Chapter I

INTRODUCTION
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Costus pictus D. Don is commonly known as Spiral Ginger, Stepladder or Insulin Plant (Figure 1.1a and Figure 1.1b). The plant has its origin in Mexico and is a native of South and Central America. It is introduced recently in India and is now cultivated in gardens in South India, especially in the state of Kerala, as an Ornamental plant.

Costus pictus D. Don belongs to Monocotyledons. Its Order is Scitamineae, family is Zingiberaceae and genus is Costus. According to Engler, genus Costus is included in Zingiberaceae family. Main difference of genus Costus from other genera under Zingiberaceae family is that its aerial parts are not aromatic (Trease and Evans, 1987). Costus pictus is a perennial herb with Fleshy Rhizomes and Tuberous Roots. The plant grows up to the height of 2 meters in pots and up to 3 meters in fields.

Costus pictus has tropical looking wavy foliage. The stem is upright and green and may develop faint reddish vertical striations on maturity. No branching is observed in the plant. Stem bears leaves in open spiral form, enhancing its beauty (Figure 1.2a and Figure 1.2b). Bases of leaves form layers of sheath, which cover the stem (amplexicaul) but do not form pseudo stem. Macroscopically, leaves are Leathery to Coriaceous with firm, flexible Texture and are slightly Succulent. Leaves are Elliptical in shape, up to 20 cm in length and up to 7.5 cm in width, with Entire and Wavy Margin showing Parallel Venation diverging from prominent midrib with Acute to Acuminate Apex and Sub Sessile to Cuneate Base.

Costus pictus has green Inflorescence with short ligule and there is one Inflorescence per aerial shoot. Inflorescence is pedunculate with racemes of flowers or of 2--7-flowered monochasial cymes (Cincinnati); bracts of main axis enclosing or subtending flowers or Cincinnati. Costus pictus usually blooms at the top of the stem but occasionally produces basal bloom (Figures 1.3a and Figure 1.3b). The plant can be identified by its yellow flowers. Flowers have beautiful lip and red stripes on upper
portion of petals (Figure 1.4a and Figure 1.4b). Flowers do not produce aroma. Flowers are bisexual, bilaterally symmetric; Sepals and Petals differentiated, Sepals 3, connate, Petals connate, but Sepals free from Petals.

During the present study, Rhizomes were observed to develop as aerial parts at the base of mature Inflorescence and aerial plantlets developed from such Rhizomes (Figure 1.5a and Figure 1.5b). On full development, such plantlets were seen to get detached due to weight and took roots in the soil.

*Costus pictus* D. Don, besides being an ornamental plant, has become a potential source of herbal medicine for many ailments. There are indications that active compounds in *Costus pictus* display Anti-oxidant, Anti-microbial, Anti-inflammatory and Hepato-protective and Anti-diabetic effects. (Meléndez-Camargo M E *et al.*, 2006; Nandhakumar Jothivel *et al.*, 2007; Nadumane V K *et al.*, 2011; Majumdar M *et al.*, 2012). In the state of Kerala, fresh raw leaves of *Costus pictus* are eaten as a munching supplementary food for treatment of Diabetes (Jayasri M A *et al.*, 2009).

The plant kingdom has been tapped by traditional systems of medicine for the well-being of mankind. Plants belonging to almost all families are found to have been traditionally used as medicines (Trease and Evans, 1987). Before synthetic drugs came into widespread use, herbal medicines played an important role in human health. All major systems of medicine have employed plants in their pharmacopoeias even though their underlying philosophies are different. Practitioners trained in traditional systems of medicine use herbal medicines and take a holistic approach for treatment of ailments and promotion of health (Trease and Evans, 2002). Knowledge about herbal medicine has been handed down through generations and there are scriptures to support this claim.

