INTRODUCTION

Medicinal plants are the plants that have been alleged to have a medicinal property, which is effects that relate to health, or which have been proven to be useful as drugs by western standards or which contains constituents that are used as drugs (Farnsworth and Soejarto, 1991). India has about 45000 plant species, of which several thousands have claimed to possess medicinal properties.

The world health organization (WHO) reported that nearly 80% of the world’s population depends on traditional medicine of plants to meet their health care needs. These medicinal plants fall into two distinct areas, traditional system of medicine and modern system of medicine (Mazid et al., 2012).

Medicinal plants with active constituents such as alkaloids, flavonoids, glycosides, polysaccharides, peptidoglycans, hypoglycans, guanidine, steroids, carbohydrates, terpenoids and coumarins are known to cure several ailments such as to strengthen immune system, inflammation, kidney and urinary tract ailments, cancer, dengue, malaria, diabetes (Hemalatha et al., 2012), scurvy, arthritis, liver problems, cardiac diseases, laxative, asthma, obesity etc. Hence it is necessary to investigate the efficacy of the medicinal plant based on the traditional practices by indigenous people.

These plants belongs to the family such as Asteraceae, Amaranthaceae, Apiaceae, Araliaceae, Apocynaceae, Brassicaceae, Cactaceae, Cucurbitaceae, Caesalpinaceae, Labiatae, Liliaceae, Meliaceae, Palmae,
Punicaceae, Piperaceae, Rhamnaceae, Rubiaceae, Rutaceae, Solanaceae, Sapotaceae, Verbenaceae and Zygophyllacea (Verma and Singh, 2008; Bnouham et al., 2006; Hemalatha et al., 2012). Herbal medicinal system seems to be important in many developing countries for the treatment of several ailments. It is estimated that nearly 85% of Indians are using crude plant preparations for treatment of diseases.

Drugs from plants can be obtained from roots, rhizomes, tubers, bark, stem, woods, leaves, flowers, fruits, seeds etc and it act as a source of therapeutic agents such as to isolate bioactive compounds as drugs e.g digoxin, to produce semi synthetic drugs e.g metformin, to use as pharmacologic tools e.g yohimbine and to use as herbal remedy e.g garlic (Rout et al., 2009). The prescribed drugs (25%) that are currently marketed were originally isolated from plants or semi synthetic analogues of phytochemicals. Several hundreds or thousands of different chemical constituents are present in crude plant extracts that are known to interact in complex ways and produce physiological actions in body (Cock, 2015).

1.1. Diabetes Mellitus (DM)

DM is a multifactorial metabolic disease characterized by hyperglycemia, including abnormal carbohydrate, fat and protein metabolism leading to several complications and hence needs a combined therapeutic approach (Patil et al., 2011; Mohammadi and Naik, 2012). Insulin injections are given in case of total lack of insulin; the post prandial hyperglycemia is managed at digestive level by using acarbose, miglitol and voglibose drugs (Gunjan et al., 2011). In India, the number of people affected with diabetes is expected to rise from 171 million in 2000 to 366 million by 2030 (Noor et al.,
Out of the two major types of diabetes, type II diabetes is more prevalent and mainly due to the loss of pancreatic $\beta$-cell function, which results in insulin resistance. The regeneration or stabilization of $\beta$-cell must occur, in order to prevent the loss of $\beta$-cells (Verma et al., 2011).

The postprandial hyperglycemia will cause non-enzymatic glycosylation of proteins that leads to micro and macro vascular complications. Hence the control of postprandial plasma glucose level is essential in the early treatment of diabetes and also to reduce chronic complications (Ahmed et al., 2011). Eventhough there are several drugs to tightly regulate blood glucose, to reduce microvascular and macrovascular complications, the main undesirable effects of this anti-diabetic drug that are currently available are brain damage, swelling, erythema, abdominal pain, weight gain, metallic taste, vitamin B$_{12}$ deficiency, heart failure and gastrointestinal disturbances (Patil et al., 2011).

Due to these side effects of oral hypoglycemic agents and oxidative stress in complicating diabetes (Singh et al., 2013), there is growing interest in herbal remedy for the treatment of type II diabetes mellitus and oxidative stress (Kumar et al., 2011; Gunjan et al., 2011).

1.2. Role of glucose in Diabetes Mellitus

N-D-glucose, a reactive molecule and a central substance of human metabolic pathway serves as a transport and short term storage form of energy. The energy needed to sustain life is delivered by oxidation of glucose. The aldehyde group in glucose is found to be more reactive and many nucleophilic agents like alcohol, amine and carbanions are prone to attack the aldehyde
group (Pischetsrieder, 2000). Glucose sources may be exogenous (dietary carbohydrates or dextrose infusions), endogenous (from liver glycogenolysis reaction, lactate, alanine and glycerol), that get converted into glucose by gluconeogenesis mainly in liver and kidney (lesser extent).

