INTRODUCTION
1. INTRODUCTION

The diabetes mellitus is an endocrinal disorder which is growing with jet speed globally. The epidemiological studies indicate that every 3rd person in India is suffering from insulin dependent diabetes mellitus. The multifactorial or multifacet is causing a global problem and waiting for a cure from new chemical entities (NCE) either from medicinal plants or synthesis.

The prevalence of Diabetes Mellitus is increasing globally day by day. The past two decades have seen a hotheaded global increase in the number of people diagnosed as non-insulin dependent diabetics i.e., Type 2 Diabetes. In India it is estimated that 19.4 million individuals are affected by non-insulin dependent diabetes mellitus, which is likely to grow up to 57.2 million by the year 2025 and is about one sixth of the world total (IDF, 2011, Zimmet et al., 2001, Mohan, 2004, Rajendra et al., 2010 Pradeepa et al., 2002).

The established causes may be of heredity, aging, obesity, environmental and lifestyle factors like urbanization physical inactivity and refined diet etc., all these seems to contribute to this widely spread disease. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value.

Diabetes mellitus is a common metabolic and endocrine disorder characterized by chronic hyperglycemic condition and disturbance of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency of insulin secretion or insulin action (Apparao, 2003, Bennet and Joslin, 1998). However the uses of oral hypoglycemic agents are the drug of
choice in the management of diabetic in NIDDM. The major advantages associated with oral hypoglycemic agents are patient acceptability, ease of administration, decreased insulin antigenicity and frequency of administration administrated.

1.1 Justification for the project

The high costs of modern treatment and the evidence that current methods of treatment fail to achieve the normoglycaemia and the prevention of diabetic complications, indicate that alternative strategies for the prevention and treatment of diabetes must be developed. Hence almost 90% of people in developing countries still rely on traditional medicines for their primary health care and the fact that scientific investigations of traditional medicines have led to the discovery of drugs, a synthesis of traditional and modern knowledge and techniques for the management of diabetes is feasible (Wagner and Farnsworth, 1994). Therefore indigenous medicinal plant resources could prove to be the practical and cost-effective alternative that is clearly and desperately needed.

The multifactorial pathogenicity of Diabetes demands a multi-modalistic therapeutic approach. Thus, the future therapeutic strategies require the combination of various types of agents. The theories of polyherbal formulation have the synergistic, potentiative, agonistic/antagonistic, pharmacological agents, within themselves due to incorporation of plant medicines with diverse pharmacological actions. These pharmacological principles work together in a dynamic way to produce maximum therapeutic efficacy with minimum side effects. They should be formulated and prepared with individual ingredients
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(Ashok et al., 2002). Therefore, it is important that the herbal medicines and preparations need to be taken with the consideration of their holistic therapeutic approach. The multiple activities of plant-based medicinal preparations meant for control of Diabetes offer enormous scope for combating the threat of the Diabetic epidemic.

Insulin resistance and non-insulin-dependent diabetes mellitus (T2D) have reached epidemic status in industrialized societies a dramatically increased risk for developing atherosclerotic heart disease, stroke, renal disease, blindness, and limb amputations. Equally worrisome is that T2D, initially defined as a disease of adult onset, is now appearing in adolescents (Forman, 2002).

Based on the above fact the project has been designed to scientifically prove the efficacy of herbal therapy and to commercialize the outcome for the effective treatment in diabetes Mellitus.

Combination products form an important part of the phytomedicines. They are widely accepted by patients and medical professionals. The combination products have great potential and they may be explored further for fixing the specifications of their standardization parameters.

The different constituents of a combination may influence the different symptoms of a single syndrome. The indication of a single compound may be different from that of the combination, but it may enhance the efficacy of the total combination. Further the criteria could be an improvement in tolerance and compliance, a simplification of the dosage scheme or avoidance of pharmaceutical incompatibilities (Ivorra et al., 1989). Literature review reveals
that the beneficial multiple activities of medicinal plants in treatment of Diabetes is common. This is possible because of the following reasons.

