Chapter 1:

Introduction and review of literature
CHAPTER 1

1.0. Introduction and review of literature

1.1. General Introduction

Medicinal plants have a history as long as human civilization. Early Chinese, Indians, Hebrews and Egyptians have left us many written documents eulogizing the medicinal properties of plants. The drugs used by ancient civilizations were extracts of plants or animal products with a few inorganic salts. Herbal medicine is gaining prominence as the “drug of future demand” and is growing exponentially throughout the world. Majority of pharmaceutical companies are currently conducting expensive research on plant material for their potential medicinal values (Tandn et. al., 2004).

Currently numerous biochemical pathways are revealed from natural products for new therapeutic approaches. Globally medicinal plants are recognized as important resources for all major system of medicinal healthcare, neutraceuticals, phytochemicals and cosmetics. Mankind had realized very early the value of herbs as gift from the divine to heal both bodily and mental ailments. According to world health organization (WHO), 80% of the world population relies primarily on traditional medicines as source of primary health care (Franswarth et al., 1985).

Herbal formulations are advantageous over allopathic drugs which causes adverse drug reactions (ADRS). Adverse drug reactions is reported to be the fourth cause of death behind heart disease, cancer, strokes and diabetes.

Over 1000 chemical substances which are important drugs have been derived from numerous plants all over the world (Cragg and Newman, 2001). During the period of 1983 to 1994, 39% of new drugs approved were from natural products or derivatives of natural products (Harvey, 2001).
India has been recognized globally as an important resourceful area for traditional or herbal medicines. India, represented by rich culture and natural biodiversity, offers a unique opportunity for novel drug discovery (Jachak and Saklani, 2007).

About 45,000 plant species are found in India and it is one of the mega biodiversity centre (Hasan et al., 2009). About 15,000 medicinal plants are recorded in which only 7000-7500 plants are used for curing different diseases. In Ayurvedic literatures including Charak Samhita and Sushruta Samhita about 700 types of medicinal plants have been mentioned (Meena et al., 2009).

India is the largest producer of medicinal herbs and is called “Herbal Drug House” (Seth and Sharma, 2004). In India about 7,800 medicinal drugs manufacturing units are present and 2000 tones of herbs are consumed annually (Ramakrishnappa, 2002). Herbal remedies are effective because of their easy availability, safety, no side effects and low cost.

1.2. **Microbial spectra in diabetic patients**

Diabetic mellitus is a major disorder affecting a large section of population and is considered as a major public health problem (Anandi et al., 2004). Hence, Diabetes is rightly called a disease of complications and “Ice berg of disease”.

India homes 33 million diabetic cases and thus highest in the world and has a prevalence of about 8% in urban India. About 20% of all diabetic complications involve feet wounds (Gaur et al., 2007).

In diabetic patients wounds become infected 5 times more often than in non-diabetic patients and rate of infection parallels blood glucose levels. Wounds in diabetic patients are polymicrobial in nature rather than mono microbial, frequently harboring anaerobes synergistically with aerobes.
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The most common aerobic cultures identified are the Gram positive cocci including coagulase negative *Staphylococci* and *Streptococcus* species and the predominant are *Staphylococcus aureus*. The Gram negative pathogens include *Escherichia coli*, *Proteus* species and various other species belonging to the genera *Enterobacteriaceae*. *Peptostreptococcus* species, *Bacteroides melaninogenicus* and *Bacteroids fragilis* are the most commonly isolated anaerobes. Non clostridial gas gangrene due to aerobic gas forming coli forms has been seen in diabetic patients (Bessman and Wagner, 1975; Willis and Reece, 1960).

About 10-30% of diabetic patients with a foot ulcer will eventually progress to an amputation. Initial antimicrobial regimens of more severe ulcers include cefetetan ampicillin/ sulbactam or fluroquinolone (Baal *et al*., 2004). Inspite of management of wound infection in diabetic patients there is deleterious effects of infection on soft tissues and bone. Delayed diagnosis and unawareness of the extent of infections or suboptimal wound or antimicrobial therapy are the major reason for the progress of infection. Diabetic patients are prone for higher incidence of candida infections affecting tissue and organs (Stapleton, 2002, Etienne and Caron, 2007). Surgical site infections (SSI’s) have plagued surgeons’ time immemorial (Siguan *et al*., 1990). Susceptibility to infections increases due to hyperglycemia and poor vascular supply to the wounds. Hyperglycemia causes alterations in function of the phagocytic cells, such as decreased chemotaxis, poor adherence at the site of infection leading to a slower response by the immune system to control the infection (Malihe, 2008).

