CHAPTER 1
GENERAL INTRODUCTION

1.1 INTRODUCTION

The use of herbal medicine is now widespread for the treatment of various diseases and disorders, it is redundant (Schulz et al., 2001). The use of pharmaceuticals has led to unforeseen side effects such as genetic alterations, biomagnifications and even death. Unforeseen side effects often appear after a drug has been on the market for years and is taken by many. Drug testing does not find these effects, as the number of patients in trials is not generally high enough. Also, trials are controlled by the company that wants the medicine approved, they are slanted to find efficacy and safety (Nicholas et al., 2008).

On the other hand, the use of herbal medicines has several advantages. One advantage is its wide availability and simple in preparation. Plants can contain sugars, minerals, proteins and other chemicals that interact with the active chemical in a variety of ways viz. they may concentrate or intensify its effect, they may make it easier to digest or absorb, or they may lessen its harsh or toxic side effects (Jellin et al., 2002). Most herbs can be used as medicine by making decoctions. Traditional prescriptions generally include extracts and concentrated single active compound from plants (Shaw, 1998). Supporters of traditional herbal medicine feel that medicine is most effective in its natural state which contains all the active ingredients rather than the processed synthetic drug.

World Health Organisation (WHO) defines Traditional medicine is the sum total of the knowledge, skills, practices based on the theories, beliefs, and experiences indigenous to different cultures, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness (WHO, 1999).
In recent years, there has been renewed interest in the treatment against different diseases using herbal drugs, as they are generally non-toxic and World Health Organization has recommended its effectiveness rather than the precarious modern drugs. Plant derivatives with hypoglycemic properties have been used in folk medicine and traditional healing systems around the world (Yeh et al., 2003) from ancient time. Despite, the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem to people (Ravi et al., 2005). Medicinal plants used to treat hypoglycemic and hyperglycemic conditions are of considerable interest to ethnobotanical community as the plants contain valuable medicinal properties in its different parts.

In traditional medicine, diabetes mellitus is treated with diet, physical exercise and medicinal plants. Even though, more than 1200 plants were used in the control of diabetes mellitus, approximately 30% of the antidiabetic plants were pharmacologically and chemically investigated (Alarcon et al., 2002). On the other hand, potential hypoglycemic agents have also been detected in more than 100 plants which were used for antidiabetic therapy. Traditional treatments may provide valuable clues for the development of new oral hypoglycemic agents and simple dietary adjuncts. More than 100 medicinal plants were mentioned in the Indian system of medicines including folk medicines for the management of diabetes, which are effective either separately or in combinations (Kar et al., 2003). As per the ethnobotanical literature on traditional phytotherapy of Indian medicinal plants, the species like Asparagus racemosus, Butea monosperma, Catharanthus roseus, Coccinia indica, Gymnema sylvestre, Syzygium cumini and Momordica charantia are consistently used by the tribal communities for the treatment of diabetes (Rana et al., 1999) as well as in modern medicine.

1.2 DIABETES MELLITUS

1.2.1 Motivation and overview of diabetic research

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia. The World Health Organization (WHO) warns that the deaths due to diabetes will increase all over the world by 80% in some regions, over the next ten years.
Among these, India host to the largest diabetes population in the world with an estimated 35 million people, amounting to 8% of the adult population. WHO also predicts that the diabetes currently affects almost two hundred million people worldwide. International Diabetes Federation estimates that this figure will increase to 333 million people by 2025. Only 5% of the diabetes in the world is type 1 (IDDM). The remaining 95% is type 2 (NIDDM).

1.2.2 Diabetes epidemic in India

The first national study on the prevalence of type 2 diabetes in India was done between 1972 and 1975 by the Indian Council of Medical Research (ICMR, New Delhi) (Ahuja, 1979). Screening was done in about 35,000 individuals above 14 year of age, using 50 g glucose load. Capillary blood glucose level >170 mg/dl was used to diagnose diabetes. The prevalence was 2.1 % in urban population and 1.5% in the rural population while in those above 40 year of age, the prevalence was 5% in urban and 2.8% in rural areas. Subsequent studies showed a rising trend in the prevalence of diabetes across different parts of India. In 1988, a study done in a small township in south India reported a prevalence of 5% (Ramachandran et al., 1988). This study also revealed that the prevalence in the southern part of India to be higher-13.5 % in Chennai, 12.4 % in Bangalore and 16.6 % Hyderabad; compared to eastern India (Kolkata), 11.7 %; northern India (New Delhi), 11.6 %; and western India (Mumbai) 9.3 % (Mohan et al., 2007).

1.2.3 Definition of diabetes

Diabetes mellitus is a syndrome characterised by disordered metabolism and abnormally high blood sugar (hyperglycemia) resulting from low levels of the hormone insulin with or without abnormal resistance to insulin’s effects.