Medicinal systems in the Indian subcontinent and in China have developed over thousands of years (Trease and Evans, 1987). Ayurveda is practiced in India since time immemorial. Like Ayurveda, Siddha system is one of the oldest systems of medicine in India. Siddha literature is in Tamil and it is practiced largely in Tamil
Figures 1.1a and 1.1b: *Costus pictus* D.Don
Figures 1.2a and 1.2b: Terminal Inflorescence of *Costus pictus* with Buds

Figures 1.3a and 1.3b: Flowers of *Costus pictus* displaying bright yellow color with beautiful red stripes and a yellow lip
Figures 1.4a and 1.4b: Open Spiral arrangement of Leaves

Figures 1.5a and 1.5b: Aerial Rhizomes developed at the base of Mature Inflorescence
speaking part of India and abroad. Greece is a place of origin for Unani system of medicine. The foundation of Unani system was laid by Hippocrates and it developed further by inducting the best from traditional medicines in Egypt, Syria, Iraq, Persia, India, China and other Middle East countries. In India, Unani System of Medicine was introduced by Arabs (Dept of AYUSH, 2013). The Oriental system of medicine flourished in China, Japan and Tibet. Homoeopathic medicines also involve a very wide range of herbal products (Trease and Evans, 1987). Even Western medicine originated from traditional systems of Mesopotamia and Egypt. In the African Continent, various tribes hold key to traditional system of medicine. Even if Africa is a vast store of medicinal plants, much of the knowledge on usage of plants is confined to the boundaries of tribes. The very existence of such aboriginal tribes is under threat now. Traditional wisdom regarding plant based remedies is now on the radar of the World Health Organization and in response to the appeal from the WHO, many studies on plant based drugs are conducted by various countries (WHO global survey, 2005).

In recent years herbal medicines and associated research has received a big impetus. International Trade in Traditional Medicines is now a possibility due to opening of World Economy. Opportunities at global market for indigenous products can be explored by fulfilling international standard and herbal products must be presented with International Quality.

On one hand there is immense unexplored potential of plants and on the other hand, many medicinal plants are facing the threat of extinction (WHO global survey, 2005). Loss of plant diversity around the world, destruction of natural habitats due to urbanization and demands of growing population and unsustainable harvesting practices are observed to be the causes of worry (WHO, IUCN & WWF, 1993). Cultivation of medicinal plants can be one of the solutions to the problem (Handa S S et al., 1996).

The World Health Organization (WHO) has published projections on global health and related risk and the study has brought out main causes responsible for deaths
globally to be; High Blood Pressure (13%), Tobacco use (9%), High Blood Glucose (6%), Physical Inactivity (6%), Overweight and Obesity (5%). These risks manifest themselves into Chronic Diseases such as Heart Disease, Diabetes and several types of Cancer and affect all countries cutting across all sections of population. In India, 32.7% of mortality in adults in the age group 30 to 70 was by Cardiovascular Diseases and Diabetes mellitus in the year 2008 (WHO, 2012).

Diabetes mellitus (DM) is a major cause of mortality, but gets underreported as a "cause of death". DM refers to a group of common Metabolic Disorders that share the phenotype of Hyperglycemia. The metabolic dysregulation associated with DM, causes secondary pathophysio logic changes in multiple organ systems like Heart, Kidney and Liver (Harrison, 2012). Exponential increase in the population affected by DM and associated disorders, has posed the greatest challenge for the world. WHO expert committee on diabetes has recommended that traditional medicinal herbs be further investigated (WHO, 2006).

There are several types of Glucose lowering drugs including Insulin Secretagogues (Sulfonylureas, Meglitinides), Insulin sensitizers (Biguanides, Metformin, Thiazolidinedioness), α-glucosidase inhibitors (Miglitol, Acarbose). But most glucose lowering drugs may have side effects such as severe Hypoglycemia, Lactic Acidosis, Idiosyncratic Liver cell injury, permanent Neurological Deficit, Digestive discomfort, Headache, Dizziness and even death (K D Tripathi, 2008; Baby Joseph et al., 2011).

