CHAPTER 4

DISCUSSION

4.1 Collection of medicinal plants

Thailand and Nigeria share similar climatic characteristics (30^{o} of the tropic of Cancer and 30^{o} of the tropic of Capricorn) and the cultural uses of traditional (herbal) medicines. In most cases, medicinal plants are macerated in alcohol, honey, water, grounded into powder and ingested with water or food, or applied topically in both countries. In recent times however, medicinal plants in both countries have witnessed gradual development in areas of refinement and usage. This was observed notably in markets and traditional medical hospices, where traditional or alternative medicines are dispensed as tablets, caplets, capsules, syrup and other forms which mimic the allopathic or orthodox medicines. The new face that alternative, traditional or herbal medicines have today, both in Thailand and Nigeria have been as a result of Government recognition of the practice, though, Thailand was ahead in granting legal status to the herbal medicine practice with policies which encompasses regulation and practice compared to Nigeria which has only done so recently.

The Thai hypoglycemic medicinal plants investigated in this study were from the Thai/Lanna Medicinal Plant Database (MANOSROI II) in the Natural Product Research and Development Center (NPRDC), Faculty of Pharmacy, Chiang Mai University, Thailand. The database was a compilation of traditional knowledge of medicines and their uses among the Thai/Lanna people, painstakingly collected over a ten year period. The medicinal plants used for the treatment of diabetes mellitus and related ailments were selected based on popularity of usage and comparing with

similar plant species which are also used in Nigeria. The names of eight (8) hypoglycemic medicinal plants namely, *Anogeissus acuminata* (Roxb. ex DC) Guill. & Perr. (Ta Khian nu), *Catunaregam tomentosa* (DC.) Tirveng (Nam Taeng), *Dioecrescis erythroclada* (Kurz) Tirveng (Ma Khang Daeng), *Dendrophthoe pentandra* (L.) Miq (Ka Fak Ma Muang), *Mimosa pudica Linn. var. hispida* Bren (Mai Ya Rab), *Moringa oleifera* Linn. (Ma room), *Pterocarpus macrocarpus* kurz (Pra Doo), *Rauwolfia serpentina* (L.) Bth. ex. Kurz (Rayom) was selected after human and literature consultations. Two (2) other specimen which are recipes also used locally, namely; Yamed Boraped Pungchang® (containing *Stephania rotunda, Acanthus ilicifolius, Cyprus rotundus, Rhinaeanthus nasutus),* Mai Tau Lusi (*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) were also used in the study. These were processed medicinal plants prepared in capsules and caplets. The recipes were provided by Mr. Somnuek of the Traditional Thai Medicine Development Center of the Thailand Ministry of Science and Technology.

The selection of hypoglycemic medicinal plants from the North Eastern part of Nigeria was done after inquiries and co-ordinated field research to a few towns and villages within areas such as Mubi, Gwoza, Maiduguri, Yola and Jalingo (Fig 3.1). Being the first of such field study on traditional hypoglycemic medicinal plants of the area, problems such as reluctance by the traditional medical Practitioners to co-operate with the researcher and some patients' ignorance of the herbs they were using were encountered. On the part of the patients, many were able to give or identify some of the plants used in their communities or as described by oral tradition from

parents or relations though others were mostly "kept in the dark" because the medicinal plants dispensed to them are usually chopped into small pieces or dried and grounded before handing to them with instructions on usage. Unlike in Thailand where ancient written materials were collected from temples and converted to modern documents, there are no written documents in most parts of North Eastern Nigeria about the herbal medicines. Knowledge of this practice was usually transferred by oral tradition within families of practitioners or from master to apprentices. Hence, the selection of the medicinal plants was done based on popularity and the testimonies of credible patients who have been using the medicinal plants for a long time. Ten (10) medicinal plants namely, *Anisopus mannii* N.E.Br (Kashe zaki), *Anogeissus leiocarpus* (D.C) Guill & Perr. (Mareke), *Daniella Oliveri* (Rolfe) Hutch & Dalz. (Maje), *Detarium macrocarpum* Harms (Taura), *Leptedenia hastata* (Pers) Dec'ne (Ya diya), *Mimosa invisa* var. *inermis* Adelb (Idon Zakara), *Moringa oleifera* (Zogale), *Pterocarpus erinaceus* (Madobiya), *Rauwolfia serpentina* (Ganyen Ghana) and Maganin Ciwon Suga (recipe comprising of *Ficus Thonningii*. Blume, (chediya), *Raphia vinifera* P. Beauv, (kimba) and *Leptedenia hastata* (Pers) Dec'ne (Yaa diya)) were selected from among several plant species because of the reasons already stated above and coupled with the fact that they featured in different locations for the same uses. For example *Anisopus mannii* was found in all the towns visited but the plant was said to be sourced from Gwoza, Yola or Jalingo which are hundreds of kilometers apart from each other and having similar climate but dissimilar geomorphologic features.

4.2 Physico-chemical characteristics of the Thai/Nigerian hypoglycemic medicinal plants

The aqueous extracts of the various Thai/Nigerian hypoglycemic medicinal plants were determined according to standard procedures taking the traditional methods of preparation into consideration. The same was done for the dosage because a cup or two of the macerated or boiled medicinal plants was usually the recommended dosage. The percentage yield of the aqueous extracts of both Thai and Nigerian medicinal plants are shown on Table 3.1 and 3.2, while the percentage yield of the their methanol and chloroform extracts used subsequently for experiments are shown on Table 3.3.

The phytochemistry of the aqueous extracts of the Thai medicinal plants showed high intensities of tannins, saponins, xanthones, glycosides and alkaloids particularly in the *A. acuminata, R. serpentina,* Mai Tau Lusi, Yamed Boraped Punchang, *D. pentandra, D. erythroclada, M. oleifera, P. macrocarpus* and *M. pudica*, respectively (Table 3.3.1). The methanol extracts showed higher phytochemical intensities in anthraquinones, glycosides, xanthones, tannins and alkaloids by the *R. serpentina, A. acuminata, D. pentandra*, Mai Tau Lusi, Yamed Boraped Pungchang, *M. pudica, P. macrocarpus, M. oleifera, C. tormentosa* and *D. erythroclada*, respectively (Table 3.3.2). Similarly, the aqueous extracts of the Nigerian hypoglycemic medicinal plants showed lower phytochemical intensities comparing with the Thai plants. The Tannins, saponins, xanthones and flavones featured prominently and were higher in *D. macrocarpum, M. invisa, A. leiocarpus, A. mannii, D. oliveri, P. erinaceous, R. serpentina, MCS recipe, M. oleifera* and *L. hastata*, respectively (Table 3.3.3). The methanolic extracts of the Nigerian medicinal

