IN VITRO α AMYLASE, α GLUCOSIDASE INHIBITORY AND ANTIOXIDANT ACTIVITIES OF *Benincasa hispida* (Thunb.) Cogn. FRUIT AND SEED EXTRACTS

Dissertation submitted to

The Tamil Nadu Dr. M. G. R. Medical University, Chennai

in partial fulfillment of the award of degree of

MASTER OF PHARMACY

(PHARMACOLOGY)

Submitted by

RAVINDRAN S.

Under the guidance of

Mr. A.T. SIVASHANMUGAM, M. Pharm.,

Department of Pharmacology

MARCH – 2010

COLLEGE OF PHARMACY

SRI RAMAKRISHNA INSTITUTE OF PARAMEDICAL SCIENCES

COIMBATORE – 641 044.

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Place: Coimbatore

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LIST OF ABBREVIATIONS

BHFP	:	*Benincasa hispida* Fruit Powder
BHSP	:	*Benincasa hispida* Seed Powder
BHT	:	Butylated hydroxyltoluene
DMSO	:	Dimethyl sulphoxide.
DPPH	:	2,2' diphenyl 1-1- picryly hydrazyl hydrate
EDTA	:	Ethylene diamine tetra acetic acid
IC_{50}	:	50% Inhibitory concentration
mg/dl	:	milligram/decilitre
min	:	Minute
ml	:	Millilitre
NBT	:	Nitro blue tetrazolium
PCE	:	Pyrocatechol equivalents
ROS	:	Reactive oxygen species

rpm	:	Rotations per minute
S.E.M	:	Standard error of mean
TBA	:	Thiobarbituric acid
TCA	:	Trichloroacetic acid
U/L	:	Units/litre
UV	:	Ultra violet
µg	:	Microgram
µL	:	Microlitre
µM	:	Micromolar

CONTENTS

1. INTRODUCTION

The word diabetes was coined by Aretaeus (81–133 CE). The word is taken from Greek diabainein, which means "passing through." because of one of its main symptom is excessive urine production. In 1675, Thomas Willis noted that a diabetic's urine and blood has a sweet taste and added mellitus to the name (Greek mel means "honey") In 1776, it was confirmed that the sweet taste was because of an excess of sugar in the urine and blood.

Diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or a to a combination of both (Deutschlander *et al.*, 2009). It affected about 171 million people worldwide in 2000 and the number is projected to increase to at least 366 million by 2030 (Ali *et al.*, 2006).

One therapeutic approach for treating diabetes is to decrease the post-prandial hyperglycaemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes, a-amylase and a-glucosidase, in the digestive tract. Inhibitors of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise (Rhabasa-

Lhoret and Chiasson *et al,*, 2004). Examples of such inhibitors which are in clinical use are acarbose, miglitol and voglibose (Bailey *et al.*, 2003). Plants continue to play an important role in the treatment of diabetes, particularly in developing countries where most people have limited resources and do not have an access to modern treatment. The increase in demand in industrially-developed countries to use alternative approaches to treat diabetes, such as plant-based medicines, is also due to the side effects associated with the use of insulin and oral hypoglycaemic agents (Marles and Farnsworth, 1994). These authors identified species from 725 genera, 183 families of plants, and another review identified more than 800 plant species as potential treatments for diabetes mellitus (Perez *et al.*, 1998). In another recent review, (Grover *et al.*, 2002) stated that more than 1123 plant species have been used ethnopharmacologically or experimentally to treat symptoms of diabetes. There are more than 200 pure compounds from plant sources that have been reported to show blood glucose lowering activity (Ali *et al.*, 2006).

TYPES OF DIABETES MELLITUS

Diabetes sufferers fall into two broad categories – those with type 1 diabetes (formerly known as "juvenile" or "childhood" diabetes) and those with type 2 (or adult) diabetes. There is also said to be a third form of diabetes known as type 3 or gestational diabetes but, despite the fact that there are a few differences, this is basically nothing more than type 2 diabetes which occurs during, and because of, pregnancy.

In type 1 diabetes sufferers develop a problem with the insulin producing beta-cells of the pancreas and are unable to produce sufficient insulin to transfer glucose from the bloodstream to the cells of the body. This means that it is necessary to closely monitor levels in the blood and to administer insulin so that glucose can be transferred and the glucose levels in the blood returned to normal.

In type 1 diabetes the beta cell destruction is caused by the free radicals of oxygen generated by cytotoxic T lymphocytes and macrophages.

Symptoms of type 1 diabetes includes increased blood sugar, excessive thirst, frequent urination, increased appetite, weight loss, poor wound healing and blurred vision.

In type 2 diabetes the body usually continues to produce insulin normally but the body's cells develop a resistant to it and insulin levels begin to increase in the blood. In type 2 diabetes, insulin resistance includes decreased stimulation of muscle glycogen synthesis and hexokinase activity. It is believed that most subjects developing type 2 diabetes pass through a phase of impaired glucose tolerance (Yoshikawa *et al.*, 2009).

