

Extraction is a process of transfering a solute from one phase to another. In other words, this is the separation of a substance from a mixture by dissolving that substance in a suitable solvent. Extraction is one of the important parts in bioactivity guided isolation study. Figure 1.1 shows the flow chart for the study of plants used in traditional medicine (Luc Pieters & Arnold., 2005).

A successful extraction, should begin with careful selection of solvents and preparation of the plant samples. The extraction method chosen should try to minimize interference from compounds that may be co-extracted with the target compounds, and contamination of the extract obtained. The extraction method used if possible can prevent decomposition of important metabolites or avoid artifact formation as a result of extraction conditions or solvent impurities. In this study,' serial extraction' has been chosen as the method of extraction . Serial extraction refers to the use of increasing polarity of solvents in order to remove different polarity of secondary metabolites from the plants. The solvents used in this study were petroleum ether, chloroform, methanol and water.

### 1.3.2 *In-vivo* and *in-vitro* studies of *S. macrophylla* seeds extracts

#### 1.3.2.1 *In-vivo* experiment.

*In- vivo* experiment defines as experiment conducted in a living body. *In vivo* testing is often employed over *in vitro* because it provided overall effects of an experiment on a living subject (Wikipedia.org, accessed on 10 Dec. 2010). This is due to, some conditions in the *in-vitro* are unrealistic or unsimilar to the actual environment within the living organism. For instance, if the active entity of a substance is its metabolite, the substance might not show any activity if being tested using *in-vitro* method. In this study, we have chosen three different *in-vivo* experiments using the actual animal models , which includes both normal and diabetic rats. The experiments are hypoglycemic and intraperitoneal glucose tolerance tests in normal rats, and also antihyperglycaemic test in diabetic rats.

For the animal models of diabetes mellitus, the condition can be induced through pharmacological, surgical or genetic manipulations in several animal species. Most experiments in diabetes are carried out on rodents, although some studies are performed in larger animals. The classical model employed by Banting and Best was pancreatectomy in dogs (Bliss, 2000). However, majority of studies published in the field of ethnopharmacology between 1996 and 2006 employed models, where diabetes was induced through administration of chemicals such as streptozotocin (STZ, 69%) and alloxan (31%). This model has been useful for the study of multiple aspects of the disease. Both drugs exert their diabetogenic action when they are administered parenterally: intravenously, intraperitoneally or subcutaneously. The dose of these agents required for inducing diabetes depends on the animal species, route of

administration and nutritional status. According to the administered dose of these agents, syndromes similar to either type 1, type 2 diabetes mellitus or glucose intolerance can be induced (Lenzen *et al*., 1996; Mythili *et al*., 2004). Methods are available, but the pH and type of buffer employed as well as the preparation of the solution of either alloxan or streptozotocin in the day of the experiments are still arguable (Yu *et al*., 2000; Lei *et al*., 2005;; Miranda *et al*., 2006).

In this study, the diabetic rats are being induced by a single intraperitoneal (i.p) injection of 60mg/kg streptozotocin(STZ) dissolved immediately in normal saline (0.9% NaCl, pH 4.5) before used, in overnight (16 hours) fasted rats (Dominguez *et al*,. 2000). Hyperglycemia (diabetes) was confirmed by the elevated blood glucose level determined at 72 hours after STZ injection and the symptom of polyurea. The rats found with a fasting blood glucose (FBG) level above 15mmol/L were considered to be diabetic and used in the experiment.

#### 1.3.2.2 ***In-vitro* experiment**

*In vitro* is the opposite of *in vivo,* which means a biological process or experiment that is performed not in a living organism but in a controlled environment, such as in a test tube or Petri dish (Kail *et al.*, 2006). *In-vitro* test involved the use of organs, tissues, cells, cellular components, proteins, and/or biomolecules. It is a better test for deducing biological mechanisms of action compare to the *in-vivo* approach. However, the controlled conditions present in the *in vitro* system differ significantly from those *in vivo,* and may give misleading results due to the fact that living organisms are extremely complex functional systems that are made up of genes, protein molecules, RNA molecules, small organic compounds, inorganic ions and complexes in

an environment that is spatially organized by membranes and, in the case of multicellular organisms, organ systems (Alberts and Bruce., 2008). Therefore, *in vitro* studies are usually followed by *in vivo* studies. In this study, we used the isolated tissue preparation in order to determine or elucidate the mechanism of actions of the extract. Two different experiments will be carried out to determine whether the antihyperglycaemic properties of these extracts act through extrapancreatic mechanism of action. The first experiment involve the study of glucose uptake by muscles using isolated abdominal muscle preparation of Gray and Flatt, (1998) with some modification. Another experiment is to study the effect of the extracts on glucose absorption in the gastrointestinal tract by using the modified averted sac technique of Wilson and Wiseman, (1954). Increase of glucose uptake by muscles and inhibition of glucose absorption in the gastrointestinal tract are among the extrapancreatic mechanisms known to cause the lowering of blood glucose levels.