plants showed a dissimilar phytochemistry, the intensities of the phytochemicals were much stronger comparing with the aqueous extracts. The medicinal plant with the strongest intensities of phytochemical were *M. invisa, D. macrocarpum, A. leiocarpus, D. macrocarpum, P. erinaceous,* MCS recipe, *M. oleifera* and *L. hastata*, respectively (Table 3.3.4). A comparison of the Thai with Nigerian hypoglycemic medicinal plants showed higher phytochemical intensities in the Thai medicinal plants over the Nigerian plants. The reasons for these dissimilar attributes may be linked to the plant genotype, foliation, dissimilar climatic and geomorphologic characteristics, such as length of sun light, temperature, humidity and soil nutrient (Osier and Lindroth, 2001), and UV exposure (Sumathi *et al.*, 2008; Kafman *et al.,* 1999). The level of humidity in Northern Thailand may be said to be an additional factor responsible for the higher intensities observed because the Nigerian medicinal plants were collected from dry savannah areas which also enhances plant phytochemistry but atmospheric humidity has been observed to enhance the secretion or presence of these metabolites even higher (Gouinguene and Turlings, 2002). The beneficial medicinal effects of the medicinal plant materials typically results from the combination of secondary metabolites present in plants (Briskin, 2000). That the medicinal effects of plant are unique to particular plant species or groups is consistent with these concepts as the combinations of secondary metabolic products in a particular plant are often taxonomically distinct (Wink, 1999). This is observed between the Thai and Nigerian medicinal plants of same family, *Anogeissus acuminata* and *Anogeissus leiocarpus* (Tables 3.3.1- 3.3.4). This characteristic is in contrast to primary products, such as carbohydrates, lipids, proteins, heme, chlorophyll and nucleic acids, which are common to all plants and are involved in the primary metabolic processes of building

and maintaining the plant's cells (Kaufman et al., 1999; Wink, 1999). As regards the secondary metabolites in medicinal plants, it is possible that their ecological functions may enhance or affect their usefulness as potential medicinal effectors for humans. Secondary metabolites that are necessary for plant defense for example via toxicity directed at microorganisms could prove to be useful as antimicrobial medicines in humans, if not extremely toxic (Briskin, 2000). Those secondary metabolites which are involved in the defense against herbivores through neurotoxicity could have beneficial effects in human as anti-depressants, sedatives, muscle relaxants or anesthetic because of their actions on the central nervous system (Briskin, 2000). As a way of enhancing ecological adaptation and survival of plants, structures of secondary metabolites have evolved to interact with molecular targets affecting the cell tissues and physiological functions in various organisms including humans. Concerning these effects, the secondary metabolites in many plant species may exert their defensive actions especially by mimicking endogenous metabolites, ligands, hormones and neurotransmitters and hence have beneficial medicinal effects on humans due to similarities in their potential target sites such as the central nervous system, endocrine system (Kaufman *et al.*, 1999) an example which is the pancreas. Comparing with the synthetic drugs, most phytomedicines are known to exert their actions via "additive or synergistic action of several chemical compounds acting at single or multiple target sites linked to the physiological process" which are based on single chemicals (Briskin *et al.,* 2000). These additive or synergistic actions can be beneficial by eliminating some problematic side effect often associated with the predominance of a single pharmaceutical compound in the body (Tyler, 1999).

4.3 Free radical scavenging activities of the Thai and Nigerian medicinal plants crude extracts (DPPH assay)

The aqueous extracts of all the Thai medicinal plants showed varying levels of free radical scavenging activities (SC_{50}) expressed as fold activity comparing with ascorbic acid (Table 3.4.1) and tocopherol (Table 3.4.2). The SC_{50} was highest in aqueous extracts of *A. acuminata* with five (5) folds comparing with ascorbic acid. *P. macrocarpum, R. serpentina*, and *D. erythroclada* were comparable to ascorbic acid. The recipes Yamed Boraped Pungchang and Mai Tau Lusi showed about 50% scavenging activity compared to ascorbic acid, the others showed negligible activities (Table 3.4.1). The methanolic extract of the Thai medicinal plant *A. acuminata* showed higher free radical scavenging activities with seven (7) fold activity over the standard, tocopherol. *P. macrocarpum, D. pentandra* and *C. tormentosa* showed about two times the fold activities of tocopherol, while Mai Tau Lusi, Yamed Boraped Pungchang, *D. erythroclada* and *R. serpentina* were comparable to the standard. The others showed negligible scavenging activities (Table 3.4.2).

In the aqueous extracts of the Nigerian medicinal plants group, the highest free radical scavenging activity was 2 folds those of ascorbic acid showed by *D. macrocarpum* and *A. leiocarpus*, approximately two folds was shown by *P. erinaceous* and a comparable scavenging activity was shown by *D. oliveri*, MCS recipe, while 50% scavenging activity was shown by extracts of *L. hastata* and *M. invisa.* The other extracts showed negligible free radical scavenging activities (Table 3.4.3). The methanolic extracts showed higher free radical scavenging activities than the aqueous group, comparing with tocopherol. The highest free radical scavenging activity was about six (6) times the free radical scavenging activity of tocopherol and

was shown by *A. leiocarpus*. *D. macrocarpum* maintained two (2) fold activity, *D. oliveri* and *P. erinaceous* were comparable while the other extracts showed negligible free radical scavenging activities (Table 3.4.4).

The role of free radical scavengers is gaining high prominence because they are known to protect the cell against oxidative stress caused by the presence of substances such as nitric oxide (NO), superoxide anion and reactive oxygen species (ROS) which are by products of enzymatic reactions (Droge, 2002). The ROS are one of many factors responsible for gross cellular damage, mutagenesis, cancer and degenerative process of biological aging (Harman, 1956) which are characteristic symptoms observed in diabetes mellitus (Wright *et al.*, 2002). There are ample evidence which links ROS to other diseases seen in diabetic Patients such as retinopathy (Rai *et al.*, 2006), atherosclerosis and myocardial infarction (Harman, 1992). The claim was that, free radicals are formed too rapidly to be detoxified by natural defenses (Rai *et al.,* 2006). Natural free radical scavengers such as endogenous enzymes having been compromised in chronic diabetes mellitus which leads to increasing levels of ROS and other ailments observed in DM patients (Berry and Clarkson, 2000). Low molecular weight antioxidants known to act directly on free radicals have been identified to include ascorbic acid, retinoic acids, melatonin, polyphenols and carotenoids derived from dietary and herbal sources (Sati *et al.,* 2010).

The free radicals may play important role in the causation of diabetes mellitus (Dhanasekar and Sorimuthu, 2005). This is because diabetes mellitus resulting from glucose toxicity often leads to the generation of free radicals which may result in potentially irreversible damage (Abraham *et al.*, 2008; De Groot, 1994). Almost all

major classes of biomolecules are attacked by free radicals but lipids are the most susceptible. Cell membrane contains fatty acids which are readily attacked by the free radicals. Increased lipid peroxidation impairs membrane function by decreasing its fluidity and changing the activity of the enzymes and receptors (Sunil *et al.*, 2009). The free radical scavengers are therefore capable of donating protons to the free radicals which decreases their spectral absorption. The decrease in absorption is considered as a measure of the radical scavenging or mumping. This agrees with previous studies which have indicated that "strong antioxidant activities of some plants extracts may be partially responsible for many biological properties" (Saghizadeh *et al.,* 1996), diabetes mellitus related conditions, inclusive. Several crude extracts of medicinal plants have been observed to possess free radical scavenging activities (Nikolava and Dzhurmanski, 2009: Duwwiejua and Zeitlin, 1993). It was previously reported that the crude extracts of *Uncaria tormentosa* protected mice against ozone – free radical effects (Cisneros *et al.*, 2005). Several constituents of the extracts of medicinal plants have been implicated for the free radical scavenging activity of medicinal plants in *Citrullus colocynthis, Clitoria ternatea, Luffa acutangula* and *Madhuca indica (*Shekhawa *et al.,* 2010), *Polygonum multiflorun* Thunb (Luo *et al.,* 2011). This constituents includes; terpenes (Siedle *et al.*, 2004; Rackova *et al.*, 2007), phenols (Yoon and Baek, 2005: Sonboli *et al.*, 2010), flavonoids (Porath *et al.,* 2005; Ghasemzade *et al.,* 2011; Nickavar *et al.,* 2007), Tannins (Feglins *et al.,* 2004) and saponins (Zakaria 2007). Comparing the free radical scavenging activities of the Thai and Nigerian medicinal plants separately, the methanolic extracts of both groups showed higher free radical scavenging activities than the aqueous extracts. This could be linked to the fact that, the methanolic extracts

contained both polar and non polar components of the plants acting concomitantly or synergistically, while the aqueous extracts contained the polar portion only.

