CHAPTER 1

INTRODUCTION

Medicinal plants play a key role in human health care from the ancient period. Plant medicines were regarded as highly important in the lives of our ancestors. Their dependence on the plants in their surroundings made them to acquire the knowledge about the medicinal properties of many plants by trial and error. They were also aware of the commercial value of these plants. Consequently, they became the custodians of knowledge of many useful as well as harmful plants, accumulated and enriched through generations and passed it from one generation to another, without any written document (John Kennedy, 2003). Most developing countries are endowed with vast resources of medicinal plants. These plants have been used over the millennia for human welfare in the promotion of health. This close relationship between man and the environment continues even today as large proportion of people in developing countries are still living in rural areas (Tuley De Silva, 1997).

Millions of people in the third world still use herbal medicines because they believe in them and regard them as their own system of medicine (Audichya et al., 1983; Austin and Bourne, 1992). Many higher plants are known to be the main source of drug therapy in traditional medicine (Farnsworth et al., 1985; Martin, 1995). It occupies an important place in the health care systems of developing countries. The World Health Organization (WHO) estimates that 80% of the people living in developing countries are almost completely dependent on the traditional medicine as therapeutic remedies for their primary health care needs.

India, the largest producer of medicinal herbs, is rightly called the “Botanical garden of the world”. In India traditional communities like tribal and rural populations are frequently using the crude extracts of local plants for medicinal and other purposes because it is cheaper, more accessible and blends readily into the people’s socio-cultural life than Orthodox medicine (Zakaria, 1991; Sofowora, 1993; Luoga et al., 2000; WHO, 2002).
Plants consist of number of biologically active chemical compounds which are formed during the plants normal metabolic processes. These chemicals are often referred to as “secondary metabolites” of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, polysaccharides, phenols, tannins, terpenes and terpenoids (Okwu, 2004). In addition to these substances, plants contain other chemical compounds, those can act as agents to prevent considerable side effects of the main active substances or to assist in the assimilation of the main substances. Plants have an almost limitless ability to synthesize aromatic substances, mainly secondary metabolites of which 12,000 have been isolated, the number estimated to be less than 10% of the total. Secondary metabolites are sought after because they are known to exhibit numerous biological activities that promote health effects (Mallikharjuna et al., 2007).

The exploitation of plants for their medicinal property has given rise to the discovery of many useful drugs. Plants still continue to be almost the exclusive source of drugs for a majority of the world’s population. Several herbal remedies are now being intensively used in therapy. Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind (Hamburger and Hostettman, 1991).

The use of medicinal plants as anti-inflammatory and anti-arthritic drugs is a common practice in India, although in most cases the active principles of the plants are unknown. Herbal medicine is the use of plants (herbs) to treat disease and enhance well-being. It is used to treat a range of disorders including anxiety, arthritis, depression, high blood pressure, insomnia, hormonal imbalances, migraines, skin problems such as eczema and other disorders. It can act on the body as powerful as pharmaceutical drugs. Herbal medicine aims to return the body to a state of natural balance, so that it can start healing itself. Different herbs act on different systems of the body (Anonymous, 2011).

Diabetes mellitus is a multifactorial disease (Scoppola et al., 2001). It is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism (Sharma, 1993). Diabetes, a chronic disease that has no cure, is a group of disorders as a result of high levels of blood glucose resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 1999).
In 2008, the American Diabetes Association (ADA) issued new diagnostic and classification criteria. The classification of diabetes mellitus includes four clinical classes based on the cause or mode of treatments: Type 1 diabetes or insulin-dependent diabetes mellitus (IDDM) or juvenile onset diabetes, results from \( \beta \)-cell destruction, usually leading to absolute insulin deficiency. In type 2 diabetes or non-insulin-dependent diabetes mellitus (NIDDM) or insulin resistance or adult onset diabetes, the pancreas produces insulin, but in insufficient quantity or body become insensitive to insulin. Gestational diabetes mellitus (GDM) develops during pregnancy due to hormonal changes and mostly disappears after delivery. Maturity Onset Diabetes of the Young (MODY) is a series of familial disorders characterized by early onset and mild hyperglycemia. Specific genetic defects have been identified on chromosomes 7, 12 and 20.