### 1.3.3 **Single dose and repeated doses (14 days) studies**

Acute studies (single dose) are short-term studies with exposure periods usually less than 24 hours or maybe last for 48 to 96 hours. This study is usually implemented for the purpose of screening for various kinds of bio-activities in living organism.

Opposite to the acute study, is the chronic study (repeated doses). In certain conditions, a single dose study might not give positive result or exert any potential bio-activity when being tested. However, this does not conclude that the bio-activities are not there or in other words the plant or compound do not possess the bio-activity.

Chronic study (repeated dose) in animals are usually necessary to evaluate toxic characteristics of a chemical arise from repeated exposure over a period of time.

Repeated dose studies, mostly have been carried out after initial information on toxicity has been obtained from acute toxicity test. Duration of exposure normally depends on nature of chemical and circumstances.

In my study for the fourteen days treatment, the extracts were administered orally, twice daily (9.00 a.m and 9.00 p.m) for fourteen days . The animals were observed individually after dosing, daily for fourteen days. Each animal was observed for toxic sign and recorded. Body weight and fasting blood sugar level were measured once a week. The baseline (day 0, before the treatment start) and final (day 15,after two weeks treatment) weight and fasting blood sugar level were examined and recorded.

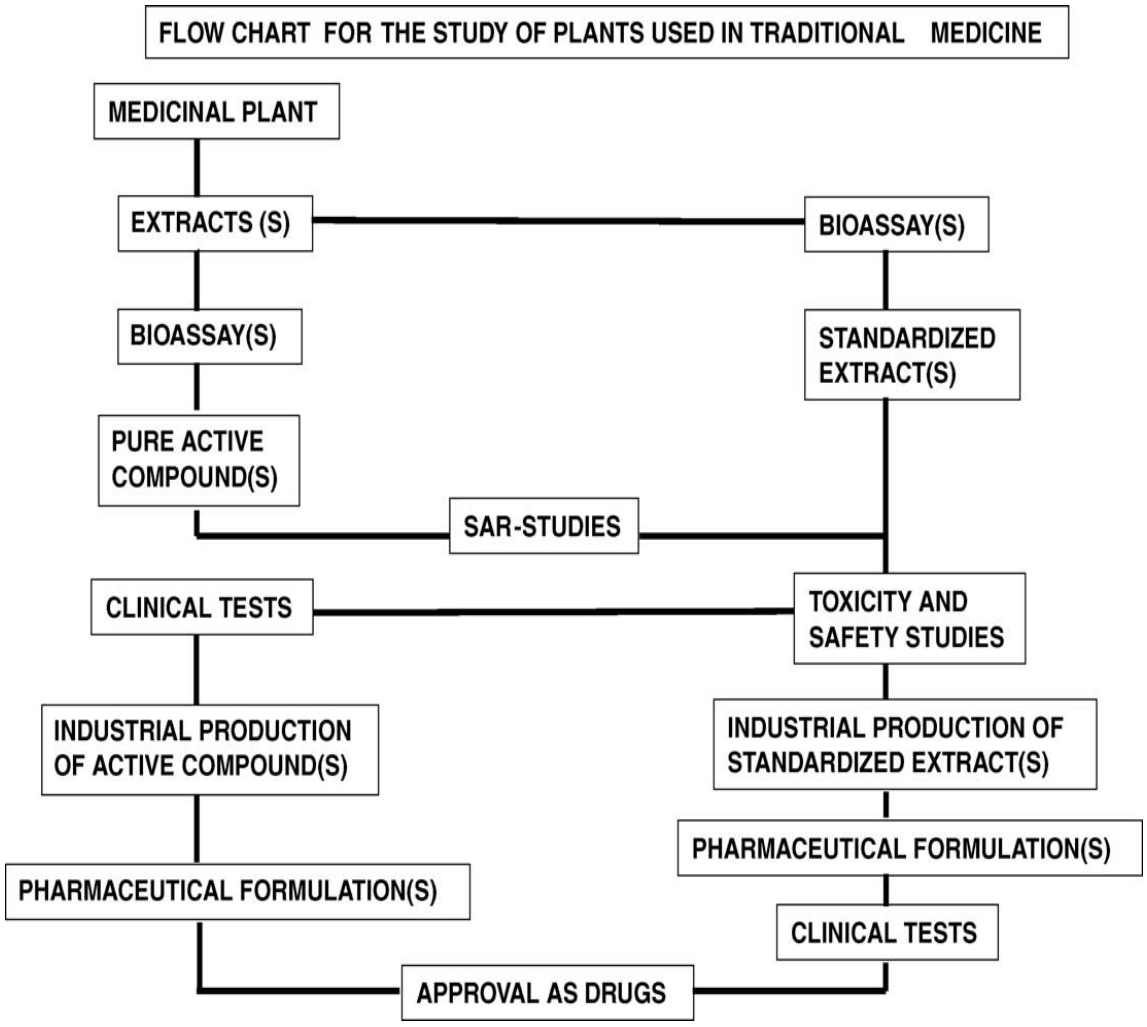
### 1.3.4 **Phytochemical screening**

Phytochemistry, or easily defined as the chemistry of plants, is one of the early subdivisions of organic chemistry. This division is importance in the identification of plant substances of medicinal value. It deals with the the study of chemicals derived from plants (phytochemicals). Phytochemicals are chemical compounds such as β-carotene that occur naturally in plants. The term is generally used to refer to those chemicals that may affect health, but are not yet established as essential nutrients (Satyanand Tyagi., 2010)

However, the use of phytochemicals comes with a caution sign. These compounds were not always beneficial under all circumstances especially in high doses. Certain biochemicals and vitamins, have been found to encourage the growth of cancer cells and their use is being discouraged in patients undergoing cancer treatments. Although it has many benefits in certain conditions, high doses of ***beta***-carotene