1. Manipulating carbohydrate metabolism by various mechanisms
2. Retarding glucose uptake in the small intestines
   a. By inhibiting digestive enzymes
   b. By inhibiting active transport of glucose across intestinal brush border membrane and
   c. By delaying the gastric emptying rate of gastrointestinal content, thereby decreasing the gastric emptying rate and suppressing\delaying the digestion and absorption of carbohydrates
3. Inhibition of carbohydrate hydrolyzing enzymes i.e. alpha amylase and alpha glucosidase and manipulation of glucose transporters
4. Preventing and restoring integrity and function of $b$-cells
5. Insulin-releasing activity (Insulin Mimetic and Insulin Sensitization Activity)
6. Improving glucose uptake and utilization
7. The antioxidant properties which offer exciting opportunity to develop them into novel therapeutics.

1.2 Aims and Objectives

The objective of the project is to evaluate polyherbal extracts from the medicinal plant *Eugenia jambolana* and *Cinnamomum zeylanicum* extracts, which are effective in the treatment of Diabetes mellitus

1) Evaluation of Phyto and Physico-chemical properties.
2) Identifying the suitable extract for the detailed evaluation
3) Preclinical Toxicity studies of Poly herbal extract
4) Pharmacological evaluation *invitro* and *invivo* studies.
5) Assessing the commercial potential.

The present study was planned keeping the above issues in mind. The objective of this project was to optimize the methodology required to screen and determine the effectiveness of the specific plants as antidiabetic potentiates, through observing if increases in glucose utilization and insulin secretion occurred under treatment. The cell lines used in this study, namely 3T3-L1 (adipocytes), C2C12 (muscle cells) and HepG2 (Chang liver) cells, were investigated as potential *invitro* evaluations for type II diabetes. Once *invitro* evaluations were achieved through this specific project, the parameters used to validate the models will in the future be able to be applied to scientifically establish the antidiabetic effects of the polyherbal extract.

### 1.3 Plan of Work

1. Selection and collection of plants having antidiabetic property based on preliminary information available in ayurvedic literature and as per folkloric claims.
2. Phytochemical analysis of selected plants for determining pharmacological property of selected plants.
3. Extraction of selected herbal plants with various polar and non polar solvents.
4. Cell culture studies of the selected herbal extracts for *invitro* screening.
5. Preclinical Toxicity Studies
6. Pharmacological studies of the selected herbal extracts on diabetogenic rats for confirming the hypoglycemic/antidiabetic activity.

7. Clinical studies to know the potentiality of the developed polyherbal formulation to control type-II diabetes.

1.4 Methodology

1. Identification of the phytochemical constituents

2. Characterizing the physiochemical properties

3. Preparation of various extracts with polar and nonpolar solvents

4. Finger printing of the extracts using HPTLC.

5. Biochemical analysis of the extracts.

6. *In vitro* and *in vivo* evaluation of plant extracts:

   Study of extracts on cell lines 3T3-L1, C2C12, and Hep G2 for glucose uptake and antidiabetic activity by using enzyme assays such as alpha-glucosidase inhibition and dipeptidyl peptidase - IV inhibition.

7. Preclinical Toxicity studies:

   - Acute and sub acute toxicity studies in wistar rats by OECD 420 guidelines.
   - Body weight, food intake daily assessment every week individually.
   - Biochemical Parameters
   - Hematological Parameters at “0” day and terminal day
   - Histopathological studies on terminal day.
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- Macroscopic and microscopic examination of the organs namely liver, spleen, kidney, pancreas, heart, lung, testes and ovaries.
- Histopathological will be evaluated by a competent pathologist.

8. Pharmacological evaluation.
   - Evaluation of Polyherbal Extract on normoglycaemic animals
   - Glucose Tolerance test in rats
   - Streptozotocin Induced Diabetes in rats

9. Influence of Polyherbal mixture on T2D patients

10. Commercial negotiations with pharmaceutical industry.

The outcome of the present study may be useful for the prevention of diabetes and add new dimension for the pharmacotherapy.