Microorganisms resistant to two or more classes of antimicrobial agents are defined as multiple drug resistant organisms (MDRO) (Siegel *et al*., 2007). Risk factors says that MDRO infection is higher in diabetic population than non-diabetic population.

In the past few decades the prevalence of methicillin resistant *Staphylococcus aureus* in both nosocomial and community acquired infections has
increased throughout the world. *Staphylococci* are one of the major group of bacteria inhibiting skin, skin glands and mucus membranes. *Staphylococci* will gain access through skin in the cases where skin barriers are breached by trauma or surgery, and enter to underlying tissues and creates characteristic lesions such as abscess, impetigo, boils, folliculitis, carbuncles, cellulitis, furuncles, osteomyelitis etc. MRSA has emerged as one of the commonest causes of hospital acquired infection (HAI) and continues to remain an important factor contributing in failure of management (Jevons, 1961). MRSA infections has been known to increase the rate of morbidity and mortality in hospitalized patients.

Extended spectrum β-Lactamase (ESBL) producing bacteria are microorganisms referred to as “Super bug” like MRSA are on the rise. It is proving to be a major healthcare problems due to paucity of effective drug (Hawkey, 2008). ESBLs are plasmid mediated β-lactamases capable of efficiently hydrolyzing and inactivating most β-lactam antibiotics including third generation cephalosporins and monobactams. The plasmid encoding ESBLs also encode for resistance to other class of antimicrobials agents such as fluroquinolones, aminoglycosides etc., that severely limit the choice of antimicrobials for treatment of these infection. ESBLs are primarily belonging to enterobacteriaceae family in particular *Klebsiella Pneumonia* and *E. coli*, amongst others (David et al., 2005). The incidence of ESBL producing strains among the clinical isolates has been steadily increasing over the past few years resulting in limitation of the therapeutic option (Baby Padmini and Appalraju, 2004).

Hence accurate laboratory detection of resistance to these antimicrobial agents is important to avoid clinical failure due to inappropriate therapy. Proper antibiotic selection directed against the commonly isolated pathogens can result in a positive favorable clinical outcome. This approach prevents unnecessary indiscriminate long term use of broad spectrum antibiotics counteracting the emergence of multi-drug resistance.
ESBL and MRSA are associated with increased morbidity and mortality in patients with diabetics. These early appropriate and judicious use of selective antibiotics can prevent complications such as amputations, surgical wounds, post surgical wounds and other co-morbidities associated with diabetic wound infections. This can shorten the duration of hospitalization thereby reducing the economical burden on the patient and family. It is necessary to know empirical therapy and avoid misuse of extended spectrum cephalosporins.

Hence the present study has been carried out to detect the microbial spectra in patients with diabetic wound infection. As data is limited in the Mysore area of Karnataka in these patients. This will help to guide the clinicians to select appropriate antibiotics in order to prevent morbidity and mortality associated with diabetic wound infections.

1.3. Antimicrobials of plant origin

Bioactive molecules produced by plants exhibit range of functional activities making them rich source in the field of medicine. Many active components are present in the plants which have medicinal values. Plant synthesizes many chemical compounds viz primary metabolites and secondary metabolites. Primary metabolites are produced for physiological functioning of the plants. Secondary metabolites like alkaloids, steroids, tannins and phenolic compounds are synthesized as self defence mechanism against many microbes, insects and herbivores (Vaghasiya et al., 2011). Due to these secondary metabolites in plants they become beneficial medicinal plants. Flavonoids are synthesized which are subclass of phenolics and flavonoids are effective antimicrobial substance against array of microbes. Their ability may be due to complex formation with extracellular and soluble proteins and also with bacterial cell wall (Cowan 1999). Terpenoids present in medicinal plants are active against bacterial fungi viruses and protozoa. Terpenoids act on cell membrane and disrupts the lipophilic compounds (Cowan 1999). Alkaloids present in medicinal plants kills Giardia and Entamoeba species as well as exhibit antidiarhoeal effect (Cowan 1999).
Plants which are known from historical era for their medicinal compound also produce emetine, berbesine and quinine which are effective against infectious microbes (Iwu et al., 1999)

Plant based antimicrobials are untapped source in medicines which have no side effects when compared to synthetic antimicrobials. Some lead molecules are developed from plants against microbes which is the development of phytomedicine (Evans et al., 2002). New research strategies have to be developed for producing antimicrobials from the plants in the near future (Bonjar et al. 2004, Aslim and Yucel, 2008). There is increasing failure of chemotherapeutic and antibiotic resistance by pathogenic microbial infectious agents so many medicinal plants are screened for potential antimicrobial agents (Imran et al., 2010) Charak and Sushruta have reported in “Ayurvedic compendium” the use of plant extract for microbial infection treatment (Chatterjee, 1994). Plants contain numerous biologically active compounds which have antibacterial properties (Branter and Grein, 1994; Perumal samy and Ignacimuthu, 1998).