Under basal conditions tissues like brain, red blood cell and renal medulla consume 50% - 80% of ingested glucose by non-insulin mediated uptake. The remaining glucose gets converted to produce energy by glycolysis pathway; in the liver and skeletal muscles it is stored as glycogen or converted as fat in the liver and adipose tissue. To maintain glucose homeostasis, this glucose metabolism involves complex metabolic reactions. Hormonal (insulin, glucagon, cortisol, catecholamines and growth hormone), neural (central and peripheral glucosensors) and hepatic autoregulatory mechanisms play an important role in controlling blood glucose concentration (Butler et al., 2005).

When there is an elevation in glucose control (fasting blood sugar in diabetes – 126mg/dl and above; random blood sugar in diabetes – 200 mg/dl and above) it leads to chronic hyperglycemia (De and Gupta, 2012). This chronic hyperglycemia is due to defects in insulin secretion, insulin action or both, that culminate to a metabolic disorder called DM. There are different types of DM: type I, type II, gestational diabetes mellitus (GDM), prediabetes, latent autoimmune diabetes of adults (LADA), congenital diabetes, cystic fibrosis-related diabetes, steroid diabetes due to high dose of glucocorticoids and maturity onset diabetes of the young (MODY).

1.3. Pancreas – An endocrine and exocrine system

Pancreas an endocrine gland situated next to the first part of small intestine between the spleen and duodenum consists of group of cells called
islets of langerhans and exocrine gland that contains acinar cells (secretes digestive enzymes) and duct cells (Fig. 1). The islets of langerhans have four types of hormone secreting cells: α cells secrete glucagon (20-30%), β cell secretes insulin, delta cell secretes gastrin and F cells produce pancreatic polypeptide. β cells occupy the central portion of the islet and are surrounded by α, delta and f cells (Nair, 2007). β cells and α cells are electrically excitable and each type of cell has unique ion channels (Wang and Bansal, 2008).

1.4. Insulin - Gate keeper for glucose

Insulin, a protein consisting of two polypeptide chains A with twenty one amino acids and B with thirty amino acid residues is synthesized in the β cells of pancreas in the form of preproinsulin (the gene is located on chromosome 11). The A and B chains are linked by disulfide bonds. C-chain (connects A and B chain) is liberated after the breakdown of proinsulin (>95%), along with insulin (Joshi et al., 2007) (Fig. 2a).

β-cells secrete insulin in response to various stimuli like glucose, arginine and sulphonylureas. GLUT-2 receptors help beta cells to take up the glucose. After entering, glucose is phosphorylated to glucose-6-phosphate (MacDonald et al., 2005), and oxidized by glucokinase. Concentration of glucose less than 90 mg/dl does not cause any insulin release (Joshi et al., 2007). At this concentration voltage gated calcium channels are closed and potassium ions efflux through open K\textsubscript{ATP} channels (composed of pore-forming K\textsuperscript{+IR} 6.2 and regulatory sulfonyl urea receptor 1 (SUR1)), thereby keeping the beta cell membrane at negative potential.

When the plasma glucose concentration increases, beta cell uptakes glucose and metabolize it by glycolysis to produce pyruvate, NADH and ATP (MacDonald et al., 2005; Alarcon et al., 2002), which leads to enhanced ATP production and closure of K\textsubscript{ATP} channels. The pyruvate is a substrate for TCA
cycle in the mitochondria and acts as a modulator for insulin secretion (MacDonald et al., 2005; Wang and Bansal, 2008). The cells take up glucose by facilitated diffusion through transporter proteins called GLUT4.

In the absence of insulin, GLUT4 glucose transporters are present in cytoplasmic vesicles and are inactive (Shepherd and Kahn, 1999). After uptake, the beta cell membrane gets depolarized, which influx calcium ions through voltage gated calcium channels (Fig. 2b). The increase in intracellular calcium concentration causes exocytosis of insulin granules (Joshi et al., 2007).