Symptoms of type 2 diabetes include polydipsia, polyphagia, polyuria, weight loss, dry mouth, itchy skin, fatigue, impotence, recurrent infections etc (Frier and Fischer, 2006).

Gestational diabetes is a form of diabetes which affects pregnant women. It is believed that the hormones produced during pregnancy reduce a woman's receptivity to insulin, leading to high blood sugar levels.

MECHANISMS OF BLOOD SUGAR REGULATION

Blood sugar levels are regulated by negative feedback in order to keep the body in homeostasis. The levels of glucose in the blood are monitored by the cells in the pancreas Islets of Langerhans. If the blood glucose level falls to dangerous levels (as in very heavy exercise or lack of food for extended periods), the Alpha cells of the pancreas release glucagon, a hormone whose effects on liver cells act to increase blood glucose levels. They convert glycogen into glucose (this process is called glycogenolysis). The glucose is released into the bloodstream, increasing blood sugar levels.

There are also several other causes for an increase in blood sugar levels. Among them are the 'stress' hormones such as adrenaline, several of the steroids, infections, trauma, and of course, the ingestion of food.

When levels of blood sugar rise, whether as a result of glycogen conversion, or from digestion of a meal, a different hormone is released from beta cells found in the Islets of Langerhans in the pancreas. This hormone, insulin, causes the liver to convert more glucose into glycogen (this process is called glycogenesis), and to force about 2/3 of body cells (primarily muscle and fat tissue cells) to take up glucose from the blood,

thus decreasing blood sugar. Insulin also provides signals to several other body systems, and is the chief regulatory metabolic control in humans.

Diabetes mellitus type 1 is caused by insufficient or non-existent production of insulin, while type 2 is primarily due to a decreased response to insulin in the tissues of the body (insulin resistance). Both types of diabetes, if untreated, result in too much glucose remaining in the blood (hyperglycemia) and many of the same complications. Also, too much insulin and/or exercise without enough corresponding food intake in diabetics can result in low blood sugar (hypoglycemia).

ROLE OF INSULIN

Insulin secretion has been through the following process. Glucose enters B cells by GLUT 2 transporters. It is phosphorylated and metabolized to pyruvate (Pyr) in the cytoplasm. Pyruvate enters mitochondria and is metabolized via citric acid cycle. The ATP formed by oxidative phosphorylation inhibits ATP-sensitive K^+ channels, reducing K^+ efflux. This depolarises the B cell, and Ca^{2+} influx is increased. The Ca^{2+} stimulates release of insulin by exocytosis. Glutamate (Glu) is also formed and this primes secretory granules preparing them for exocytosis (Ganong, 2003).

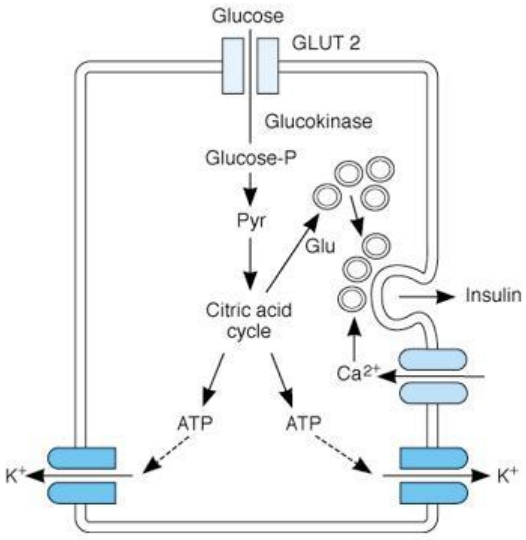

Fig. 1: Insulin secretion

The discovery of insulin is appropriately attributed to Banting and Best. Insulin consists of two peptide chains (A and B, of 21 and 30 amino acid residues, respectively) with two disulphide linkages. Insulin is required for the glucose to enter into the cells. Insulin is a hormone secreted by the Islets of Langerhans in pancreas and its main role is to transport the glucose from the blood to the different cells of the body. If the pancreas doesn't produce sufficient amount of insulin or the produced insulin does not work properly, the glucose can't enter into the body cells and hence glucose stays in the blood and cause high blood sugar level leading to diabetes. Hence body loses its main source of fuel for energy; even though blood contains a huge amount of glucose. The high level blood glucose for a longer period causes many complications to different systems of the body. Insulin is a member of a family of related peptides termed *insulin-like growth factors* (IGFs). IGFs are produced in many tissues, and they may serve a more important function in the regulation of growth than in the regulation of metabolism (Frier and Fisher, 2006).

ENZYMES INVOLVED IN CARBOHYDRATE METABOLISM

Glucose and fructose can be transported out in the intestinal lumen into the blood stream after hydrolysis of the glucosidic bonds in digestable food containing starch. Complex starches must be broken down into individual monosaccharides before being into the duodenum and the upper part of the jejunum. The digestion is facilitated by enteric enzymes,

including a-amylase and a- glucosidases that are attached to the brush border of the intestinal cells (Ortiz-Andrade *et al.*, 2006).