4.4 Hypoglycemic effects of the crude extracts of Thai and Nigerian Medicinal Plants

The selected Thai hypoglycemic medicinal plants all showed varied levels of hypoglycemic effects at different time intervals and doses comparing with distilled water, insulin and glibenclamide in aqueous and alloxan induced diabetic mice. *M. pudica* showed the highest reduction in FBG levels of 47.43% with 100 mg/kg bw at the 4 h, which was 0.77 and 1.51 fold of insulin and glibenclamide in normoglycemic mice (Fig 3.3 and 3.4). It was followed by *P. macrocarpus* with 44.71% at the 4 h with 400 mg/kg bw dose, which was 0.40 and 0.62 fold of insulin and glibenclamide (Fig 3.6 – 3.7). Others were Yamed Boraped Punchang-29.15% (Fig 3.10), *D. pentandra*- 26.46%, *R. serpentina* – 24.81%, *D. Pentandra* – 19.10%, which showed lower activities in comparing with the standards. In the alloxan induced diabetic groups, the aqueous extract of *A. acuminata* showed the highest significant ($p<0.05$) FBG reduction of 78.97% with 100 mg/kg bw at the 4 h (Fig 3.4) which was 1.10 and 1.75 of insulin and glibenclamide fold, respectively (Fig 3.5). All the other hypoglycemic medicinal plant extracts showed significant FBG reduction in the following descending order; *R. serpentina* – 66.62%, *C. tormentosa* – 57.00%, *D. erythroclada* – 55.51%, *P. macrocarpus* – 54.63%, *M. oleifera* – 50.81%, *M. pudica* – 50.35%, *D. pentandra* 49.00%, Yamed Boraped Punchang – 45.00% and Mai Tau Lusi – 36.00%, respectively (Figures 3.4; 3.5; 3.8; 3.9; 3.12; 3.13). Except probably in very limited cases, medicinal plants which have previously shown significant

hypoglycemic effects *in vivo* did so with higher percentages in diabetic than in the normoglycemic animals (Manosroi, 2011; Mahomed and Ojewole, 2003). The aqueous extract of *A. acuminata* extract showed very negligible hypoglycemic activity in the normoglycemic mice, but acted promptly and markedly on alloxan induced diabetic mice (Figures 3.2 - 3.4). Since the diabetic state in alloxan induced diabetic mice is not similar to that obtained by the total pancreatectomy, daily administration of insulin may not be required for the survival of the alloxan induced diabetic mice (Ojewole, 2002). This was indicated by the fact that most people who use the already mentioned hypoglycemic medicinal plants are mainly type II diabetic sufferers. The hypoglycemic effect of the *A. acuminata* extract is therefore most probably exerted *via* a mechanism that is not different from that of glibenclamide and/or not related to insulin secretion from pancreatic beta-cells (Ojewole, 2002). *A. acuminata* extract might improve the receptor responsiveness to insulin causing an increased glucose uptake by the tissues (Anturlikar *et al.,* 1995; Gharaibeh *et al.*, 1988) in diabetic mice which mimicked thiazolidiendiones in its reaction mechanism. It could have also enhanced secretion of insulin which may have contributed to the FBG suppression as a result of its stimulation by the oral administration of the medicinal plant extracts which the phytochemical analysis have shown to contain some amount of glycosides and other organic compounds (Tables 3.3.1 - 3.3.2) capable of stimulating insulin release (Porte *et al.,* 2003). The significant reduction in FBG levels shown by the extract may be attributed to the effects or activities of their phytochemical components, xanthones, tannins, alkaloids and saponins which were observed in previous studies to have similar effects (Khan *et al.,* 2010; Dineshkumar *et al.,* 2010). Similarly, synergistic reactions between two or more phytochemicals

may also be responsible for the observed hypoglycemic effects (Kumar and Doble, 2009; Rao *et al.,* 2003 and Alarcon-Aguilar *et al.,* 2000) which could have acted either as a single chemical or synergistically.

The results of the Nigerian medicinal plants showed that *R. serpentina* possessed the highest significant ($p<0.05$) FBG reduction of 49.74% with 100 mg/kg bw at the 3 h, which was 0.88 and 1.73 fold of insulin and glibenclamide, respectively (Fig 3.31 and 3.32). This is a newly discovered phenomena with *R. serpentina* because the plant has had a few chemical compounds isolated some of which are serpintine, sarpagine, reserpine and ajmalicine (Harisaranraj *et al.,* 2009) known to be primarily used in traditional medicine for the treatment of cardiovascular related ailments (Harisaranraj *et al.,* 2009; Genest, 1955). This was followed by *D. macrocarpum* with 44.26% with 400 mg/kg bw at the 4 h, which was 0.73 and 1.43 folds insulin and glibenclamide. Others were *P. erinaceous* – 36.38%, *A. mannii* – 21.63%, *D. oliveri* – 17.97%, *M. invisa* – 16.60%, MCS recipe – 15.72%, *A. leiocarpus* – 9.51%, *M. oleifera* – 3.00% and *L. hastata* – 0% and their various fold activities with the standard drugs, insulin and glibenclamide are shown in Figures 3.27 – 3.38). In the alloxan induced diabetic mice on the other hand, *A. mannii* showed a significant FBG reduction of 70.39% with 200 mg/kg bw at the 4 h, which was 0.98 and 1.54 fold of insulin and glibenclamide, respectively (Fig 3.29 – 3.30). It was followed by MCS recipe – 67.91% with 200 mg/kg bw at the 4 h, *A. leiocarpus* – 64.53%, *D. macrocarpum* 64.05%, *R. serpentina* – 58.88%, *P. erinaceus* – 57.83%, *L. hastata* – 55.22%, *D. oliveri* – 36.47%, *M. oleifera* – 35.95% and *M. in visa* – 24.81%, respectively (Fig 3.31 – 3.38).

The hypoglycemic effects observed in the normoglycemic mice were generally lower than in the alloxan induced glycemic mice as observed with the Thai medicinal plants. However, it is known that, alloxan monohydrate destroys the beta-cells of the islets of Langerhans, resulting in the reduced synthesis and secretion of insulin (Srinavas *et al.,* 2003). Insulin enhances tissue utilization of glucose while glibenclamide, a sulphonylurea produce hypoglycemia by increasing the secretion of insulin from the pancreas. These compounds are active in mild alloxan induced diabetes (Grodsky *et al.,* 1971). The medicinal plants extract might reduce the FBG through the insulin release by stimulating the regeneration process and revitalization of the remaining beta cells (Bolkent *et al.*, 2000; Rokeya *et al.*, 1999). This was clearly evidenced by the decreased levels in blood glucose between the zero and the fourth hour in diabetic mice (Raut and Gaikwad, 2006). Some hypoglycemic plants may exert their action by stimulating the function of the unaffected beta-cells and thus enhancing insulin release (Persuad *et al.*, 1999). Alloxan induced diabetic mice receiving *A. mannii* and *D. macrocarpum* showed rapid reduction in glycemic levels compared to the control group. This effect might be due to enhancement of insulin secretion of the unaffected beta-cells leading to glucose utilization by the tissues. Moreover, like Insulin and glibenclamide, oral administration of *A. mannii* and *D. macrocarpum* showed hypoglycemia in normoglycemic mice. This suggested that the active compounds in *A. mannii* and *D. macrocarpum* might probably mediate enhanced utilization of glucose by the tissues via action on beta-cells (Jaiswal *et al.,* 2009) considering that the fold of insulin was comparable to those of the antidiabetic plants extract (Fig 3.4 and 3.8)).