supplements are associated with an increased risk of lung cancer in male smokers. (Russel R *et al*., 1999)

With the development of new phytochemical methods, new information has become available for use in conjunction with plant taxonomy; which contributed to the modern field of chemotaxonomy, or biochemical (Britannica.com., accessed on 23 Jan 2011).




*SAR studies (Structure-activity-relationship studies)

Figure 1.1 Flow chart for the study of plants used in traditional medicine (Luc Pieters and Arnold., 2005)

## 1.4 ***Swietenia macrophylla (SM) King***

*Swietenia macrophylla* King, from family Meliaceae is also known as big-leaf mahogany or Honduras mahogany. It is a tropical timber tree that can grow to a height of 40-60 m. This tree produces a fruit commonly called "sky fruit" because the fruit seems to hang upwards from the tree. In Malaysia, the locals usually called it as '**Tunjuk Langit**'. This plant is naturally distributed from the Atlantic regions of south-east Mexico, through Central America, northern South America and across the southern Amazon Basin, in Bolivia and Brazil, but nowadays this plant is cultivated in South East Asia region for various purposes (Lugo *et al*,2003). 'Tunjuk Langit' has been processed commercially to a wide range of health foods and healthcare products.

- Scientific classification :

Kingdom: Plantae
Order: Sapindales
Family: Meliaceae
Genus: *Swietenia*
Species : *S. macrophylla*

Figure 1.2 :

a) Tree

b) Leaves

c) Fruit

d) Seeds

This plant is an umbrella-shaped crown. fast-growing perennial tree with tall straight, cylindrical bole clear of branches for 12-18m, often with high buttresses. It has pinnate leaves arranged alternately and clustered at the ends of branchlets. Each leaf consists of 3- 6 pairs of opposite or occasionally subopposite leaflets that are typically 9-14 x 3-5 cm, usually oblong to oblong-lanceolate or ovate-lanceolate (Lugo *et al.*,2003).

### 1.4.1 **The morphology of fruit and seeds of *S. macrophylla***

Figure 1.3 The fruit of *S. macrophylla*

The fruits are large (12-15 x 6-8 cm), woody, erect, capsules, oblong to slightly sub-globulus. The outer valves are thick and becoming woody with a coriaceous surface when mature. When dry, the 4 or 5 valved fruits split open from the base, or from the base and the apex simultaneously. The centre of the fruit is a thick, woody 5 angled columella extending to the apex from which the seeds hang pendulous by their wing, leaving conspicuous seed scars after their release.