ALPHA GLUCOSIDASE INHIBITORS (AGIs)

a-glucosidase and a-amylase are the key enzymes involved in the metabolism of carbohydrates. a-amylase degrades complex dietary carbohydrates to oligosaccharides and disaccharides, which are ultimately converted into monosaccharides by a-glucosidases. Liberated glucose is then absorbed by the gut and results in postprandial hyperglycaemia. Inhibition of intestinal a-glucosidases limit postprandial glucose levels by delaying the process of carbohydrate hydrolysis and absorption, making such inhibitors useful in the management of type 2 diabetes (Shinde *et al.*, 2008).

AGIs are among the available glucose-lowering medications. The a-glucosidase (systematic name: a-D-glucoside glucohydrolase) is an enzyme of the intestinal brush border that hydrolyses terminal, nonreducing 1,4-linked a-D-glucose residues and releases a-D-glucose. Inhibition of a-glucosidase diminishes glucose resorption and postprandial hyperglyacemia. While a-glucosidase catalyses the exohydrolysis of oligosaccharides, a related enzyme a-amylase (systematic name: 1,4-a-D-glucan glucanohydrolase) catalyses the endohydrolysis of 1,4- a-D-glucosidic linkages in polysaccharides with 3 or more 1,4- a-linked D-glucose units (Schafer *et al.*, 2006). The AGIs delay, but do not prevent, the absorption of ingested carbohydrates, reducing the postprandial glucose

and insulin peaks (Andrade-Cetto *et al.*, 2007).

AGIs like acarbose and miglitol are competitive inhibitors of intestinal a-glucosidases and reduces the postprandial digestion and absorption of starch and disaccharides (Ortiz-Andrade *et al.*, 2006). AGIs slow down the digestion and absorption of dietary carbohydrates either by means of dietary manipulation or by intestinal a-glucosidase inhibitory drugs like acarbose, Voglibose or Miglitol. These drugs have shown promise in reducing postprandial hyperglycaemia, hyperinsulinemia and burden of postprandial oxidative stress (Rao *et al.*, 2009). Furthermore, AGIs also offer other benefits like, reducing triglyceride levels and postprandial insulin levels. Targeting postprandial hyperglycemia may further provide advantages as it has been linked to cardiovascular mortality (Babu *et al.*, 2004).

ALPHA AMYLASE INHIBITORS (AAIs)

Starch is a polymer of glucose (polysaccharide) that is relatively insoluble. There are many enzymes in the human digestive system that help in the digestion of food (Rang *et al.*, 1999). a-amylase catalyses the breakdown of starch to maltose and finally to glucose, which is the only sugar that can be utilized by the body. (Kotowaroo *et al.*, 2005)

a-amylase (systematic name: 1,4-a-D-glucan glucanohydrolase) catalyses the endohydrolysis of 1,4- a-D-glucosidic linkages in

polysaccharides with 3 or more 1,4- a-linked D-glucose units (Schafer *et al.*, 2006).

AAIs play a major role in managing postprandial hyperglycaemia in diabetic patients. These drugs inhibit the action of the amylase enzyme leading to the reduction of the starch hydrolysis, which shows beneficial effects on glycaemic index. The known glucosidase inhibitors used in managing diabetes are acarbose and miglitol competitively and reversibly inhibits a- glucosidase enzyme from intestine as well as pancreas, but both of these drugs has the side effects such as abdominal pain, flatulence and diarrhoea. Hence there is the necessity of a natural enzyme inhibitor with high potential without any side effects (Bhat *et al.*, 2008).

ROLE OF FREE RADICALS IN DIABETES MELLITUS

The major cause of diabetes is **oxidative stress** induced hyperglycaemia which leads to reactive oxygen species (ROS) formation from a oxidative phosphorylation, glucose auto-oxidation, NAD(P)H oxidase, lipooxygenase, cytochrome P450 monooxygenase and nitric oxide synthase. Besides ROS, there are reactive nitrogen species (RNS) which causes **nitrosative stress** on diabetes patients; and also xanthine oxidase (XO) is proposed to be the major source of reactive oxygen species (ROS). ROS and RNS production depletes enzymatic and nonenzymatic antioxidants causing cellular damage to the more susceptible tissues like kidney, liver and and erythrocytes (Valko *et al.*, 2006). Glucose auto-oxidation in hyperglycaemia generates ROS, which

causes chronic oxidative stress, thereby depleting the antioxidant defense system. It also leads to an overdrive of electron transport chain, resulting in the overproduction of superoxide anions which are scavenged normally by mitochondrial superoxide dismutase. (Rachchh *et al.*, 2008)

