CHAPTER 5
SUMMARY AND CONCLUSION

Diabetes mellitus (DM) is a metabolic disorder of multiple aetiologies characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. DM is grossly reflected by profound changes in protein metabolism and by a negative nitrogen balance and loss of nitrogen from most organs. Increased urea nitrogen production in DM may be accounted for by enhanced catabolism of both liver and plasma proteins. Management of DM without any side effects is still a challenge to the medical system. There is an increasing demand by patients to use natural products with antidiabetic activity, because insulin and oral hypoglycaemic drugs have undesirable side effects. Medicinal plants are a good source of natural antioxidants believed to exert their effect by reducing the formation of the final active metabolite of the drug-induced systems or by scavenging the reactive molecular species to prevent their reaching a target site. In order identify the herbal based plant drug, the present study was focused on pharmacognostical, isolation, purification, characterization of antidiabetic compounds and evaluation of antidiabetic activity of rhizome extracts of Costus igneus in streptozotocin induced diabetic rats.

5.1 Pharmcognosy

Microscopic analysis and qualitative parameters are carried out on plant samples in order to establish appropriate data that can be used in identifying crude drugs particularly those supplied in powder form. They are standard pharmacognostic parameters that can be used to differentiate closely related plant species or varieties with similar constituents or pharmacological activities.
The Costaceae plants were identified to have different morphological and anatomical characters of leaf, rhizome and root. Among them Costus igneus has a characteristic anatomy that could be used to distinguish it from other members of Costaceae. Costus igneus studied, however, appear to be a homogenous entity united by series of leaf, rhizome and root anatomical characters, including epidermal cells, mesophyll cells, the sclerenchyma cells, vascular bundle, cortex, xylem, phloem, starch grains, stelar bundle. In addition rhizome of Costus igneus consist of large well shaped vascular bundle, cortex and other chemical compound storage part then leaf and root.

The proximate values shows that the protein content is relatively low but it can contribute to the formation of hormones which control a variety of body functions such as growth, repair and maintenance of body protein. The relatively high carbohydrate content can be used as energy source and also it is necessary in the digestion and assimilation of other foods. The moisture and ash content is useful in assessing the quality of grading the plant and also gives an idea of the amount of minerals present in the samples. This investigation suggest that various parts of Costus igneus can be differentiated by characteristic anatomy and proximate analysis that could be used to distinguish it from other members of Costaceae.

Fluorescence studies of powder with various reagents revealed the presence of green fluorescence with concentrated HCl and NaOH, under UV and day light. The plant powder were treated with various chemicals such as concentrated sulphuric acid, aqueous ferric chloride solution, iodine solution, ammonia solution, aqueous potassium hydroxide solution (5%) for preliminary chemical analysis of Costus igneus indicates that the presence of steroids, tannin, starch, and anthraquinone. Based on analysis the primary metabolite such as protein, carbohydrate, starch and secondary metabolite such as steroid, tannin, anthraquinone were found to be higher in rhizome due to the presence of larger chemical storage parts like vascular bundles and cortex in rhizome.
5.2 Phytochemistry

Fresh leaves, stems and rhizomes from *Costus igneus* were extracted with different solvents such as petroleum ether, hexane, ethanol, methanol and water. Among them the ethanol extract contain most of the compounds such as tannins, phlobatannins, saponin, flavonoids, terpenoids and cardiac glycosides compared to other extracts. Quantitative phytochemical analysis revealed that saponin, flavonoids were high concentration present in rhizome than leaf and stem.

Present study focused on isolation, purification and characterization of bioactive compounds from *Costus igneus*. The flavonoid and sapogenin compounds were isolated, purified, quantified and characterized. HPTLC analysis not only revealed that sapogenin extract consisted of diosgenin, betasitosterol and other sterol, but also had higher levels in rhizome of *Costus igneus* than leaf and stem. Similarly the concentration of quercetin and kaempferol are also higher in rhizome compared to leaf and stem. Saponins and flavonoids were also observed in high concentration of rhizome of this plant. These phytochemicals exhibit various pharmacological and biochemical actions when ingested by animals.

Chemical constituents identified by GC-MS in the essential oils of stems, leaves and rhizomes of *Costus igneus* were studied. The major constituents identified in the leaf of *Costus igneus* were hexadecanoic acid (19.53%), 9,12-octadecadienoic acid, ethyl ester (6.58%), tetradecanoic acid (5.22%), ethyl oleate (4.13%), oleic acid (21.60%), octadecanoic acid (6.48%), 1,2-benzenedicarboxylic acid, diisooctyl ester (18.44%), and squalene (8.02%). The rhizome contained hexadecanoic acid (12.70%), 9,12- octadecadienoic acid, ethyl ester (6.84%), tetradecanoic acid (1.48%), oleic acid (16.63%), octadecanoic acid (5.58%) and 1,2-benzenedicarboxylic acid, diisooctyl ester (49.61%) as important constituents; whereas n-hexadecanoic acid (35.29%), 1,6-octadiene,5,7-dimethyl-,(R) (11.76%) and di-n-octyl phthalate (52.94%) were the main compounds in the essential oil of stem. The essential oils of stems, leaves and rhizomes of the medicinal plant *Costus igneus* contained hexadecanoic acid (palmitic acid) as the major constituent.
5.3 Pharmacology

