CHAPTER 1

INTRODUCTION

1.1 Statement and significance of the problem

Diabetes mellitus (DM) is a disease in which the pancreas produces insufficient amounts of insulin, or in which the body's cells fail to respond appropriately to insulin. In people suffering from DM, glucose levels build up (hyperglycemia) in the blood and urine, causing excessive urination, thirst, hunger, and problems with fat and protein metabolism with a myriad of secondary complications such as cardiovascular disease, stroke and kidney failure (ADA, 2002) due to what is now referred to as "glucose toxicity", leading to significant levels of morbidity and mortality (Covington, 2001). The world wide prevalence of DM is expected to be more than 366 million by the year 2030 (WHO, 2000). In developing countries, the prevalence of diabetes is increasing. This is the phenomenon in countries such as Thailand and Nigeria (Wild *et al.,* 2004; Aeplakorn *et al.,* 2003). The factors responsible for the high prevalence of diabetes are genetic predisposition, ignorance, improving socioeconomic status, age, sex, life style and the environment (Zimmet, 1983).

Among the treatment option for DM, the most popular ones are the conventional/orthodox medicines beginning with the leader drug insulin and then synthetic derivatives of plant phytochemicals and others medicines. Other treatment

options exist for DM ranging from conventional orthodox or modern medicines to the traditional ones. Modern therapeutic agents like sulfonylurea and biguanides are valuable in the treatment of DM but they are often unable to lower the blood glucose to desirable levels or to reinstate a normal pattern of glucose homeostasis (Senthivet *et al.*, 2006). Some anti-diabetic drugs have limitations arising from insensitivity after long term use to organ damage as a result of the switching from one drug to another, low dose to higher doses and combinatorial therapy (UKPDS, 1998), and the fact that, the cost of DM treatment are mostly lifelong and requires multiple dosing which leads to non compliance by most patients. Hence, therapeutic agents totally lacking or with no noticeable aforementioned draw backs are sought after or often complemented with the use of traditional medicines (Pari and Saravana, 2004). Many patients switch to the use of natural products, predominantly those derived from plants, for their well being because of the growing recognition that the natural products are non toxic, have lesser side effects and are accessible, and affordable (Chandrasekaran *et al.*, 2010). These medicines comprise of single plants or multiple plants simply referred to as Complementary and Alternative Medicine (CAM) or Herbal or Traditional Medicine.

Recently, the switch from conventional orthodox medicines to CAM has been met with both success and skepticism. Though, the number of medicinal plants said to have hypoglycemic effects could be in their thousands, only a few have been subjected to scientific evaluation to verify such claims. A few of these medicinal plants now in use for the DM treatment around the world however have been tested and found to possess significant hypoglycemic effects resulting in the isolation of several novel compounds which have successfully been clinically tested and

commercialized for the DM treatment such as Berberine and Metformin (Wang *et al.*, 2010; Bailey and Day, 2004)

1.2 Objectives

The objectives of this study are as follows:

1. To screen the aqueous extracts of 20 anti-diabetic medicinal plants/recipes used traditionally in the treatment of DM for their hypoglycemic effect in Northern part of Thailand and North Eastern Nigeria in normoglycemic and alloxan induced diabetic mice.
2. To extract with methanol and chloroform, and fractionate with suitable solvents 2 of the most active anti-diabetic medicinal plants from 1 above (Thai and Nigerian) and screen each sub-fraction for its hypoglycemic activity in normoglycemic and alloxan induced diabetic mice.
3. To purify, isolate, elucidate and characterize the chemical structure of the most active hypoglycemic sub-fraction(s) from No. 2 above.
4. To determine the hypoglycemic effect of the isolated compound(s) in diabetic mice.

1.3 Scope of the study

1) Sample selection

Medicinal plants were collected from two main sources. 10 medicinal plants were selected from the Thai-Lanna Traditional Medicine Database in the Raw Material and Natural Products Research Database (MANOSROI II) at the Faculty of Pharmacy, Chiang Mai University, Thailand. The remaining 10 were selected from

28 medicinal plants during a field research trip covering the North Eastern States of Nigeria.

2) Preparation of the crude extracts

The crude extracts of the medicinal plants were prepared based on the traditional methods of preparation before usage. This was by maceration in hot water (aqueous extracts). The crude extracts were screened for their hypoglycemic effects in normoglycemic and alloxan induced diabetic mice, phytochemistry and the free radical scavenging activities by DPPH.

3) 2 of the medicinal plants (1 from each group) with the highest hypoglycemic effect were selected, methanol and chloroform was used to partition the plant extracts and suitable organic solvents of different polarity were used to fractionate them further by various separation techniques (TLC, Column chromatography and liquid phase partitioning).

4) Hypoglycemic screening of the sub-fractions

The various sub-fractions of two of the medicinal plants were screened *in vivo* in normoglycemic and alloxan induced diabetic mice and the sub-fractions with the highest hypoglycemic effect were selected for further experiments.

5) The selected sub-fractions from 4 above was subjected to further experiments to perform the isolation and purification of the bioactive compounds from the sub-fractions by using open column chromatography, thin layer chromatography (TLC), evaporative light-scattering detectors (ELSD), high performance liquid chromatography (HPLC), ultraviolet spectrometer (UV) and refractive index (RI) detectors.

6) Structure elucidation of the compounds

The isolated compounds were elucidated for their chemical structures by appropriate techniques using nuclear magnetic resonance (NMR) and mass spectroscopy (MS).

7) Bioactivity studies of the isolated compounds

The isolated compounds were evaluated for their hypoglycemic effects in alloxan induced diabetic mice.

1.4 Literature review

Medicinal plants or phytomedicines can be defined as the parts of a plant, namely its seeds, root, leaves, bark, flowers the whole plant itself which is employed for their medicinal or therapeutic purposes. This practice has long been in existence since man first worked the face of the earth. The first generally accepted use of plants as healing agents was depicted in the cave paintings discovered in Lascaux cave in France, which have been radio dated to between 13 000 to 25 000 BC. Medicinal herbs were found in the personal effects of an "ice man", whose body was frozen in the Swiss Alps for more than 5 300 years, which appeared to have been used to treat the parasites found in his intestines (Hirst, 1997). Anthropologists theorized that animals evolved a tendency to seek out bitter plant parts in response to illness. This behavior arose because bitterness is an indicator of secondary metabolites (Harker, 2008). The risk benefit ratio favored animals and protohumans that were inclined to experiment in times of sickness. Over time and with insight, instinct, and trial and error, a base of knowledge would have been acquired within early tribal communities. As this knowledge base expanded over generations, the specialized role of herbalist emerged. The process would likely have occurred in varying manners within a wide

diversity of cultures. A reasonable conjecture is that these discoveries were traditionally collected by the traditional medicinal practitioners (medicine men) of the indigenous tribes who then passed on safety information and caution. Medicinal plants have now come to be defined as "phytotherapeutic agents" or "phytopharmaceuticals". The use of these materials for such purpose of healing in contemporary times, now that evidence and rational prove are a condition needed for explaining phenomena has lead to the coinage of the term "phytotherapy" (Pulock 2006).

Many of the drugs in use today by physicians have a long history of use as herbal medicines, examples include; opium, aspirin, digitalis and quinine. The use and search for drugs and dietary supplements from plants accelerated the high patronage and believe in medicinal plants. However, with the understanding and innovations in scientific methods, ideas and opinion about medicinal plants changed, thus more patronage was seen even among the unbelieving elites. The cases of lethal and terrible side effects reported after the administration of some synthetic drugs, and the unethical drug trials carried out by some drug companies in parts of Asia, Africa and South America (Bard, 2011) endeared medicinal plants to more people. The World Health Organization (WHO, 1999), reported that over 80% of the world's population uses herbal medicines as their first form of health care. Though, cautious and encouraging further research into medicinal plants, the world body affirmed that 25% of modern drugs in use in the United States for example have actually been derived from plants. A significant number of these drugs came to the attention of researchers because of their use as medicinal plants (in traditional medicines).

China and India having raised the status and acceptability of their traditional medicines to great heights, Scientists around the world have recorded tremendous successes in research on medicinal plants, an example of which is the discovery of the anticancer drug "taxol" from the pacific yew tree (*Taxus brevifolia*) in 1993 (Tuma, 2003). Several ailments are today either managed successfully or have been cured using medicinal plants processed into herbal medicines,, such as cold, fever, microbial infection and some forms of metabolic ailments notably diabetes mellitus. The active ingredients which are exploited by man in medicinal plants are a variety of chemicals referred to as "phytochemicals", synthesized within the plants to achieve certain biochemical objectives, for example:

- Alkaloids are made up of a nitrogen ring, it has a dramatic effect on the central nervous system of higher animals. Caffeine, also an alkaloid produce mild lift but, opium containing alkaloid can cause severe intoxication and death.
- Phenols are present in anthocyanins that are responsible for the purple color in grapes, the isoflavones, phytoestrogens from soy and the tannins that give tea its astringency.
- Terpenes are the building blocks of Terpenoids. They are made up of two isoprenoid units. The aroma or fragrances which emanates from flowers (e.g. rose and lavender), are as a result of the presence of units of terpenes (monoterpenes). The colors, red, yellow, and orange of the pumpkin, corn and tomatoes are as a result of carotenoids.
- Glycosides are made up of glucose units attached to an "aglycone". These compounds are of special importance to plants in their defense mechanism.

Substances like those mentioned above, and many more, such as; *phytoenzymes* and other metabolites constitute the body of active compounds which possess the medicinal (healing) characteristics sought after by scientists today and were exploited by the herbalists before them. The last few decades recorded a huge growth in the demand for herbal medicines and their proprietary products in the pharmaceutical industry and medicinal markets, especially in China, Japan, and countries in Europe (and Africa), and North America. (Ying *et al*, 2007). This is largely because of the belief that herbal medicines may have fewer side effects and can enhance the effects of conventional agents or could serve as an alternative treatment, as such cutting the high cost of medicinal use. Also, the belief is that, the therapeutic effect of herbal medicine is based on the synergic effect of its mass constituents, which is different from that of western medicines (Van Beek, 2003). Some common uses of medicinal plants today are treatment for infections, cold stomach aches, diabetes among others.

1.4.1 Diabetes mellitus (DM)

The term DM on a broad scale describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action or both. The effect of DM includes long term damage, dysfunction and failure of various organs. It may present characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and in absence of effective treatment, death. Often, symptoms are not severe, or may be absent and

consequently hyperglycemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made. The long time effects of DM include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction including sexual dysfunction. People with DM are at risk of cardiovascular, peripheral vascular and cerebrovascular disease.

Individuals with diabetes mellitus can be grouped according to clinical stage which is achievable in all circumstances. Glycemic stage may change with time depending on the level of the underlying disease processes (Table 1). The disease may be present but its extent may not be high enough to cause hyperglycemia. The etiological classification reflects the fact that the defect or process which may lead to diabetes may be identifiable at any stage in the development of DM at the stage of normoglycemia. Therefore, the presence of islet cell antibodies in an individual with normal glycemia levels could serve as an indicator of diabetes insipidus. Unfortunately, there are few sensitive or highly specific indicators known for type 2 diabetes mellitus, although these are likely to be revealed as the etiology is more clearly defined. The number of people who suffer from diabetes mellitus around the world is alarming and the estimates of those who are predicted to suffer from it in the future is equally so (Anu *et al.*, 2007). It is therefore important that all forms of treatment and prevention methods be studied against this ailment.

Table 1.1 the three main types of diabetes are: Type 1, Type 2 and gestational diabetes (Source: www.abcdiabetes.org_)

Type 1 Diabetes  	In Type 1 diabetes, the cells that produce insulin are damaged or destroyed so the pancreas is no longer able to make any insulin. People with Type 1 diabetes need to be injected with insulin and learn to balance their insulin with their food choices.
Type 2 Diabetes  	In Type 2 diabetes, the pancreas may not make enough insulin or the body is unable to use it properly. People with Type 2 diabetes may use food choices, exercise, diabetes pills or insulin to help treat their high blood glucose levels. Insulin levels may be normal, elevated, or depressed (hyperinsulinemia) and insulin resistance characterize most patients (insulinopenia) may develop as the disease progresses. It is not insulin-dependent or ketosis-prone under normal circumstances, but may use insulin for treatment of hyperglycemia. Onset is predominantly after age 40 years but can occur at any age. It occurs in approximately 50% of men and 70% of women who are obese. Its etiology probably strongly genetic as 60%-90% of monozygotic twins suffers from it.

Table 1.1 the three main types of diabetes are: Type 1, Type 2 and gestational diabetes (continued)

Gestational Diabetes	Glucose intolerance that has its onset or recognition during pregnancy. It is associated with old age, obesity, family history of diabetes. It conveys increased risk for the woman for subsequent progression to diabetes mellitus. Associated with increased risk of *macrosomia.* Most pregnant women need two to three times more insulin than they do when they are not pregnant. This happens because of normal hormonal changes during pregnancy. Gestational diabetes occurs when a woman's body cannot make enough insulin to meet these demands.

As additional subtypes are discovered it is anticipated that they will be reclassified within their own specific category. Though hyperglycemia at a level diagnostic of diabetes is also observed to be present in instances of any of the following: Pancreatic disease, hormonal disease and in etiologic relationship between DM and the other conditions such as drug or chemical exposure and Insulin receptor abnormalities and certain genetic syndrome. Diabetes secondary to another condition can only be established by clinical workup or medical history to determine the presence of the other condition. and for Impaired Fasting Glycemia >=6.1 (>=110) and <7.0 (<126) and if measured, 2-h <8.9 (<160). For epidemiological or population screening purposes, the fasting or 2-h value after 75 g oral glucose may be used alone. For clinical purposes, the diagnosis of diabetes

1.4.2 Diagnosis

The clinical diagnosis of DM is often prompted by symptoms such as increased thirst and urine volume, recurrent infections, unexplained weight loss and, in severe cases, drowsiness and coma; high levels of glycosuria are usually present. Single blood glucose estimation in excess of the diagnostic values indicated in Figure 1.1 (black zone) establishes the diagnosis in such cases. Figure 1.1 also defines levels of blood glucose below which a diagnosis of diabetes is unlikely in non-pregnant individuals (WHO, 1999). For clinical purposes, an oral glucose tolerance test (OGTT) to establish diagnostic status need only be considered if casual blood glucose values lie in the uncertain range (between the levels that establish or exclude diabetes) and fasting blood glucose levels are below those which establish the diagnosis of diabetes. If an OGTT is performed, it is sufficient to measure the blood glucose values while fasting and at 2 hours after a 75 g oral glucose load (Table 1.1). For children the oral glucose load is related to body weight: 1.75 g per kg. The diagnostic criteria in children are the same as for adults. Diagnostic interpretations of the fasting and 2-h post-load concentrations in non-pregnant subjects are shown in Table 1.1.