Diabetic hyperglycaemia causes a variety of pathological changes in small vessels, arteries and peripheral nerves. Vascular endothelial cells become partially vulnerable targets of hyperglycaemic damage as glucose continuously flows through them. Hyperglycaemia increases the production of reactive oxygen species (ROS) inside the aortic endothelial cells and oxidative stress acts as a contributing factor in the pathogenesis of diabetes. Diabetes increases the production of tissue damaging reactive oxygen species (ROS) by glucose auto-oxidation and nonenzymatic protein glycosylation. (Tiwari & Rao, 2002)

For the normal functioning of the beta cells of the pancreas active oxygen metabolism is required. Long term exposure of pancreatic β cells to high glucose levels can cause β cell dysfunction, while the consequent production of ROS potentially suppresses the insulin gene promoter their by impairing the insulin synthesis and its secretion. Oxidative stress is not itself a disease but a condition that can lead to or accelerate disease. Oxidative stress occurs when the available supply of the body's antioxidants is insufficient to handle and neutralize free radicals of different types. The result is massive cell damage that can result in cellular mutations, tissue breakdown and immune compromise. Free radicals are formed from the

molecules through the breakage of the chemical bonds and by collision of the non radical species by a reaction between a radical and a molecule. Free radicals react with DNA bases enhancing base alterations causing mutations and DNA rearrangements (Aruoma *et al.*, 2007).

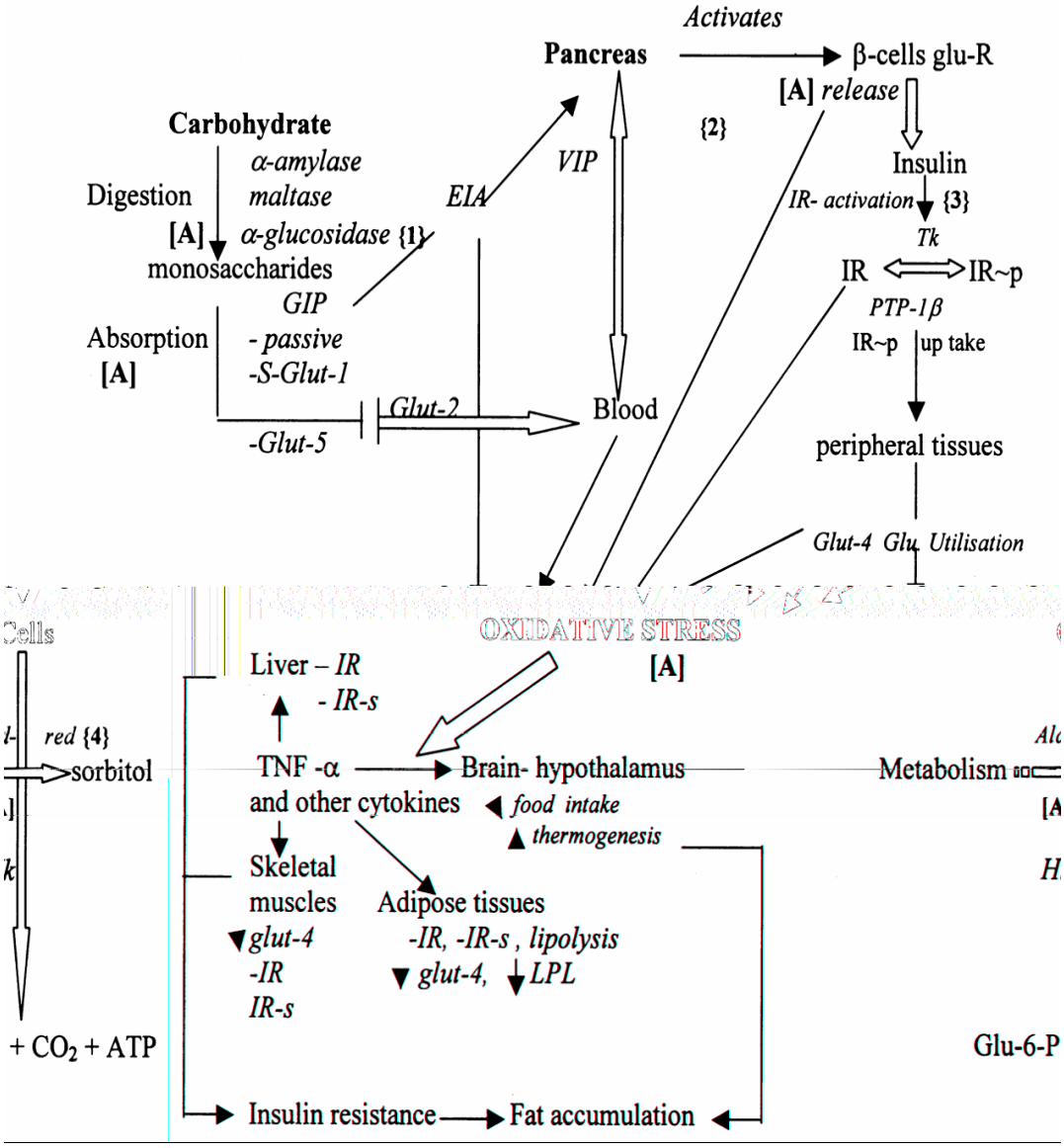

Fig. 2: Pathways of carbohydrate metabolism and targets where imbalance leads to hyperglycaemia and resultant diabetic syndrome