At least 382 million people worldwide in between the age group of 20-79 years suffered from diabetes in 2013. The number is expected to grow to 592 million by 2035. Each year another 7 million people develop diabetes (IDF, 2013).

The largest age group currently affected by diabetes is between 40-59 years. By 2030 this “record” is expected to move to the 60-79 age groups with some 196 million cases. By 2030 while most people with diabetes in developed countries will be aged 65 years or more, in developing countries the majority will be in the 45-64 year age bracket and are affected in their most productive years. Unlike in the West, where older persons are most affected, diabetes in Asian countries is disproportionately high in young to middle aged adults (Ramachandran et al., 2010).

Diabetes is one of the major causes of premature illness and death. It is a non-communicable disease, account for 60% of all deaths worldwide. The number of deaths attributable to diabetes in 2010 shows a 5.5% increase over the estimates for the year 2007. In developing countries, less than half of people with diabetes are diagnosed. Undiagnosed diabetes accounted for 85% in South Africa, 80% in Cameroon, 70% in Ghana and over 80% in Tanzania. Without timely diagnoses and adequate treatment, complications and morbidity due to diabetes would rise exponentially. It is the seventh leading cause of death in the United States (IDF, 2011).
The top 10 countries, in the numbers of sufferers are India, China, USA, Russia, Brazil, Germany, Pakistan, Japan, Indonesia and Mexico. More than 70% of the current cases of diabetes occur in low and middle income countries. The largest increase will take place in the regions dominated by developing economics (IDF, 2011).

India has been named the diabetic capital of the world, followed by China with 43.2 million. The total number of people in India with diabetes is around 50.8 million in 2010, rising to 87.0 million by 2030 (IDF, 2011). The global increase in the prevalence of diabetes is due to population growth, ageing, urbanization, food habits like consumption of food with processed carbohydrates, saturated fats and insufficient fiber rich whole foods along with physical inactivity and an increase of obesity (Chan et al., 2009). According to the WHO criteria, the prevalence of known diabetes was 5.6% and 2.7% among urban and rural areas, respectively (Mohan and Pradeepa, 2009). Moreover, the prevalence of diabetes was also found to be increasing rapidly in rural areas, as a result of the recent socio-economic transitions (Ramachandran and Snehalatha, 2009).

Some of the common diabetic complications like kidney failure, nerve damage, heart disease, stroke, non-traumatic lower limb amputations and blindness are associated with long term damage (Hungley Audrey, 1997). Dysfunction and failure of various organs, especially in eyes, kidneys, nerves, heart and blood vessels are due to chronic hyperglycemic condition (Franz and Ratner, 2003).

Free radicals and other reactive species are thought to play an important role in many human diseases. Radical scavenging activities are very important due to the deleterious role of free radicals in biological systems. Increased free radical formation in the body is known as “oxidative stress”. Oxygen free radicals and lipid peroxidation have been implicated in the pathogenesis of a number of diseases such as diabetes mellitus and atherosclerosis. It also plays a pivotal role in the development of diabetic complications both microvascular and macrovascular (Halliwell and Gutteridge, 1990).

Reactive oxygen species generated in the cells are scavenged by antioxidant enzymes. Diabetes also induces changes in the tissue content and activity of the antioxidant enzymes (Genet et al., 2002). Many secondary metabolites serve as sources of antioxidants and do scavenging activity (Ghasemi et al., 2009; Doss et al., 2010).
In modern medicine, no drug is available with satisfactory and effective therapy for the management of diabetes mellitus (Piedrola et al., 2001). Insulin therapy and oral hypoglycemic agents are available for treatment of diabetic patients. But they are unable to lower glucose concentration to within normal range and reinstate a normal pattern of glucose homeostasis permanently. Use of these therapies is restricted by their pharmacokinetic properties, secondary failure rates and accompanying serious side effects. People with diabetes may need to take these medicines for the rest of their lives (Bhargava and Singh, 1981). Hence there is a need to search for newer antidiabetic agents having high therapeutic efficacy with minimum side effects. This may be fulfilled by treating diabetes mellitus with plant derived antidiabetic agents.