Plant derived drugs constitutes complex chemical compounds which are responsible for therapeutic effect and are called active constituents. These bioactive compounds found in medicinal plants are used for therapeutic purpose or as precursors for synthesis of useful drugs. The active principle differs from plants to plants due to their biodiversity and they produce a definitely physiological action on the human body (Mukharjee, 2000). Researchers are now looking at folk medicine to develop new leads in drug against viral and microbial infections (Galal et al., 1991).

Antibiotics are employed for curing microbial infections caused by bacteria or fungi. Researchers thought that infectious diseases will no longer exist after discovery of antibiotics. However, irrational usage of antibiotics has led to the development of multidrug resistance in a number of bacterial strains (Khan et al.,
2009), which has resulted in the death of millions of people every year due to infectious diseases (Dubey et al., 2012). About 50,000 deaths occur annually around the world due to infectious disease (Ahmad and Beg, 2001). Antibiotics are not that safe as plant extract and their products (Kelmanson and Staden, 2000; Srinivasan et al., 2001). They are associated with side effects on the host which include hypersensitivity, depletion of beneficial gut and mucous microbes, immune suppression and allergic reactions (AlJabri, 2005).

Scientists have realized that life span of antibiotic is limited, though antibiotics are derived from microbes every year. Public are aware of the antibiotic usage and their problems worldwide and also of bacterial drug resistance. Hence, WHO recommends that drug from medicinal plants are the best source (Nascimento et al., 2000)

1.4. Antioxidants

Now a days in modern discussions on disease metabolisms common terms are used like free radicals, oxidative stress and antioxidants (Mc Cord, 2000). Biological functions are performed by basic, essential element for life ‘oxygen’ such as metabolic biomolecules in order to generate energy for growth and other activities. However, the same oxygen is involved in the generation of various kinds of reactive oxygen species (ROS) that cause oxidative damage of vital organs. ROS are formed due to chemicals through the action of ionizing radiation during the metabolism also due to endogenous and exogenous causes. Some non-oxygenated radicals are also generated in biological systems (Ex. Alkyl radical, R-H2O free radicals) and sulphur centered radical (ex. Thiols radical R–S) which are produced by the attack of free radicals on hydrocarbons and the oxidation of glutathione, respectively (Ivanova and Ivanova, 2004; Cheeseman and Slatter, 1993). Free radicals are highly reactive substances formed in the body cells as a result of metabolic processes (Niki, 1992).
Dr. Gerschman, first proposed the theory of free radical formation (Gerschman et al., 2001, Gutteridge and Halliwell, 2001). Free radical is a chemical species with one or more unpaired electrons which are highly unstable and to attain stability they react radically with adjacent molecules via a variety of reactions including hydrogen capturing, electron donation and electron sharing. A new free radical is formed in its place. This newly formed radicals takes electron and try to return to its ground state with antiparallel spin from cellular structures or molecules. Then the chain reaction continues (Mc Cord, 2000). Different types of ROS are generated as by-products of biological reaction or from exogenous factors like tobacco, smoke, ionizing radiations, air pollution, organic solvents and pesticides. ROS are divided into radicals and non-radicals. Radicals like superoxide, hydroxyl, Thioxyl, Alkoxyl, Nitric oxide and non-Radical like H₂O₂ hydrogen peroxide, Lipid peroxide, Singlet oxygen are produced. (Buyukokuroghu et al., 2001).

Excessive amount of ROS are harmful because they initiate biomolecular oxidation which leads to cell death and creates oxidative stress. It causes enzyme activation and oxidation damage to cellular system (Wiseman and Halliwal, 1996). Oxidative stress causes irreversible oxidation of DNA proteins and lipids. Many chronic and degenerative diseases are caused by oxidative stress viz atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immune suppression, neurodegenerative disease and others (Young and woodside, 2001). When there is a imbalance between generation of ROS and antioxidant defense mechanism of a cell or tissue leads to oxidative stress. The antioxidants play important role in protection against the damage caused by ROS in the living organisms.

Antioxidants are substance which at low concentration as compared to oxidizable substrates, significantly delays or prevents oxidation. The term oxidizable substrates includes DNA, Lipids, Proteins and Carbohydrates. Based on the nature of antioxidants the human antioxidant system is categorized into broad
classes ie., Enzymatic antioxidants and non-enzymatic antioxidants (Jakus, 2000). The major primary intracellular endogenous antioxidants defense are the enzyme system viz superoxide dismutase, catalase, glutathione peroxidase and non enzymatic antioxidants are divided into two groups viz., endogenous antioxidants like ceruloplasmin, transferrin, hepatoglobin and albumin and other non-proteins endogenous antioxidants are uric acids glutathione, bilirubin, ubiquinone and lipoic acid (Shahidi, 1997). Many effective exogenous antioxidants are generally of dietary origin. The best known are Ascorbic acid, Vitamin K, Caratenoids, Quinines and polyphenols.