(Tiwari & Rao, 2002)

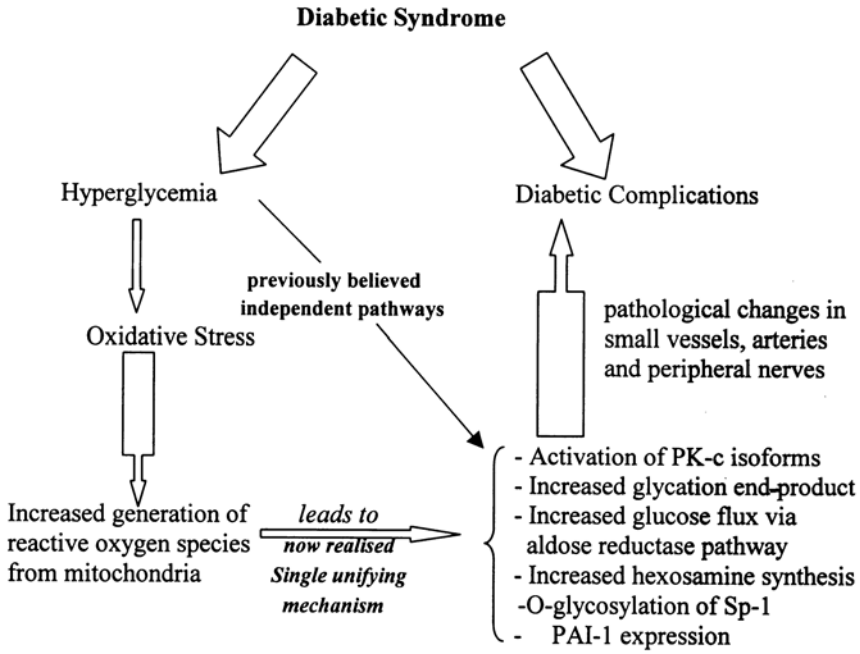

Fig. 3 Single unifying mechanism of oxidative stress due to persistent hyperglycaemia, which leads to overt generation of ROS in mitochondria (Tiwari & Rao, 2002)

ROLE OF ANTIOXIDANTS DEFENSE SYSTEM

Antioxidants are substances when present at lower concentrations compared with those of an oxidisable substrate significantly delays or prevents oxidation of the substrate. The harmful effect of free radicals causing potential biological damage is called as oxidative stress and nitrosative stress. This occurs in biological system when there is imbalance in physiological homeostasis i.e. ROS/RNS over production and deficiency to enzymatic and nonenzymatic antioxidants. The balance between harmful and beneficial effects of free radical in living organisms is

maintained by "redox regulation" which protects various oxidative stresses and maintains redox homeostasis. Pathogenesis of various neurodegenerative disorders, cancer, ischemia / reperfusion injury, inflammatory diseases and ageing are due to the involvement of cellular oxidants The human body has several mechanisms to counteract the oxidative stress by producing antioxidants, which are either naturally produced or externally supplied through foods. Endogeneous and exogeneous antioxidants act as free radical scavengers by preventing and repairing the damages caused by the ROS and RNS (Huy *et al*, 2008). Use of antioxidants provides therapeutic benefits in diabetes related endothelial dysfunction as well as oxidative damage to the pancreatic cells (Gokce and Haznedaroglu, 2008).

ETHNOBOTANICAL HERBS

During the past decade, traditional systems of medicine have become a topic of global importance. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in the developing countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Concurrently many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs.

Most of the drugs which are now available are expensive and also precipitate adverse effects such as hypoglycaemia, obesity, etc. Diabetes management without any toxic side effect is a challenge in diabetic research. So there exists a need for a thorough research on more effective and safer hypoglycaemic agents. Investigations on antidiabetic agents derived from medicinal plants have gained momentum, as recommended by WHO for research on medicinal plants containing hypoglycaemic activity (Sharma *et al.,* 2007). Numerous medicinal plants have been reported in the folkloric history worldwide for the treatment of diabetes. Traditional ethno botanicals provide novel leads to diabetic research due to rapidly increasing interest in finding newer medicines with better efficacy and safety. Research is carried out in traditional herbs to prove its described activity with the focus on evidence based traditional medicine (Verpoorte, 2007).