Plant materials which are being used as traditional medicine to treat diabetes are considered to be one of the best sources for new drugs or a lead to make new drugs. Plant extracts or different folk plant preparations are being prescribed by the traditional practitioners and are accepted by the users for diabetes, like for any other disease, in many countries especially in third world countries (Nahar, 1993; Smith and Reynard, 1995).

Now-a-days, more than 800 plants are being used in different forms for hypoglycemic effects. All the claims of the practitioners or users are baseless. Therefore, a proper scientific evaluation and screening of plants by pharmacological tests followed by chemical investigations are necessary (Nahar, 1993; Smith and Reynard, 1995). So, scientific evaluation of the pharmacological effects viz., antidiabetic, hepatoprotective, antihyperlipidemic, antifertility and immunomodulatory activities of the herbal crude extracts can still be used as a logical research strategy in the search for new drugs.

In the traditional system of Ayurvedic treatment, medicines consist of plant products, either single or in combination with other products are considered to be less toxic and free from side effects compared to synthetic drugs (Ratnakar and Murthy, 1996). Eighteen percentages of all prescribed users take medications concurrently with herbal remedies and/or high dose vitamins (Gordon et al., 2005). The medicinal value of these plants lies in some chemical substances especially phytochemicals. Most of them seems to act directly on pancreas and stimulate insulin levels in blood. Some have extra pancreatic effect by acting directly on tissues like liver, muscle etc., and alter favourably,
the activities of the regulatory enzymes of glycolysis, gluconeogenesis and other pathways (Shukia et al., 2000).

Pietro Simone Filitto (2012) classified herbal medicines for diabetes into four categories according to their mode of action. Drugs acting like insulin, acting on insulin secreting β-cells, acting by modifying glucose utilization and miscellaneous mechanisms. Most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc., which are frequently implicated as having antidiabetic effects (Malviya et al., 2010).

Many herbal medicines, as single agent or in different combined oral formulations have been recommended for the treatment of diabetes mellitus. Extracts of drugs from plant sources such as *Allium sativum* (garlic), *Azadirachta indica* (neem), *Vinca rosea* (nayantara), *Gymnema sylvestre* (meshashringle), *Trigonella foenum-graecum* (fenugreek), *Momordica charantia* (bitter gourd), *Ficus benghalensis* (banyan), *Eugenia jambolana* (black berry), *Ocimum sanctum* Linn. (tulsi) and *Eclipta alba* (karisalaankanni) reported to possess antihyperglycemic activity in experimental animals (Shukia et al., 2000). Antidiabetic herbal therapy is less expensive and have oral route of administration when compared to insulin preparations (Ponnachan et al., 1993; Chattopadhyay, 1993).

The lesions in the pathophysiology of diabetes are multiple and therefore it would require more than a single drug agent to reverse all or majority of the aspects of the disease. Polyherbal therapy is considered as the preferred therapeutic approach in the management of diabetes mellitus which has multifactorial pathogenicity (Tiwari and Rao, 2002). In the traditional system of Indian medicinal plant formulations and several cases, combined extracts of plants are used as drug of choice rather than individual (Santhosh Kumari and Devi, 1993).

**Ethnobotanical claims of *Trichosanthes dioica* Roxb. and *Clitoria ternatea* L.**

*Trichosanthes dioica* Roxb. (Plate I) and *Clitoria ternatea* L. (Plate II) are being used as popular remedy for the treatment of diabetes mellitus in Ayurveda and Siddha medicine. In the present study, the above mentioned plant materials are used individually and in combination to evaluate the phytochemical profile, antidiabetic, antihyperlipidemic
and antioxidant effectiveness in STZ-induced diabetic rats and compared to the effect of standard drug glibenclamide.

