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INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder that affects human body in terms of physical, psychological and social health. It is defined as a group of disorders characterized by hyperglycaemia, altered metabolism of lipids, carbohydrates and proteins (Patel *et al*., 2012a and Warjeet, 2011). Inappropriate hyperglycaemia is caused by a relative or absolute deficiency of insulin or by resistance to the action of insulin at the cellular level (Revathi *et al*., 2014)

Diabetes is often linked with the development of micro and macro vascular diseases which include long term damage, dysfunction and failure of various organs especially the eye, nerves, kidney, heart and blood vessels (Amreen *et al.,* 2012) The complications related to diabetes pose a significant health care burden and a deterrent to overall quality of life (Viswanathan *et al*., 2013).

The prevalence of Diabetes Mellitus is increasing throughout the world, especially in developing countries, including India due to changing lifestyles of people and genetic background (Dhanwal *et al*., 2014).India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the “Diabetes capital of the world” (Das *et al*., 2015). According to International Diabetic Federation, (2013) Type 2 Diabetes is one of the most significant global health problems of modern time; more than 317 million people have been diagnosed with diabetes and about 187 million are living undiagnosed. Recently published Indian Council of Medical Research–India Diabetes (ICMR–INDIAB) national study reported that there are 62.4 million people with Type 2 Diabetes and 77 million people with pre-diabetes in India (Anjana *et al*., 2011).

Diabetes is a complex, chronic illness requiring continuous medical care with multifactorial risk-reduction strategies beyond glycaemic control (American Diabetes Association, 2015). Progress in understanding the metabolic staging of diabetes over the past few years has led to significant advances in the regimen for treatment of this devastating disease. Management of diabetes without any side effects is still a challenge for medical system (Patil *et al*., 2011).

The primary goal of the management of Diabetes Mellitus is to maintain normal blood glucose level (Ramachandran *et al.,* 2010*).* Different methods are available for treatment of diabetes such as diet, exercise and medications. The treatment of DM is based on parenteral insulin and oral antidiabetic drugs. Oral hypoglycaemic agents include sulphonylureas, biguanides, and other drugs like acarbose (Wadkar *et al*., 2008).

Enzyme inhibitors can be a potential target in many areas of disease control and treatment, as enzymes catalyze the most important biochemical pathways. Controlled kinetics of carbohydrate digestion and monosaccharide absorption could be of great value in the avoidance of conditions such as diabetes, obesity, hyperlipoproteinaemia and hyperlipidaemia. In this aspect, amylase and glucosidase inhibitors are of particular importance (Alagesan *et al*., 2012).

One of the therapeutic approaches to treat Type 2 Diabetes is to lower the postprandial blood glucose level by inhibition of carbohydrate hydrolyzing enzyme such as alpha amylase (Gopinath *et al.*, 2013). Pancreatic α-amylase enzyme plays an important role in early breakdown of complex carbohydrates into simple molecules. Modulation of α-amylase activity affects the utilization of carbohydrates as an energy source and stronger this modulation, more significant is the reduction in the breakdown of complex carbohydrates (Fatemeh, 2014).

Acarbose, voglibose and miglitol are few pharmaceutical glucosidase inhibitors currently in use that have shown considerable value in controlling hyperglycaemia. These synthetic drugs have strong inhibitory effects on both α- amylase and α-glucosidase activities; however, their several side effects have been reported, such as liver disorders, flatulence, abdominal pain, renal tumours and diarrhoea (Fujisawa *et al*., 2005 and Thinkratok *et al*., 2014).

To manage diabetes without any side effect is still a challenge. Therefore, research is been switched towards the natural resources like plants and herbs which possess antidiabetic activities with low or no side effects (Kaur *et al*., 2013). The evaluation of alpha amylase inhibitory activity is not only limited to traditional herbs or spices but also to diverse food extracts (Etxeberria *et al.,* 2012).

Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian literature mention the use of plants in the treatment of various human ailments. India has about 45000 plant species and among them, several

thousands have been claimed to possess medicinal properties (Vadivelan *et al*., 2011). World Health Organization (WHO) study shows that 80% of the world population solely relies on medicinal plants for their primary health care needs (Ngugi *et al.,* 2012).

Herbal treatments have been used in patients with insulin dependent and non-insulin dependent diabetes, diabetic retinopathy, diabetic peripheral neuropathy and other complications of diabetes (Nashte *et al*., 2013).Diabetes has been treated with several medicinal plants or their extract based on the folklore medicine (Malpani and Manjunath, 2013). The mechanism is most often not completely understood and so more and more studies are being conducted to elucidate the mechanism of action of different plants and natural compounds (Cristina *et al.,* 2012).