Cumulative research over the years in plant based remedies has reported many plants to be effective for treatment of Diabetic Mellitus. An ethno botanical study of plants revealed that 14 plant species were used by traditional healers, herbalists and rural dwellers for treatment of diabetes mellitus in the Eastern Cape Province (P Erasto et al., 2005). Twenty one plants for their hypoglycaemic effects were reported in traditional remedies in Palestine (N Jaradat, 2005). In Morocco, several dozen botanical species are widely used for treatment of diabetes (Mohamed Bnouham et al., 2006). More than 400 plant species with hypoglycaemic activity have been reported in literature (Neelesh Malaviya et al., 2010; Singh Ayodhya et al., 2010).
Indian plants which are very effective and the most studied in relation to diabetes and the associated complications are *Allium cepa*, *Syzygium cumini*, *Mormodica charantia*, *Ocimum sanctum*, *Tinospora cordifolia*, *Pterocarpus marsupium* etc. (Elder C, 2004; Stabitha T Isaac et al., 2011).

Many conventional drugs for treatment of DM have been derived from Prototype Molecules in medicinal plants. Metformin exemplifies an efficacious oral glucose lowering agent. Its development was based on the use of *Galega officinalis* to treat diabetes as *Galega officinalis* is rich in Guanidines, the hypoglycemic component (Baby Joseph et al., 2011).

A wide range of plant derived principles belonging to different compounds have demonstrated bioactivity against Hyperglycemia (Rokeya Begum et al., 2008). In many instances, Hypoglycemic Natural Products are presented in a wide variety of Chemical Classes indicating that a variety of mechanisms must be involved in lowering of Blood Glucose levels. Some of these compounds may have therapeutic potential, while others may produce hypoglycemia, as side effects of their toxicity, which is also another important concern. Table 1.1 contains the Number of plant derived, medicinally active anti-diabetic compounds belonging to broad Chemical Classes of Secondary Metabolites (Rokeya Begum et al., 2008).

<table>
<thead>
<tr>
<th>S No</th>
<th>Chemical Class</th>
<th>Number of Active Compounds</th>
<th>S No</th>
<th>Chemical Class</th>
<th>Number of Active Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>38</td>
<td>10</td>
<td>Peptides and Amines</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates</td>
<td>66</td>
<td>11</td>
<td>Phenolics (simple)</td>
<td>04</td>
</tr>
<tr>
<td>3</td>
<td>Coumarins</td>
<td>01</td>
<td>12</td>
<td>Phenolpropanoid</td>
<td>01</td>
</tr>
<tr>
<td>4</td>
<td>C. glycosides</td>
<td>01</td>
<td>13</td>
<td>Steroids</td>
<td>07</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoids</td>
<td>07</td>
<td>14</td>
<td>Stilbenes</td>
<td>01</td>
</tr>
<tr>
<td>6</td>
<td>Glycopeptides</td>
<td>20</td>
<td>15</td>
<td>Terpenoids</td>
<td>17</td>
</tr>
<tr>
<td>7</td>
<td>Inorganic salts</td>
<td>03</td>
<td>16</td>
<td>Sulfur compounds</td>
<td>02</td>
</tr>
<tr>
<td>8</td>
<td>Iridoids</td>
<td>04</td>
<td>17</td>
<td>Vitamins</td>
<td>02</td>
</tr>
<tr>
<td>9</td>
<td>Lipids</td>
<td>06</td>
<td>18</td>
<td>Xanthenes</td>
<td>02</td>
</tr>
</tbody>
</table>

*Table 1.1:* Number of Active Compounds with Known Anti-Diabetic Activity Belonging to Broad Chemical Classes of Secondary Metabolites
When any plant is to be screened, studied and established as a potential source of a novel medicine, issues regarding standardization of various aspects have to be addressed. As a plant is tapped for developing its metabolites into a commercially viable drug, the Pharmaceutical Industry would require constant inflow of standardized raw material of assured quality with least variance and in large quantity. In practice, such large scale supply may not be possible because the plant may be difficult to cultivate, may have slow growth, may vary in chemical constituents due to environmental factors and due to variability found in wild variety or may even become endangered (WHO, IUCN & WWF, 1993; WHO, 1998; WHO global survey, 2005).