1.4.3 Treatment of Diabetes

A treatment for DM may not really be defined as such, rather it is much easier to accept that it can only be managed for very longtime, thus removing it from the category of terminal ailments. The choice of treatment depends on the severity and the disposition of the health practitioners.

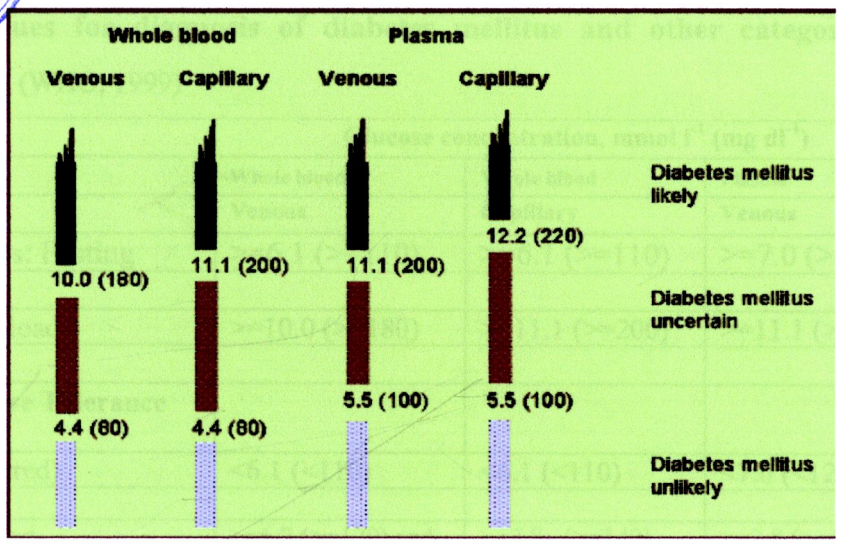

Figure 1.1 Unstandardized (casual, random) blood glucose values in the diagnosis of diabetes in mmol l^{-1}(mg dl^{-1}). Taken from the 1985 WHO Study Group Report (9) (WHO, 1999)

In some diabetics, glycemia levels can be lowered with weight reduction, exercise and oral agents such as drugs in the form of tablets or herbal medicines (mixtures). While some individuals may re-attain normal glycemic levels of the former, other individuals however would require insulin to lower their glucose levels, but they can still survive without it. Such individuals are said to have residual insulin secretion (type 2). Those whose beta cells have been destroyed, and have entirely zero insulin secretion would require insulin shots for survival (type 1). The extent of metabolic abnormality can reduce (with weight reduction), increase (with weight gain) or remain the same (David *et al.*, 2002).

Table 1.2 Values for diagnosis of diabetes mellitus and other categories of hyperglycemia (WHO, 1999)

	Glucose concentration, mmol l^{-1} (mg dl^{-1})		
	Whole blood	**Whole blood**	**Plasma***
	Venous	**Capillary**	**Venous**
Diabetes mellitus: Fasting	>=6.1 (>=110)	>=6.1 (>=110)	>=7.0 (>=126)
Or			
2-h post glucose load	>=10.0 (>=180)	>=11.1 (>=200)	>=11.1 (>=200)
or both			
Impaired Glucose Tolerance (IGT):			
Fasting (if measured)	<6.1 (<110)	<6.1 (<110)	<7.0 (<126)
And			
2-h post glucose load	>=6.7 (>=120) and	>=7.8 (>=140) and	>=7.8 (>=140) and
	<10.0 (<180)	<11.1 (<200)	<11.1 (<200)
Impaired Fasting Glycemia (IFG):			
Fasting	>=5.6 (>=100) and	>=5.6 (>=100) and	>=6.1 (>=110) and
	<6.1 (<110)	<6.1 (<110)	<7.0 (<126)
and (if measured)			
2-h post glucose load	<6.7 (<120)	<7.8 (<140)	<7.8 (<140)

* Corresponding values for capillary plasma are: for Diabetes Mellitus, fasting >=7.0 (>=126), 2-h >=12.2 (>=220); for Impaired Glucose Tolerance, fasting <7.0 (<126) and 2-h >=8.9 (>=160) and <12.2 (<220); should always be confirmed by repeating the test on another day unless there is unequivocal hyperglycemia with acute metabolic decompensation or obvious symptoms (WHO, 1999).

Irrespective of the underlying cause, diabetes mellitus can be sub-divided into three groups :(i) those that require insulin for survival, e.g. C-peptide deficient. (ii) Those that require insulin for metabolic control rather than survival. Here some endogenous insulin secretion are observed but are insufficient to achieve normal glucose levels without added exogenous insulin; and, (iii) Those that do not require

insulin, i.e. they may control glucose levels satisfactorily by non pharmacological methods or drugs other that insulin. Among all the various therapeutic methods employed for the management of diabetes mellitus, the drug of choice for the condition is insulin. Since diabetes was discovered and attempts made to really define it, Scientists have come up with various possible explanations to the frequently asked questions that diabetes poses. This ranges from the isolation and purification of insulin from animal source, such as, ox, pig, cattle and so on, to laboratory synthesis by recombinant biotechnology. Example of such drugs obtained from synthesis: thiazolidiendiones, sulfonylureas, biguanides, and alpha- glucosidase inhibitors and incretins.

The most important pathophysiologic feature in type 2 diabetics is insulin resistance. Obesity and physical inactivity contributes to insulin resistance, characterized by inability to use glucose by the skeletal muscles where insulin action is abnormal. Glyco- genolysis fails in the liver, creating an inadequacy for the insulin secretion to compensate for insulin resistance. Because of all these complications, must patients require more therapy as time passes, hence the need for more drugs.

1.4.4 Drugs used for diabetes mellitus treatment

Currently five groups of oral hypoglycemic agents are among the most popular being prescribed for DM patients. The introduction of new drugs may possibly render the achievement of better glycemic control with drug therapy. However, in instances of renal failure, care must be taken in their use, while a host of them are to be avoided altogether (William and Roshan, 1999). These groups of drugs are designed to do the following, **i.** act by enhancing the production of insulin

(Sulfonylureas and incretins), **ii.** Enhance peripheral insulin action (thiazolidiendiones), **iii.** Inhibit hepatic glucose production (Metformin), **iv.** Slow intestinal absorption of glucose (alpha-glucose inhibitors).

a. Insulin

Insulin is an animal hormone whose presence informs the body's cells that the animal is well fed, signaling the liver and muscle cells to absorb glucose and get it stored in the form of glycogen, and fat and blood lipids converting them into triglycerides. Insulin is produced in the pancreas, and released upon stimuli. In target cells they initiate a signal transduction that gives effects of increasing glucose uptake and storage. Finally, insulin is degraded, terminating the response.

The major actions of insulin lead to increase synthesis of protein, glycogen, and fat, thereby promoting muscle strength and energy reserves when food is available. The signaling systems that mediate these anabolic responses to insulin regulate key controlling enzymes in these respective metabolic pathways. In general, these regulated enzymes are stimulated or inhibited by insulin through phosphorylation of protein serine/threonine residue or by dephosphorylation of such sites. Because the insulin receptor is a tyrosine kinase, the general underlying feature of insulin signaling is switching the initial receptor-mediated tyrosine phosphorylation to modulation of protein serine/threonine protein kinase and protein serine/threonine phosphate phosphatases (Klarlund *et al.*, 2003).

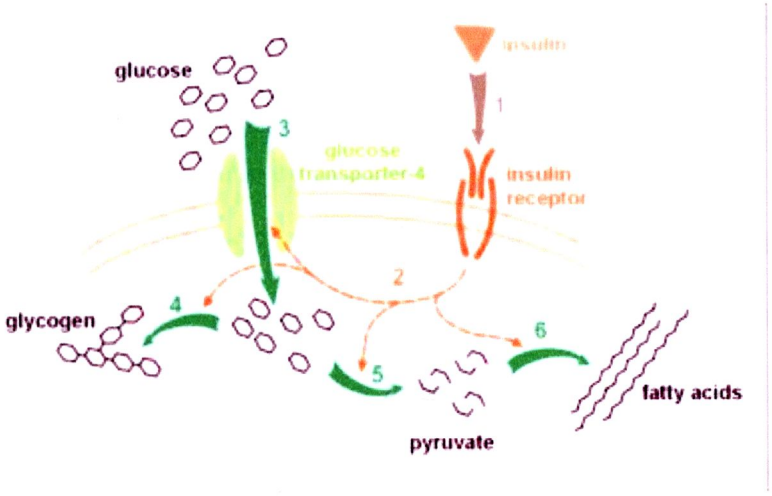

Figure 1.2 Effect of insulin on glucose uptake and metabolism. Insulin binds to its receptor (1) which in turn starts many protein activation cascades (2). These include: translocation of Glut-4 transporter to the plasma membrane and influx of glucose (3), glycogen synthesis (4), glycolysis (5) and fatty acid synthesis (6) (Source: N/A)

Another switching mechanism involves the activation of a lipid kinase, phosphatidylinositol-3-kinase (PI-3-Kinase), which catalyzes the formation of 3' polyphosphoinositides that in turn initiate recruitment and activation of the serine/threonine protein kinases Akt (protein kinase B) and certain protein kinase C isoforms. Many of the metabolic effects of insulin appear to be regulated by this pathway (Klarlund *et al.*, 2003). By increasing the uptake of glucose by cells and reducing the concentration of glucose in the blood, insulin prevents or reduces the long-term complications of diabetes, including damage to the blood vessels, eyes, kidneys, and nerves. Insulin is administered by injection under the skin (subcutaneously). The subcutaneous tissue of the abdomen is preferred because absorption of the insulin is more consistent from this location than subcutaneous tissues in other locations. Regular insulin has an onset of action (begins to reduce

blood sugar) within 30 minutes of injection, reaches a peak effect at 1-3 hours, and has effects that last 6-8 hours.

Biochemically, insulin is made up two distinct peptide chains referred to as the A and B chain, linked together by two disulfide bonds, and an additional disulfide is formed with the A chain. In most species, the A chain consists of 21 amino acids and the B chain of 30 amino acids. Although the amino acid sequence of insulin varies among species', certain segments of the molecule are highly conserved, including the positions of the three disulfide bonds, both ends of the A and the C terminal residues of the B chain. These similarities in amino acid sequence of insulin lead to a three dimensional conformation of insulin that is very similar among species, hence its intra usage from pigs or ox by human patients (Bowen, 1999).

Side effects

Insulin may cause minor and usually temporary side effects such as rash, irritation or redness at the injection site. Too much insulin can cause low blood sugar (hypoglycemia). The symptoms include cold sweat, shaking, rapid heart rate, weakness, headache and fainting which, if untreated, may lead to slurred speech and other behaviors that resemble drunkenness. Too little insulin on the other hand can cause symptoms of high blood sugar (hyperglycemia) which include confusion, drowsiness, rapid breathing, fruity breath odor (alkalosis), increased urination or unusual thirst and worsening of diabetic retinopathy, changes in the distribution of body fat (lipodystrophy), allergic reactions, sodium retention, and general body swelling. Symptoms of an allergic reaction include: rash, itching, swelling, dizziness,

trouble breathing. Treatments with human insulin have also been observed to produce anti-bodies in the diabetic human patient (Lahtela *et al.*, 1997).

b. Incretins

The incretins (or glucagon like proteins – GLP) represent one of the earliest classes of hormones to be described and their study, especially as they relate to normal and abnormal glucose tolerance has a long history. The two major characteristics used to define an incretin are: (a) that it is released following nutrient, particularly carbohydrate ingestion and (b) that concentrations reached after meals stimulate insulin secretion (Creutzfeldt and Nauck, 1992). There are currently two known hormones that meet these criteria namely: glucagon-like peptide 1 (GLP) and gastric inhibitory peptide (also known as: glucose-dependent insulinotropic polypeptide or GIP). Recent studies suggest that both of these peptides are necessary for normal glucose tolerance.

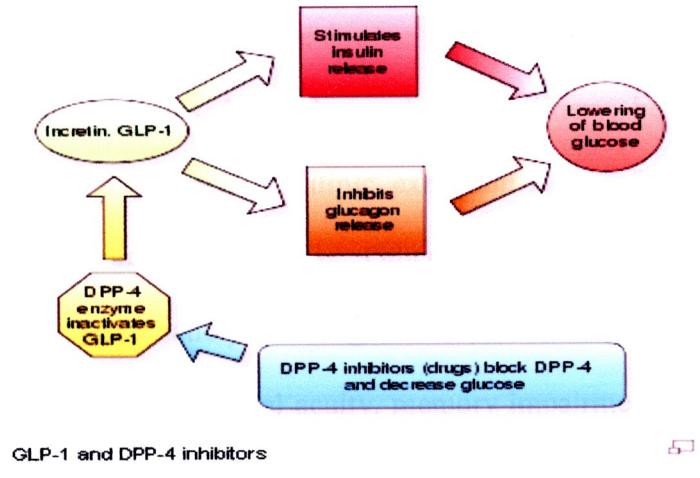

Figure 1.3 Mechanism of incretin action (Drucker and Nauck, 2006)

The GLPs and glucagon are formed by alternative tissue-specific cleavages in the L cells of the intestine, the a-cells of the endocrine pancreas, and neurons in the

brain. Glucagon-like peptide-1 (GLP-1) is now known to be a potent glucose-dependent insulinotropic hormone, which has important actions on gastric motility, on the suppression of plasma glucagon levels, and possibly on the promotion of satiety and stimulation of glucose disposal in peripheral tissues independent of the actions of insulin. GLP-2 was recognized only recently to have potent growth-promoting activities on intestinal epithelium. (Kieffer and Habener, 1999).

Drugs that mimic's incretin action but are long lasting than the natural incretin have been developed and three examples are exenatide (Byetta) and liraglutide (Victoza). Their merits had been that they must be administered by subcutaneous injection. While the third which inhibits the enzyme that inactivates GLP-1 and GIP, DPP-4, is sitagliptin (Januvia). Several DPP-4 inhibitors that can be taken orally as a tablet have also been developed.