Figure 1.4 : The seeds of *S. macrophylla*

The seeds are chestnut colored and 7.5-12 cm in length with wings. There are arranged in irregular forms inside the fruit. There are usually about 35-45 winged seeds per fruit and it takes around 13,000 to 20,000 seeds to reach a kilogram weight. This seeds ripen from the end of January to beginning of March and varies according to location.

### 1.4.2 **Phytochemistry of *Swietenia macrophylla***

Phytochemical study of *Swietenia macrophylla* King has revealed various compound exist in this plant. The first phytochemistry study on *S. macrophylla* was reported in 1951 by Guha Sircar *et al* where they successfully isolate two tetranotriterpenoids known as swietenine (figure 1.5) and swietenolide. Later, Mootoo *et al* in 1999, reported that, upto fifteen limonoids have been isolated from *S. macrophylla*, which are 7-deacetoxy-7-oxogedunin, andirobin (figure 1.8), and thirteen bicyclononanolides. In 1999, they successfully discovered another two new compounds which are augustineolide and 3$\beta$,6-dihydroxydihydrocarapin from the same plant . In addition, they also mentioned another nine known compounds that have been identified

from *S. macrophylla* which are; proceranolide, 6-O acetylswietenolide, 3,6-O,O-diacetylswietenolide, Khayasin T, Swietemahonins E-G, 2-hydroxyswietenine and 6-deoxyswietenine also known as febrifugin (figure 1.10).

Further phytochemical investigations have revealed the presence of limonoids such as 8, 30-epoxyswietenine acetate, swietenine acetate, swietenolide diacetate (figure 1.6), and swietenolide tiglate, in the seeds of *S. macrophylla* (Chakravarty & Chatterjee, 1955; Chakravarty, Chatterjee, & Krishnagar, 1957; Chan, Tang, & Toh, 1976; Connolly, Henderson, McCrindle, Overton, & Bhacca, 1964; Connolly & Labbe, 1980; Ghosh, Chakrabartty, & Chatterjee, 1960).

Other parts of the plant, which is the leaves of *S. macrophylla*, have been reported to yield essential oils which contain himachalene, germacrene D, germacrene A, cadina-1,4-diene, hexadecanoic acid, and ethyl hexadecanoate (Marisi *et al*., 2003). Seok-Keik Tan *et al* in 2009, also mentioned four new phragmalin-type limonoids, named swietephragmin H-J and swietemacrophine have been isolated from the leaves of *S. macrophylla* King. The barks of *S. macrophylla* have been reported to possess triterpenoids, limonoids, flavonoids and tannins according to Guha Sircar *et al* in 1951 and Mootoo *et al* in 1999.

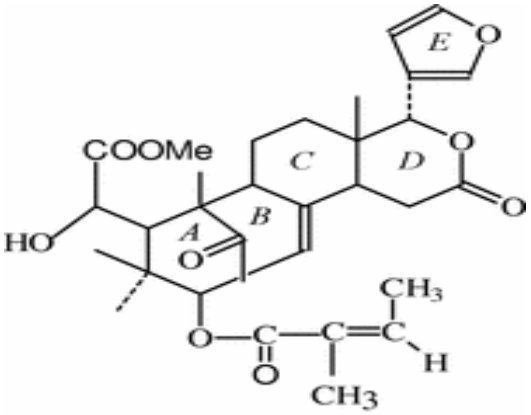



Figure 1.5 *Swietenine*
(I)