Plant Tissue Culture Technique has become an extremely dependable technique that meets all the expectations of Pharmaceutical Industry regarding consistency and sufficiency of plant based raw material (George and Sherrington, 1984). The current ability of modern Biotechnology to manipulate living organisms is the key and principal tool in the hands of researchers. Such manipulations with high level of complexity and precision make the biological processes controllable, predictable and precise.

Chemical Profiling of Pharmacetically important plant as well as Isolation and Identification of Phytoconstituents present therein, are other essential steps in drug development.

Fingerprinting of Phytoconstituents extracted from a specific part of a plant, establishes authenticity of raw material. Extraction procedure, Solvent used for extraction and the part of the plant used, determine the Chemical Profile of Phytoconstituents and the Fingerprints. For Identification of Phytoconstituents, the Phytoconstituents have to be isolated first. Fractionation of extract is achieved by one of the Chromatographic Techniques. Such Isolated fractions are subjected to Modern Techniques for Structure Elucidation.
To establish any plant material as a potential source of medicine for some ailment, a thorough investigation of Toxicity and associated side effects of the test material has to be undertaken. Various \textit{in vivo} methods are employed for testing Toxicity. It is also known as Safety testing and is always conducted for every test material before its Efficacy is tested (OECD Guideline No 420).

Once the test material is found to be non-toxic and safe for consumption, \textit{in vivo} study is employed to test Efficacy of the test material. Animals, mostly rodents, are the preferred models. Disease under study is induced in animals and then the animals are treated with test material. To evaluate Efficacy, various physical (e.g. Body Weight, Water consumption) and biochemical (e.g. Blood serum parameters) parameters are monitored during the study period.

It is an accepted fact that for Quality Control, basic tests must be undertaken and Good Manufacturing Practices must be followed while developing an herbal drug (WHO, 1996; WHO, 1998).

Aim of the present study was to find herbal remedy for the ever expanding killer disease, Diabetes mellitus. Even though a large number of plants are found to be effective against Diabetes, looking at the vast proportion and wide spread of diabetes affected population, there is always a scope for a new potent herbal drug. Hence, anti-diabetic and Hepatoprotective activity of \textit{Costus pictus} was the main focus of investigations during the present study.

\textit{Costus pictus} D. Don is a relatively new entrant in the research field in India and is currently being explored for its Phytoconstituents, Toxicity and Efficacy of potential drugs against various diseases. As \textit{Costus pictus} is not in use traditionally for long time, comprehensive investigation of the plant was undertaken during the course of the present study.

Plant Tissue Culture is the first step towards commercial utilization of the plant. There are no reports that Plant Tissue Culture was carried out for \textit{Costus pictus} D.
Don despite it being a potential source of drug against multiple ailments. Plant Tissue Culture of *Costus pictus* D. Don is a maiden effort reported in the present study.

It has been reported that anti-diabetic and Hepatoprotective activities are shown by Phytoconstituents belonging to many broad Chemical classes (Table1.1). The present study focused on Alkaloids, Flavonoids, Saponins and Triterpenoids Classes present in Methanolic Extract of dried leaves of *Costus pictus*. Fingerprinting of *Costus pictus* with respect to Alkaloids, Flavonoids, Saponins and Triterpenoids is carried out and exhaustive analysis of Chromatograms so obtained is done in order to study the plant comprehensively. Fingerprinting with respect to Alkaloids, Flavonoids, Saponins and Triterpenoids carried out in the present investigation is a maiden effort. Phytoconstituents from Chemical class ‘Triterpenoids’ were isolated using HPTLC technique. Such separated fractions were subjected to FTIR analysis.

Following the Chemical Analysis, the present study aimed at investigating the therapeutic potential of *Costus pictus*, as anti-diabetic and Hepatoprotective drug.