1.5. Complications in Diabetes Mellitus

Insulin release (5 mmol L\textsuperscript{-1}) is tightly regulated in the fasting blood glucose concentration. With normal consumption of food, the digested carbohydrates lead to increase in blood glucose level. This triggers insulin to tightly regulate blood glucose concentration at 4-10 mmol L\textsuperscript{-1} (Sonksen and Sonksen, 2000). In diabetic patients, uncontrolled glucose concentration (Chronic hyperglycemia) is associated with microvascular complications (peripheral neuropathy, retinopathy and nephropathy) and macrovascular complications (atherosclerotic cardiovascular, peripheral vascular and cerebrovascular diseases). It may also lead to osmotic diuresis, fluid and electrolyte imbalance, worsening skeletal muscle catabolism, hyperosmolar nonketotic coma, impaired wound healing and immune function, premature death and increased susceptibility to infections (Butler et al., 2005). Therefore it is necessary to reduce hyperglycemia in order to decrease the onset and progression of various complications.
1.6. **Prevalence of Diabetes Mellitus**

The Indian Council of Medical Research (ICMR) conducted a study which revealed that in Northern India, Chandigarh (0.12 million), Jharkhand (0.96 million), Maharashtra (9.2 million) and Tamilnadu (4.8 million) people are affected with DM (Kaveeshwar and Cornwall, 2014). In India it is predicted that nearly 79.4 million individuals will be affected with DM by 2030. Of which Type II diabetes is more prevalent form all over the world.

The combination of genetic factors (impaired insulin secretion and insulin resistance) and environmental factors (obesity, over eating, lack of exercise, stress and aging) is the major cause for type II diabetes (Kaku, 2010). Type II diabetes is due to progressive insulin secretory defect, insulin resistance in the liver and skeletal muscle, increased glucose production in the liver and over production of free fatty acids by fat cells (Loghmani, 2005).

1.7. **Hypoglycemic agents**

There are several synthetic drugs like biguanides, sulfonylurea, insulin, insulin analogues, meglitinides, thiaazolidinediones, alpha glucosidase inhibitors, GLP-1 inhibitors, pramlintide and DPP-4 (Loghmani, 2005) inhibitors are available but due to their high cost and side effects (Table 1), traditional treatment with plants having medicinal potential becomes an alternative option for health conscious and for financially deprived populations.

1.8. **Mode of action of medicinal plants on Diabetes Mellitus**

The antidiabetic activity of plants is due to phytoconstituents like polysaccharides, alkaloids, peptides, glycopeptides, triterpenoids, aminoacids, steroids, xanthone, flavonoids, lipids, phenols, coumarins, iridoids, alkyl
disulphides and guanidines (Jarald et al., 2008). These phytoconstituents may have different sites of action and different mechanism of action within the body, including the mechanism of actions of synthetic oral hypoglycemic drugs. Based on its mode of action herbal medicines can be classified into drugs acting like insulin, drugs acting on insulin secreting beta cells, drugs modifying glucose utilization, drugs showing adrenomimeticism, pancreatic beta cell potassium channel blockers, cAMP stimulators, renal glucose resorption inhibitors, herbal drugs providing certain necessary elements like calcium, zinc, magnesium, manganese and copper for the beta cells, drugs regenerating or repairing pancreatic beta cells, effectors of size and number of cells in the islets of langerhans, glycogenesis and hepatic glycolysis stimulators, drugs preventing pathological conversion of starch to glucose by inhibition of beta galactosidase and alpha amylase and drugs preventing oxidative stress that is involved in pancreatic beta cell dysfunction (Arora et al., 2013). The insulin secretion in normal beta cell can be controlled by six possible sites of action (Table 2) (Henquin, 2004).

1.9. Plants as antioxidants

More than 1000 plant species have been reported as folk medicine for diabetes due to restricted access to health care system (Socaciu et al., 2012; Noor et al., 2013), of which only a small number have received scientific and medical evaluation to assess their efficacy. These plants are known for their perceived effectiveness, fewer side effects, acceptability and low cost (Rao et al., 2010; Kumar et al., 2011).

Many hypoglycemic compounds are known to have antioxidant activities (Socaciu et al., 2012). Oxygen free radicals such as O$_2^-$, H$_2$O$_2$ and OH can induce tissue damage due to peroxidation to biomembranes that leads
to several diseases such as autoimmune disease, arthritis, ageing, diabetes, cataract, inflammatory disease, neurodegenerative disease, retinopathy and rheumatism (Milos et al., 2006; Singh et al., 2013). Antioxidants can scavenge ROS by inhibiting their formation, by binding transition metal ions, preventing the formation of OH, by repairing damage or combination of above. Plants having vitamins, flavonoids, flavonones, anthocyanins and polyphenol are reported to have antioxidant activity (Sharma and Gupta, 2006).

1.10. Plants with pro-healing effects

Due to the increased resistance of microorganisms to antimicrobial drugs, it become necessary to search for new cost effective antimicrobial drugs either natural or synthetic (Hofling et al., 2010). The plants secondary metabolite serves as defense mechanism against predation by microorganisms, insects and herbivores. Terpenoids gives plant their odors, quinones and tannins are responsible for plant pigment, simple phenols, terpenoids, essential oil, alkaloids, lectin, polypeptides and phenolic acids are shown to be toxic to microorganisms (Cowan, 1999).