Antioxidants inhibit oxidative reaction by scavenging free radicals while certain compounds may chelate redox active metals or inhibit particular oxidative enzymes (Paker, 2001). Some of the synthetic antioxidants like Butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) which are used in the industries and in processed foods are having side effects and are carcinogenic (Branen, 1975; Ito et al., 1983). So from the last two decades there is a scientific research in the field of antioxidants from natural source mainly from plants for dietary cosmetic and pharmaceutical uses. The natural antioxidants are safe nutritionally and therapeutically if they are derived from food and other biological materials (Ajila et al., 2007).

1.4.1. Uses of medicinal plants as antioxidants

Medicinal plants have therapeutic potentials like antioxidant which recently interest has been developed in this field and are now investigated for novel antioxidants (Koleva et al., 2002; Oke and Hamburger, 2002). Indian traditional system (Ayurveda) uses some plants as Rasayana to treat diseases and these have shown to have potent antioxidant activity (Kaur and Kappor, 2002; Aqil et al., 2006). Medicinal plants are screened throughout the world in the light of recent scientific development due to their potent antioxidant activities, low toxicity and economic viability (Auddy et al., 2003).
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Natural antioxidants play an important role in human health and nutrition (Aruoma et al., 1994). The secondary metabolites like phenolic and flavonoids from plants have been reported to be free radical scavengers (Mathew and Abraham, 2006). The redox properties which allow them to react as reducing agents, as an electron donor, an oxygen quenchers or chelating metal. Antioxidants either in the form of crude extracts or their active chemical compound are very effective to prevent the destructive processes caused by oxidative stress (Zengin et al., 2011; Sayed et al., 2012).

1.5. Diabetic mellitus and its classification

Diabetes mellitus is the most common endocrine disorder, it is a progressive disease characterized by insulin deficiency and insulin resistance or both. The elevated fasting and post prandial blood glucose leads to acute and chronic complications (Micro and macro vascular) resulting in blindness, kidney failure, heart disease, stroke and amputations (Pankaj, 2007).

1.5.1. Classification of diabetes (Expert committee 2003)

The disease states underlying the diagnosis of diabetes mellitus are classified into four categories

1) **Type 1, (Insulin dependent diabetes)**: Here there is selective beta cell destruction and severe or absolute insulin deficiency, susceptibility appears to involve a multifactorial genetic linkage, but only 10-15% of patients have a positive family history. Insulin replacement therapy can be life threatening and can result in diabetic ketoacidosis or death.

2) **Type 2, (non-insulin dependent diabetes mellitus)**: It is characterized by tissue resistance to the action of insulin combined with a relative deficiency in insulin secretion. The impaired insulin action also affects fat metabolism, resulting in increased free fatty acid flux and triglycerides levels and reciprocally low level of high density lipoprotein. They may progress slowly to type 1 called Latent Autoimmune Diabetes of Adults (LADA).
3) **Type 3, (Other types of diabetes mellitus):** It refers to multiple other specific causes of elevated blood glucose like pancreatectomy, pancreatitis, non-pancreatic diseases, drug therapy etc.

4) **Type 4, (Gestational diabetes mellitus):** Gestational diabetes mellitus is defined as any abnormality in glucose levels noted for the first time during pregnancy. Approximately it is seen in 4% of all pregnancies. The placenta and placental hormones during pregnancy cause insulin resistance which pronounce in the last trimester (Bertram, 2009).

Diabetes is also classified etiologically by American diabetic association (2004) into following types

- Immune mediated
- Idiopathic insulin resistance genetic defects of beta cell
- Genetic defect in insulin action
- Disease of exocrine pancreas
- Endocrinopathies
- Drug or chemical induced
- Other genetic syndromes

Pathogenicity of diabetes mellitus increases due to environmental factors like diet, style of eating and sedentary lifestyle.

**1.5.2. Clinical features:**

Hyperglycemia causes wide variety of symptoms in diabetes mellitus. The classical symptoms of thirst, polyurea, nocturia and rapid weight loss are predominant in type 1 diabetes but are often absent in patients with type 2 diabetes, many of whom are asymptomatic or have non-specific complaints such as chronic fatigue malaise and increased susceptibility to infections.