1.2.4 Types of Diabetic mellitus

The World Health Organisation recognises three main forms of diabetes: Type 1, Type 2 and gestational diabetes (occurring during pregnancy), which have different causes and population distributions. Type 1 is usually due to autoimmune destruction of the pancreatic beta cells. Type 2 is characterised by insulin resistance in target tissues. This causes a need or abnormally high amounts of
insulin and diabetes develops when the beta cells cannot meet its demand (Ahmed et al., 1989).

1.2.5 Diabetic complication

Diabetes can cause many complications. Acute complications (hypoglycemia, ketoacidosis or non ketotic hyperosmolar coma) may occur if the disease is not adequately controlled. Serious long term complications include cardio vascular disease, chronic renal failure, retinal damage, nerve damage, and micro vascular damage, which may cause erecting dysfunction and poor healing (Bondy et al., 1949).

1.2.6 Carbohydrate metabolism

Insulin is the principle hormone that regulates uptake of glucose into most of the cells from the blood (primarily muscle and fat cells, but not central nervous system cells). Deficiency of insulin or the insensitivity of its receptor plays a central role in all forms of diabetes mellitus (Czech, 1977). Much of the carbohydrate in food is converted within few hours to the monosaccharide glucose, the principle carbohydrate found in blood. Insulin is released into the blood by beta cells in the pancreas in response to rising levels of blood glucose (eg. after a meal). Insulin enables most body cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules or for storage. Insulin is also the principle control signal for conversion of glucose to glycogen for internal storage in liver and muscle cells (Czech, 1976). Reduced glucose levels result both in the reduced release of insulin from beta cells and in the reverse conversion of glycogen to glucose when glucose levels falls, although only glucose thus recovered by the liver re-enters the blood stream as muscle cells lack the necessary export mechanism (Chakravathy et al., 1981).

Higher insulin levels increase many anabolic processes such as cell growth and duplication, protein synthesis, and fat storage. Insulin is a principle signal in converting many of the bi-directional processes of metabolism is catabolic to an anabolic direction and vice versa. If the amount of insulin available is insufficient, body cells can not utilise the glucose properly or not stored appropriately in the
liver and muscles (Josiah, 1979). The net effect is persistent high levels of blood glucose, poor protein synthesis and other metabolic rearrangements, such as acidosis.

1.2.7 Lipid metabolism

One major role of insulin is to stimulate the storage of food energy following the consumption of a meal. This energy storage is in the form of glycogen in hepatocytes and skeletal muscle. Additionally, insulin stimulates hepatocytes to synthesis triglycerides and storage of triglycerides in adipose tissue. In opposition to increased adipocyte storage of triglycerides is insulin-mediated inhibition of lipolysis (Suzuki, 1990). In uncontrolled noninsulin dependent diabetic mellitus there is a rapid mobilisation of triglycerides leading to increased levels of plasma free fatty acids. The free fatty acids are taken up by numerous tissue (however, not the brain) and metabolised to provide energy. Free fatty acids are also taken up by the liver.

Normally, the levels of malonyl-CoA are high in the presence of insulin (McGarry et al., 1978). These high levels of malonyl-CoA inhibit carnitine palmitoyl transfersase I, the enzyme required for the transport of fatty acyl–CoA into the mitochondria were they are subject to oxidation for energy production (McGarry and Foster, 1980). Thus, in the absence of insulin malonyl-CoA levels fall and transport of fatty acyl–CoA’s into the mitochondria increases. Mitochondrial oxidation of fatty acids generates acetyl-CoA, which can be further oxidised in the TCA cycle. However, in hepatocytes the majority of the acetyl-CoA, is not oxidised by the TCA cycle but it is metabolised into the ketone bodies, aceto acetate and β-hydroxy butyrate. These ketone bodies leave the liver and were used for energy production by the brain, heart and skeletal muscle (Suzuki, 1990).

In diabetic mellitus the increased availability of free fatty acids and ketone bodies exacerbates the reduced utilisation of glucose further the ensuing hyperglycemia. Production of ketone bodies, in excess of the organism’s ability to utilise them leads to ketoacidosis can be easily diagnosed by smelling the breath. The plasma triglycerides are acted upon by lipoprotein lipase (LPL) to store in
adipocytes. The activity of LPL requires insulin and its absence resulted in hypertriglyceridemia (Kameshwara et al., 2003).

1.2.8 Protein metabolism

Insulin regulates expression of many genes, either positively or negatively that then affect overall metabolism. Insulin has a global effect on protein metabolism-increasing the rate of protein synthesis and decreasing the rate of protein degradation. Thus, insulin deficiency will lead to increased catabolism of protein (Fluckey, 1996). The increased rate of proteolysis leads to elevated concentrations in plasma amino acids. These amino acids serve as precursors for hepatic and renal gluconeogenesis. In liver, the increased gluconeogenesis further contributes to the hyperglycemia seen in noninsulin dependent diabetic mellitus (Karuayaka et al., 1984).