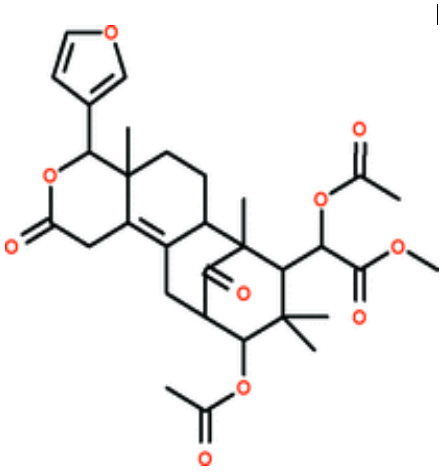


Figure 1.6 Swietenolide diacetate

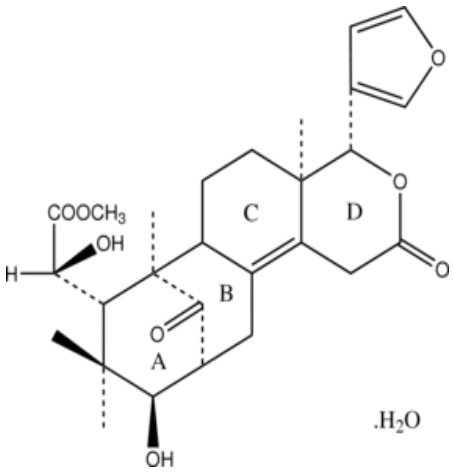



Figure 1.7 Swietenolide monohydrate

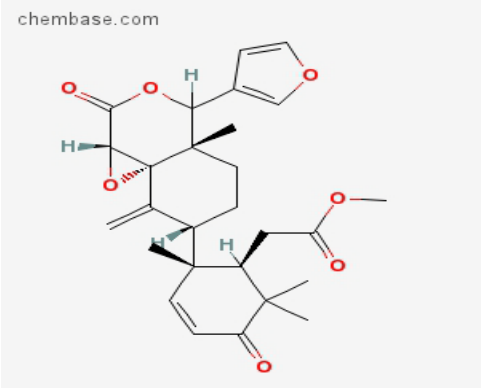



Figure 1.8 Andirobin

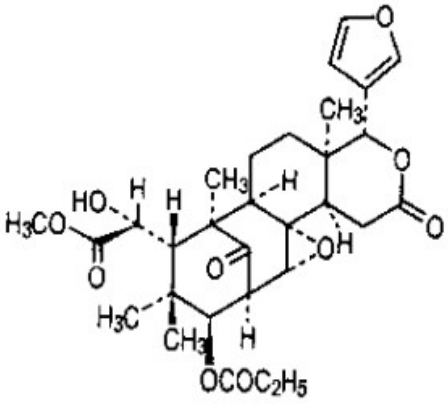



Figure 1.9 Swietemahonin A

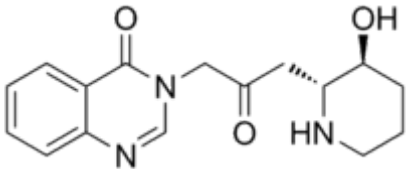



Figure 1.10 Febrifugin

### 1.4.3 **Medicinal value of *S. macrophylla***

Herbal medicines have been used since the dawn of civilization to maintain health and to treat disease. There is a tremendous historical legacy in folklore uses of plant preparations in medicines. Scientific studies on plants used in ethnomedicine led to the discovery of many valuable drugs (Shylesh BS and Padikkale J, 2000).

The seeds of *S. macrophylla* have been used for Leishmaniasis and abortion medicine by an Amazonian Bolivian ethnic group (Bourdy *et al*.,2000) and for treatment of hypertension, diabetes and malaria as folk medicines in Indonesia (Kadota *et al*., 1990a). Murningsih *et al* in 2005, reported anti-malarial activity of water extract of *S. macrophylla* seeds against *Plasmodium falciparum* . The seeds also have antibabesial (Murningsih *et al*.,2005) and anti-diarrhoeal activities (Maiti *et al*.,2007). The seeds also have been reported to possess antiinflammatory, antimutagenicity and antitumor activity (Maiti *et al.*, 2007).

In Malaysia, the seeds of *S. macrophylla,* are used as a folk medicine for treating diabetes and high blood pressure (Chan *et al*, 1976). A decoction of the crushed seeds of this tree is also used to treat skin ailments and wounds (Munoz *et al*, 2000). The seeds are also used for diarrhea and a survey of literature showed that the organic and aqueous extracts of *S. macrophylla* seeds possess a wide array of biological properties including anti-diabetic (Maiti *et al*, 2007a) . In the same year, Maiti *et al* also discovered that *S. macrophylla* also possess anti-diarrhoeal (Maiti *et al*,2007b) and antibabesial activities (Maiti *et al*,2007c). Anti-microbial and anti-malarial activities have also been reported with this plant (Soediro *et al*,1990, Jean *et al*, 2000).