**1.5.3. Prevalence of Diabetes mellitus:**

Diabetes in the year 2007 has affected 246 million individuals and in the future it is expected to increase by 55% i.e. 380 million by the year 2025 (Sicree et
Type 2 diabetes is prevalent in 95% of diabetic population in United States (Narayan et al., 2003). By 2025, in developed countries it is going to increase by 42% from 51-72 million and in developing countries by 170% increase from 84-228 millions.

India has over 41 million diabetic population and every fifth diabetic in the world is Indian (Figure 1.1), so it is called “Diabetes Capital of the World” (Joshi and Parikh, 2007). Indians are more prone to diabetic due to unique clinical and biochemical abnormalities which include greater abdominal adiposity i.e., higher waist circumference. Despite lower body mass index and increased insulin resistance. Most disturbing trend in diabetes is shift in age group to a younger age in the recent years.

Diet, obesity and sedentary life style are some of the environmental factors which are increasing the risk of diabetes. Major risk factors like high family aggregations, insulin resistance, nutritional status, urbanization also play an important role in diabetes mellitus (Deepashree and Prakash, 2007).

Management of diabetes is a challenge as there is no treatment successfully discovered till date (Malviya et al., 2010).

![Figure 1.1: Estimated number of diabetic subjects in India (Mohan et al., 2007)]
1.5.4. **Diagnostic criteria for diabetes mellitus**

WHO issued following criteria:

- Symptoms of diabetes plus random blood glucose concentration $\geq 11.1$ mmol/L (200 mg/dl) or
- Fasting plasma glucose $\geq 7$ mmol/L (126 mg/dl) or
- Two hours plasma glucose $\geq 11.1$ mmol/L (200 mg/dl) during an oral glucose tolerance test (Expert committee, 2003).

Determination of blood glucose is done by glucose oxidase peroxidase (Trinder’ method). The glucose concentration depends on the intensity of the pink color formed and can be measured photometrically between 500 to 540 nm (Teitz, 1976).

- Oral glucose tolerance test (OGTT): Fasting plasma glucose 6.1-7 m mol/L (110-126 mg/dl)
- Random plasma glucose: 7.8-11 m mol/L unrestricted carbohydrate diet for 3 hours before the test.
- Fasting over night (For atleast 8 h)
  Rest before test (30 minutes).
- Plasma glucose measured before and 2 h after 75 gms of glucose taken by the subjects with impaired glucose tolerance having an increased risk of type 2 diabetes.
- Glycohemoglobin assay (HbA1c) (Devies et al., 2010).

HbA1c is the tool for monitoring complications of diabetes, hence WHO technical guideline development group awaited it but likely to enclose HbA1c as the diagnostic criteria (Table 1.1).
Table 1.1a: Diagnostic criteria of HbA1c in diabetes

<table>
<thead>
<tr>
<th>Disease status</th>
<th>HbA1c %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
<td>≥ 6.5</td>
</tr>
<tr>
<td>Pre diabetic</td>
<td>≤ 5.7-6.4</td>
</tr>
<tr>
<td>Normal</td>
<td>≤5.6</td>
</tr>
</tbody>
</table>

C-peptide and insulin test are the tests used to assess the beta cell capacity and function (Wiwanitkit, 2009). C-peptide level helps in the classification of diabetes mellitus (Power, 2008). Insulin released from the pancreas is known by C-peptide test. C-peptide level in normal persons will be 0.8 – 3.1 ng/ml, however in hypoglycemic and abnormal endogenous insulin secretion level goes to ≥ 1.2 ng/ml. In hypoglycemic and high circulating insulin level due to administration of insulin will be < 1.2 ng/ml (Robert and Hwians, 2009).

1.5.5. **Complications of diabetes mellitus:**

Diabetes mellitus leads to acute and chronic complications. Acute complications like diabetic ketoacidosis (diabetic coma) and some chronic complications are microvascular/neuropathic (Frier and Fisher, 2006).

1. Retinopathy, cataract: Impaired vision
2. Nephropathy: Renal failure
3. Peripheral neuropathy: Sensory loss and Motor weakness
4. Autonomic neuropathy: Altered bowel habit
5. Foot disease: Ulceration and Macrovascular
6. Coronary circulation: Myocardial ischaemia (heart attack)
7. Cerebral circulation: Stroke
8. Peripheral circulation: Ischaemia

1.5.6. **Experimental diabetic animal model:**

STZ-NAD (Streptozotocin and Nicotinamide) induced diabetic rat model is used for our study where it is characterized by moderately decreased β-cell mass compared with STZ induced diabetic animals. Rats with STZ-NA induced diabetes
are relatively mild hyperglycemia compared to diabetes induced by STZ alone. The dosage selection of NA and STZ depends upon the protection of β-cell from necrosis (Masiello et al., 1988).