Side effects

The main side effects of exenatide use are gastrointestinal in nature, including acid or sour stomach, dizziness, headache, and feeling jittery, belching, diarrhea, heartburn, indigestion, nausea, and vomiting; exenatide is therefore not meant for people with severe gastrointestinal disease. Other side effects such as thrombosis, arthralgia, haematuria, early satiety, burning sensation, choking, multiple allergies, sleep apnoea syndrome, reduced visual acuity, memory impairment were reported in female diabetic patients (Ogburu and Mark, 2011).

Liraglitude has potential for inhibiting apoptosis (Vedtofte *et al.*, 2010) and stimulating regeneration of beta cells in animal studies (Sturis *et al.*, 2003). It has only mild and transient side effects, mainly gastrointestinal (Nauck *et al.*, 2008).

Side effects reported for sitagliptin eosinophilia, erythema, skin exfoliation, haemorrhage, leukaemia, diabetes mellitus inadequate control, diabetic retinopathy, diabetic vascular disorder, obesity, pancreatitis, anaemia, cardiac failure, pallor, pyrexia, renal failure, blood pressure increased, rhabdomyolysis, diarrhoea, lip swelling, swollen tongue among others (Nauck *et al.*, 2008).

c. Sulfonylureas

The Sulfonylureas stimulate insulin secretion thus making it the first choice for oral glycemic control, because all type of DM are relatively deficient in insulin. These compound enables beta cell insulin secretion directly through high affinity receptor via a mechanism similar to glucose itself, involving *beta* cell ion flux and membrane polarization. The sulfonylurea receptor present in pancreatic beta cells regulates adenosine triphosphate – dependent potassium channels. Sulfonylurea binding leads to inhibition of the channels; the decrease in potassium efflux causes beta cell depolarization, calcium influx and insulin secretion. The next result is to augment glucose- induced insulin secretion. Sulfonylureas also have extra pancreatic effects to improve glucose use. Improved varieties of this compound have been proven to be 100% times more potent than earlier versions with respect to receptor binding (Feldman, 1985). This is attributed to the differences derived from their chemical structures, thus enhancing its absorption, metabolism and dosing.

Sulfonylureas are used almost exclusively in DM (type 2). Sulfonylureas are ineffective where there is absolute deficiency of insulin production such as in type 1 diabetes or post-pancreatectomy. In about 10% of patients, sulphonylureas alone are

ineffective in controlling blood glucose levels. Addition of metformin or a thiazolidinedione may be necessary, or (ultimately) insulin.

Some sulfonylureas are metabolized by liver metabolic enzymes (cytochrome P_{450}) and inducers of this enzyme system (such as the antibiotic rifampicin) can therefore increase the clearance of sulphonylureas. In addition, because some sulfonylureas are bound to plasma proteins, use of drugs that also bind to plasma proteins can release the sulfonylureas from their binding places, leading to increased clearance (RxList, 2007).

Side effects

Generally, the drug has no known contraindications with regards to kidney or the liver. It is normally inactivated by the liver which serves as the main route of excretion along with varying amounts of the parent drug. It is expected that sulfonylurea lowers blood glucose by approximately 20%. One third of the patients who use this drug may not respond adequately. Over 50% of patients in clinical trials achieve normal or near normal blood glucose levels, i.e. "good" glycemic targets. In nearly one forth, initial or "primary" failure occurs, most commonly due to insulin insufficiency. It is observed that, the drug's efficiency diminishes with time (5% patient yearly), usually owing to progressive *beta*-cell impairment, obesity, illness, pregnancy, decreased patient activity or noncompliance. Other side effects known to have been caused by sulfonylureas are notably hypoglycemia. Hypoglycemic episodes are reported in about 20% of treated patients over six months. Most are mild, but sulfonylureas are the major cause of drug related hypoglycemia requiring hospitalization, e.g. chlorpromide or glyburide. Additional infrequent side effects that

can occur with all sulphonylureas include nausea, stomach upset, weight gain is observed unless diabetic diet and exercise program are constantly encouraged, skin reaction and abnormal liver function are also observed. Data on adverse side effect in patients with renal failure are not available. Other side effects recognized with sulphonylureas are cardiovascular problems, the cause being probably directly related to the mechanism by which Sulfonylureas are effective in treating type 2 diabetes. Sulfonylureas in pancreatic *beta* cells bind to the subunit of adenosine triphosphate (ATP) sensitive potassium channels that keeps the channels closed. This causes an influx of calcium ions into the cell that result in an increased release of insulin via exocytosis of insulin-containing granules. Unfortunately, traditional sulfonylurea drugs are not specific for pancreatic *beta*-cells and also bind to ATP-sensitive potassium channels in cardiomyocytes and vascular smooth-muscle cells (Bell, 2006). Channel binding by sulfonylureas in cardiac tissues prevents 3 otherwise beneficial mechanisms: the vascular smooth-muscle cell relaxation that improves coronary blood flow; the limitation of myocardial damage during ischemia; and the protection in cardiomyocytes of energy-generating mitochondria. The phenomenon by which myocardium develops a tolerance to brief periods of ischemia (after which an episode of prolonged ischemia will cause less damage than might otherwise be expected) is collectively known as ischemic preconditioning. Examples of this preconditioning are the decrease in size of a myocardial infarction when it is preceded by angina, the ability to exercise at the same rate without chest pain after an anginal attack ("warm-up" or "first hole" angina) and the decrease in anginal pain and ST-segment depression that accompanies the second balloon inflation during angioplasty (Bell, 2006). Because

of its affordability, Sulfonylureas remain the oral hypoglycemic agents of choice for patients with diabetes mellitus.

d. Thiazolidinediones (TZDs)

Thiazolidinediones (TZDs) are a promising new class of orally active compounds that can reverse insulin resistance by improving insulin dependent glucose disposal (Nolan *et al.*, 1994). A form of TZD, Troglitazone, works primarily by facilitating insulin mediated glucose uptake. Given alone in diabetes mellitus patients, it achieves normal blood glucose levels after meal tolerance test (Maggs *et al.*, 1998). TZDs primarily affects glucose disposal in skeletal muscle, by suppressing the production of basal hepatic glucose observed in high dosage conditions. As evident from clinical trials, this effect is seen as early as one week after initiation with the lowest possible point at the sixteenth week (Schwartz *et al.*, 1998). About 60% of patients responded positively, those who do not respond much still show signs of improvement. These show the lowest level of insulin secretion. Examples of TZDs are; Rosiglitazone (Avandia), Pioglitazone (Actos), and Troglitazone (Rezulin).

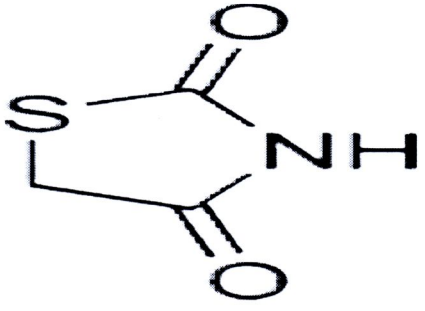

Figure 1.4 Thiazolidinedione
(*http://news.softpedia.com*)

The action of TZDs can be summarized in the following ways;

i. **Insulin sensitivity and secretion**: TZDs lowers fasting and postprandial glucose concentration as well as free fatty acid concentrations in clinical trials. Insulin concentration also decreases in the study. Such changes are indications that TZDs act as insulin sensitizers, which has been confirmed by direct measurement in in-vitro studies in humans. It also increases hepatic insulin sensitivity (the ability of insulin to suppress endogenous glucose production) and insulin sensitivity in the adipose tissues. Somehow, this improvement are accompanied by weight gain and an increase in the subcutaneous adipose tissue mass (Miyazaki *et al.*, 2001).

ii. **Enhancement of insulin sensitivity:** Peroxisomes Proliferators Activated Receptors gamma (PPARy) is essential for normal adipocyte differentiation and its proliferation as well as fatty acid uptake and storage. TZDs increase the number of small adipocytes and the subcutaneous adipose tissue mass in studies carried out on animal models (Picard and Auwerx, 2002). These observations plus the high level of PPARy expression in adipose tissues lead to the conclusion that TZDs exert their insulin sensitizing action either directly or indirectly by means of altered adipokine release, modulating insulin sensitivity outside adipose tissue. According to the fatty acid steal hypothesis, TZDs promote fatty acid uptake and storage in adipose tissue. In this way, they increase adipose tissue mass and spare other insulin sensitive issues such as skeletal muscle and liver, and possibly pancreatic beta cells, from the harmful metabolic effect of high concentrations of free fatty acids. TZDs thus keep fats in their appropriate places. Furthermore, with respect to this hypothesis, TZDs lower circulating free fatty acid concentration and triglyceride content in the liver but not in skeletal muscle, in patients with type 2 diabetes (Bajaj *et al.,* 2003). Pioglitazone

reduces hepatic fat content and augments splanchnic glucose uptake in patients with type 2 diabetes.

Side effects

Cardiovascular disease is a leading cause of death worldwide and a major complication of type 2 diabetes (Haffner *et al.*, 1998; DeNoon, 2010). The Thiazolidiendiones lead to an increase in body weight of 2 to 3 kg for every 1 percent decrease in Glycosylated hemoglobin values. The magnitude of the increase is similar during monotherapy and combination therapy with insulin or metformin in type 2 diabetes (UKPDS, 1998). The increase in body weight has been attributed to expansion of the subcutaneous fat depot, and in some patients to edema, whereas the mass of visceral fat remains unchanged or decreases. The clinical significance of these changes for patients with cardiovascular disease remains to be established. Systematic reviews of the literatures have found no notable benefits of thiazolidiendiones in regard to blood pressure (Hannele, 2004). The enzyme, alanine aminotransferase level was observered in 1% of patients using troglitazone to increase by about three times its normal values. This effect was observed only in this member of TZD. This finding is an indication of the drug being possibly hepatotoxic (Lebovitz *et al.*, 2002).

e. Alpha glucosidase inhibitors (AGIs)

These are a group of treatment options for DM patients. AGIs are recommended to patients alongside their meals and it works by inhibiting alpha glucosidase which then slows down the digestion of complex carbohydrates into glucose. The end results are a delayed glucose absorption in the small intestine and

thus lowering blood sugars after meals. AGIs can be used alone or along with other medications for diabetes. Examples of available brands of AGIs are, Glyset (Maglitol), and Precose (Acarbose) and Prandase. Alpha-glucosidase inhibitors are used to establish greater glycemic control over hyperglycemia in diabetes mellitus, particularly with regard to postprandial hyperglycemia. They may be used as monotherapy in conjunction with an appropriate diabetic diet and exercise, or they may be used in conjunction with other anti-diabetic drugs. Alpha-glucosidase inhibitors are saccharides that act as competitive inhibitors of enzymes needed to digest carbohydrates: specifically alpha-glucosidase enzymes in the brush border of the small intestines. The membrane-bound intestinal alpha-glucosidases hydrolyze oligosaccharides, trisaccharides, and disaccharides to glucose and other monosaccharides in the small intestine.

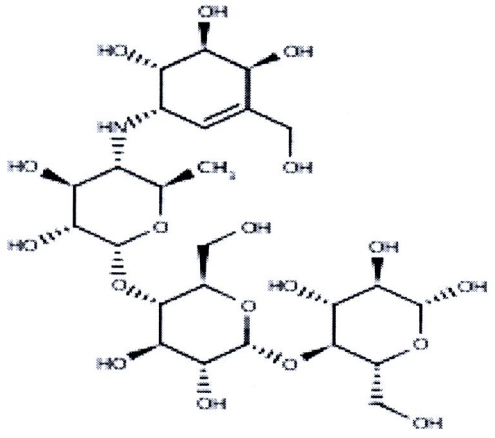

Figure 1.5 Chemical structure/formula: C_{25} H_{43} NO_{18}
(Drug Bank, 2007)

Acarbose binds to and inhibits alpha amylase and alpha glucoside hydrolases, this enzyme inhibition results in delayed glucose absorption and a lowering of postprandial hyperglycemia (Drug Bank, 2007). Acarbose also blocks pancreatic

alpha-amylase in addition to inhibiting membrane-bound alpha-glucosidases. Pancreatic alpha-amylase hydrolyzes complex starches to oligosaccharides in the lumen of the small intestine. Inhibition of these enzyme systems reduces the rate of digestion of carbohydrates. Less glucose is absorbed because the carbohydrates are not broken down into glucose molecules. In diabetic patients, the short-term effect of these drugs therapies is to decrease current blood glucose levels: the long term effect is a small reduction in hemoglobin $_{A1c}$ level (Venable and Aschenbrenner, 2006).

Side effects

The side effects commonly observed with this drug are as follows: temporary digestive symptoms, abdominal discomfort, excessive gas (flatulence) and diarrhea. Though these conditions were observed also in the safety studies carried out over one year period, they abated with time. Patients are normally advised in a bid to reduce these side effects to lower the amount of high carbohydrate foods they eat, e.g. bread, pasta (noodle) and rice. Where blood glucose levels are observed to have dropped very low, the patient can be advised to take glucose tablets as against the use of fructose, lactose or sucrose. In other circumstances, the patient is advised to simply eat more food. In some other instance, a drop is observed in the packed cell volume (PCV) of patients treated with AGIs, but this was not linked to reduction in hemoglobin. A reduced serum calcium and plasma vitamin B6 were linked to AGI therapy but were concluded to be of no clinical significance.

f. Biguanides

These are described as hypoglycemic agents used in the treatment of non insulin dependent diabetes mellitus which showed no response to dietary modification. It improves glucose levels by improving insulin sensitivity and decreasing intestinal absorption. For example, Glucophage (metformin hydrochloride tablets) and Glucophage XR (metformin hydrochloride extended-release tablets), as monotherapy, are indicated as an adjunct to diet and exercise to improve glycemic control in patients with type 2 diabetes. Glucophage is indicated in patients 10 years of age and older. Metformin may be used concomitantly with a sulfonylurea or insulin to improve glycemic control in adults (17 years of age and older) (Drug Digest, 2006).