Phytoalexins are antimicrobial compounds in which after elicitation of biosynthetic pathway requires the de novo expression of the enzymes. Low molecular weight antimicrobial compounds that are produced after infection are called phytoanticipin. They both are used as antimicrobial agents in human medicine (Bouarab et al., 2009).

Diabetic foot disease is most common complications of diabetes and it is the leading cause for hospital admission among patients in India. Any break in the cutaneous barrier, extending through the full thickness of the dermis is called diabetic foot wound. The main goal involved in this disease is wound
healing and tissue repair. Plant products such as extracts, vitamins and minerals either phytoalexins or phytoanticipin are known to possess pro-healing effects. They encourage blood clotting, fight infection and accelerate the wound healing (Viswanathan et al., 2011). The new hypoglycemic agents derived from plants not only reduce blood glucose but also improve secondary complications of diabetes.

1.11. **Plant extracts in synthesizing NPs**

Due to its versatility, Nanotechnology has been highly used in various applications. Nanoparticles are used to treat various diseases including Diabetes Mellitus. Due to nanoparticles variable size, shape, chemical composition and controlled dispersity, it is gaining importance in drug discovery and tissue engineering. Nanoparticles can be produced through chemical, physical and biological methods. However, biological method (plant extracts) for synthesizing nanoparticles is an ecofriendly method (Daisy and Saipriya, 2012).

Particle with a size less than 100nm are called nanoparticles. Nobel metals such as silver, gold and platinum are applied in electronic, magnetic and optoelectronic field. One such metal is silver which is harder than gold, act as antimicrobial agent and silver colloids are used to treat various diseases in medicine (Sengottaiyan et al., 2015; Das et al., 2014). They are also used in shampoos, soaps, detergents, toothpastes, cosmetics and pharmaceutical products and hence it can be come into contact with human system (Das et al., 2014) and has good conductivity, chemical stability, catalytic activity, anti-inflammatory, antifungal and antiviral activities (Ikram et al., 2016).
Plant mediated synthesized NPs are biodegradable, non-toxic and biocompatible that show quick action by entering into cell membrane and act as an alternative system of herbal medicine to treat diabetes (Kalakotla et al., 2015).

With this background, the present research study has been initiated with an overall objective to analyze the possible effects of crude extracts of C. anisata and extract mediated synthesized Nanoparticles for its antioxidant, antibacterial and hypoglycemic activity under \textit{in vitro} and \textit{in vivo} conditions. Histopathological study was also carried out on pancreatic tissue of experimental rats to analyse the possible mechanism for hypoglycemic activity.

\textbf{1.12 AIM AND OBJECTIVES}

As per traditional claims, \textit{C.anisata} was reported to possess insulin stimulating activity. Eventhough the plant is explored for antidiabetic activity but no reports are available on the mechanisms of their hypoglycemic effects. Hence based on the traditional indication, the present study was aimed to evaluate their effects on glucose adsorption, diffusion and glucose uptake by yeast cells using \textit{in vitro} and \textit{in vivo} techniques that will elucidate the plausible mechanism of its activity. The antioxidant, antibacterial and hypoglycemic activity of ethanolic SNP extracts (leaf and root) of \textit{C. anisata} has not been reported till date under \textit{in vitro} and \textit{in vivo} conditions. Evaluating their potential will provide an effective utilization as therapeutic agents for Type II diabetes.
In this context, the present study has been taken up with the following objectives:

1. To carry out phytochemical screening of leaf and root extracts obtained by using solvents of varying polarities such as hexane, chloroform, ethylacetate, acetone, ethanol and aqueous.

2. To study the DPPH – free radical scavenging activity, antibacterial activity, *in vitro* hypoglycemic effects such as α-amylase inhibition assay and glucose uptake by yeast cell assay for the obtained leaf and root extracts.

3. To evaluate *in vitro* hypoglycemic effects for ethanolic leaf and root extracts of *C. anisata* – Glucose diffusion inhibitory assay and glucose adsorption assay.

4. To optimize and biosynthesis SNPs from ethanolic leaf and root extracts.

5. To characterize SNPs obtained from ethanolic leaf and root extracts.

6. To evaluate *in vitro* hypoglycemic activity, DPPH-free radical scavenging assay and antibacterial activity of ethanolic SNP leaf and root extracts.

7. Hypoglycemic study under *in vivo* using Wistar strains of albino rats.

8. Histopathological studies on pancreas.