STZ is a Glucose analogue which is procured from cultures of *Streptomyces achromogenes*. The glucose transporter-2 (GLUT. 2) uptake into pancreatic β-cells through the STZ leads to fragments of DNA through alkylation (Elsner et al., 2000). This mechanism causes activation of poly ADP ribosylation and depletion of cellular NAD⁺. Further, STZ liberates ROS particularly high amount of NO (Nitric oxide) that participates in DNA damage and necrosis of β-cell takes place. Nicotinamide is a biochemical which exhibit antioxidant properties and a precursor of nicotinamide adenine dinucleotide (NAD) which also improves metabolic activities. Nicotinamide scavenges free radicals and reduces DNA damage. It inhibits the apoptosis of pancreatic β-cells against STZ by inhibiting nitric oxide (NO) mediated damage. Hence it is a best model for induction of type 2 diabetes mellitus. (Szkudelski, 2001).

1.5.7. Treatment of diabetes mellitus

Currently available oral agents in the treatment of diabetes mellitus are presented in table 1.1 (Tripathi 2003).
Table 1.1b: Advantages and disadvantages of available oral agents to treat diabetes

<table>
<thead>
<tr>
<th>Hypoglycemic Agents</th>
<th>Activity and advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphonylureas</td>
<td>Increase endogenous insulin secretion; generally well tolerated; inexpensive</td>
<td>No effects on insulin sensitivity, blood pressure, lipids or lipoproteins, adverse effects include hypoglycemia (particularly in the elderly) and body weight gain; no positive effects on cardiovascular parameter; drug interaction</td>
</tr>
<tr>
<td>Biguanides</td>
<td>Decreases hepatic production of glucose, peripheral insulin levels, total LDL-C, free fatty acids and triglycerides levels and slightly increases muscle insulin sensitivity; low risk of hypoglycemia; beneficial effects on cardiovascular parameters doesn’t cause body weight</td>
<td>Contraindicated in patients with renal impairment (risk of lactic acidosis) liver disease, respiratory insufficiency, hypoxaemia, severe infections, cardiac failure; associated with gastrointestinal adverse effects; neither decrease blood pressure nor increase.</td>
</tr>
<tr>
<td>Metformin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha glycosidase Inhibitors</td>
<td>Inhibits carbohydrate absorption from the gastrointestinal tract; serious adverse effects are rare; hypoglycemia is not at risk; no effects on body weight</td>
<td>Gastrointestinal adverse effect; hepatic toxicity (acarabose); less effective than sulphonl urease or metformin; contraindicated in patients with inflammatory bowel disease or renal or hepatic impairment.</td>
</tr>
<tr>
<td>Miglitol</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1.5.8. Use of medicinal plants for the treatment of diabetes mellitus

In developing countries use of conventional medicines is a burden to the population so traditional medicines are used for the treatment of diabetes (Saravanan and Pari, 2008). Diabetes is successfully managed by the indigenous Indian medicinal plants since medicinal plants are easily available and have little side effects. They play a very important role in the medical field. Many plants have shown anti-diabetic activity which are presently proved by available experimental techniques (Jafri et al., 2000). Table 1.2 depicts the list of plants known for their effect in treating diabetes. For the past few years bio-active drugs isolated from plants are showing promising results for anti-diabetic treatment which are more potential than oral hypoglycemic agents used in clinical therapy.
Table 1.2: Plants recognized for their anti-diabetic effect