Oxidative stress (formation of free radicals) is generated due to hyperglycaemic status through both enzymatic and non-enzymatic processes. These free radicals would damage cellular proteins as well as mitochondrial DNA (Brajendra *et al*., 2006).Overproduction of free radicals or reactive oxygen species (ROS) contributes to oxidative stress, that is associated with chronic degenerative diseases, including cancer, coronary artery diseases, hypertension and diabetes (Santharam *et al*., 2015 and Gafrikova *et al*., 2014).Free radicals are formed disproportionately during diabetes due to glucose oxidation and the subsequent oxidative degradation of glycated proteins (Mehta *et al.,* 2006).

Most of the ROS produced are scavenged by endogenous defense system under normo glycaemic status (Gauresh *et al*., 2012). An imbalance between ROS and the inherent antioxidant capacity of the body, direct the use of dietary and or medicinal supplements particularly during the disease attack (Gulcin, 2012). Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells (Nunes *et al*., 2012). They prevent the destruction of β-cells by inhibiting the peroxidation chain reaction and preserve β-cells function preventing diabetes induced ROS formation (Aslan *et al.,* 2010 and Fenercioglu *et al.,* 2010).

Antioxidant compounds can offer a possible solution for curing serious diseases like diabetes, cardiovascular and female reproductive diseases (Gupta *et al.,* 2009). Modern physicians are increasing their use of pure natural antioxidants extracted from plants to treat many important common diseases due to their proven ability to restrain specific enzymes, to stimulate a number of hormones and neurotransmitters and to scavenge free radicals (Asif, 2015)

Plant secondary metabolites are important sources of many food ingredients and phytochemicals (Doss *et al.,* 2012). Plants produce several secondary metabolite compounds including phenols, alkaloids, cyanogenic glycosides, glucosinolates, flavonoids, saponins, steroids and terpenoids to protect themselves from the attack of naturally occurring pathogens, insect pests and environmental stresses (Kumar *et al.,* 2009 and Premkumar *et al.,* 2011).

Phytochemicals have various health benefits such as antioxidant, anti-microbial, antiinflammatory, cancer preventive, antidiabetic and antihypertensive effects (Savithramma *et al*., 2011). They are present in virtually all of the fruits, leaves, vegetables, legumes (beans and peas) and grains (Bamishaiye *et al*., 2011).Complementary and alternative approaches to diabetes management such as isolation of phytochemicals with anti-hyperglycemic activities from medicinal plants are therefore imperative (Elekofehinti, 2015).

In vitro methods play an important role for the pre clinical studies for any activity that may support *in vivo* studies (Sathish *et al.,* 2011). Animal models of diabetes are greatly useful and advantageous in biomedical studies because they offer promise of new insights into human diabetes (Srinivasan and Ramarao, 2007). Identification and quantification of active constituents in the plant material may be useful for proper standardization of herbs and their formulations (Sushma *et al.,* 2013).There is a need for isolation, characterization, determination of bioactivity of the lead compound for its pharmaceutical exploitation (Mariswamy *et al.*, 2011).

Structure-based computational methods, including molecular docking, have increasingly been used in the study of biomolecular structure and function, as well as in the design of structure-based rational drugs. In particular, molecular docking contributes to the development of several inhibitors and inhibitor candidates that have been advanced to clinical trials (Kufareva and Abagyan, 2008; Torktaz *et al*., 2013 and Zhang *et a*l., 2014).

Natural products from plants are excellent sources of human pancreatic alpha amylase inhibitors. Computational molecular docking of natural products with interesting biological properties and structural diversity have often served as valuable lead drug candidates for the treatment of human diseases and also they replace the chemically synthesized drugs which cause side effects (Maanvizhi *et al*.,2014).

Momordica charantia L., belonging to the Cucurbitaceae family, also known as bitter gourd, bitter melon, karela and paharkai in Tamil is an economically important medicinal plant (Dipesh *et al*., 2015). It has been widely consumed as a vegetable and also used as herbal medicine particularly for diabetic patients, in India, Asia and South America. Bitter gourd has received growing attention among all vegetable crops nowadays because it contains an abundance of phytochemicals that is associated with antioxidants, antidiabetic, anti microbial, anticancer and anti hypertensive properties (Saeed *et a*l., 2010).

Momordica charantia is a powerful nutrient-dense plant composed of bioactive chemicals, vitamins, minerals and antioxidants all of which contribute to its remarkable versatility in treating a wide range of illnesses (Joseph and Jini, 2013 and Bakare and Magbagbeola, 2010). The plant contains hypoglycaemic or insulin-like principle designated as plant insulin which has been found highly beneficial in lowering the blood and urine sugar levels. Bitter and non-bitter cucurbitane triterpene aglycones and glycosides have been isolated from the plant. *Momordica charantia* is a good source of natural antioxidants (Amira *et al.,* 2013).