As mentioned earlier there are three types of diabetes i.e., Type 1, Type 2 and gestational diabetes. This study focuses on Type 2 diabetes and its control by treating with the Costus igneus extracts.

1.2.9 Type 2 Diabetes Mellitus

1.2.9.1 Definition

Type 2 diabetes is a chronic, lifelong disease that is due to insulin resistance or reduced insulin sensitivity, combined with reduced insulin secretion. Insulin is a hormone released by the pancreas in response to the increased glucose level in the blood. The abnormality is reduced insulin sensitivity, characterised by reduced level of insulin in the blood.

1.2.9.2 Signs and symptoms

The symptoms of diabetes are polyuria (frequent urination), polydipsia (increased thirst and consequent increased fluid intake) and polyphagia (increased appetite). In Type 2 diabetes the symptoms develop slowly and may be subtle or completely absent.
1.2.9.3 Treatment and its disadvantage

Type 2 diabetes can be cured by one type of gastric bypass surgery in 80-100 percent of severely obese patients. The pattern of secretion of gastrointestinal hormones is changed by the bypass and removal of the duodenum and proximal jejunum, which together form the upper part of the small intestine. The main drawback of this surgery is its expensiveness. Many commercial tablets are available for controlling the Type 2 diabetes mellitus, it may cause side effects or high cost. Hence, in this study the rhizome extract of *Costus igneus*, which has the ability to revitalize the beta cells of the Islets of langerhans and activate insulin production and also to activate the inactive forms of the insulin present. This activity is due to the sapogenin and flavonol diglycoside contents in the rhizome extract of *Costus igneus*.

1.3 Description of *Costus igneus*

The large, smooth, dark green leaves of this tropical evergreen plant have light purple undersides and are spirally arranged around stems, forming attractive, arching clumps, arising from underground root stocks (Fig. 1.1). Plants reach to about two feet tall, with the tallest stems falling over and lying on the ground. Beautiful, 1.5 inch diameter, orange flowers are produced in the warm months, appearing on cone-like heads at the tip of branches.

**Plant Details**

Scientific Name: *Costus igneus*, Common name(s): Spiral-Flag, Fiery costus

Family: Costaceae, Plant type: perennial, Soil tolerances: occasionally wet, slightly alkaline; Drought tolerance: moderate.
Fig. 1.1 *Costus igneus* (N.E.Br) grown in jute bags under shady net at PMU nursery (90 days old)
1.4 REVIEW OF LITERATURE

1.4.1 Pharmacognostical studies

Costaceae, a pantropical family of monocots consisting of approximately 120 species, is one of the most easily recognizable groups within the order Zingiberales. It is distinguished from other families including bananas (Musaceae) and gingers (Zingiberaceae) by its well-developed and sometimes branched aerial shoots that have a characteristic spiral monostichous (one-sided) phyllotaxy (Kirchoff and Rutishauser, 1990). The floral structure of Costaceae is also unique
within the Zingiberales in that only a single fertile stamen develops while the remaining five infertile stamens fuse together to form a large, petaloid labellum that dominates the floral display (Troll, 1928; Kirchoff, 1988).

Costaceae, predominantly Neotropical in species diversity, is sister to the mainly old world family Zingiberaceae. Nakai (1941) cited the non-aromatic vegetative body, spirally arranged leaves and anther appendages to separate the Costaceae from the Zingiberaceae. Anatomically, the monophyly of Costaceae and its separation from Zingiberaceae is supported by multicellular, uniseriate, unbranched hairs with the base never sunken as found in the Zingiberaceae (Tomlinson, 1956). In addition, the hypodermis is always well developed with one or more layers below each surface in contrast to the Zingiberaceae, which either lacks a hypodermis or has only a single hypodermal layer below each surface. The leaf axis in Costaceae has one poorly developed system of air canals that are situated adaxially and often absent at certain levels, whereas the petiole of Zingiberaceae has a well-developed abaxial arc of air canals. The silica bodies of Costaceae are found in all examined species to be stellate or druse-like in shape, whereas the silica bodies found in the Zingiberaceae are frequently (but not universally) present and are spherical in shape. In Costus, the silica bodies never occur in the epidermis but rather are adjacent to the vascular bundles whether in the lamina, in which they are least common or in the petiole, sheath, stem or rhizome (Tomlinson, 1956). Finally, the Costaceae completely lack oil cells, which are abundant in all parts of the Zingiberaceae. These characters indicate the uniqueness of the Costaceae lineage and provide morphological and anatomical synapomorphies for the family. Additionally, the well-developed, sometimes-branched aerial stem, the distinctive monostichous spiral phyllotaxy and the fusion of five staminodes into a labellum (Kirchoff, 1988) versus three staminodes in Zingiberaceae, form the suite of synapomorphies most commonly sited as defining Costaceae. Maas (1977) divided the neotropical species of Costus subgenus Costus into two sections, Costus and Ornithophilus, Ornithophilus being hummingbird-pollinated. The first section is characterized as having a labellum with a short, rather broad tube, and a distinct, exposed limb; its colour varies from white to yellow, but the lateral lobes are often striped with red to
purple. The bracts of this group are typically green. The second section is comprised of species with a small, tubular labellum of yellow, orange, or reddish colour: the bracts are of the same colour, or rarely green. Edeoga and Okoli (1997) state that the anatomy of lamina, rhizome and root of three taxa of *Costus* was investigated with a view of establishing interrelationships among the previous confused species of the genus. Similarly the hexarch, nonarch and polyarch vascular bundle of the root of *C. afer*, *C. lucanusianus* and *Costus* hybrid respectively, are all distinguishable attributes of these taxa that are important in systematic.