The significant FBG reduction exhibited by *A. acuminata* (Thai medicinal plants), *D. macrocarpum*, *A. leiocarpus, P. erinaceus* (Nigerian medicinal plants) suggested that its free radical scavenging activity may also play an important role in the hypoglycemic effect as shown in the results obtained which also supports previous claims observed in other medicinal plant extracts (Saghizade *et al.,* 1996). The other medicinal plant extracts in this study which showed significant hypoglycemic activity in diabetic mice also supported this idea for example *D. erythroclada, R. serpentina, P. macrocarpus, D. pentandra* (Thai plants) and *L. hastata,* Maganin Ciwon Suga (Nigerian recipe) due to their antioxidants activity. This may however not be the case with for other medicinal plant extracts especially *C. tormentosa* (Thai Plant) and *A. mannii, R. serpentina* and *D. oliveri* (Nigerian plants) which showed a negligible scavenging activities but significant hypoglycemic effect. This is not a conclusive deduction however, because the concentration of extracts used for the determination of free radical scavenging studies may play some role in the scavenging activity as observed for *A. mannii* by Musa *et al.*, (2009).

4.5 Extraction, fractionation and hypoglycemic activities of the Thai and Nigerian medicinal plant fractions

4.5.1 Thai Medicinal Plant *A. acuminata*

The methanol extract obtained from the Soxhlet extraction of *A. acuminata* powder gave a yield 18.05% w/w. After partitioning, the yields of methanol and chloroform fractions were 1.39% and 16.59% w/w, respectively (Fig 3.25). In silica gel column chromatography eluted with chloroform, it gave three separate sub-fractions of chloroform (0.91%), chloroform-methanol (0.15%) and methanol

(0.30%), sub-fractions, respectively. For the methanolic fraction, after liquid partitioning, the following sub-fractions ethyl acetate (0.42%), chloroform (0%), n-butanol (13.63%) and methanol (2.49%) were obtained.

4.5.2 Hypoglycemic activities of the methanolic fractions of *A. acuminata*

The hypoglycemic effects of the methanolic and chloroform sub-fractions of *A. acuminata* in normoglycemic mice comparing with DW and the standard drugs (insulin and glibenclamide) are shown in Figures 3.26 – 3.33. The 400 mg/kg *bw* of ethyl acetate, methanol and down to 100 mg/kg *bw* of n-butanol gave significant ($p<0.05$) FBG reductions at the 4 h. The highest FBG reduction of 35.15% was observed in the ethyl acetate at 400 mg/kg *bw*, which was 0.53 and 0.82 folds of insulin and glibenclamide, respectively. All hypoglycemic effects observed in the methanolic sub-fractions were lower than insulin and glibenclamide (Fig 3.27 and 3). Figures 3.28 and 3.29, showed the hypoglycemic effects of the chloroform sub-fractions of *A. acuminata* in normoglycemic mice. Only chloroform at the dose of 400 mg/kg *bw* showed significant ($p<0.05$) hypoglycemic effect of 21.63% FBG reduction at the 4 h, which was 0.33 and 0.51 fold of insulin and glibenclamide, respectively.

In the alloxan induced diabetic mice group (Figures 3.30 and 3.31), ethyl acetate (100 mg/kg *bw*), n-butanol (100 mg/kg *bw*) and methanol (400 mg/kg *bw*) showed significant ($P<0.05$) decreases in FBG levels in dose dependent order, which were higher than or comparable to glibenclamide but lower than insulin. Methanol indicated a 41.10% reduction in FBG at the 4 h, which was 0.47 and 0.91 fold of insulin and glibenclamide, respectively. The hypoglycemic effects of the chloroform sub-fractions in alloxan induced diabetic mice in comparing to the standard drugs

(insulin and glibenclamide) and DW are shown in Figures 3.32 and 3.33 methanol at the dose of 400 mg/kg *bw* showed significant ($p<0.05$) hypoglycemic effect of 29.96% at the 4 h which was 0.34 and 0.67 fold of insulin and glibenclamide, respectively. chloroform-methanol at 400 mg/kg *bw* also showed significant ($p<0.05$) hypoglycemic effect but was lower than insulin and glibenclamide. Other sub-fractions which showed no or reverse hypoglycemic effects are not presented. The methanol sub-fraction of the methanol *A. acuminata* crude extract which gave the most effective hypoglycemic activity at 400 mg/kg *bw* and was used for further experiments.

The hypoglycemic effects of the methanol sub-fraction in postprandial hyperglycemia in normal mice fed with various carbohydrates were shown in Fig 3.34 Oral administration of the methanol (250 mg/kg *bw*) suppressed postprandial blood glucose (PPBG) (<150 mg/dl) in all the groups at various time intervals (Fig 3.34 a - d). The most efficient effect of methanol sub-fraction was on the glucose group only. Failure to suppress PPBG as the standard protocol recommended (2 h) by any of the sub-fractions indicated ineffectiveness of the compound. Thus, the mechanism of methanol sub-fraction on the hypoglycemic effect might be from glucose oxidase inhibition activity mainly.

The hypoglycemic efficiency of the methanol sub-fraction of *A. acuminata* was altered as a result of the mode of administration as shown in Figure 3.25. The intraperitoneal administration of the extract showed higher efficiency in suppressing FBG levels than the oral administration. The effects (ip or po) were comparable to glibenclamide only.

In the mammals, the secretion of insulin is activated by a number of stimulus with glucose being the most important (Olefsky and Kruszynska, 2003). This is archived via a K^+ ATP dependent way (Sato and Henguin, 1998; Segher *et al.*, 2000), in which the closure of the cell surface ATP – sensitive K^+ channels and the resulting opening of the voltage dependent Ca^+ channels facilitates extracellular calcium influx into beta cell thereby stimulating the exocytosis of insulin (Wu *et al.*, 2011). In testing the effects of Isosorbide dinitrate, a K^+ channel opener and nifedipine, a Ca^+ channel blocker on the hypoglycemic activity of the methanol sub-fraction in other to set a possible mechanism for its insulin stimulating effect. The result showed that, the FBG levels were reduced significantly when the methanol sub-fraction in normal saline (AMS) was used to treat the mice comparing to the control group (Fig 3.26). The efficiency of the sub-fraction was however altered when the mice was co-treated with Isosorbide (AMI) or nifedipine (AMN). The co-treatment with the Isosorbide dinitrate and nifedipine lowered the BG levels slightly at the 2 h though the effect of the fraction was deeper, but the levels were comparable at the 4 h and it remained that way unto the 6 h. It suggested that, the hypoglycemic effect of the methanol fraction was probably by the cell surface ATP – sensitive K^+ closure or voltage dependent Ca^+ channel opening. Hence, the conclusion that any insulin stimulation by this sub-fraction was by the cell surface K^+ channel closure and voltage dependent Ca^+ channel opening, which indicated that the sub-fraction's mechanism of suppressing FBG levels in the blood was by stimulating insulin secretion.