Systematic position, plant identification and habitat, botanical description and ethnopharmacological uses of the above said two plants taken for the study are as follows:

(i) *Trichosanthes dioica* Roxb.

**Systematic position**

<table>
<thead>
<tr>
<th>Botanical Name</th>
<th>Trichosanthes dioica</th>
</tr>
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<tbody>
<tr>
<td>Common Name</td>
<td>Pointed Gourd, Parwal</td>
</tr>
<tr>
<td>Kingdom</td>
<td>Plantae</td>
</tr>
<tr>
<td>Division</td>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida</td>
</tr>
<tr>
<td>Order</td>
<td>Cucurbitales</td>
</tr>
<tr>
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<td>Cucurbitaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Trichosanthes</td>
</tr>
<tr>
<td>Species</td>
<td>dioica</td>
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<tr>
<td>Vernacular names</td>
<td>Patol (Tamil), Parval/Parwal (Hindi, Telugu, Malayalam, Kannada, Marathi), Wild snake gourd (English), Potol (Assamese, Oriya and Bengali), Chhachindra (Oriya), Patola (Sanskrit).</td>
</tr>
</tbody>
</table>

**Plant identification and habitat**

*Trichosanthes dioica* Roxb. is a dioecious (male and female) vine (creeper) perennial herb distributed in tropical Asia, Polynesia and Australia. It is found in wild in the plains of North India from Punjab to Assam (Chakravarthy, 1982). It is also extensively cultivated as a vegetable crop in the Eastern part of India, particularly in Orissa, West Bengal, Assam, Bihar, Uttar Pradesh Tripura and also in Tamil Nadu (Haines, 1961; Hooker, 1973; Kanjilal, 1997). In India, it is often called green potato. Young and unripe fruit is valued by Europeans next to potatoes and brinjals.

**Botanical description**

The fruits are green with white or no stripes. Size can vary from small and round to thick and long 2-6 inches (5-15 cm), oblong or nearly spherical acute and smooth (Kirtikar and Basu, 1956). The leaves are 7.5 cm long, ovate-oblong, cordate, acute, sinuate denate, rigid and rough on both surfaces. They are produced and harvested from
PLATE - I
Trichosanthes dioica Roxb.

PLATE - II
Clitoria ternatea L.
the beginning of July and continued to the middle of October. The fruits are an affluent source of protein and minerals and are used in conventional system of medicine since ancient times (Singh, 1989; Sharma et al., 1989).

**Ethnopharmacological uses**

The leaves of the plant has been used for constipation, fever, skin infection, convalescents, cancer like conditions, dysentery, diarrhoea, bronchitis and diuretics. The fruits are used as a remedy for spermatorrhoea, cooling and laxative (Kirtikar and Basu, 1975), diuretic, antiulcerous effects (Som et al., 1993), improves appetite and digestion (Chopra et al., 1956), haemagglutinating activities (Asolkar et al., 1992).

The reported pharmacological properties of *Trichosanthes dioica* Roxb. include antidiabetic (Chandrasekar et al., 1988; Rai et al., 2008a), antihyperlipidemic (Sharma and Pant 1988; Sharmila et al., 2007), hepatoprotective (Ghaisas et al., 2008), anti-stress, anxiolytic, antidepressant, anti-convulsant, tranquilizing and sedative agent (Mukherjee et al., 2008), antioxidant (Shivhare et al., 2010) and wound healing activities (Shivhare et al., 2010a).

(ii) *Clitoria ternatea* L.