Momordica charantia seeds possess potent antioxidant activity, which may be directly or indirectly responsible for their hypoglycaemic property (Sathishsekar and Subramanian 2005). *Momordica charantia* increases the glucose uptake in liver by promoting glucose -6- phosphate dehydrogenase activity and declining glucose -6- phosphatase activities. In addition, it could also increase the mRNA expression of Glucose transporter 4 (GLUT4) proteins in skeletal muscles (Mahomoodally *et al*., 2007).

Bitter gourd reduces the amount of glucose that is released into blood by inhibiting the enzyme which breakdown disaccharides into monosaccharides. The blood glucose lowering effects of *Momordica charantia* were closely associated with its inhibitory activity against disaccharidase (Oishi *et al.*, 2007). The exact ingredients responsible for the hypoglycemic effect and the underlying molecular mechanisms of their actions have not been systematically investigated (Cheng *et al.*, 2008).

Trigonella foenum graecum also known as fenugreek and vendayam in Tamil is an annual leguminous bean and belongs to Fabaceae family (Devasena *et al*., 2014). Its seeds and green leaves used as food posses medicinal applications and is an old practice of human history (Thomas *et al*., 2011, Parildar *et al.,* 2011 and Vaidya *et al*., 2013).

Latest research reports indicate that fenugreek possesses immunomodulatory, anti-carcinogenic, anthelmintic, anti-nociceptive, antioxidant, anti-microbial, anti-ulcer, gastro- and hepatoprotective, anti-obesity, anti-hyperglycemic, antidiabetic and hypocholesterolemic effects (Kumar *et al.*, 2013). Leguminous plants are a rich source of proteins and peptides that are involved in plant defense, including proteinaceous amylase inhibitors (Khan, 2011).

The herb has an enormous potential to prevent or cure diabetes more than other plant species especially due to the presence of unique chemical constituents including quercetin, diosgenin, trignolline, galactomanin and unusual amino acid 4- hydroxy isoleucine. However, due to lack of enough scientific or clinical studies the use of fenugreek as hypoglycaemic official drug remains to be explored (Laila and Murtaza, 2015).

A huge challenge lies in developing new approaches for treating diabetes. Pancreatic alpha amylase inhibitors might be used for the design of novel functional foods with blood-glucose-lowering potential, which could be used as a complement of other antidiabetic drugs. Research work should be focused mainly on the isolation of the principal active compounds and more clinical studies are essential in order to draw concise conclusions regarding the safety and efficacy of acute and long- term administration of the extracts and their bioactive compounds in Type 2 diabetic patients (Etxeberria *et al*., 2012).

Even though numerous scientific articles have reported the hypoglycaemic and pharmacological properties of these two edible plant sources, the constituents responsible for glucose lowering activity and their mechanisms are still not fully understood. Hence, the present research work was initiated to evaluate the antidiabetic potential of alpha amylase inhibitory activity of locally available edible plant sources namely *Momordica charantia* and *Trigonella foenum-graecum.* The present investigation was designed with the following objectives:

- *In vitro* alpha amylase inhibitory and antioxidant activities of *Momordica charantia* and *Trigonella foenum graecum*
- Antidiabetic potential of the selected plant extracts on streptozotocin induced diabetic rats
- Phytochemical analysis, isolation and characterization of active principles responsible for alpha amylase inhibition

- *In silico* molecular docking studies of compounds identified with pancreatic alpha amylase enzyme

The study was conducted in four phases. In phase I, the *in vitro* antidiabetic activity of *Momordica charantia* and *Trigonella foenum graecum* were studied by assessing alpha amylase inhibition activity of the selected plant sources. The antioxidant potential of the plant extracts was studied by total antioxidant activity, free radical scavenging assays and inhibition of lipid peroxidation. In phase II, the *in vivo* antidiabetic potential of the plant extracts in streptozotocin induced diabetes rats was studied. In phase III, qualitative and quantitative assessment of selected phytochemicals present in the plant extracts were attempted. Thin layer Chromatography (TLC) was performed for the separation of active compounds and alpha amylase inhibitory activity was studied. High Performance Thin Layer Chromatography (HPTLC), Fourier Transform Infra Red Spectroscopy (FT-IR) and Gas Chromatography Mass Spectrometry (GCMS) analysis were performed to identify the compounds responsible for alpha amylase inhibitory activity. In phase IV, *in silico* molecular docking was performed to study the interaction between the compounds identified with human pancreatic alpha amylase and porcine pancreatic alpha amylase enzymes which might help in understanding the mechanism of antidiabetic action.