The plants are capable of synthesizing various secondary metabolites with varied antioxidant potential which can probably afford protection against the molecular damage induced by ROS. Various indigenous medicinal plants have been used in the treatment of diabetes from time immemorial and WHO Expert committee 1980 has also recommended attention towards plants in investigating the traditional methods of diabetes mellitus. (Sridevi *et al.*, 2008)

Our country is proud to have a rich and varied source of traditional medicine and it is each one of our researchers judicious mind that should

be prepared to study the potential of this knowledge in the chain of drug discovery process. Our country also has a rich herbal and medicinal plant wealth with suitable geographical climatic conditions and has well documented practical knowledge for the traditional herbal medicines (Grover *et al.*, 2002). Belief in the curative powers of the plants rested exclusively upon the traditional knowledge *i.e*, empirical information not subjected to critical examination.

Herbal alternatives are proven to provide symptomatic relief and assist in the prevention of secondary complications. Few herbs are also proven to help in the regeneration of the beta cells improving insulin resistance. Herbal alternatives are necessary due to the inabilities of the current therapies to sustain normoglycaemia and prevent diabetic complications. Due to the enormous costs of the modern medicine rural populations in the developing countries still rely upon the traditional medicines as their primary health care; moreover herbs have the cultural acceptability among them.

PHYTOCHEMICALS IN THE MANAGEMENT OF DIABETES

The side effects from prolonged administration of conventional drugs have necessitated the search for safe and effective alternatives. Also, it has been observed that certain resistant cases of diabetes that do not respond well to conventional drugs often responds well after supplementation with natural remedies.

Many plant extracts and isolated phytochemicals have been

examined for antidiabetic activity. Medicinal plants and herbs are of great importance to the health of individuals and communities. The World Health Organization (WHO) estimates that currently 80% of the world's population uses the botanical medicine for their primary healthcare needs. (Sharma *et al.*,2007). Despite the existence of herbal medicines over many centuries, only relatively small number of plant species has been studied for their application. However, in the recent past, an increasing research evidence is getting accumulated, which clearly indicate the positive role of traditional medicinal plants in the prevention or control of some metabolic disorders like diabetes. The great advantage of these medicinal plants is that these are easily available and have no side effects.

Now the reputation of the herbal medicines is growing globally wider, even though their active compounds are unknown, which is only due to its therapeutic efficacy and safety (Hakkim *et al.*, 2007). Mechanisms of action of most of the indigenous medicinal plants are still unknown and most of the antioxidant herbs have a significant role in the management of diabetes and most of the antidiabetic herbs are reported for its antioxidant activity also.

Herbal remedy, the ancient healing system from India has steadily increased its popularity in the western countries. The botanicals in the Ayurvedic Materia Medica have been proven to be safe and effective, through several hundreds to thousands years of its use. This proves the positive role of traditional medicinal plants in the prevention or control of

some metabolic disorders like diabetes, heart disease and certain types of cancer. The great advantages of these medicinal plants are easily availability and lesser or no side effects (Majeed and Prakash, 2007). Herbal preparation alone or in combination with other hypoglycaemic agents may produce a good therapeutic response in certain resistant individuals were allopathic treatment fails (Ghosh, 2004). Traditional herbs can be supplemented in the dietary management of diabetic patients along with certain non pharmacological measures such as diet, exercise & weight loss (Gayathri and Kannabiran 2008).

PLANT PROFILE

Plant name	:	*Benincasa hispida* (Thunb) Cogn.
Family	:	Cucurbitaceae.
Synonym	:	*Benincasa ceriferae.*

Vernacular names

English	:	Ash gourd, White gourd melon
Tamil	:	Pusanikkai.
Malayalam	:	Kum palam.
Telugu	:	Budidagummadi.
Kannada	:	Budikumbala.
Hindi	:	Petha, Raksa.
Sanskrit	:	Kusamandah.

Biological Source: : Fresh juice and dried seeds of *Benincasa hispida* (Thunb) Cogn.

Part Used : Fruits and seeds.

Distribution

Cultivated throughout India in plains and hills.

Description

A large trailing gourd climbing by means of tendrils. **Leaves** large, hispid beneath. **Flowers** yellow, unisexual, male peduncle 7.5 – 10 cm long, female peduncle shorter. **Fruits** broadly cylindrical, 30 – 45 cm long, hairy throughout, ultimately covered with a waxy bloom. **Seeds** consists of hard hull, thin internal seed coat and cotyledon (Krithikar K.R. and Basu R.D. 1988).

Medicinal uses

The fruits are sweet, cooling, styptic, laxative, diuretic, tonic, aphrodisiac and antiperiodic. They are useful in asthma, cough, diabetes, haemoptysis, hemorrhages from internal organs, epilepsy, fever and

vitiated conditions of pitta. The seeds are cooling and anthelmintic and are useful in dry cough, fever, urethrorrhea, syphilis, hyperdipsia and vitiated conditions of pitta (Krithikar K.R. and Basu, R.D. 1988).