The literature survey reveals that there is no work done for morphological, anatomical, proximate and fluorescent analysis in *Costus igneus* leaf, rhizome and root. It is essential to standardize plant anatomy and morphological study of *Costus igneus*. Therefore an attempt has been made to pharmagnostical study of *Costus igneus* in first chapter which is useful for the plant standardization.

### 1.4.2 Phytochemical Analysis

Medicinal plants continue to be a major source of drugs and natural products on the basis of their therapeutics (Lown, 1993) in virtually all cultures (Anwannil and Atta, 2006). The plants possess potent bioactive compounds capable of preventing and treating most oxidative related diseases, diabetic, cancer (Dahanuka *et al.*, 2000) and have often been used in folkloric medicine (Wang *et al.*, 2007). In developing countries, the use of medicinal plants in the treatment of infectious disease and diabetic are rife and reasons include the high cost of effective drugs (Okeke *et al.*, 1999). However, potential indigenous plants exploited for medicinal purposes have to undergo basic phytochemical screening and bioassay as first step towards the ultimate development of drugs (Odebiyi and Sofowora, 1998).

#### 1.4.2.1 Plant constituents - Saponin and Sapogenin compounds

The saponins are naturally occurring surface-active glycosides. They are mainly produced by plants and also by lower marine animals and some bacteria (Riguera, 1997; Yoshiki *et al.*, 1998). Saponins consist of a sugar moiety usually
containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be triterpenoid or steroid in nature. The aglycone may contain one or more unsaturated C–C bonds. The oligosaccharide chain is normally attached at the C₃ position (monodesmosidic), but many saponins have an additional sugar moiety at the C₂₆ or C₂₈ position (bidesmosidic). The great complexity of saponin structure arises from the variability of the aglycone structure, the nature of the side chains and the position of attachment of these moieties on the aglycone. Experiments demonstrating the physiological, immunological and pharmacological properties of saponins have provoked considerable clinical interest in these substances.

Saponins and their aglycone such as 3-methoxy-lanost-9(11)-ene, diosgenin, yamogenin, 3-O-Dglucopyranosyl-24(S)-ethyl-22E-dihydrocholesterol, 3-O-D-glucopyranosyl-24(R)-ethyl-22E-dihydrocholesterol, dioscin were isolated from butanolic extract of *Brachiaria decumbens* by Viviane et al., (2002).

The 95 % ethanolic extract of the rhizome of *Costus lacerus* yielded β-sitosterol-β-D-glucoside, prosallogenin A, dioscin and gracilin. Partial hydrolysis of gracillin gave the two saponin such as 3-O-{β-D-glucopyranosi-(1-3)-β-D-gluco pyranosyl}diosgenin (Uma Prawat, 1989). A spirostanol glycoside, (25R)-spirost-5-en-3β-ol 3-O-α-L-rhamnopyranosyl-(1→2)-O-[β-D-gluco pyranosyl-(1→4)]-β-D-glucopyranoside were isolated from wild yam (*Dioscorea villosa*) extract with reversed-phase preparative HPLC. The structure was identified by LC-MS, ¹H- and ¹³C-NMR (Chao Chin Hu, 2007).

New steroidal saponin like diosgenin-3-O-d-glucopyranosiduronic acid methyl ester, diosgenin, diosgenin 3-O-d-glucopyranosiduronic acid, diosgenin 3-O-α-rhamnopyranosyl-(152)-glucopyranosiduronic acid, diosgenin-3-O-α-L-l-rhamnopyranosyl-(152)-glucuroniduronic acid methyl ester from *Solanum lyratum* were isolated and characterized by Li et al., (2006). The structural diversity of spirostanol saponins lies mainly in their sugar moieties. In general, the sugar moieties are oligosaccharides with 2-4 kinds of sugar units, e.g. D-glucose, D-galactose, D-xylose and L-rhamnose. The first sugar attached to diosgenin
usually is D-glucose or D-galactose, while D-xylose and L-rhamnose generally occur at the terminal positions. (Chuan Chun Zho et al., 2003)

The sarsasapogenin was extracted from rhizomes of *Anemarrhena asphodeloides* and applied to inhibit HepG2 human hepatoma cells. Flow cytometry analysis showed that sarsasapogenin-induced cell apoptosis was through arrest of cell cycle in G2/M phase (Wenna Bao, 2007). Sterols, triterpenes, volatiles, polar and other constituents in aerial parts of *Carthamus lanatus* were analyzed by gas chromatography-mass spectrometry. Sitosterol and stigmasterol were the most abundant of 10 sterols identified in the sterol fraction. Taraxasterol, α- and amyrine prevailed in the triterpene fraction. Volatiles, sterols and a fraction of the dichloromethane extract showed strong cytotoxicity (Maya, 2003).