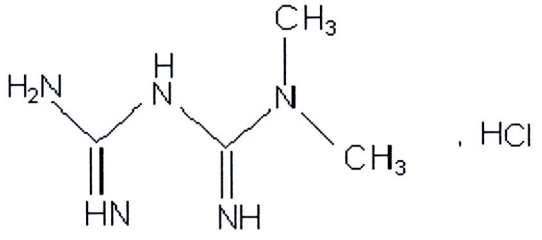

Figure 1.6 *N*, *N*-dimethylimidodicarbonimidic diamide
Formula: $C_4H_{12}N_5Cl$ (Shalmashi, 2008)

The exact mechanism of action of metformin is uncertain, though it appears to act mainly by reducing hepatic gluconeogenesis, but it also decreases absorption of glucose from the gastrointestinal tract and increases insulin sensitivity by increasing peripheral utilization of glucose (RPSGBBMA, 2000). Evidence suggests that increased peripheral utilization of glucose may be due to improved insulin binding to insulin receptors since metformin is not effective in patients who no longer have any residual insulin production (Bailey and Turner, 1996). The 'average' person with type

2 diabetes has three times the normal rate of gluconeogenesis; metformin treatment reduces this by over one third (Hundal *et al.*, 2000).

Side effects

Metformin is absorbed un-metabolized. The most serious potential side effect of metformin is lactic acidosis; this complication is very rare, and seems limited to those with impaired liver or kidney function. Phenformin, another biguanide was withdrawn because of an increased risk of lactic acidosis (up to 60 cases per million patients). However, metformin is safer than phenformin, and the risk of developing lactic acidosis is not increased by the medication so long as it is not prescribed to known high-risk groups (Salpeter *et al.*, 2003). The most common adverse effect of metformin is gastrointestinal upset, including diarrhea, cramps, nausea and vomiting; metformin is more commonly associated with gastrointestinal side effects than most other anti-diabetic drugs. In a clinical trial of 286 subjects, 53.2% of the 141 who were given immediate-release metformin (as opposed to placebo) reported diarrhea, versus 11.7% for placebo, and 25.5% reported nausea/vomiting, versus 8.3% for those on placebo (Drug Facts, 2005). Gastrointestinal upset can cause severe discomfort for patients; it is most common when metformin is first administered, or when the dose is increased. The discomfort can often be avoided by beginning at a low dose (1 to 1.7 grams per day) and increasing the dose gradually. Gastrointestinal upset after prolonged, steady use is less common. Long-term use of metformin has been associated with increased homocysteine levels and malabsorption of vitamin B_{12} (Andres *et al.*, 2000). Higher doses and prolonged use are associated with increased

incidence of vitamin B_{12} deficiency, and some researchers recommend screening or prevention strategies (Ting *et al.*, 2006).

There was an initial report; involving four patients with impaired thyroid function, that metformin can suppress the TSH level with no accompanying symptoms of hyperthyroidism or changes in measured thyroid hormone levels. The mechanism is currently unknown (Vigersky *et al.,* 2006). Conditions such as epigastric discomfort, nausea and vomiting followed by diarrhea, drowsiness, weakness, dizziness, malaise and headache may occur when Metformin is used (Drug Bank, 2007).

1.5 Hypoglycemic medicinal plants

Conventional anti-diabetic medicinal use has been a success to some patients while a series of side effects were reported among many others and on certain occasion deaths (Juurlink, 2010; Monami *et al.,* 2008; Nissen and Wolski, 2007). The reports of side effects and deaths from anti-diabetic drugs has not resulted to a total rejection of these drugs but has made health care givers to come up with innovative schemes for combinatorial drug therapies to alleviate some of the problems earlier reported and also to improve on the efficiency of some of the drugs. Though this has recorded some successes also, it is not devoid of its own draw backs (Bell, 2002). On the other hand, other patients often resort to combining orthodox with CAM to manage their blood glucose levels and some use only CAM for theirs. This phenomenon is still found in developing countries such as Thailand and Nigeria where the indigenous population still relies to a great extent on traditional healers and medicinal plants to meet their healthcare needs due to cultural inclinations, perceived

effectiveness, cultural inclination, presumed safety with minimal side effects and affordability (Vliathan, 1998).

In Thailand, medicinal plants used for the treatment or management of symptoms of DM, (*Bao wan* –Thai word) were known in traditional medicine practice. Though the western terminology "diabetes" was not the term used for it. Studies have shown that the belief in the efficacy of medicinal plants for diabetes mellitus treatment is firm to the extent that such plants were given to diabetic patients in local hospitals (Arthorn *et al.*, 1993). Some medicinal plants used in Thailand for the treatment of DM are *Coccinia indica* or the Ivy gourd. This is a unique tropical plant that is a member of the family of *Cucurbitaceae.* It grows well in Thailand and India. The ivy gourd is classified as a medical herb in traditional Thai and Ayurvedic medicine (McWhorter, 2005). The parts used in diabetes treatment are the leaves, but the primary use of the ivy is culinary and it is considered a vegetable. It contains beta carotene, a major vitamin A precursor from plant sources. It is considered a good source of protein and fiber.

Scientific studies of the hypoglycemic activity of the Ivy gourd explained that, the mechanism of diabetes control is that ivy gourd extract may suppress the activity of certain enzymes involved in glucose production, such as glucose -6- phosphatase (McWhorter, 2005). Other plants used in Thailand are *Piper sarmentosum* Roxb. (Piperaceae, Thai name: Chaplu), (Peungvicha, 1998); Noni "Yor" or *Morinda citrifolia* (Nandhasri, 2007); Mormodica charantia (Tiangda *et al.*, 1987); Barleria lupulina (Suba *et al.*, 2004) and several others.

In traditional African societies, phytotherapy is highly valued and widely utilized. Majority of the population use plant materials as their sources of primary

healthcare (Farnsworth *et al.*, 1985). The existence of traditional medicine depends on plant species diversity and related knowledge of their use as herbal medicines (Svarstad and Dhillion, 2000 and Tabuti *et al.,* 2003).

The western part of Africa has among other features has the highest number of tribes and ethnic groups in the whole of the continent. It is semi arid in nature with a stretch of rain forests on the coastal parts. The importance of medicinal plants in this part of Africa cannot be overemphasized, especially when the population density is known for high level of poverty and a slow but growing economy. In western Nigeria for example, Jayesimi *et al.*, (2007) published a compendium of diabetes mellitus treatment in south western Nigeria. In the areas visited by the researchers, mainly rural areas, 100 respondents were interviewed (96 males and 4 females), Eighty-three (83%) of the respondents gave a 20 or more years experience in TM practice. Furthermore, 83% of respondents attributed experience in managing diabetes mellitus to family and clan traditions, i.e. ancestral. Only 14 (14%) considered dietary changes as part of management of diabetes mellitus. Some examples of anti-diabetic medicinal plants in Western Nigeria are as follows: *Bares pectoris* L. (Ojuologbo), *Colocynthis citrullus* L. (Baara), *Vernonia amygdalina* L. (Ewuro), *Capscicum frutescens* L. (Ata Ijosi), *Gladiolus psittaccinus* Hook f (Baaka) (Jayesimi *et al.*, 2007). In northern Nigeria, various plants are used as medicines for diabetes mellitus most of which have still not been documented. Among the few that have been studied are *Nauclea latifolia* (family: *Rubiaceae*) (Gidado *et al.*, 2005), *Moringa oleifera Lam,* (Dièye *et al.*, 2004). *Tetrapleura tetraptera* (Komlaga, 2004), *Lannea welwitschii* (Okine, 2004) among others.

1.5.1 Hypoglycemic medicinal plants studied

1.5.2 Thai hypoglycemic medicinal plants

a. ***Anogeissus acuminata*** (Roxb. ex DC.) Guill. & Perr. (*Combretaceae*)

Local Name: Ta khian Nu

Location: Doi Thong, Chiang Mai

A. acuminata is a moderate sized tree with small leaves which falls earlier in the dry seasons before falling the foliage of these turns a beautifully yellowish red. It is found in countries of South Eastern Asia. The leafy branches are pendulous and hang down gracefully. Leaves up to 3 cm appear in nearly opposite pairs. Tiny yellowish green flowers occur in spherical heads about 1 cm wide. Protruding stamens are prominently visible like fuzz on the round heads. Fruits are sort of circular, clustered in round heads.

Uses in traditional medicine: The aqueous extract of the bark of *A. acuminata* are used to treat painful inflammatory conditions in India (Hemamalini *et al.*, 2010), while it is used in some parts of Thailand for treating DM. A perusal of literature shows that the plant has been scientifically tested for anti-HIV activity (Rimando *et al.*, 1994), anti-inflammatory and analgesic activity (Hemamalini *et al.*, 2010), used as anti-snake venom (Dahare and Jain, 2010).

Figure 1.7 *Anogeissus acuminata* (Ta Khian Nu)
(Source: http://plantspedia.wikia)

Rimando *et al.*, (1994), reported the isolation of compounds such as Anolignan A and B, dibenzylbutadiene lignans and confirmed that anolignan A and dibenzylbutadiene lignans possess active HIV-1 reverse transcriptase inhibitory activity.

b. *Catunaregam tormentosa* (DC.) Tirveng (*Rubiaceae*)

Local Name: Nam Teang

Location: Nong Bua

The *C. tormentosa* plant is also known as indigo berry is a Neotropical genus of shrub or small trees in the *Rubiaceae* family. Small tree, 4-8 m high; branches long thorny. Leaves simple, opposite, broadly ovate or ovate-elliptic, 3-5 cm wide, 5-7 cm long,

pale coloured and pubescent beneath; stipules interpetiolar. Flower solitary, axillary; corolla whitish, fragrant. Fruit dry, dehiscent, ovate.

Uses in traditional medicine: The *C. tormentosa* is used in the treatment of several infections in the early childhood care, in the form of decoction (Chuakul *et al.*, 2002).

Figure 1.8 *Catunaregam tormentosa* (DC.) Tirveng
(Source http.vmtd.net)

c. ***Dendrophthoe pentandra* (L.) Miq (*Loranthanceae*)**

Local Name: Ka Fak Ma Muang

Location: Faculty of Pharmacy, Chiang Mai University

Epiphytic parasite with simple leaf, alternate, elliptic-oblong or lanceolate, 2-4 cm wide, 5-8 cm long. Inflorescence in axillary short raceme or fascicle; flowers yellowish green. Fruit berry, oblong-ovoid, turn pinkish red when ripe.

Uses in traditional medicine: The aqueous extracts of *D. pentandra* are used in folk medicine to treat highpertension (Salaelanone, 1998).

Figure 1.9 *Dendrophthoe pentandra*
(Source: http://garden-frenzy.blogspot.com)

It is reported to have strong anti-inflammatory properties and two anti-oxidants have been isolated from the plant namely flavonol glycoside, quercitrin (quercetin- 3- O- rhamnoside) (Artanti *et al.,* 2006).

d. *Mimosa pudica Linn. var* hispida Bren (*Fabaceae*)

Local Name: Mai Yaa Rab

Location: Faculty of Pharmacy Botanical Garden, Chiang Mai University.

M. pudica is low, much branched, generally perennial, and slightly woody at the base, from 15 to 100 cm high and has either an upright or a low trailing habit. Its stiff reddish-brown or purple stems bear scattered thorns. The hairy leaves are

alternate, bipinnate and compound. They are sensitive to the touch, the petiole dropping and the leaflets being rapidly drawn back and folded. The pink flowers form small globular heads, each borne on a short hairy stalk arising from a leaf axil. Seeds are produced in pods which split into single-seeded segments bearing bristles, which aid dispersal by animals. In tropical countries the weed flowers all year and each plant may produce up to 700 seeds.

Uses in traditional medicine: The aqueous extracts of the roots of *M. pudica* are used in the treatment of leprosy, dysentery, vaginal and uterine complaints, inflammation, burning sensation, asthma, leucoderma, and fatigue and blood diseases. In the Unani Healthcare System its root is resolvent, alternative, and useful in the treatment of diseases arising from blood impurities and bile, bilious fevers, piles, jaundice, and leprosy. Decoction of root is used with water to gargle to reduce toothache. It is very useful in diarrhea, amoebic dysentery, bleeding piles and urinary infections (Rajkumar *et al.*, 2011). It arrests bleeding and hastens the wound healing process. It is mainly used in herbal preparations for gynecological disorders.

Figure 1.10 *Mimosa pudica var. hispida.* Bren.
(Source http://herbal-ayurveda-remedy.com

M. pudica contains Mimosine which is a toxic alkaloid. Adrenalin like substance has been identified in the extract of its leaves. Some workers have reported the presence of Crocetin dimethyl Easter in the extract of the plant (Kumar, 2010). Roots contain tannin up to 10 per cent. Seeds contain a mucilage which is composed of d-xylose and d-glucuronic acid. The plant extract contains green yellow fatty oil up to 17 per cent. The plant is reported to contain tubuline and a new class phytohormone turgorines is found to be active in the plant. The periodic leaf movement factors are reportedly the derivatives of 4-o-(β-D-glucopyranosyl-6-sulphate) gallic acid.

e. *Moringa oleifera* (*Moringaceae*)

Local Name: Ma Room

Location: Faculty of Pharmacy Botanical Garden, Chiang Mai University.

Moringa (Moringa spp.) is one of the world's most useful plants. This fast-growing tree is grown throughout the tropics for human food, livestock forage, medicine, dye, and water purification. It is known by several names in different countries, but is popularly called the "drumstick tree" for its pods that are used by drummers and the "horseradish tree" for the flavor of its roots.

Uses in traditional medicine: antihypertensive, anti-inflammatory, antimicrobial and anti diabetic. In India it is traditionally used for anemia, anxiety, asthma, blackheads, blood impurities, bronchitis, catarrh, chest congestion, cholera, conjunctivitis, cough, diarrhea, eye & ear infections, fever, glandular swelling, headaches, abnormal blood pressure, hysteria, pain in joints, pimples, psoriasis, respiratory disorders, scurvy, semen deficiency, sore throat, sprain, tuberculosis.