The body and organ weights of mice fed with the methanol sub-fraction at the doses of 2,000 and 5,000 mg/kg *bw* were shown in Tables 3.4.5 and 3.4.6. There were no deaths of mice in any of the groups. Significant body weight gains were observed

in the 5,000 mg/kg *bw* treated groups at day 3 to 14. The body weight gain might be from the effect of the methanol sub-fraction. The 2,000 mg/kg *bw* groups gave a significant ($P<0.05$) decrease in organ weights in comparing to the control group while the reverse was the case with the 5,000 mg/kg *bw*. BUN levels decreased in both the 2,000 mg/kg *bw* and 5,000 mg/kg *bw* groups while creatinine decreased in the 2,000 mg/kg *bw* but increased in the 5,000 mg/kg *bw* treated groups (Fig 3.37a and b). However, a dose dependent elevation was observed for AST, ALT and total bilirubin levels of the 2,000 and 5,000 mg/kg *bw* though the 2,000 mg/kg *bw* group was higher (Figs 3.37c - e).

4.5.3 The Nigerian medicinal plants *A. mannii*

The percentage yield of the Nigerian medicinal plant after reflux with methanol was 19.89% (Fig 3.38). After separation by liquid phase partitioning using chloroform 52.62% and 46.98% of chloroform and methanol fractions were obtained, respectively. The liquid phase partitioning of the methanol fraction yield were as follows; ethyl acetate – 0.83%, ethyl acetate: acetic acid – 40.14%, ethyl acetate: acetic acid: methanol – 53.09% and methanol residual fraction – 5.64%. The silica gel column chromatography used to separate the chloroform fraction gave the following yields; chloroform – **1**: 21.74%, chloroform – **1I**: 5.00%, chloroform – **III**: 2.53%, chloroform: methanol – 27.83% and chloroform: acetic acid: methanol – 46.28%, respectively.

4.5.4 Hypoglycemic activities of the methanolic fractions of *A. mannii*

After the reflux, filtration and evaporation of the methanol crude extract of *A. mannii*, the percentage yield was 19.90%. After partitioning, the chloroform fraction was 10.47% and (Fig. 3.28) the methanolic fraction was 9.35%. For the elution with the silica gel column chromatography of the chloroform fraction, there were five separate sub-fractions as follows chloroform I 1.87% (22.77 g), chloroform II 0.52% (6.39 g), chloroform III 0.30% (3.24 g). Three separate bands on the column were observed by using chloroform as an eluant and were eluted and collected separately as chloroform I, chloroform II and chloroform III, while chloroform-methanol 2.91% (8:1) (35.54 g), chloroform-acetic acid-methanol (8:1:1) 4.84% (59.10g) were eluted with chloroform/methanol (8:1) and chloroform/acetic acid/methanol (8:1:1), respectively. For the methanol residue, it gave the sub-fractions of ethyl acetate 0.08% (0.95 g), ethyl acetate-acetic acid (7:2) 3.75% (45.77 g), ethyl acetate-acetic acid-methanol 4.96% (7:2:2) (60.54 g) and methanol 0.53% (6.43 g) by liquid phase partitioning.

The hypoglycemic effects of methanol fractions of *A. mannii* sub-fractions in normoglycemic mice compared with DW and standard drugs insulin and glibenclamide are shown in Figure 3.39. The 100 mg/kg *bw* of ethyl acetate, ethyl acetate-acetic acid-methanol and 400 mg/kg *bw* of ethyl acetate, and methanol showed significant ($p<0.05$) FBG reductions at 3 and 4 h, respectively. The highest reduction of 27.36% by 400 mg/kg bw, which was, 0.41 and 0.64 fold activity of insulin and glibenclamide was observed with the methanol sub-fraction (Fig 3.40). All hypoglycemic effects observed in this group were low when compared with insulin and glibenclamide. Figure 3.41 showed the hypoglycemic effects of the chloroform

sub-fractions of *A. mannii* in normoglycemic mice compared with DW and standard drugs, insulin and glibenclamide (Glb). 100 mg/kg *bw* of chloroform II, chloroform-methanol; 200 mg/kg *bw* of chloroform-methanol, ethyl acetate - acetic acid - methanol and 400 mg/kg *bw* of chloroform-methanol and chloroform-acetic acid-methanol showed significant ($P<0.05$) hypoglycemic effect at various time intervals but was lower than glibenclamide and insulin. CM-100 mg/kg *bw* indicated 25.01% reduction which was 0.38 and 0.58 fold activity of insulin and glibenclamide (Fig 3.42). Other fractions not represented showed no or reverse hypoglycemic effects.

In the alloxan induced diabetic group (Fig 3.43), *A. mannii* methanol sub-fractions, 100 mg/kg *bw* of ethyl acetate-acetic acid, 200 mg/kg *bw* of ethyl acetate - acetic acid - methanol and 400 mg/kg *bw* of ethyl acetate-acetic acid, ethyl acetate - acetic acid - methanol and methanol showed significant ($p<0.05$) decrease in FBG levels which were higher than glibenclamide but lower than insulin. The methanolic sub-fraction exhibited a 65.57% decrease in FBG which was 0.75 and 1.50 fold activity of insulin and glibenclamide, respectively (Fig 3.44). The hypoglycemic effects of *A. mannii* chloroform fractions in alloxan induced diabetic mice comparing with the standard drugs, insulin and glibenclamide and distilled water shown in Fig 3.45, 100 mg/kg *bw* of chloroform-methanol, showed significant ($p<0.05$) hypoglycemic effect of about 40% comparable with glibenclamide at the 3 and 4 h. 200 mg/kg *bw* of chloroform-methanol showed significant ($p<0.05$) hypoglycemic effect on FBG levels with the highest effect of 23%, while the 400 mg/kg *bw* of chloroform III, chloroform-methanol and chloroform-acetic acid-methanol showed hypoglycemic effects about 39% in peak values which were comparable to glibenclamide but lower than insulin (Fig 3.46). The chloroform III sub-fraction was

the most effective dose with 39.98% FBG reduction, which were 0.52 and 1.06 folds of insulin and glibenclamide (Fig 3.46). Other fractions not represented showed zero or reverse hypoglycemic effects. A comparison of all the results showed that, the 400 mg/kg *bw* methanol fraction of *A. mannii* was the most effective and was used for further experiments.

These results establishes the fact that, the non polar constituents of *A. mannii* has significant hypoglycemic activity inspite of the plant been used traditionally in aqueous form by maceration or boiling in water which utilizes more of its polar constituents. In some cases though, some patients grin the dried plant and use prescribed amount on food. The fraction from the methanolic *A. mannii* crude extract which gave the highest FBG reducing effect confirms a previous study of the aqueous extract of *A. mannii* for treating DM (Manosroi *et al.*, 2011). The methanol fraction showed a tendency for a longer hypoglycemic activity beyond the 4 h when compared to the standard drugs. This was an advantage over the standard hypoglycemic drugs.