### 1.4.2.2 Flavonoids

Flavonoids are a group of polyphenolic compounds of low molecular weight that present a common benzo-γ-pyrone structure. They are categorized into various subclasses including flavones, flavonols, flavanones, isoflavonones, anthocyanidins, and catechins. Flavonoids are often hydroxylated in positions 3, 5, 7, 3', 4' and/or 5'. Frequently, one or more of these hydroxyl groups are methylated, acetylated, prenylated or sulphated. In plants, flavonoids are often present as *O*- or *C*- glycosides; *O*- bonding occurs in flavonoids far more frequently than *C* bonding. The *O*-glycosides have sugar substituents bound to a hydroxyl group of the aglycone, usually located at positions 3 or 7, whereas the *C*-glycosides have sugar groups bound to a carbon of the aglycone, usually 6-C or 8-C. The most common carbohydrates are rhamnose, glucose, galactose and arabinose. Flavonoid-diglycosides are also frequently found. Two very common disaccharides contain glucose and rhamnose, 1→6 linked in neohesperidose and 1→2 linked in rutinose. The sugars are often further substituted by acyl residues such as malonate and acetate (Davies and Stumpf, 1981). Flavonol, Kaempferol, Fisetin and Naringenin are the four flavonoid compounds extracted, separated and detected by spectroscopy methods (*1*HNMR, *13*C-NMR, Mass and IR) from *Chrysanthemum parthenium* (Shafaghat and Salimi, 2008). Two flavones were
isolated from the leaves of *Marchantia convoluta* by silica gel column and preparative high performance liquid chromatography (PHPLC). These compounds were identified through spectral analysis (IR, UV, $^1$H-NMR, $^{13}$C-NMR, MS) as 5-hydroxyl-7-methoxyl-2-methylchromone and a flavone glycoside, Apigenin-7-O-$\beta$-D-glucuronide (Xiao, 2006). Phytochemical investigation of the whole plants of *Crotalaria sessiliflora* led to the isolation of four flavonoids. The structures of these compounds were identified as 2', 4', 5, 7-tetrahydroxyisoflavone, 2',4',7-trihydroxyisoflavone, 4',7-dihydroxyflavone, and isovitexin using spectroscopic analysis (Hun, 2004).

Antioxidant flavonoids have been isolated from the flower of *Rhododendron yedoense*. One new flavonoid and three known flavonoids, quercetin-5-O-$\beta$-D-glucopyranoside, quercetin and quercitrin, were isolated from the butanol and ethyl acetate extracts of the plant. The new flavonoid was identified as myricitrin-5-methyl ether (Santosh, 2008). Three flavonoids, apigenin, quercetin and rutin, have been isolated from waste tobacco leaves and their identities have been confirmed by UV-visible, $^1$H-NMR and $^{13}$C-NMR spectroscopy. The amount of rutin present in the before and after fermentation of tobacco leaves and also in waste tobacco leaves, has been determined by HPLC as 1.5, 0.5 and 0.6%, respectively (Fatemeh *et al.*, 2006).

Kaempferol 3,7-O-L-dirhamnoside and quercetin 3,7-O-L-dirhamnoside (II) were isolated from the leaves of *Tilia argentea* (Tiliaceae). The structure elucidation of the isolated compounds was performed by spectroscopic techniques (Gulnur *et al.*, 2004). The major flavanols in the litchi fruit pericarp are reported to be procyanidin B4, procyanidin B2 and epicatechin, while cyanindin-3-rutinside, cyanidin-3-glucoside, quercetin-3-rutinoside and quercetin-3-glucoside are identified as the important anthocyanins (Jiangrong and Yueming, 2007)