<table>
<thead>
<tr>
<th>Name</th>
<th>Family</th>
<th>Anti-diabetic and beneficial properties</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aegle marmelos</em> L</td>
<td>Rutaceae</td>
<td>Decreases in liver glycogen and also in blood urea and serum cholesterol.</td>
<td>Ponnachan <em>et al.</em>, 1993</td>
</tr>
<tr>
<td><em>Acacia arabica</em> L</td>
<td>Mimosaceae</td>
<td>Secretagogue to release insulin from pancreatic β-cells.</td>
<td>Wadood <em>et al.</em>, 1989</td>
</tr>
<tr>
<td><em>Andrographis paniculata</em></td>
<td>Acanthaceae</td>
<td>Increase glucose metabolism, decreases blood glucose level (hypoglycemic) due to its anti-oxidant properties.</td>
<td>Dandu and Inamdar, 2009; Zhang and Tan, 2000</td>
</tr>
<tr>
<td><em>Azadirachta indica</em></td>
<td>Meliacea</td>
<td>Hypoglycemic activity</td>
<td>Waheed <em>et al.</em>, 2006</td>
</tr>
<tr>
<td><em>Biophytum sensitivum</em></td>
<td>Oxalidaceae</td>
<td>Insulino tropic effect</td>
<td>Puri, 2001</td>
</tr>
<tr>
<td><em>Casearia esculenta</em> L</td>
<td>Flacourtiaceae</td>
<td>Anti-hyperglycemic activity, increase in liver hexokinase activity</td>
<td>Prakasam <em>et al.</em>, 2002</td>
</tr>
<tr>
<td><em>Embelica officinalis</em> L</td>
<td>Euphorbiaceae</td>
<td>Alpha amylase and alpha glucosidase inhibitor</td>
<td>Nampoothiri <em>et al.</em>, 2010</td>
</tr>
<tr>
<td><em>Enicostema littorale</em> L</td>
<td>Gentianaceae</td>
<td>Decrease in glycosylated hemoglobin and increase in serum insulin level</td>
<td>Maroo <em>et al.</em>, 2003</td>
</tr>
<tr>
<td><em>Helicteres isora</em> L</td>
<td>Sterculiaceae</td>
<td>Glucose uptake active comparable with insulin</td>
<td>Gupta <em>et al.</em>, 2009</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Family</td>
<td>Effect</td>
<td>Reference</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>------------------</td>
<td>------------------------------------------------------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Momordica charantia L</td>
<td>Cucurbitaceae</td>
<td>Suppresses plasma glucose and insulin, inhibits alpha glucosidase.</td>
<td>Vebanso et al., 2007</td>
</tr>
<tr>
<td>Pterocarpus marsupium L</td>
<td>Papilionaceae</td>
<td>Insulinogenic enhancing insulin release and conversion of proinsulin to insulin in-vitro.</td>
<td>Ahmad et al., 1989</td>
</tr>
<tr>
<td>Salacia reticulate</td>
<td>Celastraceae</td>
<td>Lowering of the plasma insulin level and elevation of intestinal alpha glucosidase activities in type 1 diabetic mice.</td>
<td>Yoshino et al., 2009</td>
</tr>
<tr>
<td>Syzigium cumini L skeels</td>
<td>Myrtaceae</td>
<td>Reduction in blood glucose and an increase in total hemoglobin.</td>
<td>Prince et al., 1998</td>
</tr>
<tr>
<td>Trigonella foenum Graecum L</td>
<td>Papilionaceae</td>
<td>Improves glucose metabolism.</td>
<td>Gupta et al., 1999</td>
</tr>
</tbody>
</table>
1.6. *Rotula aquatica* plant description and Uses

**Figure 1.2: Photograph of Rotula aquatica Lour**

1.6.1. Plant Profile

Plant: *Rotula aquatica*  
Synonyms: *Carmona viminea*  
*Ehretia viminea*  
*Rhabdia viminea*  
Botanical name: *Rotula aquatica* Lour.  
Family: Boraginaceae

1.6.2. Vernacular names:

Sanskrit: Pasanabhedah, Asmabheda, Mootrala  
Kannada: Pasanabhed  
Malayalam: Kallurvanci  
Telgu: Pasanabheda  
Tamil: Seppunerinji  
Hindi: Pashanabhed  
Parts used: root, stem tuber
1.6.3. **Botanical description:**

The tree is a small much branched shrub, 60-180 cm in height with numerous short lateral arrested branchlets often rooting. Leaves are simple, nearly sessile, spatulate, rounded at the apex, more or less hairy, crowded branches. Flowers are pink or reddish, shortly pedicellate, single or 2-3 together on the short lateral branches, stamens exerted beyond the corolla tube. Fruits are subglobose, orange red drupes, tipped with the remain of the style. Roots are 2-4 layered cambium and predominant wood which help in the vegetative propagation.

1.6.4. **Geographical distribution**

*Rotula aquatica* Lour is a rare rheophyte belonging to the family Boraginaceae which is represented by about 100 genera and 2000 species. The plant is a member of lotic ecosystem of streams in India. The plant is scattered throughout peninsular and western ghats of India in the sandy and rocky beds of streams and rivers and are occasionally submerged in floods. In Kerala region of western ghats, the plant occurs attached to rocks along running water in Parappa, Payyanus, Kannoth, Mattanur and aralam of Kannur district, Vazhachal, Chalakkudy and Athirabbilly of Thrissur district, Mukkali, Parambikulam, Poovanchola and Bhavani river of Pallakad district. It is also distributed in Srilanka, China, Africa, Brazil and Latin America.

1.6.5. **Traditional uses:**

The tribal communities residing near to river in Kerala were more knowledge and experience about the usage of *Rotula aquatica*. Root, stem and leaf are used for different ailments like psychostimulent, to dissolve stone in kidney and bladder, urinary tract infections and as analgesic for body pain.