The present study is undertaken to test *in vivo* Acute Toxicity of test materials with Albino Swiss mice as animal model. Methanolic Extract of dried leaves is used as the test chemical for Toxicity study (OECD Guideline No 420, 2001). Toxic effects, if any, due to the Powder of dried leaves were compared with those due to Methanolic Extract of leaves. The study protocol was authenticated by Institutional Ethics Committee.

Medicinal herbalists firmly believe that there is synergy between the molecules effective as medicine and other molecules present in the plant. The presence of all constituents together, eliminates the side effects. When Hypoglycemic Natural Products are spoken about, they are presented in a wide variety of Chemical Classes indicating that a variety of mechanisms must be involved in lowering of Blood Glucose levels and influencing effects due to others (Trease and Evans, 2002).
Previously reported Toxicity and Efficacy studies on laboratory animals for a variety of ailments, on many plants including *Costus pictus* D. Don, have all been on plant extracts (Neveen Helmy *et al.*, 2007; Qi Xiang-Yang *et al.*, 2008; Fujii *et al.*, 2009; Bhanot Abhishek *et al.*, 2010; Paul S. *et al.*, 2011).

In order to test the Theory of Synergy put forth by medicinal herbalists, Powder of dried leaves is used as a test material in the present study as Methanolic Extract of leaves may not contain all the Phytoconstituents originally present in the leaves. The present study reports use of Powder of dried leaves as a test substance for the first time and it is a maiden effort.

The present investigation is designed to establish Efficacy of *Costus pictus* D. Don against diabetes using Powder of dried leaves and Methanolic Extract of dried leaves as test dosing materials. Efficacy study was carried out using Albino Wistar female Rats. Diabetes was induced in animals by injecting Streptozotocin. Predetermined doses of test substances were fed daily to diabetic animals for 14 days. Blood biochemical parameters were used as indicators for monitoring the trend towards normalcy from abnormal levels.

Physical parameters like body weight, food and water consumption were also recorded and analyzed which further supported the Efficacy of the test materials.

Comparative evaluation was made for Efficacy of the two test substances, Powder and Methanolic Extract of dried leaves by plotting appropriate graphs and using Statistical tools. Experiment was performed with different Dose Levels and dependence of Efficacy on Dose level was also analyzed.

It can thus be stated emphatically that the present study is a comprehensive investigation of many aspects of the plant, *Costus pictus* D. Don. The aims and objectives of the study are listed below:
• Develop efficient protocol for Rapid \textit{in vitro} Propagation of the plant
• Establish optimum protocol for Extraction of Phytoconstituents
• Obtain Chemical Fingerprinting with respect to four major Chemical Classes
• Isolate and investigate some fractions from profile of Triterpenoids from plant leaf extract
• Study \textit{in vivo} Toxicity of the plant leaves (in the form of Powder and Methanolic Extract)
• Study \textit{in vivo} Efficacy of the plant leaves (in the form of Powder and Methanolic Extract) as anti-diabetic and Hepatoprotective agents

It may be worth mentioning here that

• \textbf{Plant Tissue} Culture of \textit{Costus pictus} D. Don is reported for the first time
• Fingerprinting of the Methanolic Extract of leaves is reported as a maiden effort.
• Use of High Performance Thin Layer Chromatography by employing Analytical and \textbf{Preparative} TLC plates to separate Triterpenoids has not been reported earlier.
• Use of Powder of leaves of \textit{Costus pictus} D. Don as test material for Efficacy studies against diabetes is not reported earlier.
• Comparative evaluation of Efficacy of Powder and Methanolic Extract of dried leaves \textit{Costus pictus} D. Don is also a maiden effort.

It may hence be stated that the present study is undertaken to Micropropagate \textit{Costus pictus} D. Don and establish protocol for rapid propagation on large scale. Isolation of Bioactive Molecules from Methanolic Extract of Leaves and Study of Medicinal properties of leaves of \textit{Costus pictus} D. Don has been undertaken to establish \textit{Costus pictus} as a potential source of drug against Diabetes.