In the OGTT with the methanolic sub-fraction in normoglycemic mice, the blood glucose levels were significantly ($p<0.05$) reduced to the levels similar to those of the standard without the aid of the fraction because of the activity of natural enzymes in healthy animals (Fig 3.47). Usually, in the successful OGTT, the FBG levels were suppressed at the second hour (WHO, 1999). This phenomenon was observed in the glucose and lactose group which implied that, the methanol sub-fraction may possess glucose oxidase and lactase (β-galactosidase) inhibition activity. A single dose treatment of this sub-fraction (ip or po) was able to decrease FBG levels at similar rates, thus, the fraction showed efficiency in both fasting and postprandial states, which lead to the conclusion that *A. mannii* methanolic sub-fraction may have

been acting on the beta cells of the pancreas to stimulate insulin secretion and also enhanced insulin sensitivity by the cells (Manosroi *et al.,* 2011).

Both oral and intraperitoneal administration of the methanolic sub-fraction showed similar outcome when administered orally or by intraperitoneal routes (Fig 3.48). The effect of a single dose of *A. mannii* methanolic sub-fraction (62.5 mg/kg *bw* and 120 mg/kg *bw*) were comparable with the standard drugs insulin (0.5 iu/kg *bw- iv*) and glibenclamide (1.0 mg/kg *bw*- po). The extract showed higher efficiency than glibenclamide but lower than insulin (Fig 3.48).

It has been broadly reported that glucose stimulates insulin secretion *via* K^+ ATP channel pathway (Yang *et al.*, 2007; Segher *et al.*, 2000) by closure of the cell surface ATP-sensitive K^+ channels and the resulting opening of cell-surface voltage-dependent Ca^{2+} which facilitates the extracellular Ca^{2+} influx into beta cells and triggers the exocytosis of insulin (Wu *et al.*, 2011). This phenomenon was supported by the hypoglycemic effect of the methanol sub-fraction of *A. mannii*, which was observed in mice co-treated with isosorbide dinitrate and nifedipine. It indicated that, the hypoglycemic activity shown by the methanol sub-fraction of *A. mannii* was not dependent on the closure of K^+ and opening of the Ca^{2+} channel. It could therefore be speculated that the methanol sub-fraction of *A. mannii* may have enhanced insulin activity as a way of FBG reduction in the diabetic mice (Fig 3.49).

In the acute toxicity studies with the methanol sub-fraction of *A. mannii*, no sign of behaviour changes, toxic signs as shown by the normal appearance of respiration pattern, colour of body surface, frequency of movement both voluntary and involuntary were observed. However, both body and organ weights were significantly (p <0.05) altered (Table 3.47 and 4.48). Surprisingly, weight gains were

higher in the mice treated with the methanol sub-fraction of 2,000 mg/kg *bw* (6.7%) dose of *A. mannii* than in 5,000 mg/kg *bw* (4.4%) group. A similar characteristic was also reported by Sani *et al.,* (2009). Evidences of the likely impaired glomerular function were observed by the alteration of BUN and creatinine in the serum (Fig 3.50a and b). However, a reduction in serum level of blood urea could indicate that the methanol sub-fraction may be tolerable to the body as observed with other medicinal plant extracts such as *Bridelia ferruginea* (Kolawole and Sunmonu, 2010). It was assumed therefore that, the kidney was able to clear the waste products from the blood. The elevation of AST and ALT in the methanol sub-fraction treated mice may be an indication of temporary liver dysfunction. These increased levels were possibly due to the leakage of these enzymes from the liver cytosol or other organs into the blood stream. The results concords with the observation of Singh *et al.*, (2001), that the administration of some medicinal plant extracts resulted in the elevated levels of AST and ALT in the animal serum. On the other hand, the severe elevation of bilirubin by 50% or 75% in the treated groups was probably the result of the excess destruction of hemoglobin or that the liver was not actively treating the haemoglobin it is receiving (Audullu and Varardycheryalu, 2001; Tietz, 2000). The histopathological results however did not show any damage to the cells (Fig 3.50c - e), which lead to the conclusion that the abnormality observed in AST, ALT and total bilirubin may be from some unknown factors, probably some other phytochemical substances (Pereira *et al.,* 2010). These were characterized by a reduction in the animal's body and organ weights (Jahn and Gunzel, 1997; Huo *et al.,* 2003). The significant ($p<0.05$) increase in body weight, however was probably due to the effect of the fraction on anti-diuretic hormone resulting in fluid retention similar to other

medicinal plants (Goyal *et al.,* 2003). It was possible that, since the traditional herbalists understood polydipsia and polyuria as the symptoms of DM (Abo *et al.*, 2008), this characteristic (fluid retention) was exploited for reducing the craving for water (taste) and polyuria, while it enhanced insulin secretion for the metabolic utilization of the blood glucose.

4.5.5 Isolation, purification and structural elucidation of the methanolic fraction of *A. acuminata* and *A. mannii*

The study of the both the Thai and Nigerian hypoglycemic medicinal plants, which comprises the screening of the aqueous crude extracts to the sub-fractions and other hypoglycemic activities has shown that the methanolic sub-fractions of the two plants gave the highest efficiency in diabetic mice. They were observed to have good free radical scavenging activity, in the Thai plants (more), and non-toxic to the mice on a short term basis. Some chemical constituents and other medicinal properties, such as anti-inflammatory, analgesic, anti-HIV, anti-cholesterolemic and antibacterial for *A. acuminata* have previously been reported by Hemamalini *et al.,* (2010); Yoshida et al., (2010); Rimando *et al.,* (2005); Rimando *et al.,* (1994) and Akpata and Akinrimisi (1977). *A. mannii* have also been reported to have anti-inflammatory, analgesic and anti-microbial properties along side known compounds by Tsopmo *et al.,* (2009); Sani *et al.*, (2009) and Musa *et al.,* (2009). All the above mentioned properties of the two medicinal plants are advantageous to the diabetic patient because they are susceptible to one or more of these symptoms.

Considering the scanty scientific information on these two medicinal plants, it is very interesting to find out that they both possess very distinctive medicinal

properties as mentioned above. However, it is worthy of note that, though several species of *A. acuminata* exist both in Thailand and Nigeria, a sample of the Nigerian (*Anogeissus leiocarpus*) equivalent has shown very promising medicinal properties (Table 3.3.3; 3.3.4; 3.4.3; 3.4.4 and Fig 3.18; 3.19; 3.20; 3.21). The *A. mannii* being a seasonal plant in Nigeria was not easily sourced and neither was a closely related species found as medicine in Northern Thailand (NPRDC database). The *A. mannii* showed a negligible free radical scavenging activity in this study but previous study attributed strong anti-inflammatory activity with high doses (Musa *et al.*, 2009). Though both methanolic fractions were selected, they had similar difficulties with regards to their isolation, purification and structure elucidation because of their polar nature. These observations were made with similarities in their NMR spectral analyzes hence the need for further purification.