This study has been undertaken to evaluate the activities of crude ethanolic, sapogenin extracts and isolated diosgenin from *Costus igneus* rhizome on, glucose tolerance test, antihyperglycemic, hypolipidemic, carbohydrate metabolic enzyme (glucokinase, glucose-6-phosphatase, and fructose-1, 6-bisphosphatase in liver), creatinine, urea, uric acid, protein (total protein, albumin, globulin), hepatoprotective enzyme (aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in plasma and liver) and antioxidative enzymes (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Gpx), reduced glutathione content (GSH), total sulfhydryl groups (TSH), lipid peroxides (LPO) in liver, kidney and pancreas) in streptozotocin (STZ) induced diabetic rats. Glucose tolerance test studies indicate reduction of blood glucose level to 32%, 27%, 24% in rats that were administered *CiREE* (200mg/kg), *SECiR* (30mg/kg) and isolated diosgenin (10mg/kg) respectively and maximum effect was recorded at the 120min after administration. The *CiREE* at a dose of 100, 200mg/kg, *SECiR* at a dose of 20, 30mg/kg, isolated diosgenin at a dose of 5,10mg/kg were orally administered as a single dose per day to diabetes induced rats for a period of 30 days. The results showed that gluconeogenic enzymes (glucose-6-phosphatase and fructose-1, 6-bisphosphatase), AST, ALT, ALP, LPO, LDL, total cholesterol, triglyceride, urea, uric acid were found to be significantly (p<0.05) increased, whereas glycolytic enzyme glucokinase, SOD, CAT, GPx TSH, HDL, total protein, albumin and globulin levels were significantly (p<0.05) decreased in the diabetic rats. Oral administration of *CiREE, SECiR*, isolated diosgenin and reference drug (commercial diosgenin and glibenclamide) to diabetic rats for 30 days significantly (P<0.05) reversed their values to normal (section 4.3.3.1). In diabetic rats, the destruction of the liver, kidney and pancreas architectures, cytoplasmic vacuolation, nuclei of many cells revealed clear signs of necrosis, leucocytic infiltration, fibrosis, inflammation in central vein and blood vessels, the portal veins appeared congested with blood with fibrosis after treatment remarkable improvement in histological structure of liver, kidney and pancreas sections of diabetic rats (sections 4.3.3.2 and 4.3.3.3).
Quercetin, kaempferol, betasitosterol and diosgenin are the bioactive compounds were isolated from *Costus igneus* rhizomes were discussed. The result indicates that, in the presence of all this bioactive compounds exhibits the hypoglycaemic, hypolipidimic, antioxidant activity through the recovery of oxidative stress in liver, pancreas, kidney of diabetic rat and stimulated glycolytic enzyme and controlled gluconeogenesis in diabetic animals.

In conclusion glucose lowering activity observed in the diabetic rats may be due to the stimulation of the β- cells of the pancreatic islets, and 200mg/ kg/day of *CiREE*, 30mg/kg/day of *SECiR*, 10mg/kg/day of isolated diosgenin were exhibited better antidiabetic activity by enhancing the peripheral utilization of glucose by correcting the impaired liver and kidney glycolysis, glucogenesis (increased glycogen in liver) and by limiting its gluconeogenic formation. Histopathological and Electron Microscopical studies clearly indicate that the number of beta cells and number of insulin granules in beta cells are increased in STZ induced diabetic treated rats. *Costus igneus* has exerted a rapid protective effect against lipid peroxidation by scavenging free radicals and elevating both nonenzymic and enzymic antioxidants and thus alleviating the adverse complications of diabetes mellitus. In addition, *Costus igneus* could influence protein, lipid metabolism and marker enzymes in STZ-induced diabetic rats. This effect may be due to the presence of steroidal sapogenin such as diosgenin, betasitosterol, flavonoids such as quercetin, kaempferol and other constituents presence in the rhizome, which could act synergistically or independently in enhancing the activity of glycolytic, glucogenisis and gluconeogenic enzymes.

The present study warrants further research to isolate and characterise the potent molecules for diabetic mellitus and its lipid associated complication. To further study the impact of *CiREE*, *SECiR* and isolated diosgenin in the gene expression associated with carbohydrate, protein and lipid metabolism in normal and STZ induced diabetic rats, the present study clear form the basics for understanding the mode of action of *Costus igneus* extracts in STZ induced diabetic rats.