In Kollam district of Kerala, the malapandram tribes in achenkovil forest use root to treat skin diseases and against body healing and prickles (George *et al.*, 2007), for treating urinary disorders (Yesodharan *et al.*, 2007) and also for treating stomach ulcers by preparing root decoction.
Rotula aquatica roots are bitter, astringent, cooling, diuretic and used in diabetes and venereal diseases (Prasad et al., 1993; Warries et al., 2000).

1.6.6. **Chemical constituents:**

The plant contains bauerenol m.p. 282°C, αβ amyrin (Rastogi and Mehrotra, 1970). The diuretic action of root is due to the presence of allantoin (0.5% in air dry root). A sterol named rhabdiol (C_{35}H_{60}O, mp 210°C) has also been isolated from the roots (The wealth of India, 1972).

1.6.7. **Formulations:**

The roots are important constituents in about 10 preparations like poothikaranjasava, matsuayakshyadi, kashaya, veeratharadi kashaya, vrikshadanyadi kashaya, dadhikaghritra, vasthyamayanthaka gritha, varahyadi gritha and veeratharadi gritha.

1.6.8. **Pharmacological applications:**

1.6.8.1. **Antiurolithiasis effect:** In males there is a recurrent disorder called renal stone formation (urolithiasis). When rats were induced with lithiasis by ethylene glycol, administration of the *R. aquatica* Lour extract prevented the growth of urinary stones. Recovery of renal damage may be due to protective effect of plant against oral-induced lipid peroxidation (Umesh et al., 2010).

1.6.8.2. **Antidiarrhoeal property:** Antidiarrhoeal effect may be due to alkaloids and flavonoids present in alcoholic extract of *R. aquatica* (Singh et al., 2012).

1.6.8.3. **Anti-inflammatory property:** Anti-inflammatory activity at dose of 200 mg/kg body weight was shown by the alcoholic extract of *R. aquatica* Lour. The whole plant of *R. aquatica* Lour in petroleum ether, ethyl acetate and ethanolic extract on acute inflammation and sub-acute inflammation in
animal model revealed that petroleum ether and ethyl acetate extract of whole plant at dose of 200 mg/kg possessed significant anti-inflammatory activity (Kamurthy et al., 2014).

1.6.8.4. **Antidiabetic property:** In normal Streptozotocin induced diabetic rats the *R. aquatica* Lour aqueous extract showed antidiabetic and hypoglycemic effect (Ashwini et al., 2012). The methanolic extract of the roots showed antidiabetic activity which was evaluated by Shyam et al., (2013).

1.6.8.5. **Antibacterial effect:** Methanolic extract of *R. aquatica* lour showed antibacterial activity against food related bacteria like *E. coli* and *Salmonella typhi* (Aswathanarayan et al., 2013). Prashanthi et al., (2012) revealed in their study that urinary tract infections associated with kidney stone which is common in nephrolithiiosis vice-verse. So antibacterial property was shown against Gram positive bacteria viz., *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus* and four Gram negative strains viz., *Pseudomonas aeroginusa, E. coli, Klebsiella pneumonia, Salmonella abony*. Due to it potency as antibacterial property, the plant can be used as natural preservative in food industry and pharmaceutical for treatment of urolithiasis and urinary tract infection.

1.6.8.6. **Anticancer property:** Due to polyphenols especially tannins present in *R. aquatica* Lour extract helps in the treatment of cancer by efficiently preventing cell proliferation of the pancreatic cancer cell lines (Patil et al., 2004).

1.6.8.7. **Antihelmintic property:** *Rotula aquatica* Lour as a source of novel antihelminitic drug was shown by Lakshmi et al. (2012). Leaves and bark of *R. aquatica* Lour revealed to be antihelminic, which was reported by Zade
et al., (2013). Dose dependent activity of aqueous extract showed antihelminthic effect on adult earthworm.

1.7. **Plan of present investigation**

With the detail study about the diabetes prevalence, failure and side effects of synthetic chemical drugs and the complications associated, the present study was undertaken initially to understand the prevalence of diabetes in Mysore and characterize the predominant pathogens associated with wound infection. Diversity and wider application of herbal drug as alternative effective medicine for the treatment of diabetes motivated the present investigation to analyze the plant *Rotula aquatica* Lour for its hypoglycemic effect. In this view the following objectives were framed.

1. Isolation and Identification of microbial spectra from diabetic patients.
2. Isolation of crude extracts of medicinal plants and *in vitro* antimicrobial activity of the extracts.
3. Determination of *in vitro* antioxidant properties.
4. Study on *in vitro* anti-diabetic properties
5. *In vivo* antidiabetic activities of the extract on adult Wister rats.