Fig. 4: *Benincasa hispida* (Thunb) Cogn. Fruit and Seeds

2. REVIEW OF LITERATURE

Bhalodia *et al.*, (2009) suggested that reactive oxygen species play a role in the pathophysiology of renal ischemia/reperfusion (I/R) injury. This study was designed to investigate renoprotective activity of methanolic fruit extract of *Benincasa cerifera* in I/R induced kidney failure in rats. Renal I/R caused significant impairment of kidney function. Six-day administration of *Benincasa cerifera* minimized this effect. Rats with renal I/R showed significantly decreased activity of superoxide dismutase, catalase, and reduced glutathione compared with sham operated rats. These declining trends were significantly less in the group treated with *Benincasa cerifera* compared with those in the I/R only group. Renal I/R produced a significant increase in malondialdehyde level, while pretreatment with *Benincasa cerifera* was associated with a significantly lower malondialdehyde level ($P < 0.001$). These findings imply that ROS play a crucial role in I/R-induced kidney injury and *Benincasa cerifera* exerts renoprotective activity probably by the radical scavenging activity.

Rachchh *et al.*, (2008) investigated antiulcer activity of *Benincasa hispida* (Thunb.) Cogn. fruit extract in rats against ethanol-induced gastric mucosal damage, pylorus ligated (PL) gastric ulcers, and cold restraint-stress (CRS)-induced gastric ulcer models. Petroleum ether and methanol extracts were administered orally at 300 mg/kg and omeprazole at the dose of 20 mg/kg. Ulcer index was the common parameter studied in all the models. Vascular permeability was evaluated in ethanol model, effect

on lipid peroxidation, viz. malondialdehyde content, superoxide dismutase and catalase levels were studied in CRS model. Both extracts produced significant reduction in ulcer index ($P < 0.05$) in all the models. Significant reduction in vascular permeability ($P < 0.05$) was observed. In CRS model malondialdehyde content was significantly reduced along with increase in catalase levels as compared to control groups which confirms that petroleum ether and methanol extracts of *Benincasa hispida* possess significant antiulcer activity as well as antioxidant property.

Moon *et al.*, (2008) investigated the mechanism of anti-vascular inflammatory activity of an aqueous extract of *Benincasa hispida* (ABH) in human umbilical vein endothelial cells (HUVECs). The study was performed on HUVECs that were pretreated with various concentrations (1-20 μg/ml) of ABH before exposure with high glucose (25 mM) for 48 h. Cell ELISA and Western blot analysis showed that ABH reduced cell adhesion of U937 monocytes. ABH also inhibited the mRNA expression level of monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8). High glucose induced ROS production was inhibited by treatment of ABH. It was observed that pretreatment with HUVECs with ABH blocks NF-κB activation via blocking phosphorylation and degreadation of its inhibitory protein I κB-α. ABH also reduced NF-κB promoter activity. These results suggested that ABH reduces high glucose-induced CAMs activation by inhibiting monocyte adhesion, ROS, and NF-κB in HUVECs.

Mathad *et al.*, (2005) suggested that the methanolic extract of fruit

extract of *Benincasa hispida* (BHFE) was evaluated for its antidiarrheal potential against several experimental models of diarrhea in rats. BHFE treated animals showed significant inhibitory activity against castor oil induced diarrhea and inhibited PGE2 induced enter pooling in rats. It also showed significant reduction in gastrointestinal motility following charcoal meal in rats. The results confirmed the efficacy of BHFE as an antidiarrheal agent.

Huang *et al.*, (2004) investigated the abilities of antioxidation and inhibition of angiotensin-converting enzyme (ACE) activity of *Benincasa hispida* pulp, core, seed and peel prepared by different extraction methods. The fresh weights required to reach 50% inhibition of linoleic acid oxidation were higher in fresh extracts compared to other extraction methods. Fresh weights required to reach 50% inhibition were the lowest in seed. The seed had the lowest Cu^{2+} -induced low-density lipoprotein (LDL) oxidation percentage and inhibition level of ACE activity among all parts. The higher antioxidant capacity of the seed may result from the higher total phenolic contents and superoxide dismutase activity. The abilities of antioxidation and ACE activity inhibition may provide protective effects against cardiovascular diseases and cancers.

Kumar *et al.*, (2004) investigated the anorectic effect of the methanolic extract of *Benincasa hispida* (MEBH) in Swiss albino mice. Fasted mice were administered with various doses of MEBH (0.2-1 g/kg,i.p.), and the food intake was measured hourly for a period of 7 h. In

another experiment, the percentage of gastric emptying at 4th h was determined after the administration of MEBH (0.2-1 g/kg, i.p.) in different set of mice which had free access to preweighed food for either 1, 2 or 4 h. MEBH significantly reduced the cumulative food intake over a 7 h period in a dose-dependent manner. The percentage reduction of cumulative food intake at 7th h for MEBH with 0.2, 0.6 and 1 g/kg was 27%, 38% and 54% respectively. The 4 h gastric emptying was not significantly influenced by MEBH when compared to control. The present study reveals for the first time a possible anorectic activity of *Benincasa hispida*, most probably mediated through the CNS without affecting the gastric emptying. However, further studies are required to find its potential as an antiobesity agent.