Guevera AP *et al* in 1996, mention that the seed of *S.macrophylla* had been reported to have anti-inflammatory, antimutagenicity, and anti-tumor activities. *S.macrophylla* also have been shown to exhibit insect antifeedant activities (Nsiama *et al.*, 2008), anti-fungal (Govindachari *et al*., 1999) and in Malaysia, the seeds are used as a folk medicine for treatment of hypertension (Chan, Tang, & Toh, 1976). Munoz *et al* (2000) reported that this plant also has anti-inflammatory activity. The barks of *S. macrophylla* also reported to possess anti-HIV, antimicrobial, antimalarial, and antitumor activities (Munoz *et al*, 2000) .

### 1.5 **Diabetes mellitus, (DM) disease of the millennium**

Diabetes is one of the oldest diseases known to mankind. It was first mentioned in the Ebers Payrus (Egypt 1500BC) and also called as 'honey urine' by Sushrutha in India in 400 BC (Larry A. Distiller.,1980). The Latin word 'mel' which means honey was used, and the disease came to be known as diabetes mellitus that is, the passing of honeyed urine. This is still the full name of the disease.

Diabetes occur when the quantity of insulin produced is too little to allow for glucose to be used or to be stored. In other words, this disease will appear when a person's body does not make enough insulin or cannot use insulin properly. Therefore, the hallmark of diabetes, which is due to insulin deficiency, is a rapid increase in the level of glucose in the blood to more than normal levels.

Diabetes can be divided into two groups : Type 1 which is known as insulin dependant diabetes mellitus (idDM) and also Type 2 ; non-insulin dependant diabetes mellitus (nidDM).

According to World Health Organization (WHO) data, based on a survey conducted in 1996 in Malaysia , it was estimated that around 700,000 to 900,000 adults aged 30 and above were having diabetes or there are 8 diabetic patients in every 100 adults.

### 1.5.1 **What causes diabetes?**

The causes of diabetes mellitus are rather ill understood even now. However, as mentioned previously, the most important cause of diabetes mellitus is the failure of beta cells in the pancreas to secrete the insulin hormone sufficiently to the body needs. Causes of type 1 diabetes differs from type 2 in many aspects . Type 1 diabetes is mainly due to the destruction of insulin-producing beta cells of the pancreas with resulting loss of insulin production. The actual process causing the damage of the beta cells is still unknown although there are many theories.

As for type 2 diabetes, which usually develops gradually, differs greatly from the type 1. The cause is unknown. One type of type 2 is associated with obesity with marked insulin resistance. Many factors are blamed for the cause of this type of diabetes but recently more interest has been shown in the possibility of faulty neural mechanisms of metabolic control. Unlike type 1 diabetes, islet cell antibodies are not found in type 2 diabetes. Table 3 shows some of the causes of diabetes mellitus.

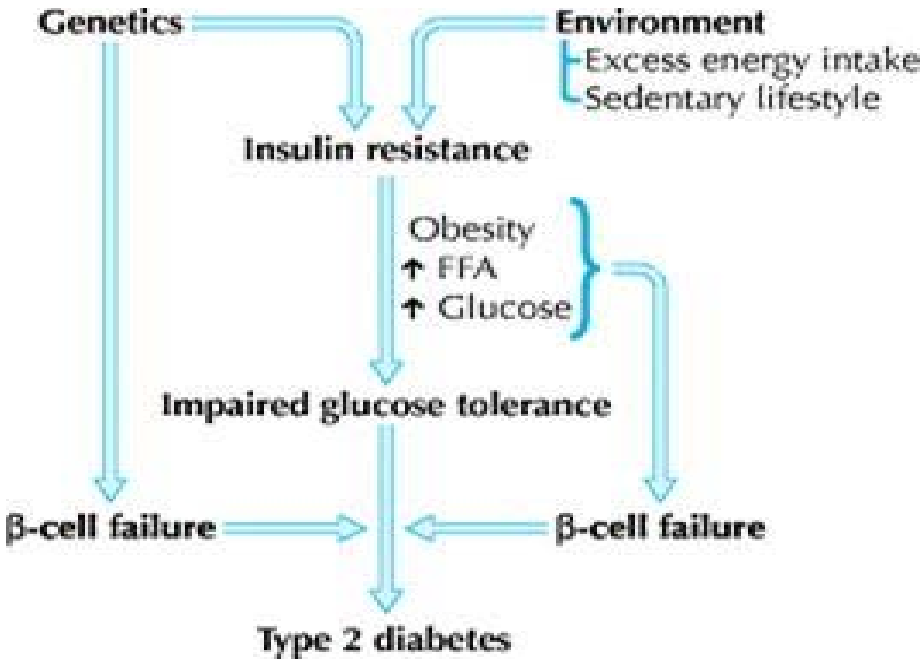
| CAUSES OF DIABETES MELLITUS | |
|---|---|
| TYPE 1 : | |
| a) Viral Infection | ?mumps<br>?Coxsackie B4 virus<br>? Rubella |
| b) Hereditary<br>c) Auto- immunity<br>d) Toxin to Islet cells. | |
| TYPE 2 : | |
| a) Hereditary + | i ? diet<br>ii ? exercise |
| b) Obesity | |