Figure 1.11 *Moringa oleifera Linn.*
(*Source: http://healthforfree.com*)

Native to South Asia, this tree is becoming a vital source of nutrition in this region, where most of the world's poor people live. Some components of Moringa preparations that have been reported to have hypo-tensive, anticancer, and antibacterial activity include 4-(4'-O-acetyl-α-L-rhamnopyranosyloxy)benzyl isothiocy-anate [1], 4-(α-L-rhamnopyranosyloxy)benzyl isothiocy-anate [2], niazimicin [3], pterygospermin [4], benzyl isothiocyanate [5], and 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate (Fahey, 2005; Jed, 2005). The plant pigments (flavonoids) such as rutin and quercetin, and other beneficial phytochemicals (lutein, caffeoylquinic acids) to name just a few, are present in Moringa. These substances act as antioxidant, anti-aging or protect various tissues (retina, liver, blood vessels) from age-related damage and disease.

f. *Pterocarpus macrocarpus* (*Fabaceae*)

Local Name: Pra Doo

Location: Doi Suthep

P. macrocarpus is a large-sized tree species, seasonal shedding of leaves, crown large umbrella-shaped, with buttrees at the stem base, stem straight, up to 25-35m high, diameter 0.7-0.9m or over. Bark grey, peeled in large patches, cracks along the stem; inner bark yellow, 1-1.5cm thick, containing solid sap, brightly red. The flowers are yellow, produced in racemes 5–9 cm long. The fruit is a pod surrounded by a round wing 4.5–7 cm diameter, containing two or three seeds. According to taxonomical documents, *P. pedatus* is also present in Laos, Cambodia and Vietnam and *P. cambodianus* is present in Cambodia.

Uses in traditional medicine: In recent years, herbal teas and pills made from *P. indicus* extracts a closely related tree have been popularized in the Philippines for treating a wide range of diseases and ailments including leprosy, menstrual pain, flu, rheumatoid arthritis, and diabetes (Thompson, 2006).

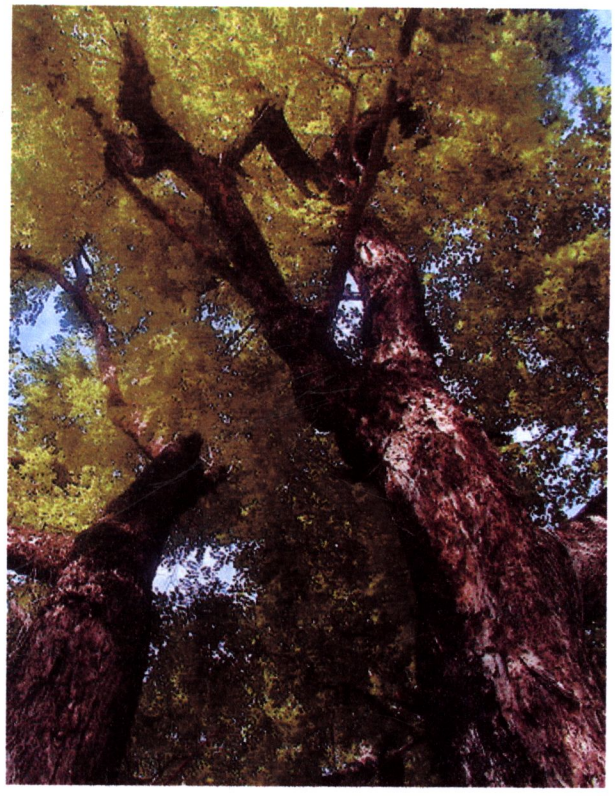

Figure 1.12 *Pterocarpus macrocarpus*
(Source: http://flickr.com)

g. *Dioecrescis erythroclada* (Kurz) Tirveng (*Rubiaceae*)

Local Name: Ma Khang Dang

Location: Faculty of Pharmacy Botanical Garden, Chiang Mai University

Is a monotypic tree genus (*D. erythroclada*; cf. Tirvengadum, 1983), with paired, straight thorns. Often, several tiers of such thorn bearing branch clusters can be observed. The life-span of these branches is limited, so that very old individuals may lack thorns altogether. The presence of these thorn-bearing branches along the lower part of the trunks could be interpreted as a protection against browsing animals.

Uses in traditional medicine: It is used in traditional medicine to treat stomach ache and also used as an anti-pyretic (Kaewkrud *et al.,* 2007) and laxative (Bunyapraphatsara, 1999).

Figure 1.13 *Dioecrescis erythroclada* (Kurz) Tirveng
Source: http://www. Phargarden.com

Six compounds were previously isolated from *D. erythroclada* namely: apodathoside, mussaenoside, gardenoside, benzylalcohol O–β– D-apiofuranosyl- (1 – 6)- β-D-glucopyranoside, phen-ethyl alcohol O – β- D- apiofuranosyl-(1 – 6) – β-D-glucopyranoside and Oct-1-en-3-ol-α-L-arabinopyranosyl-(1-6)-β-D- glucopyranoside (Kaewkrud *et al.,* 2007).

h.Yamed Boraped Pungchang (YBP)

Local Name: Yamed Boraped Pungchang

Location: Pratoomthani

Like several traditional medicines around the world, YBP is a multi herbal mixture of pulverized medicinal plants made up of *Stephania rotunda, Acanthus ilicifolius, Cyprus rotundus and Rhinaeanthus nasutus.*

i. *Stephania rotunda*

Local Name: (Kwao Kreu Dang) (*Menispermaceae*)

Location: NA

S. rotunda is a genus native to Eastern and Southern Asia and Australasia. They are herbaceous perennial vines growing to around four metres tall, with a large, woody caudex. The leaves are arranged spirally on the stem, and are peltate, with the leaf petiole attached near the centre of the leaf. It is used to treat fever and dysentery in asthma (Hout *et al.,* 2006; Perry, 1980).

Three major alkaloids: cepharanthine (1), tetrahydropalmatine (2) and xylopinine (3) isolated from *Stephania rotunda* tuber were investigated for their cytotoxic activity in a panel of human cancer cells (HT29, LS174T, SW620 and HepG2) using MTT assay (Bun *et al.,* 2009). The antioxidant activity of cepharanthine and fangchinoline (Gülçin *et al.*, 2010) and were observed to have strong effects.

Figure 1.14 *Stephania rotunda*
Source: http://forums.gardenweb.com

j. *Acanthus ilicifolius*

Local Name: Ngueak-Plae-Moo) (*Acanthaceae*)

Location: NA

Unlike some mangrove plants, Sea Holly does not exclude salt at the root level. In fact, their sap is salty and excess salt is secreted through the leaves, to be removed by rain or wind. Sometimes, the salt can be seen as a white crystalline layer on the upper surface. The leaves of *A. ilicifolius* are used to treat rheumatism, neuralgia and poison arrow wounds (Malaysia). It is widely believed among mangrove dwellers that chewing the leaves will protect against snake bite. The pounded seeds of *A. ebracteatus* are used to treat boils, the juice of leaves to prevent hair loss and the leaves themselves to ward off evil (Malay). Both species are also

used to treat kidney stones. The whole plant is boiled in fresh water, and the patient drinks the solution instead of water, half a glass at a time, until the signs and symptoms disappear (Thailand). Water extracted from the bark is used to treat colds and skin allergies. Ground fresh bark is used as an antiseptic. Tea brewed from the leaves relieves pain and purifies the blood (*Ethnobotanical Leaflets, 2009*). The plant is reported to contain phytochemicals including alkaloid and wide range of glucosides (lignan and phenylethanoid).

Figure 1.15 *Acanthus ilicifolius*
Source: http://*tidechaser.blogspot.com*

Laboratory investigations on extracts of the plant have demonstrated significant pharmacological activities like antioxidant, anticarcinogenic, anti-osteporotic and hepatoprotective (Singh *et al.,* 2009**).**

k. *Cyprus rotundus*

Local Name: Ya Khon Mu

Location: NA

C. rotundus belongs to family *Cyperaceae.* a perennial, *stolaniferous, rhizomatous*, halophytic sedge. Rhizome, many, slender; tuber-white, succulent when young, hard and black when mature; stem-leafy at base arising from a tuber. Culm-dark green, glabrous. Leaf is dark green above, with reddish brown sheaths, clustered at the base of stem. Inflorescence 3-9 spreading rays bearing tassels of few, large spikelets; spikelet 20-40 flowered, red brown to almost black. Fruit, oblong ovate. According to the Ayurveda, root is pungent, acrid, cooling, astringent, appetizer, stomachic, antihelmintic and useful in treatment of leprosy, thirst, fever, blood diseases, biliousness, dysentery, pruritis, pain, vomiting, epilepsy, opthalmia, erysipelas. The essential oil from the plant contains at least 27 components comprising sesquiterpene hydrocarbons, epoxides, ketones, monoterpene and aliphatic alcohols and some unidentified constituents, (+) copadiene and (+) epoxyquaine. The rhizomes contain sitosterol, cyperene, seniline, cyperenone and sesquiterpenes cyperone is obtained from the tubers.

The leaves contain luteolin and auresidin. Cyperene – 1 and cyperene- 2 isolated from tubers. Muskatone and patchoulenone isolated from essential oil. Structure of rotunol and rotunol, two norsesquiter penoides – kobusone and isokobusone identified. From the rhizomes cyperene, selinene, cyperenone and cyperone isolated. A new saponon-olenolic acid -3-0 neohesperidoside (I) isolated form tubers and characterized (Singh and Singh, 1980).

Figure 1.16 *Cyprus rotundus*
Source: http://australian-insects.com

l. *Rhinaeanthus nasutus*

Local Name: Thong Phan Chang

Location: NA

R. nasutus, a small shrub of *Acanthaceae* family, has long been used in Thai traditional medicine for treatment of *Tinea versicolor*, ringworm, pruritic rash, abscess pain, and skin diseases (Panichayupakaranant, 2009). The plant is a slender, erect, branched, somewhat hairy shrub 1-2 m in height. The leaves are oblong, 4-10 cm in length, and narrowed and pointed at both ends. The inflorescence is a spreading, leafy, hairy panicle with the flowers usually in clusters. The calyx is green, hairy, and about 5 mm long. The corolla-tube is greenish, slender, cylindric, and about 2 cm long. The flowers is 2-lipped; the upper lip is white, erect, oblong or lance like, 2-toothed at the apex, and about 3 mm in both length and width; and the lower lip is

broadly obviate, The fruit (capsule) is club-shaped and contains 4 seeds (Puttarak *et al.*, 2010).

Figure 1.17 *Rhinacanthus nasutus*
Source: http://flowersofindia.in

It has been reported that rhinacanthin-C, rhinacanthin-D and rhinacanthin-N isolated from *R. nasutus* possessed antifungal, antibacterial, antiviral, anti-inflammatory, anti-allergic and cytotoxic activities (Panichayupakaranant, 2009).

m. Mai Tau Lusi (MTL)

Local Name: Mai Tau Lusi

Location: NA

MTL was also a multi herbal traditional anti-diabetic medicine made up of the following medicinal plants namely *Curcuma zedoria* (Berg) Roscoe, *Eugenia caryophyllum* Bullock &Harrison, *Piper chaba* Hunt, *Abroma malvaceae*, *Piper*

nigrum Linn, *Myristica fragans* Houtt, *Amomum krevanh* Pierre, *Zingiber cassumunar* Roxb.

n.*Curcuma zedoria* (Berg) Roscoe

Local Name: Kha Min Dan

Location: NA

C. zedoria is a rhizome that grows in tropical and subtropical wet forest regions. It is a perennial herb and member of the genus Curcuma Linn., family *Zingiberaceae*. The plant is native to South East Asia. It was introduced to Europe by Arabs around the sixth century, but its use as a spice in the West today is extremely rare, having been replaced by ginger. The plant is used in traditional medicine to treat coronary heart disease, liver cancer, anemia, chronic pelvic inflammation and helps prevent leucopenia due to cancer therapies (Dharmananda, 2010) and also used as an anti-venom for the Indian cobra (Daduang *et al.*, 2005).

According to Pharmacology and Applications of Chinese Materia Medica and Chinese Herbal Medicine Materia Medica (Bersky, 1999), in laboratory animal studies, turmeric has been shown to: reduce blood lipids, improve blood circulation to the heart, lower blood pressure, reduce platelet aggregation and promote fibrinolysis, increase bile formation and secretion, reduce inflammation, and alleviate pain.

Figure 1.18 *Curcuma zedoria* (Berg) Roscoe
Source: http: blog.taradkaset.com

o. ***Eugenia caryophylum*** Bullock and Harrison

Local Name: Kan Plu

Location: NA

E. caryophyllum or clove belongs to the plant family *Myrtaceae*. The use of clove as a food ingredient spice is common in oriental foods. It has also been used in folklore treatment of toothaches, insect bites, gastroenteritis and intestinal parasites. It has been used as a sedative and as a cement material in dentistry (Markowitz *et al.*, 1992). Furthermore, antioxidant (Atsumi *et al.*, 2005) and anti-herpes simplex virus (Tragoolpua and Jatisatienr, 2007) activities of eugenol an essential oil from *E. caryophyllum* have also been reported (Puangpronpitag *et al.*, 2009; Atsumi *et al.*, 2005). The essential oil from clove has been reported to have antimicrobial activity against food-borne pathogen bacteria (Yano *et al.*, 2006).

Figure 1.19 *Eugenia caryophyllum*
Source: http://viable-herbal.com

p. *Piper chaba* Hunt

Local Name: Prik Thai Deeplee

Location: NA

The plant *Piper chaba* Hunter (*Piperaceae)* is a climbing, glabrous shrub available in various parts of India and Malay Islands (Kirtikar and Basu, 1980). In Bangladesh, it grows in plenty in Khulna division and more specifically in the Satkhira–Bagerhatt area. Like other plants of *Piper* genus, the plant enjoys vast folklore uses, as traditional medicine. The root is alexiteric; useful in asthma, bronchitis, consumption.

and female sexual organs, respiratory organs, neck, back and limbs, skin diseases, fever; to regulate appetite and sleep. And also used as a uterine stimulant, produces an even menstrual flow and decreases pain in menstruation, the condition known as dysmenorrheal.

Figure 1.21 *Ambroma malvaceae*
Source: http://envis.frlht.org

The decoction, powdered root or distilled water of the same is effective in diseases women suffer from, especially in cases where women have pain before or during the menstrual period. Report on the hypoglycemic effect on *A. augusta* was reported by Halim *et al.,* (2001).

r. *Piper nigrum* Linn

Local Name: Prik Thai Dam

Location: NA

The plant *P. nigrum* is a climbing plant indigenous to South East Asia. The plant produces a pendulous spike of sessile fruits, which is collected as soon as the lower fruits change in colour from green to red, and dried. The fruits are spherical, dark brown, inferior, and about 5 millimeters in diameter. The surface is deeply and coarsely reticulately wrinkled; at the apex the remains of the sessile stigma are visible. The pericarp is thin, and contains a single seed, completely filling the cavity. The kernel consists almost entirely of perisperm, the small endosperm surrounding the minute embryo at the apex of the fruit. The odour is aromatic, and the taste pungent. The chief constituents of black pepper are a crystalline alkaloid, piperine (5 to 8.25 per cent), volatile oil (1 to 2.3 per cent.), and a pungent resin called chavicin, of which little definite is known. Black pepper has in a high degree the stimulating and carminative properties of the volatile oils, causing a reflex flow of saliva, with increased secretion of gastric juice and improved appetite. Gastro-intestinal movements are augmented, with consequent eructation of gas and relief of colic. In sufficient doses, the peppers dilate the superficial vessels of the skin, causing a feeling of warmth, followed by diaphoresis and some reduction of temperature. Pepper is a diuretic, and is sometimes used in place of cubebs in gonorrhea and urethritis. It is also used for haemorrhoids.