4.5.5.1 The purified and elucidated compound from *A. acuminata*

The compound identified as **Castalagin** ($C_{41}H_{26}0_{26}$) or **1, 2, 3, 5-nonahydroxytriphenoyl-4, 6-hexahydroxydiphenoyl-glucose** was isolated from the methanolic sub-fraction of *A. acuminata* (Appendix E). The identification of other pure compounds was still in progress at the time of this report. Castalagin belongs to the family of ellagitannins. Some members of this compound are known to be water soluble, as such they can be found in foods, edible fruits and medicinal plants. Castalagins are closest chemical relations to castlins, vescalin, vescalagins and stachyurins. The ellagitannins are plant phenols whose building blocks are gallic acid moieties esterified to D-glucose core. The ellagitannins are a sub-group of the secondary metabolites of plants called tannins. These groups of secondary metabolites

are unique from other phenolic compounds of other plants such as lignans and lignins because of their ability to precipitate and bind alkaloids, gelatin and mostly proteins (Okuda, 1999). These polyphenols are hydrolysable by water and have molecular weights between 500 and 300 (Haslam and Cai, 1994). The tannins are generally classified into two groups namely the "condensed tannins" and the "hydrolysable tannins", on the basis of their distinctive structures. The structural units of the condensed tannins are made up of flavan -3-ol units (Hergert, 1988), glucose and gallic acid (3, 4, 5-trihydroxybenzoic acid) which serves as the building block of the hydrolysable tannins (Haslam and Cai, 1994). The hydrolysable tannins which are considered to be derived from β-1, 2, 3, 4, 6 – pentagalloylglucose (PGG 2) are in fact, esters of gallic acid and glucose (Lei, 2002). It is the cleavage of the ester bonds under mild condition which renders these groups of tannins hydrolysable. As a result of the modification of the galloyl groups on the glucose core, the hydrolysable tannins are further divided into gallotannin and ellagitannins (Lei, 2002). The ellagitannins are reclassified into several groups namely; monomeric ellagitannins, with only single glucose core, examples of which are strictinin, tellimagradin I and monomeric ellagitannins with acyclic aromatic glycosides such as vescalagin and Castalagin. These aromatic glycosides have 4, 6 – HHDP coupling and a unique flavogalloyl group which are composed of three galloyl groups linked together with carbon – carbon bonds. These are the most predominant ellagitannins found in oaks (Viriot *et al.*, 1994). They are known to constitute about 10%w/w in oaks or heartwood. This property of the wood renders it important both physiologically and economically (Lei, 2002). Several medicinal properties have been attributed to ellagitanins from anti-microbial (Taguri *et al.,* 2004), antioxidant and antiviral (Yoshida *et al.,* 2007), anti

tumor and anti HIV (Okuda *et al.,* 1989), antihypertensive (Cheng *et al.,* 1993), suppress human colon carcinoma cells (Fridrich *et al.*, 2008) and it was reported to have very strong inhibitory activity against lipid peroxidation and free radical scavenging activity compared to tannins (Fridrich *et al.,* 2008). The castalagins are particularly known to have strong antimicrobial property (Shuaibu *et al.*, 2008). Ellagitannins from other plant species have previously been reported to have hypoglycemic activity (Klein *et al.*, 2007 and Maria *et al.,* 2010).

Castalagin are also known as glucosidic tannins, C – glucosidic tannins have a carbon - carbon linkage between an aromatic residue and the carbon 1 of the glucose chain. The castalagin is a 33 beta – isomer of vescalagin, which was first reported as extract of oak wood in the aging process of wine and it can be transformed by chemical reaction into acutissimin A (Puech *et al.,* 1999). This polyphenol has been reported to be 250 times more effective than the anticancer drug Etoposide in stopping the growth of cancerous tumors (Kuo *et al.*, 2009).

The significant hypoglycemic activity exhibited by the castalagin in this study, opens a new area in the pharmacological application of the drug as a suitable agent for the treatment of diabetes mellitus for the first time. The various medicinal properties which the entire family of this compound are attributed to have are all ailments which the diabetic patients are susceptible to. The most important of these properties are the fact that Castalagin is closely related to acutissimin A, which not only have antioxidant, antibacterial, anticancer activity but also but is known to be more potent than etoposide, a known pharmaceutical agent for cancer treatment with anti-HIV activity. The hypoglycemic effect observed from this known compound for the first

time, should elicit further investigation on the full potentials of Castalagin on in diabetes mellitus and related diseases as well as anti-cancer studies.

The hypoglycemic effect exhibited by Castalagin when the first dose 0.32 ug/kg was administered to the mice was 2.45% reduction while an increase in the dose to 1.6 ug/kg bw produced no hypoglycemic response. However, the 4 ug/kg bw dose gave 65.06% FBG suppression. This effect is comparable to the insulin effect but is significantly higher than the glibenclamide effect. Though Castalagin has never been mentioned for the treatment of diabetes mellitus, its mother compound tannins present in tea have been reported to be an efficient insulin enhancer (Anderson and Polansky, 2002). Later studies have also reported the role of tannins in efficiently controlling diabetes mellitus whether used as drugs or in foods (Nyunai *et al.*, 2009; Klein *et al.*, 2007; Broadhurst *et al.,* 2000; Iwu, 1983). Therefore, the new role of castalagin as a hypoglycemic agent agrees with the previous findings. There was no abnormal behaviour exhibited by the mice during the trials. Earlier speculation about the possible mechanism of the drug action was by insulin secretion from the pancreas, stimulated by the K^+ channel closure and voltage dependent Ca^{2+} channel opening. In the testing of the pure compound, it appeared that the lower doses (0.32 and 1.6 ug/kg bw) were negligible to exert insulin response as compared to the 4 ug/kg bw, which was 100 ng/0.5 ml, that gave a sharp reduction noticed from the second hour post treatment.

4.5.5.2 The purified and elucidated compound from *A. mannii*

The structural elucidation of the isolated compound from the methanolic sub-fraction of *A. mannii* on the basis of the interpretation of ^{1}H- and ^{13}C-NMR and MS

data (Gao *et al.,* 2003), and comparative references estimation and verified by web NMR predictor (2011), gave a probably new compound called **3, 23, 28 Trihydroxy-12-oleanen-3-O-(β-Dglucopyranosyl-(1,6)-β-D-glucopyranosyl(1,6)-β-D-xylopyranosyl)-28-O-β-D-glucopyranosyl-(1,6)-β-D-glucopyranoside (Manosrin).** The estimation of the pure compound was identified by comparison with the corresponding compounds in the following literatures with similar compounds: **1.** Pentacyclic triterpene esters, 3β, 23, 28-trihydroxy-12-oleanane-23-caffeate (Yun *et al.,* 1998). **2.** Longispinogenin 3-*O*-β-D-glucopyranoside, (Ye *et al.,* 2000). **3.** sitakisoside XI – XX, 3-*O*-β-D-xylopyranosyl (1-6)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (Yoshikawa *et al.*, 1997), **4.** Platycoside H, 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-2β, 3β, 16α, 23-tetrahyxyolenane-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl-(1-4)-α-L-rhamnopyranosyl-(1-2)-α-L-arabinopyranoside (Fu *et al.*, 2006), The molecular formula of the compound was determined by high resolution MS data. The molecular formula of the compound is $\mathbf{C_{59}H_{94}O_{29}}$. In the total ^{13}C NMR, 59 signals were detected and the ^{13}C NMR and DEPT experiment indicated **6** - C, **30** - CH, **17** - CH_2 and **6** - CH_3 (Yun *et al.*, 1998). Hence the conclusion of the aglycone structure as '3β, 23, 28-trihydroxy-12-oleanene' (Yun *et al.,* 1998), which is $\mathbf{C_{30}H_{48}O_3}$. Comparing with the ^{13}C NMR data of the compound 7-3-4 is very similar in the aglycone part with slight dissimilarities due to difference in the arrangement of the sugar moieties observed in compound 7-3-4. Five sugar moieties were detected in the compound with reference to 2, 3 and 4, which were estimated to be four hexose ($\mathbf{C_{24}H_{41}O_{21}}$) and one pentose ($\mathbf{C_5H_9O_5}$) giving a total of $\mathbf{C_{29}H_{50}O_{26}}$, accordingly (Yoshikawa *et al.*, 1997). The fraction was obtained as a clear brownish film. Furthermore, a D absolute