1.4.3 Pharmacological study

1.4.3.1 Antidiabetic potential of some important herbal plants

Repeated daily oral administrations of aqueous extract of *Orthosiphon stamineus* (0.5 g/kg) for 14 days, the extract significantly reduced plasma glucose concentration in diabetic rats at days $^{7}$th and $^{14}$th (Sriplang *et al.*, 2007). By the
end of the study, plasma triglyceride concentration was lower in the extract-treated diabetic rats than untreated ones. Furthermore, plasma HDL-cholesterol concentration was significantly increased in treated diabetic rats. Wild *Panax ginseng* leaf extract (WGLE) supplementation was found involved in suppressing a sudden increase in blood glucose levels and a consequent decrease in thiobarbituric acid reactive substances (TBARS) levels in diabetic rats. TBARS levels in the liver, kidney and spleen of WGLE-fed diabetic groups were also significantly lower than in the control diabetic group indicating that oral administration of WGLE effectively suppresses lipid peroxidation that occurs in the organs of diabetic rats. Antioxidant activities of WGLE supplementation further extend in suppressing activities of antioxidant related enzymes, such as glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD), in the organs of diabetic rats (Chang *et al.*, 2005). The treatment of diabetic rats with oral administration of *Phlomis anisodonta* ethanolic (PAE) at doses of 100, 200 and 400 mg kg\(^{-1}\) for 10 days resulted in a significant reduction in fasting blood glucose, and an increase in serum insulin levels in comparison with diabetic control group. Hepatic ferric reducing ability of plasma (FRAP) increased and lipid hydroproxide (LPO) in diabetic rats decreased after treatment by PAE at doses of 200 mg/kg and 400mg/kg. PAE-treated diabetic rats at three doses indicated a significant increase in hepatic SOD, CAT and GPx activities (Parisa *et al.*, 2007).

Oral administration of the aqueous extract of *Retama raetam* (RR) (20 mg/kg) on lipid metabolism in normal and streptozotocin-induced diabetic rats was studied by Maghrani *et al.*, 2004. In normal rats, the aqueous extract of RR induced a significant decrease of the plasma triglycerides concentrations one week after repeated oral administration. This reduction was maintained two weeks after once daily repeated oral administration. The *Rhus chirindensis* stem-bark aqueous extract (RCE, 50–800 mg/kg) also significantly inhibited fresh egg albumin-induced acute inflammation and caused dose-related, significant hypoglycaemia in normal and diabetic rats (Ojewole, 2007).
Smallantus sonchifolius 10% decoction produced a significant decrease in plasma glucose levels in normal rats when administered by intraperitoneal injection or gastric tube. In a glucose tolerance test, a single administration of 10% yacon decoction lowered the plasma glucose levels in normal rats (Manuel, 2001). The aqueous extract of Vitis vinifera was fractionated through successive solvent extractions and the acute effect of different doses of its subfractions, 25 mg/kg for ethylacetate fraction, 80 mg/kg for n-butanol fraction and 375 mg/kg for remaining aqueous fraction were investigated using normal, glucose-hyperglycaemic and streptozotocin-induced diabetic rats. Blood glucose levels were measured according to the glucose oxidase method (Nilufer, 2006). After the oral administration of water and ethanolic extracts of Helichrysum plicatum at doses of 500 mg/kg body weight prepared from the capitulums of plant, blood glucose levels were reduced into normal level (Mustafa et al., 2007).

The treatment with Hygrophila auriculata extract (HAEt) significantly increased the glutathione (GSH), glutathione peroxidase (GPx), glutathione S-transferase (GST) and catalase (CAT) in the drug-treated group, comparable to the control group. HAEt and glibenclamide-treated rats also showed decreased lipid peroxidation associated with increased activity of superoxide dismutase (SOD) and catalase (Vijayakumar et al., 2006). Plasma glucose concentrations in STZ-diabetic mice were reduced by the administration of extracts of Momordica charantia (MC) (200 mg/kg), Eugenia jambolana (EJ) (200 mg/kg), Mucuna pruriens (MP) (200 mg/kg) and Tinospora cordifolia (TC) by 24.4%, 20.84%, 7.45% and 9.07%, respectively (P<0.005 for MC, EJ, MP and P<0.05 for TC). Urine volume was significantly higher (P<0.005) in diabetic controls and MC, EJ, MP and TC treatment prevented polyuria (P<0.001, 0.0001, 0.01 and 0.001, respectively). After 10 days of STZ administration urinary albumin levels (UAE) were over 6 fold higher in diabetic controls as compared to normal controls. Treatment with MC, EJ, MP and TC significantly prevented the rise in UAE levels from day 0 to 40 in comparison to diabetic controls (P<0.0001, 0.0001, 0.05, 0.05, respectively) (Grover, 2001). Oral administration of Ichnocarpus frutescens leaf methanolic extract (IFLMExt) daily for 45 days to diabetic rats significantly reduced the FPG (54.5%) to near normal. After 7 days of
streptozotocin administration plasma insulin decreased in diabetic controls compared to normal controls. Treatment with IFLMExt significantly prevented the decrease in plasma insulin levels from day 0 to 45 in comparison to diabetic controls. Histopathological examination showed that IFLMExt extract protected the pancreatic tissue from streptozotocin-induced damage enormously (Subash et al., 2008).