Black pepper is used as *Confectio Piperis,* often with confection of senna, as a stimulating laxative in haemorrhoids, anal fissure. In conjunction with opium and other carminatives it is employed as *Pulvis Opii Compositus* (*The British Pharmaceutical Codex,* 1911).

Figure 1.22 *Piper nigrum* Linn
Source: http://www.*jacexim.com*

s. *Myristica fragrans* Houtt. (*Myristicaceae*)

Local Name: Chan-Thed or Jun Ted

Location: NA

M. fragrans (nutmeg) is a spreading aromatic evergreen tree usually growing to around 5 to 13 metres high, occasionally 20 metres. The bark contains watery pink or red sap. The pointed dark green leaves (5 to 15 cm × 2 to 7 cm) are arranged alternately along the branches and are borne on leaf stems about 1 cm long. Upper leaf surfaces are shiny. Flowers are usually single sexed; occasionally male and female flowers are found on the same tree. Flowers are pale yellow, waxy, fleshy and bell-shaped. The fruits are fleshy, drooping, yellow, smooth, 6 to 9 cm long with a longitudinal ridge. Seeds (nutmegs) are broadly ovoid (2 to 3 cm long), firm, fleshy, whitish and transversed by red-brown veins (Purseglove, 1968). *M. fragrans* Houtt. (Nutmeg) is used in traditional Ayurvedic medicine for impotency,

skin diseases, insomnia, hyperdipsia, cardiac disorders, fever and general debility (Kumar, 2009).

Figure 1.23 *Myristica fragrans* Houtt
Source: www.uni-graz.at/~katzer

Scientific studies have shown that it improves sexual functions in rats (Tajuddin *et al.*, 2005) and that it possesses strong antioxidant activity (Olaleye *et al.*, 2006).

t. *Amomum krevanh* Pierre (*Zingiberaceae*)

Local Name: Mak Naeng

Location: NA

The Round Siam cardamom or Comphor seed is a large herb up to 300 cm tall, with superficial rhizome. The leaves are lanceolate, up to 60 cm x 12 cm. Inflorescence cylindrical to conical, up to 11 cm long, bracts about 4 cm long, bracteoles tubular at the base. Flowers with corolla tube about as long as calyx,

labellum elliptical, rounded at apex, white with a yellow patch in the centre and yellow at margins, anther with a 3-lobed appendage. Fruit is about 1.5 cm in diameter, slightly ribbed.

Figure 1.24 *Amomum krevanh* Pierre
Source: http://*biogang.net*

It is used in TCM to promote qi circulation and transform dampness and to warm the spleen and stomach and stop vomiting. It is also used as an analgesic, decongestant, bronchial dilator, stimulant, antihypertensive, digestive and skin cleanser (Blake, 2004).

u. *Zingiber cassumunar* Roxb. (*Zingiberaceae*)

Local Name: Plai

Location: NA

Z. cassumunar is synonymous with *Zingiber purpureum* Roscoe and has long been regarded by Thai massage therapists as one of those oils necessary to have in

their kit to combat joint and muscle problems. Plai is of the same family as ginger (Zingiber officinale Roscoe) but has different properties and more intense actions (Chamratpan and Homchuen, 2010). Considered analgesic, anti-neuralgic, anti-inflammatory, antiseptic, antispasmodic, antitoxic, anti-viral, carminative, digestive, diuretic, febrifugal, laxative, rubefacient, stimulant, tonic and vermifuge, it has been used for aches and pains, asthma, catarrh, chronic colds, colic, constipation, diarrhoea, fevers, flatulence, heartburn, immune problems, inflammation, influenza, joint problems, muscle spasms, nausea, respiratory problems, sprains and strains, torn muscles and ligaments (Chamratpan and Homchuen, 2010).

Figure 1.25 *Zingiber cassumunar* Roxb.
Source: *herbal.medicalonlinemedia.com*

The compounds that have been isolated from *Z. cassumunar* are sabinene, a–terpinene, terpinen-4-ol and (E)-1-(3'4'-dimethoxyphenyl)- butadiene (DMPBD) of which terpinen-4-ol and a–terpinene are said to have topical anti-inflammatory effect (Pongprayon *et al.*, 1997).

v. ***Rauwolfia serpentina*** (*Apocynaceae*)

Local Name: Rayom

Location: Lampang District, Thailand

It is an evergreen, perennial, glabrous and erect under shrub grows up to a height of 60 cm (rarely more than it). The roots are tuberous with pale brown cork and the leaves are in whorls of three, elliptic to lanceolate or obovate, bright green above, pale green below, tip acute or acuminate, base tapering and slender. Flowers are in many flowered irregular corymbose cymes. Flowers white, often has violet coloured tinge. Calyx glabrous, bright red and lanceolate. Corolla is longer than calyx, tube slender, swollen a little above the middle, lobes 3, and elliptic-oblong. Disc is cup shaped. Drupes are slightly connate, obliquely ovoid and purplish black in colour. It is highly rated in TCM.

Use in traditional medicine: *R. serpentina* extract is used for the relief of anxiety, agitation, epilepsy, poisonous bites from snakes and insects, corneal opacities and a variety of other conditions including hypertension (Genest, 1955). Some pure compounds have been successfully isolated from *R. serpentina* namely serpentine, sarpagine, reserpine and ajmalicine (Harisaranraj *et al.*, 2009).

Figure 1.26 *Rauwolfia serpentina*
Source: http://*www.motherherbs.com*

1.5.3 Nigerian Medicinal Plants used

a. *Anisopus mannii* N. E. Br. (*Asclepiadaceae*)

Local Name: Kashe Zaki

Location: Jimeta, Yola

A. mannii is a perennial herb with leaves spread and petiole 1.3 - 2 cm long, bearing a distinct gland at the apex, blade about 5.7 - 7.6 cm long and stem twining to a height of 3.7 - 4.6 m (Hutchinson and Dalziel, 1963). It is known as 'Kashe zaki' (Hausa) meaning "destroying sweetness".

Use in traditional medicine: *A. mannii* is a familiar herb in the traditional medicinal preparations in northern Nigeria, where a decoction of the whole plant is used as a remedy for *diabetes*, diarrhea and pile. The plant is also used traditionally to treat diseases like fevers, (Boye, 1983) rheumatism, and urinary tract diseases (Deepak *et al.*, 1996). Some compounds have been successfully isolated from the plant such as 1,

7-naphthyridine alkaloid- named anisopusin, 5-hydroxy-lup-20(29)-en-3_-yl eicosanoate, [6]-gingerdione, [6]-dehydrogingerdione and ferulic acid from acetone extract of the stem bark have been reported (Tsopmo *et al.,* 2009).

b. *Anogeissus leiocarpus* (D.C) Guill &Peer

Local Name: Mareke

Location: Mubi

A. leiocarpus is a tall evergreen tree native to savannas of Tropical Africa. It is the sole West African species of the genus *Anogeissus*, a genus otherwise distributed from tropical central and East Africa through tropical Southeast Asia. *A. leiocarpus* germinates in the new soils produced by seasonal wetlands and grows at the edges of the rainforest, although not in the rainforest, in the savanna, and along riverbanks forming gallery forests. The tree flowers in the rainy season, from June to October. The seeds, winged samaras, are dispersed by ants.

Use in traditional medicine: Small branches with leaves are crushed to make one of the yellow dyes (Arbonnier, 2004). The inner bark of the tree is used as a human and livestock anthelmintic for treating worms, and for treatment of a couple of protozoan diseases in animals, nagana an animal *trypanosomiasis*, and *babesiosis* (Bizimana, 1994). The inner bark used as a chewing stick in Nigeria and extracts of the bark show antibacterial properties (Mann *et al.,* 2008). The stem barks contains castalagin (Shuaibu *et al.*, 2008). It is also used to treat asthma, cough, and tuberculosis, worm killer, gonorrhea (Mann *et al.,* 2008).

Figure 1.27 *Anogeissus leiocarpus* (D.C) Guill & Perr.
Source: http://*www.prota4u.org*

c. *Daniella oliveri* (Rolfe) Huctch & Dalz

Local Name**:** Maje

Location**:** Jimetta**,** Yola

D. oliveri (Rolfe) Hutch and Dalz is commonly known as African copaiba balsam or Ilorin balsam (Ahmadu *et al.,* 2004). It belongs to the family *Fabaceae*. It is important both as timber and forest enrichment tree. It is a species of great economic value being used as pole, fuel wood, charcoal, food, shade and shelter (Bojang, 2000).

Figure 1.28 *Daniella oliveri* (Rolfe) Hutch & Dalz
Source: *http://www.westafricanplants.senckenberg.de*

Uses in traditional medicine: *D. oliveri* is used in treating gastrointestinal ailments (Ahmadu *et al.,* 2003), as anti-aborifacients in pregnancy, pain-killer, skin *mucosae* and as sedative (Burkill, 1985), for the cure of rheumatism/ pains (MacDonald and Olorunfemi, 2000) and active as antimicrobial agent (Ahmadu *et al.,* 2004). Other uses are as stimulant, local cosmestics (Lawal *et al.,* 2010).

d. *Detarium macrocarpum* Harms (*Caesalpiniaceae*)

Local Name: Taura

Location: Mubi

D. macrocarpum is a tree found in both temperate and tropical areas. It belongs to one of the largest families of flowering plants, numbering 630 genera and 18,000 species. The leaves are stipulate, nearly always alternate, and range from bipinnately or simple. The petiole base is commonly enlarged into a pulvinus that commonly functions in orientation of the leaves. The flowers are usually bisexual, actinomorphic to zygomorphic, slightly too strongly perigynous, and commonly in racemes, spikes, or heads. The perianth commonly consists of a calyx and corolla of 5 segments each. The pistil is simple, often stipitate, comprising a single style and stigma, and a superior ovary with one locule containing 2-many marginal ovules. The fruit is usually a legume, sometimes a samara, loment, follicle, indehiscent pod, achene, drupe, or berry. The seeds often have a hard coat with hourglass-shaped cells , and sometimes bear a u-shaped line called a pleurogram.

Use in traditional medicine: The extracts of the bark, leaves and roots of *Detarium macrocarpum* are widely used throughout its distribution area because of their diuretic and astringent properties. They are prepared as infusions or decoctions to treat rheumatism, venereal diseases, urogenital infections, haemorrhoids, caries, biliousness, stomach-ache, intestinal worms and diarrhoea including dysentery. They are also used against malaria, leprosy and impotence. A decoction of the powdered bark is widely taken to alleviate pain, e.g. headache, sore throat, back pain and painful menstruation. The fresh bark or leaves are applied to wounds, to prevent and cure infections. In Mali the bark is also used to treat measles, nocturia, hypertension, itch and tiredness, while a decoction of the leaves or roots is taken against paralysis, meningitis, tiredness, cramps and difficult delivery (Abreu, 1999).

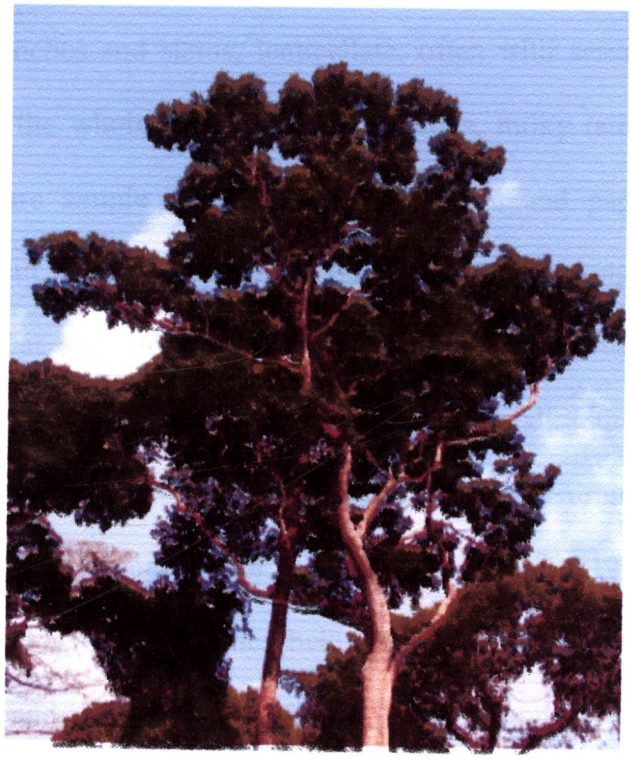

Figure 1.29 *Detarium macrocarpum* Harms
Source: http://*phyto-afri.ird.fr*

e. Maganin Ciwon Suga –Recipe

Local Name: Maganin Ciwon Suga

Location: Mubi, Nigeria

f. *Ficus thonningii*

Local Name: Cééréériíyáá

Location: Mubi

F. thonningii is an evergreen tree 6-21 m, with a rounded to spreading and dense crown. Sometimes epiphytic, often a strangler; trunk fluted or multistemmed. Bark on young branches hairy, with a stipular cap covering the growth tip, but smooth

and grey on older branches and stems, lenticellate, often with aerial roots hanging down from branches; the whole plant exudes a copious, milky latex often turning pinkish. The leaves are simple, glossy, dark green, thin and papery or slightly leathery, margin smooth, elliptic or obovate, sometimes rather elongated or slightly oblanceolate, grouped at ends of twigs, 3-20 x 1.5-10 cm, glabrous, puberulous or pubescent; with 6-12 pairs of upcurving main lateral veins; stalk rather slender, 1-7.5 cm; base cuneate or obtuse (sometimes subcordate); apex rounded or obtuse, sometimes shortly and bluntly acuminate. The stipules are about 12 mm long, soon falling off. Figs in leaf axils, sometimes below the leaves, enclosing many small flowers, mostly hairy and borne in the leaf axils, sessile or on peduncles to 10 mm long, yellow or red, globose or ellipsoid, 7-14 mm in diameter, smooth or warted, glabrous or pubescent, basal bracts 2-4 mm long, persistent.