**Systematic position**

<table>
<thead>
<tr>
<th>Common name</th>
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<tbody>
<tr>
<td>Botanical name</td>
<td><em>Clitoria ternatea</em> Linn.</td>
</tr>
<tr>
<td>Family name</td>
<td>Fabaceae</td>
</tr>
<tr>
<td>Local names</td>
<td>Sangu pu (Tamil), Aparajita (Sanskrit and Bengali), Koyala (Hindi), Sankhu pushpam (Telugu), Sangu pushpam (Malayalam), Nagarhedi (Kannada), Gokarna (Marathi), Butterfly pea and Blue pea vine (English).</td>
</tr>
</tbody>
</table>

**Plant identification and habitat**

*Clitoria ternatea* L. is a perennial twining herbaceous plant, belonging to the Fabaceae family. It is distributed throughout tropical equatorial Asia and later was distributed widely in South and Central America, East and West Indies, China, Bangladesh and India, where it has become naturalized (Bank et al., 2007). It is used as ornamental plant. It can be grown as a forage legume either alone or with perennial fodder grasses in Punjab, Rajasthan, Uttar Pradesh, Gujarat, Maharashtra, Madhya
Pradesh, Andhra Pradesh, Karnataka and Tamil Nadu. It is now widely distributed throughout the humid, low land tropics, occurring both naturally and in cultivations (Gupta Girish Kumar et al., 2010).

**Botanical description**

*Clitoria ternatea* L. has twining fine stems, 0.5-3.0 m long. The leaves are pinnate, with 5-7 elliptic to lanceolate leaflets, 3-5 cm long and shortly pubescent underneath. Flowers are axillary, solitary, white or standard deep blue to blue mauve with a white centre (Kulkarni et al., 1988).

**Ethnopharmacological uses**

*Clitoria ternatea* L. was used traditionally to cure sexual ailments like infertility and gonorrhoea, to control menstrual discharge and also as an aphrodisiac (Fantz and Paul, 1991). It has been traditionally used as a remedy for various disease like urinogenital disorder, bronchitis, purgative, diuretic, anthelmintic, rheumatism, diabetes mellitus (Parrotta, 2001; Prajapati et al., 2003; Khare, 2004; Kapoor, 2005), antipyretic, anti-inflammatory, analgesic (Devi et al., 2003), memory enhancer, nootropic, antistress, anxiolytic, anti-depressant, anti-convulsant, tranquilizing and sedative agent (Jain et al., 2003; Mukherjee et al., 2008), anticancer activity (Balachandran and Govindarajan, 2005), antihelminthic (Khadatkar et al., 2008; Nirmal et al., 2008), neurological disorders (Kamkaen and Wilkinson, 2009), hypoglycemic (Sharma and Majumdar, 1990; Daisy et al., 2009; Daisy and Rajathi, 2009), hepatoprotective and anti-stress agent (Gupta Girish Kumar et al., 2010).

The present work was undertaken to investigate the antihyperglycemic effect of ethanolic extracts of these two different herbs, each of which have been individually used as antihyperglycemic agents in traditional medicine. The added advantage is that the combination of these two plant extracts may act at multiple levels to bring about the therapeutic effects.
SCOPE OF THE STUDY

The present study, entitled “Phytochemical profile and antidiabetic activity of ethanolic extracts of leaf and fruit of *Trichosanthes dioica* Roxb. and leaf of *Clitoria ternatea* L. in streptozotocin-induced diabetic rats” was an attempt undertaken with the following objectives:

- To study the pharmacochemical characterization and qualitative organic analysis of leaf and fruit of *Trichosanthes dioica* Roxb. and leaf of *Clitoria ternatea* L..

- To determine the *in vitro* free radical scavenging activities of ethanolic extracts of leaf and fruit of *Trichosanthes dioica* Roxb. and leaf of *Clitoria ternatea* L..

- To analyse antidiabetic, antihyperlipidemic and *in vivo* antioxidant activities of ethanolic extracts of leaf and fruit of *Trichosanthes dioica* Roxb. and leaf of *Clitoria ternatea* L. against streptozotocin-induced diabetic rats.

- To study the histopathology examination of pancreas, liver and kidney tissues of the animal models.

- To compare all the parameters with standard drug glibenclamide treated diabetic rats.