Oral inhabitation of 50 mg per day of lyophilised Eugenia jambolana fruit-pulp extract for 41 days showed no observable difference in body weight, food or water intake, urine volume, glycaemia, urinary urea and glucose, hepatic glycogen, or on serum levels of total cholesterol, HDL cholesterol or triglycerides. No change was observed in the masses of epididymal or retroperitoneal adipose tissue or of soleus or extensor digitorum longus muscles. This lack of any apparent effect on the diabetes may be attributable to the regional ecosystem where the fruit was collected and/or to the severity of the induced diabetes (Pepato et al., 2005). After a acute (single dose) or chronic (15 daily repeated administration) oral treatments, the aqueous Lepidium sativum extract (LS) (20 mg/kg) produced a significant decrease on blood glucose levels in STZ diabetic rats. The blood glucose levels were normalised 2 weeks after daily repeated oral administration of aqueous LS extract (20 mg/kg) (Eddouks et al., 2005). Oral application of water extracts of Malmea depressa at doses of 40 and 80 mg/kg, ethanolic (112 mg/kg) and butanolic (80 mg/kg) extracts significantly lowered the plasma glucose levels in diabetic rats within three hours. Glibenclamide and metformin were used as references and showed similar hypoglycemic effects like the extracts (Adolfo, 2005). Administration of the bark extract of Helicteres isora (100 and 200 mg/kg b.w.) for 21 days resulted in significant reduction in serum and tissue cholesterol, phospholipids, free fatty acids and triglycerides in STZ diabetic rats. In addition to that, significant ($p < 0.05$) decrease in high-density lipoprotein (HDL) whereas significant increase ($p < 0.05$) low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were observed in STZ diabetic rats, which were normalized after 21 days of bark extract treatment (Kumar and Murugesan, 2008). Supplementation of this aqueous extract of Tamarindus indica by gavage at the dose of 80 mg/0.5 ml distilled water/100 g body weight per day
in STZ-induced diabetic rat resulted a significant diminution of fasting blood sugar level after 7 and 14\textsuperscript{th} days. Moreover, this supplementation produced a significant elevation in liver and skeletal muscle glycogen content, activity of liver glucose-6-phosphate dehydrogenase in respect to diabetic group. Activities of liver glucose-6-phosphatase, liver and kidney glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities were decreased significantly in the aqueous extract supplemented group in respect to diabetic group (Maiti \textit{et al.}, 2004). 150 mg/kg and 300 mg/kg of extract daily for 14 days. At 300 mg/kg, the two extracts (\textit{Terminalia superba} and \textit{Canarium schweinfurthii}), significantly showed at least 67.1\% and 69.9\% reduction in blood glucose level, respectively, while insulin (three units) given subcutaneously and once daily, had 76.8\% reduction compared to diabetic untreated control rats (Kamtchouing \textit{et al.}, 2006).

Oral administration of water extracts of \textit{Tournefortia hirsutissima} at doses of 20 and 80 mg/kg, and butanolic extracts (8 and 80 mg/kg) significantly lowered the plasma glucose levels in diabetic rats within 3 h. Glibenclamide was used as reference and showed similar hypoglycaemic effect (Adolfo, 2007). Ethanolic extract of \textit{Zingiber officinale} (200 mg/kg) fed orally for 20 days produced, significantant hyperglycaemic effect ($P<0.01$) in diabetic rats. Further, the treatment also lowered serum total cholesterol, triglycerides and increased the HDL-cholesterol levels when compared with pathogenic diabetic rats ($P<0.01$). STZ-treatment also induced a statistically significant increase in liver and pancreas lipid peroxide levels ($P < 0.01$) as compared to normal healthy control rats (Uma \textit{et al.}, 2005). Oral application of water extracts at doses of 20 and 200 mg/kg and of butanol extracts of \textit{Acosmium panamense} at doses of 20 and 100 mg/kg significantly lowered the plasma glucose levels in diabetic rats within 3h. Glibenclamide was used as reference and showed similar hypoglycemic effect like the extracts (Adolfo and Helmut, 2004).