Uses in traditional medicine: *F. thonningii* commonly known as fig tree is used for therapeutic purposes against a number of diseases (Coker *et al.*, 2009). The extracts of *F. thonningii* is used in traditional medicine to treat cold, sore throat, diarrhea malaria, yellow fever and also used as secretagogues (Danthu *et al.,* 2002). A survey of 100 households from two rural settlements of Fulani in northeastern Nigeria showed that "Cééréériíyáá or Cediya" (*F. thonningii*) was among those classified as "good sources of protein, fat, calcium, iron, copper and zinc" (Locket and Grivetti 2000).

Figure 1.30 *Ficus thonningii*

Source: http://ipmimages.org

g. *Raphia vinifera* P. Beauv

Local Name: Tukurwa

Location: Mubi

They grow up to 16 m tall and are remarkable for their compound pinnate leaves. The plants are either monocarpic, flowering once and then dying after the seeds are mature, or hapaxanthic, with individual stems dying after fruiting but the root system remaining alive and sending up new stems.

Figure 1.31 *Raphia vinifera* P. Beauv
Source: http://*virboga.de*

Uses in traditional medicine: There are no known traditional medicinal uses other than its addition into this recipe.

h. *Leptedenia hastata* (Pers) Dec'ne:

Local Name: Yaa'diya

Location: Mubi

L. hastata belongs to the family *Asclepiadaceae*, used as food by many African populations (Hutchinson and Dalziel, 1937). It is commonly used as a vegetable and is considered as a famine food due to its high content of valuable nutrients in Niger (Sena *et al.*, 1998).

Figure 1.32 *Leptedenia hastata* (Pers) Dec'ne (Ref: NA)

The major chemical compounds found in *L. hastata* were: triterpenes, fatty acids, amino acids, poly-oxypregnane, lutein, -carotene (Nikièma *et al.*, 2001).

Uses in traditional medicine The aqueous extract of *L. hastata* (Pers.) Dec'ne is used to treat gonorrhea, stomach ache, tertiary syphilis (Odugbemi, 2008). It is also used in herbal medicine against milk drying (galactogogue), sex-impotence, trypanosomosis, acute rhinopharyngitis and wounds (Tamboura *et al.*, 2005). The leaves are often chewed by shepherds against polydipsia and mouth dryness (Olivier-Bover, 1986). In some part of northern Nigeria, leaves extract is used for the treatment of stomach upset in children (Aliero *et al.*, 2001).

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Figure 1.33 *Leptedenia hastata* (Pers) Dec'ne (Ref: NA)

j. *Mimosa in visa var.* inermis Adelb

Local Name: Idon Zakara

Location: Mubi

A conspicuously 4 angled, branching profusely 2-4 m long, occasionally 6 m, with numerous recurved prickles 3-6 mm long, occurring at intervals on the angles of the stems. Bright green; bipinnate 10-20 cm long, consisting of 4-9 pairs of primary segments, each with 10-20 pairs of opposite leaflets without any stalks, tapering 6 -12 mm long by 1.5 mm wide. Leaflets fold when touched, and at night. Pinkish violet, numerous in globular heads about 1.2 cm diameter, 1-3 heads arising on separate stalks from the leaf axils. Petal tube four lobed with 8 pinkish-violet stamens that protrude beyond the petals. Robust branched taproot to 1-2 m, with nitrogen-fixing nodules amongst the fine fixing roots (Akobundu and Agyaka, 1987).

Uses in traditional medicine: The aqueous extracts of *M. invisa* are locally used for the treatment of cough mostly for children and often for adults. While some use it for lowering their blood sugar. It is also used for the same purpose (blood sugar lowering) in combination with other herbs. The roots of *M. invisa* are used in ayuvedic medicine to treat piles. The juice of leaves is useful in vaginal diseases, as a topical application. Being styptic and a wound healer, it is extremely beneficial in wounds and ulcers, fistula, scrofula, hydrocele, conjunctivitis. The whole plant is used externally for edema, rheumatism and myalgia.

Figure 1.34 *Mimosa in visa var.* inermis Adelb
Source: http://*library.kiwix.org*

k. *Moringa oleifera*

Local Name: Zogale

Location: Mubi

Moringa (*Moringa spp.*) is one of the world's most useful plants. This fast-growing tree is grown throughout the tropics for human food, livestock forage, medicine, dye, and water purification. It is known by several names in different countries, but is popularly called the "drumstick tree" for its pods that are used by drummers and the "horseradish tree" for the flavor of its roots.

Use in traditional medicine: The aqueous extracts of *M. oleifera* are a known antihypertensive, anti-inflammatory, antimicrobial and anti diabetic in North Eastern Nigeria. In India it is traditionally used for anemia, anxiety, asthma, blackheads, blood impurities, bronchitis, catarrh, chest congestion, cholera, conjunctivitis, cough, diarrhea, eye & ear infections, fever, glandular swelling, headaches, abnormal blood

pressure, hysteria, pain in joints, pimples, psoriasis, respiratory disorders, scurvy, semen deficiency, sore throat, sprain, tuberculosis (Ferreira *et al.,* 2008). It is locally used as vegetable by all the communities in Northern Nigeria and other regions.

Figure 1.35 *Moringa oleifera Linn.*
Source: http://*moringa.blogspot.com*

l. *Pterocarpus erinaceus*

Local Names: Madobiya

Location: Jalingo

P. erinaceus Poir. (*Leguminosae*, subfamily *Papilionoideae*) is a small to medium-sized tree 12–15 m tall with a diameter of 1.2–1.8 m. In the drier part of its range it has an open, spreading form and is low-branching, but under favorable rainfall and soil conditions, much larger specimens with clean straight boles 6–8 m long or more can be found. Exceptionally tall trees reaching 35 m height have been reported (von Maydell 1983). The bark of the trunk is dark gray and rough, with scales that curl up at the ends. Its branches are light gray and smooth. The leaves are

once-compound, imparipinnate, and 30 cm long. There are 10–15 alternate or sub-opposite leaflets, 6–11 cm long and 3–6 cm wide. The flowering tree is showy and very attractive, with masses of golden-yellow flowers that completely cover the canopy. In its native range, *P. erinaceus* flowers from December to February. The fruit is 4–7 cm in diameter, indehiscent, and broadly winged, giving it a "flying saucer" appearance.

Uses in traditional medicine: The medicinal uses of *P. erinaceus* are many, including the use of leaves as a febrifuge, the bark for tooth and mouth troubles, and bark and resin as astringent for severe diarrhea and dysentery (Marcu, 2010). The grated root is mixed with tobacco and smoked in a pipe as a cough and asthma remedy. The resin, kino, is used for dyeing cloth to produce a dark purple color.

Figure 1.36 *Pterocarpus erinaceus*

Source: http://metafro.be

m. *Rauwolfia serpentina*

Local Name: Gayen Ghana

Location: Jimetta, Yola

R. serpentina is a shrub or small tree grow up to 8 m. Older parts of the plant contain no latex. The branches are whorled and the nodes enlarged and lumpy. The leaves are in threes, elliptic-acuminate to broadly lanceolate. The flowers are minute, sweet-scented, branches of inflorescences are distinctly puberulous with hardly any free corolla lobes. Fruits are fleshy and red in colour.

Uses in traditional medicine: It is locally used as an aphrodisiac, treatment of high blood pressure, diabetes and cardiovascular problems.

Figure 1.37 *Rauwolfia serpentina*
Source: *http://www.virboga.de*

1.5.4 Biological activity assay

1.5.5 DPPH free radical scavenging activity

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be considered as fragments of molecules once formed are highly reactive radicals and can start a chain reaction. Free radicals are very unstable and react quickly with other compounds, trying to capture the needed electron to gain stability. Generally, free radicals attack the nearest stable molecule, "stealing" its electron. When the "attacked" molecule loses its electron, it becomes a free radical itself, beginning a chain reaction. Once the process is started, it can cascade, finally resulting in the disruption of a living cell. Some free radicals arise normally during metabolism. The kinetic stability of all biological molecules in an oxygen-rich atmosphere results from the unique spin state of the unpaired electrons in ground state molecular (triplet) oxygen in the atmosphere. This enables atmospheric oxygen relatively inert to reduced, carbon based biomolecules. Therefore, the reaction between oxygen and protein, lipids, polynucleotides and carbohydrates proceeds at very slow rates except they are catalyzed. However, once a free radical chain reaction is initiated, the free radicals generated rapidly propagate and interact directly with various targets also to yield hydroperoxides. These hydroperoxides are steadily attacked by reduced metals, leading to host decomposition products. Some of these products could be deleterious to the tissues also, and some formed via self propagation are themselves free radicals; thus oxidation is reinitiated (Frankel, 1991; Porter *et al.*, 1995). Lipid peroxidation for example formed during lipid oxidation decomposes to short-chain aldehydes, ketones and alcohols (Frankel, 1991). These products as well as free radicals, compromise health in several ways such as (1). The

direct oxidation of susceptible molecules can result in loss of function. An example is the oxidation of membrane lipids alters membrane integrity and promotes red blood cells fragility and membrane leakage. The oxidation of proteins results in loss of enzyme catalytic activity and/or regulation. (2). Reaction of some of these products leads to adduct formation with loss of native functions. The oxidative modification of the apolipoprotein B molecule on LDL prevents uptake by the LDL receptor and stimulates uptake by the scavenger receptor. (3). Oxidation can cleave DNA and cause point, frameshift, and deletion mutations and base damage. This oxidative cleavage impairs or destroys normal functionality. (4). Oxidative reaction can liberate signal molecules or analogs that elicit inappropriate response such as the activation of platelet aggregation, promotion of cell proliferation and down regulation of vascular relaxation by leukotoxin and eicosanoid analogs (Olearczyk, 2006). The susceptibility and overall rate of oxidation of a lipid molecule is related to the number of double bonds on the fatty acids. The rate of oxidation is determined by the ease of hydrogen abstraction. An increase in the number of double bonds increases the oxidation rate; for example, the fatty acids 18: 1, 18: 2, 18: 3 and 20: 4 have relative oxidation rates of 1, 50, 100 and 200, respectively. These relative rates of oxidation as a function of number of double bonds may be important to rates of deterioration of biological molecules *in vivo.* Diets high in poly unsaturated fatty acids require more antioxidant nutrients to prevent oxidation and rancidity (Fritsche and Johnston, 1988). Consumption by animals of diets with high amounts of poly unsaturated fatty acids appears to increase the antioxidant requirements to prevent tissue damage (Muggli, 1989). The molecular basis for this increased requirement is not known. Several reports on the increased in vivo oxidative damage are based on crude measures of

lipid oxidation such as thiobarbituric acid-reacting substances. This protocol dose not differentiates between oxidation among different dietary fats as it responds differently to the same amount of oxidation in polyunsaturated fatty acid with different numbers of double bonds. A diet enriched in highly unsaturated fatty acids would appear to increase the tendency to oxidation and increase the incidence of oxidation-associated chronic degenerative diseases, however, this has not been observed. Studies have shown that replacement of diets high in saturated fat with highly unsaturated fat diets frequently reduces atherosclerosis, thrombosis and other chronic diseases (Keen *et al.*, 1991).

Antioxidant defense falls in two main categories, those whose role is to prevent the generation of free radicals and those that intercept any radicals that are generated. These can be in the form of enzymes or non enzymes. Various in vivo studies have shown that antioxidants delay or protect against the oxidative damage produced by the free radical reaction and a protective role against ailments mediated by free radicals is now well established (Cheeseman and Slater, 1993). The natural antioxidant system can be classified into two major groups: endogenous enzymes and low – molecular weight antioxidants. Endogenous enzymes include extensively studied enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase, DT diaphorase and gluthione-regenerating enzyme systems (Sies, 1991). Some enzymatic systems such as SOD and catalase are specific against reactive oxygen species (ROS), while certain other enzyme systems reduce thiols. The low molecular weight antioxidant can further be classified into direct acting antioxidants (scavengers and chain breaking antioxidants) and indirect acting antioxidants (chelating agents). The directly acting antioxidants are important for defense against

oxidative stress. Direct scavenging of ROS is one of the many antioxidant actions required to restore oxidative equilibrium once it is lost in different pathologies. This subgroup of antioxidants currently contains hundred compounds including ascorbic acid (vitamin C), retinoic acid (vitamin A), melatonin, lipoic acids, polyphenols and carotenoids, being derived from dietary and herbal sources in phytochemicals. The hypothesis that restoring redox equilibrium through activation of intracellular signals is also an important step of the antioxidation process is gaining increasing support. It is likely that the trapping of excess free radicals could restore redox equilibrium in the initial states of cellular oxidative stress (Mukherjee and Houghton, 2009).

The potential health effects of phytochemicals as antioxidant protector must be considered in the context of the overall response of living organisms to oxidation. Many complex biochemical pathways have evolved to respond to oxidation namely: **1)**. Prevention of oxidant formation. Mitochondria and peroxisomes are specialized cellular organelles that contain the generation and transfer of electrons and that dispose of toxic intermediates and products of metabolism. Transferrin and ceruloplasmin are capable of initiating oxidation. **2)**. Scavenging of activated oxidants. Primary chain reaction-breaking antioxidants include alpha tocopherol, ubiquinone, ascorbate, uric acid, polyphenolics, various flavonoids and their polymers, amino acids and protein thiols. **3)**. Reduction of reactive intermediates. Higher animals possess enzyme systems that scavenge active oxygen and detoxify reactive intermediates, including catalase, a scavenger of hydrogen peroxide, gluthione peroxidase, a remover of hydroperoxide, and superoxide dismutase, which reduces superoxides, and superoxide dismutase, which reduces superoxide anion. Natural food constituents with antioxidant activity can also act as free radical

scavengers, antioxidants, and/or protectors/regenerators of other antioxidants (Buettner, 1993). Synergistic and antagonistic effects among mixtures of antioxidant compounds are possible based on the nature of redox couples formed by the actual redox potential of these compounds present in tissues. As such, the final participation in a redox chain would vary among different tissues and according to dietary intakes. Phenolic antioxidants stabilize some enzymes, enhance some activities, and limit others. **4)**. Induction of repair systems. Proteases, lipases, RNAase and many others, constantly turn over cellular constituents, and degenerative enzymes often have higher affinities for modified molecules. Substrate affinities of synthetic enzymes discriminate against oxidative forms of lipids, proteins and nucleotides. This discriminatory process removes damaged molecules from the cell (Suzuki *et al.*, 1995). **5)**. Apoptosis. Cells that are un-repairable are removed by the process of "programmed cell death" or apoptosis. This process, in some cases involving activation of oxygen-response elements, is used by higher organism to selectively reshapes tissues during normal growth and development and when undergoing sub necrotic stress. During apoptosis, cells are dissembled in a highly regulated and coordinated fashion. Apoptosis also limits damage as it prevents the release of reactive and toxic compounds from lysosomes and peroxisomes. Phytochemicals can effectively participate in several antioxidant defenses, inhibit platet aggregation, and spare alpha tocopherol; they may protect sensitive targets such as proteins or DNA. The molecular composition of naturally occurring antioxidants is not fully understood. It was proposed that altered disease risk in specific populations could be explained by a mechanism involving antioxidant polyphenolics in fruits and vegetables and other plant products, and this possibility focuses considerable attention

on the actions of these compounds in human health. This interest has produced epidemiological studies, hypotheses for mechanisms of actions, and testing of oxidants/antioxidants effects in the progression of several diseases that can be classified by the aberration in oxidant balance that is believed to cause them (Walzem *et al.,* 1995).