Table 1.3 : Causes of diabetes mellitus (Lee Y.S., 1991).

### 1.5.2 **Pathophysiology of diabetes mellitus (DM)**

Glucose is a simple sugar found in food. Glucose is an essential nutrient that provides energy for the proper functioning of the body cells. Carbohydrates are broken down to glucose in the small intestine, and the glucose is then absorbed by the intestinal cells into the bloodstream, and is carried by the bloodstream to all the cells in the body where it is utilized. However, glucose cannot enter the cells alone and needs **insulin** to aid in its transport into the cells. Without insulin, the cells become starved of glucose energy despite the presence of abundant glucose in the blood stream. The abundant, unutilized glucose is wastefully excreted in the urine.

Insulin is a hormone that is produced by specialized cells (beta cells) of the pancreas. (The pancreas is a deep-seated organ in the abdomen located behind the stomach). In addition to helping glucose enter the cells, insulin is also important in tightly regulating the level of glucose in the blood. After a meal, the blood glucose level rises. In response to the increased glucose level, the pancreas normally releases more insulin into the bloodstream to help glucose enter the cells and lower blood glucose levels after a meal. When the blood glucose levels are lowered, the insulin release from the pancreas is turned down. As outlined above, in patients with diabetes, the insulin is either absent, relatively insufficient for the body's needs, or not used properly by the body. All of these factors cause elevated levels of blood glucose (hyperglycemia). Over a period of time, high glucose level in the bloodstream can lead to severe complications, such as eye disorders, cardiovascular diseases, kidney damage and nerve problems.




* FFA = free fatty acid

Figure 1.11: Overview of the pathogenesis of type 2 diabetes mellitus ( adopted from (http://www.pharmainfo.net )

### 1.5.3 **Treatment for diabetes mellitus (DM)**

When diet and exercise unable to lower the raised blood sugar level to an acceptable value, the use of drugs became a must. Many Type 2 diabetics will require the use of hypoglycaemic drugs to lower the blood sugar. However, almost all Type 1 diabetics will not respond to oral hypoglycaemic drugs, hence the only way to overcome this type of diabetes is through administration of Insulin. Generally, there are four main groups of oral hypoglycaemic agents (OHA) available for the treatment of diabetes, which are classified according to their mode of actions. They are Insulin secretagogues, insulin sensitizers, α-glucosidase inhibitors and biguanide (Lebovitz 2001).

1.5.3.a **Insulin Secretagogues.**

Insulin secretagogues can be divided into 2 subclasses: **sulfonylureas** and non-sulfonylureas. The basic action of sulphonylureas is normalizing the release of insulin by some healthy β-cells of the pancreas (Lee Y.S.,1991). It also reduces the production of glucose in the liver and the absorption of glucose from the gut. Some sulphonylureas are said to enhance insulin sensitivity in the peripheral tissues. Sulfonylureas that are currently available are glipizide, gliclazide, **glibenclamide**, glimerpide and glyburide. As for the non-sulphonylureas, the mechanism of action of these drugs is similar to that of the sulfonylureas , except they bind to the sulfonylurea receptor at a different site and with different kinetics than the sulfonylureas. Nateglinide and repaglinide are some of the drugs belong to this group.

1.5.3.b **Insulin Sensitizers**

Thiazolidinediones (TZD) are the main insulin sensitizers currently available. Examples of TZDs are rosiglitazone and pioglitazone. TZDs are selective agonists for the peroxisome proliferator-activated receptor gamma (PPAR γ), which is most highly expressed in adipocytes. These nuclear receptors, which are ligand-activated transcription factors, play an integral part in the regulation of the expression of a variety of genes involved in carbohydrate and lipid metabolism (Lebovitz.,2001). TZDs require insulin to be present for their activity, where they increase the insulin sensitivity, particularly in the peripheral tissues.