configuration of these sugar residues was assumed, consistent with the stereochemistry of naturally occurring monosaccharides. A comparison of the ^{13}C-NMR spectral data with those of the sugar moieties of saponin in *Gymnema sylvestre* (Ye *et al.,* 2000) indicated a glycosylation shift at the C-3 and C-28 positions while C-23 had no shift, leading to the conclusion that the glucosidic residue was attached to the C-3 and C-28 position of the compound. The ^{1}H- and ^{13}C-NMR spectra of the compound exhibited five sugars at the 1-6 linkages in the δ 105.7, 105.3, 105.5, 104.8 and 104.8, respectively (Table 3.4.10). This estimation agrees in totality with the formula obtained from the MS data (Appendix F). Hence the name of Fr. 7-3-4 was deduced as "3, 23, 28–Trihydroxy-12-oleanen-3-*O*-(β-D-glucopyranosyl-(1,6)-β-D-glucopyranosyl-(1,6)-β-D-xylopyranosyl)-28-*O*-β-D-glucopyranosyl-(1,6)-β-D-glucopyranoside" (Manosrin), a triterpene saponin glycoside. The specific positions of the other individual Carbon spectra observed in the sugar moieties are still inconclusive namely: CH-78.2, CH-78.0, CH-77.9, CH-77.8, CH-77.7, CH-77.6, CH-76.9, CH-76.7, CH-75.6, CH-75.1, CH-75.9, CH-74.8, CH-71.6, CH-71.5, CH-71.3 and CH-71.1(some carbon atoms overlapping).

The triterpene saponin belongs to the family of saponin compounds. The chemical building blocks of triterpene saponins are four or five ring configuration of 30 carbons with oxygen atoms attached. Triterpenes are synthesized from five carbon isoprenoid units which create a steroidal structure such as cholesterol ($C_{27}H_{46}O$). They are mostly found in plants saponin glycosides which indicate the linkages of various sugar moieties to the triterpene unit (aglycone). The sugar moieties which could be either hexose or pentose can be easily detached from the triterpene in the mammalian gut by intestinal microflora. Thus, they are easily inserted into the cell membranes

and modified in its molecular arrangement and affect the membrane fluidity. As pharmaceutical agents, several triterpene saponins have been observed to possess enormous biological activities, for example, triterpene saponin such as phytosterol have been observed to decrease the absorption of cholesterol from the gastrointestinal tract and enhance its excretion (Moreau *et al.*, 2002), inhibit the action of enzymes responsible for the synthesis of cholesterol (Carr and Jesch, 2006; Craig, 1999). Vast majority of triterpene saponins have been reported to enhance the modulation of the mammalian immune system (Helal and Melzig, 2011; Sun *et al.*, 2010). Other effects pharmaceutical effects reported are anti-inflammatory and improved blood circulation (Kwak *et al.,* 2003; Gao *et al.,* 2003; Navarro *et al.*, 2001; Just *et al.,* 1998), antimicrobial and hypocholesterolemic (Karimi *et al.,* 2011; Iorizzi *et al.,* 2002), antihelmitic (Maghraby *et al.,* 2010), anti snake venom (Viana *et al.,* 2004), antihypertensive (Somova *et al.*, 2003), anti-carcinogenic effect (Oskoueia *et al.,* 2011; Feng *et al.*, 2011; Ryu et al., 2010; Alessiae *et al.*, 2009; and Hanausek *et al.*, 2001), hypolipidaemic (Morikawa *et al.*, 2008) and hypoglycemic (Xi *et al.*, 2010; Li *et al.,* 2010; Morikawa, *et al.*, 2008; Ha *et al.*, 2006; Yoshikawa *et al.*, 2001; Lee *et al.*, 2000; Kako *et al.*, 1997). Several triterpene saponins were previously isolated and found to be hypoglycemic among which Senegins II – IV is desmethoxy senegin from the rhizome of *Polygala senega var latifolia* (Kako *et al.,* 1997). Yoshikawa and Matsuda, (2000) attributed the inhibition of intestinal α-amylase to the hypoglycemic activity of triterpene saponin. They attributed the hypoglycemic effect of the triterpene saponin to the 3-O-glucoronide moiety and the 28-carbonyl group of the oleanolic acid glycoside as responsible for the exertion of the effect. Other characteristics observed with triterpene saponin was the suppression of gastric

emptying by stimulation of the release of dopamine to act through the dopamine 2 receptor which stimulates the release of prostaglandis (Yoshikawa and Matsuda, 2000). The oleanolic acid constitutes the core of the discovered triterpene saponin. The oleanolic acid (saponins) have been reported to possess strong wound healing activity (Gustavo *et al.*, 2006) which is one of the vital needs for diabetic patients. The elucidation of this new compound (Manosrin) is only one of few compounds that have been isolated from *A. mannii* as a minor novel 1,7-naphthyridine alkaloid with an unprecedented skeleton, named anisopusine (1), was previously isolated while four other known compounds namely: 5α-hydroxy-lup-20(29)-en-3β-yl eicosanoate (2), [6]-gingerdione (3), [6]-dehydrogingerdione (4), and ferulic acid (5) were also isolated from the plant (Tsopmo *et al.*, 2009). In an attempt to treat diabetes mellitus permanently, several plants were reported to yield triterpene saponins as active principles which were observed to possess potent fasting blood glucose reduction (FBG) selected on the basis of their traditional usage as was *A. mannii*, among these medicinal plants are Mormodica charantia (Hague *et al.,* 2011), *Glycyrrhiza glabra* (Duwiejua and Zeitlin, 1993), *Panax ginseng* (Shibata, 2001), *Platycodon Radix* and *Bellis perennis* (Morikawa *et al.,* 2008). Thus the addition of *A. mannii* to the scientific group of hypoglycemic medicinal plants should trigger more research into other possible compounds that may be isolated from the plant with other biological characteristics. The compound manosrin showed a 45.15% FBG suppression in mice with the highest effect observed at the 4 h when administered with **3.2** ug/kg bw. Though the concentration used to archive this was very high, the main aim of this research had been archived. However, a higher increase in the dosage of the pure compound **32** ug/kg bw administered to other groups of diabetic mice lead to a further

suppression of the FBG to 67.97% and a further increase in dosage **320** ug/kg bw lowered the hypoglycemic effect to 29.03% with hyperactivity observed in the two groups of mice for reasons not well understood. Though, earlier results from the biological effect of the *A. mannii* sub-fraction lead to the speculation that the mechanism of action of the active principle may have enhanced insulin activity (Thiazolidinedione action) as a way of FBG reduction in the diabetic mice (Fig 3.46), the effect of the pure compound could be said to suggest alternative mechanism in addition to that which was earlier speculated, because the lowering of the FBG suppression may have been exerted as a result of the effect of the excess dose of the compound leading to a negative feedback inhibition of the available amount of insulin present in the blood stream. This implied that, the suppression of FBG in the diabetic mice may also be as a result of the action of the pure compound on the beta cells of the pancreas (sulfonylurea action), leading to hyper-secretion of insulin and a possible negative feedback inhibition leading to the observed reduction in hypoglycemia effect. There are reports which implicated triterpene saponins for gastric emptying and the stimulation and release of dopamine to act on dopamine 2 receptors (Yoshikawa and Matsuda, 2000) which may have been responsible for the hyperactivity observed in the mice groups as the expectations of the dopamine levels are supposed to be reduced as observed by Ishida *et al.*, (1997).