\textbf{1.4.3.2 Biological activity of saponin}

Saponins form a heterogeneous group of triterpene or steroid glycosides which occur in many hundreds of plant species (Tschesche and Wulff, 1973).
Many of these are staple items of the human diet (Oakenfull, 1981) and examples particularly rich in saponins are soya beans (*Glycine max*), chickpeas (*Cicer arietinum*), navy beans (*Phaseolus vulgaris*) and lucerne (*Medicago sativa*) (Fenwick and Oakenfull, 1983). Isolated saponins and foods containing saponins have been shown to lower plasma cholesterol concentrations in a number of animal species (Malinow et al., 1977) and it has been suggested that foods containing saponins could be important in formulating hypocholesterolaemic diets for human consumption (Potter et al., 1980). Thus the mechanism of the hypocholesterolaemic activity of saponins is of considerable interest. Saponins remain within the gastrointestinal tract (Birk, 1969). Some interact directly with cholesterol, producing an insoluble complex which prevents cholesterol absorption (Malinow et al., 1977). Others appear to affect cholesterol metabolism indirectly by interacting with bile acids and increased faecal excretion of bile acids was observed in response to feeding saponins of this type (Oakenfull et al., 1981). Bile acids thus diverted from the enterohepatic cycle would be replaced by hepatic synthesis from cholesterol (Heaton, 1972). *Polyscias filicifolia* saponins effectively reduced the carrageenan, formaldehyde induced edema and cotton pellet induced granuloma. The saponin extracts from these plants were found to be a potent inhibitor of hydroxyl, superoxide, peroxide and nitric oxide radicals in invitro studies (Madhu, 2010). Treatment of Type 2 diabetic mice with saponins of *Momordica Cymbalaria* (175mg/kg/30 days) and metformin (350mg/kg/30 days) produced a significant fall in blood glucose (p<0.001), cholesterol (p<0.001), triglycerides (p<0.001) and an increase in the serum insulin level (p<0.001). Pancreatic islets and beta cells showed an increase in numbers (Firdous, 2009).

Recent studies have found that diosgenin can be absorbed through the gut and plays an important role in the control of cholesterol metabolism (Roman et al., 1995). Other authors have reported that it has estrogenic effects (Aradhana et al., 1992) and antitumor activity (Moalic et al., 2001; Corbiere et al., 2003). Studies have also revealed that diosgenin produce changes in the lipoxygenase activity of human erythroleukemia cells and also responsible for morphological and biochemical changes in megakaryocyte cells (Beneytont et al., 1995; Nappe
et al., 1995). Furthermore, diosgenin was found to be the most effective cell death inducer compared to the other two plant steroids (hecogenin and tigogenin) in the human osteosarcoma 1547 cell line (Corbiere et al., 2003). Diosgenin is generally used as a starting material for partial synthesis of oral contraceptives, sex hormones, and other steroids (Zenk, 1978). The partial synthesis of steroids from plant-based precursors has been a boon because of the increasing demand for corticosteroids, contraceptives, sex hormones, and anabolic steroids (Hall and Walker, 1991).

1.4.3.3 Biological activity of Flavonoids

Flavonoids are phenolic phytochemicals that represent substantial constituents of the non energetic part of the human diet and are thought to promote optimal health, partly via their antioxidant effects in protecting cellular components against reactive oxygen species (ROS) (Poli and Parola, 1997). Quercetin (3,5,7,3,4-pentahydroxy flavon) is one of the most distributed flavonoids, semi essential food components, in certain species of plants (Manach et al, 1999). Earlier studies have shown that quercetin and other flavonoids have a broad range of pharmacological properties, including carcinostatic and antiviral activities, suppression of cell proliferation, modification of eicosanoid synthesis, protection of LDL from oxidation, prevention of platelet aggregation, stabilization of immune cells, and relaxation of cardiovascular smooth muscle (Formica and Regelson, 1995; Park, 2003). Laboratory studies show it may have anti-inflammatory, and it is being investigated for a wide range of potential health benefits (Laura et al., 2008; Mark et al., 2009) Quercetin has been shown to increase energy expenditure in rats, but only for short periods (fewer than 8 weeks) (Laura et al., 2008). Effects of quercetin on exercise tolerance in mice have been associated with increased mitochondrial biogenesis (Mark et al., 2009). Several studies reported that chronic oral treatment with quercetin reduces blood and restores endothelial function in hypertensive animal models (Ajay et al., 2005). In addition, quercetin has been reported to elicit blood glucose lowering effect (Vessal, 2003), to show vasodilator effect in isolated aortas (Roghani et al., 2005), to reduce the oxidative stress in experimental animal (Anjaneyulu, 2004).
With this background this study focuses on the most common disease called diabetes mellitus and its control by medicinal plant is an alternative treatment, because of less side effect and low cost. The plant species *Costus igneus* is a widely found weed in tea plantations of India. The decoction prepared from the dry leaves of this plant is used to alleviate the symptoms of diabetes in folk medicine. The objectives are as follows

**1.5 Objectives**

- Plant Standardization (Morphological, anatomical study of *Costus igneus*)
- Phytochemical analysis of *Costus igneus*.
- Evaluation of antidiabetic activity of *Costus igneus* crude extracts in animal model.
- Evaluation of antidiabetic activity of bioactive compound of *Costus igneus* in animal model

**1.6 Work plan**

To achieve the objective of the present study, the following Viz. pharmacognostical, phytochemical and pharmacological activity of *Costus igneus* are studied as follows.

**Chapter I**: General introduction and review of literature

**Chapter II**: Pharmacognosy: Morphological, anatomical and proximate analysis of leaf, root and rhizome of *Costus igneus*

**Chapter III**: Phytochemistry: Analytical study of *Costus igneus*

**Chapter IV**: Pharmacology: Antidiabetic study of *Costus igneus* rhizome in streptozotocin induced diabetic rats

**Chapter V**: Summary and conclusion