1.5.6 Hypoglycemic activity tests

The interest in the assaying of blood glucose has been growing since insulin was first discovered to be responsible for glucose metabolism. DM being a disease with multiple etiologies ranging from genetic predisposition cum environmental factors to the induced model for the purpose of study and therapeutics. For example, in a European study of DM based on genetic predisposition (EPIC, 2011) using data from existing large scale multi – cohort observational study to identify genes and lifestyle factors that interact in their influence on the risk of DM, observed that the prevalence of type 2 DM varies from one country to another which suggests that an interaction between genes and lifestyle is central to a person's risk of developing diabetes, and that the number of people with type 2 DM is lowest in rural areas of developing countries, and is highest in in certain ethnic groups who follow Western lifestyles (Carulli *et al.*, 2005; Kahn and Porte, 1999). The testing of DM is synonymous with the determination of blood glucose in a specified amount of blood. These are expressed as 3.6 to 5.8 mM/L or 64.8 to 108.4 mg/dl. Though this level fluctuates throughout the day but are usually lowest in the morning before the first meal of the day (fasting levels) and rises an hour or two later. It is considered therefore that, the blood glucose levels outside the normal range is an indication of a

disease condition. If these levels are constantly on the high side, it is considered as 'hyperglycemia', and lower levels are considered as 'hypoglycemia'. The persistence of hyperglycemia in an individual from any cause of DM is the most conspicuous disease symptom related to the failure of blood glucose regulation. Hyperglycemia may also result from severe stress, such as trauma, stroke, myocardial infarction, kidney and liver problems. The consumption of high amount of alcohol raises blood glucose levels, which eventually drops. Likewise, certain drug such acetaminophen, anabolic steroids, monoamine oxidase inhibitors (MAOIs) and pentamidin decrease blood glucose levels.

The ability of the mammalian body to regulate or keep the blood glucose levels within a narrow range (homeostatic mechanism), comprises of several interacting systems, such as hormone regulation is depended upon for the hypoglycemic testing . The main hormone is insulin. The assay of insulin thus serves as a vital tool in the assessment of DM. The insulin assay is to ascertain the following i. Insulin concentrations in the blood, from which glucose levels could be deduced. ii. The efficiency of the beta cells of the pancreas, from which insulin is secreted. iii. The genetic makeup of the insulin i.e. its amino acid constituents, which is essential for its efficiency (sensitivity and responsiveness).

The clinical features of DM such as seen in obesity, hypertension, polyuria, nocturia, weight loss, fatigue and malaise are used alongside the confirmatory tests which is the blood glucose level assay. The results that may likely be the outcome of such a study establishes the glycemic level of the mammal. And in the choice of diagnosis, the requirements are different for a known case in humans, for example (one with severe symptoms) and the asymptomatic individuals severe hypoglycemia

detected under conditions of acute infective, traumatic circulatory or other stress may be transitory and is not considered a perfect diagnostic criteria or confirmatory neither is a single test in the asymptomatic case (WHO, 1999). In the asymptomatic case, additional blood glucose test results will be required in the diabetic range, either in the fasting state, post prandial or from the oral glucose tolerance test (OGTT). In humans, factors such as ethnicity, family history, age, adiposity and concomitant disorder are looked into prior to a diagnostic or therapeutic course of action.

The OGTT serves as an alternative to blood glucose estimation and has for a long time been used to simplify the diagnosis of DM. In the fasting state, the blood glucose levels in the plasma are a very sensitive indicator of beta cell function. For example, partial pancreatectomy resulting in the loss of two thirds of the beta cells produces no significant changes of the fasting glucose levels (Ward *et al.,* 1988; Bonner-Weir *et al.*, 1983). Equally, elevated insulin levels which is normally observed in the obese dose not lower fasting blood glucose levels but a severity in beta cell function such as total pancreatectomy or autoimmune or chemical destruction of most of the beta cells, results in fasting hyperglycemia. It is evident therefore that , the fasting blood glucose level reflects only impairments of the beta cell function that are severe enough to prevent the increase of insulin secretion that usually compensates for the losses of beta cell mass or defects in insulin action (Ahren and Taborsky, 2003). In the post prandial state (after carbohydrate ingestion), the insulin secreted aids in the clearance of the absorbed glucose from the plasma. Therefore, in the OGTT, plasma glucose levels at specified time after a standard oral glucose load (usually 75 g) are used to assess the adequacy of insulin secretion. The relationship between the insulin and glucose levels is time dependent, because the

insulin released early in the test influences the glucose values later in the test. Therefore, only the early part (2 h) of the OGTT can be used to estimate beta cell function (Ahren and Taborsky, 2003) insulin resistance and sometimes reactive hypoglycemia or rarer disorders of carbohydrate metabolism such as lactose and fructose intolerance, galactosemia and glycogen storage disease.

A closely related estimation to the OGTT is the 'insulinogenic index', which is the early increment of plasma insulin divided by the early increment of plasma glucose. This index provides a crude measure of beta cell function useful in epidemiologic studies (Hanson *et al.*, 2000). Low index values does not necessarily connotes beta cell defects or malfunction because part of the insulin released at the early stage is as a result of stimulation from the parasympathetic nervous system and the release of gastrointestinal hormones that occurs during digestion. A deficiency in the gastrointestinal function can affect both insulinogenic index and the OGTT by decreasing gastrointestinal hormone release and the rate of glucose absorption. The glucose clearance from the plasma is dependent on the sensitivity of the liver and muscle to the insulin secreted. The impaired glucose tolerance in the face of a normal or enhanced insulin response could be brought about by stress and obesity. However, only when the impairment of glucose tolerance is severe as in diabetes, can the OGTT be used to infer an impairment of the pancreatic beta cell's ability to recognize glucose (NDDG, 1979). A compensatory insulin secretion, the type seen in OGTT can be produced by some drugs such as, growth hormone, glucocorticoid and nicotinic acid in what is termed 'drug induced insulin resistance'. The growth hormone and corticoids have direct inhibitory effects on the beta cell that prevent complete

compensation and thus produce mild hyperglycemia. In contrast, nicotinic acid has minimal direct effects on the beta cells and markedly reduces insulin action.

Animal models of type 2 diabetes are used generally for hypoglycemic activity tests, because diabetes research in humans is impeded by obvious ethical considerations because provocation of disease is strictly impermissible in man. Most of the available models are based on rodents because of their small size, short generation interval, easy availability and economic consideration (Srivasan and Ramarao, 2007). There are five classifications of type 2 diabetes in animal models namely **i.** Spontaneous or genetically derived diabetic animals. **ii.** Diet/nutrition induced diabetic animals. **iii.** Chemically induced diabetic animals. **iv**. Surgically diabetic animals and **v.** Transgenic/knock-out diabetic animals.

The chemically induced diabetic animals will be explained further because it was used in this study. Several chemicals can be used to induce DM in animal models such as; dehydrouramil hydrate, 4,5-dihydro-4,5-dihydroxyuric acid, 5-hydroxypseudouric acid, alloxan monohydrate (ALX) and streptozotocin (STZ), but two of these chemicals are the most popularly used i.e. alloxan monohydrate and streptozotocin. The advantages for the use of these chemicals are due to their ability to selectively destroy pancreatic beta cells leaving other pancreatic alpha and delta cells intact. Residual insulin secretion makes the animals live long without insulin treatment, ketosis and resulting mortality is relatively less and it is comparatively cheaper,, easier to develop and maintain (Srivasan and Ramarao, 2007). Some studies have reported that ALX induced diabetes also impacts insulin resistance in the animal models (Yili *et al.*, 2009; Reaven *et al.,* 1977).

ALX is highly unstable in water at neutral pH. It is rapidly absorbed by beta cells and has a direct effect on membrane permeability thereby altering its morphology. The mechanism of ALX action on the site is by its inhibition of glucose stimulated insulin release, thereby interfering with the generation of glucose derived energy by inhibiting glycolytic flux and pyruvate oxidation. It was thus concluded that, the deleterious effects of ALX on permeability, transport, intracellular energy generation and insulin is attributed to free radical formation (Malaise, 1987). Within the cell, the ALX is reduced to diuric acid, which then autoreoxidizes to alloxan. This results to the generation of superoxide radicals from the alloxan-diuric acid cycle and hence undergoes decomposition by the enzyme superoxide dismutase (SOD), producing H_2O_2. Extremely reactive OH radicals are formed in the presence of Fe^{2+} ions, which can be visualized by chemoluminiscence (Asayama *et al.,* 1984), and damages to various cellular constituents.

ALX is most effective after intravenous injection and damage to the beta cells occur within minutes and structural changes within hours in most rodents. The doses for ALX induced diabetes varies with differences in the species of animals to be used, for example dosage for rodents ranging from 40 – 150 mg/kg *bw* (Manosoroi *et al.*, 2011; Wang *et al.*, 2010).

STZ an antibiotic derived from *Streptpmyces achromogenes* and structurally a glucosamine derivative of nitrouria is used as a diabetogenic in rodents, mice, hamster, rabbits and dogs. Like ALX, it causes hyperglycemia mainly by its direct cytotoxic action on the pancreatic beta cells (Ozturk *et al.*, 1996). It is more effective than ALX in certain species such as guinea pigs, which shows reversible effect after alloxan injection. STZ treated animals, though insulinopenic, retain some insulin

secretion capacity, are not ketotic and do not usually require insulin support for survival. A mild diabetic state resembling an insulin type 2 DM may be induced in rats by a single low dose of 35 mg/kg *bw*. The STZ effect tends to be reversible in rats because of spontaneous recovery observed in them, hence dosage above 35 mg/kg *bw* is usually administered (Ho *et al.,* 1988). The STZ nitrosurea moiety is responsible for beta cell toxicity, while the deoxyglucose moiety facilitates transport across the cell membrane. The alpha – anomer of STZ shows higher potency (Rossini *et al.,* 1997) parallel to the greater effect of the alpha-glucose anomer on insulin secretion, suggesting the involvement of the membrane gluco-receptor in beta cell penetration.

The most likely reasons for the specific beta cell toxicity of ALX and STZ can be summed as a) Their high affinity for glucose-like moieties related to the exquisite sensitivity of beta cell to glucose, raising the concentration of ALX and STZ to levels higher than in the other cells, b) the SH groups in beta cell membrane render it especially sensitive to oxidative lesion, c) beta cells have a low capacity for the removal of oxidizing agent radicals due to specific glutathione depletion and low glutathione peroxidase activity (Mak *et al.,* 1996) and d) the NAD:DNA ratio in islet is lower than in other tissues about one half of that in the liver.

1.5.7 Limit test/toxicological studies

The toxic nature of a material can be obtained by the acute toxicity test, ensuring preliminary information which may probably not be available on the material. Such information can be used to: **a**) deal with cases of accidental ingestion of a large amount of the material (*e.g.*, for poison control information); **b**) determine possible target organs that should be scrutinized and/or special tests that should be

conducted in repeated-dose toxicity tests; **c)** and select doses for short-term and subchronic toxicity tests when no other toxicology information is available.

The limit test is primarily used when the test sample is likely to be nontoxic, i.e. having toxicity below regulatory limit doses. Though information on the test material can be obtained by comparing with similar samples in previous experiments, and comparing toxicological data obtained. The limit test is a sequential test that uses a maximum of 5 animals. A test dose of 2000, or exceptionally high dose of 5000 mg/kg *bw* may be used. (OECD/OCDE,1999). The test procedure allows some flexibility.

Rodents, especially the rats are usually the animal of choice for this test and the sex choice is female (OECD, 2000). This does not imply that other rodents' species cannot be used since literature shows that there is little difference in sensitivity between the sexes (Lipnick *et al.*, 1995**).** Healthy young adult animals of commonly used laboratory strains could be employed. The limit test is most suitable for medicinal plants especially those that will be studied because; the medicinal plants have been used by humans for many generations.

1.5.8 Mechanism of action

The mechanism of action of a drug refers to the specific biochemical interaction through which the drug produces its pharmacological effect. There are conditions which may influence the mechanism of action of a compound such as the specific molecular target to which the drug binds or the drug receptor, and a myriad of events that occurs before or after the binding.

Drugs act on the cell membrane by physical and/or chemical interactions. This is usually through specific drug receptor sites known to be located on the membrane. A receptor is the specific chemical constituents of the cell with which a drug interacts to produce its pharmacological effects. Some receptor sites have been identified with specific parts of proteins and nucleic acids. In most cases, the chemical nature of the receptor site remains obscure.

Several approaches can be utilized to study mechanism of drug action and one of such is the use of drug cues to compare with other pharmacological agents as reported in several hypoglycemic researches. Drugs such as insulin, glibenclamide, rosiglitazone and metformin were used as standards hypoglycemic agents compared with medicinal plant extracts (Manosroi *et al.*, 2011; Michael *et al.*, 2010; Chen *et al.*, 2009 and Rosecrans and Glennon, 1979). The known mechanism of action of the cue can be examined by attempting to antagonize it with various receptor antagonists (Wu *et al.*, 2010) or studying the mechanism of a drug cue by central neuropharmacological manipulations (Rosencrans and Glennon, 1979).