Kumar *et al.*, (2002) studied methanolic extract of *Benincasa hispida* (MEBH) showed excellent protection in guinea pigs against the histamine-induced bronchospasm even at a very low dose, 50 mg/kg, p.o. However, even at higher dose level 400 mg/kg, MEBH did not offer any significant protection against acetylcholine challenge. Therefore, it can be deduced that MEBH is unlikely to have muscarinic action. Thus, the results suggest that the protective effect against bronchospasm induced by histamine aerosol may be mediated by antihistaminic activity (H1 receptor antagonism).

Grover *et al.*, (2001) investigated antiulcerogenic activity of different extracts of *Benincasa hispida* (fresh juice, supernatant and

residue fraction of centrifuged juice, alcoholic and petroleum ether extract). These were studied in aspirin plus restraint, swimming stress, indomethacin plus histamine and serotonin-induced ulcers in rats and mice. The oral feeding of different doses of the extract significantly reduced the ulcer index produced by various ulcerogens. The anti-ulcerogenic effect was dose-dependent in stress induced model of ulcer and not in other models. *Benincasa hispida* probably has a CNS component in prevention of stress induced ulceration. However, antihistaminic, anticholinergic effects and prevention of disturbance in gastric micro-circulation as possible modes of action cannot be ruled out. Thus extracts of *Benincasa hispida* may be considered to be a drug of natural origin possessing anti-ulcer activity.

Deutschländer *et al.*, (2009) investigated four plants for hypoglycemic activity by evaluating inhibiting effects on carbohydrate-hydrolyzing enzymes: a-glucosidase and a-amylase. Acetone plant extracts were screened against C2C12 myocytes, 3T3-L1 preadipocytes and Chang liver cells by measuring glucose uptake. Cytotoxicity was done in preadipocytes and hepatocytes. Extract of *Euclea undulata* root bark exhibited highest activity, displaying a glucose uptake of 162.2% by Chang liver cells at 50mg/ml. An inhibition concentration of 50% for *Euclea undulata* was found to be 49.95 mg/ml for a-glucosidase and 2.8 mg/ml for a-amylase. No cytotoxicity was recorded for *Euclea undulata*, while *Schkuhria pinnata* and *Elaeo dendron transvaalense* exhibited

cytotoxicity at 2.5mg/ml. a-Glucosidase and a-amylase assays showed inhibitory activity on enzymes for three plant extracts. *Euclea undulata, Schkuhria pinnata* and *Elaeo dendron transvaalense* showed *in vitro* hypoglycemic activity. *Schkuhria pinnata* and *Elaeo dendron transvaalense* indicated cytotoxicity on 3T3-L1 preadipocytes and Chang liver cells. *Euclea undulata, Pteronia divaricata* and *Elaeodendron transvaalense* inhibited a-glucosidase and a-amylase enzymes. Screening of plant extracts scientifically validated traditional use of *Euclea undulata* for treatment of diabetes. Cytotoxicity results revealed that acetone extracts of *Schkuhria pinnata* and *Elaeo dendron transvaalense* are toxic and raise concern for chronic use.

Subramanian *et al.*, (2008) worked on *in vitro* a-glucosidase and a-amylase enzyme inhibitory effects of *Andrographis paniculata* extract and *andrographolide.* The extract showed appreciable a-glucosidase inhibitory effect in a concentration-dependent manner and a weak a-amylase inhibitory activity. *Andrographolide* demonstrated a similar a-glucosidase and a-amylase inhibitory activity. The positive *in vitro* enzyme inhibition tests paved way for confirmatory *in vivo* studies. The *in vivo* studies demonstrated that *A. paniculata* extract significantly ($P<0.05$) reduced peak blood glucose and area under curve in diabetic rats when challenged with oral administration of starch and sucrose. Further, *andrographolide* also caused a significant ($P<0.05$) reduction in peak blood glucose and area under the curve in diabetic rats. Hence a-

glucosidase inhibition may possibly be one of the mechanisms for the *A. paniculata* extract to exert antidiabetic activity and indicates that AP extract can be considered as a potential candidate for the management of type 2 diabetes mellitus.

Rao *et al.*, (2009) investigated antihyperglycemic activity of root extracts of *Derris indica* which resulted in isolation and characterization of two new furanoflavanoids (1, 2) along with thirteen known compounds (3–15). Their structures were determined on the basis of extensive spectroscopic (IR, MS, 1D and 2D NMR) data analysis and by comparison with the literature data. All the compounds were tested *in vitro* for intestinal a-glucosidase inhibitory and DPPH radical activity. New compounds (1, 2) displayed moderate intestinal a-glucosidase inhibitory as well as free radical scavenging activity. Other compounds also displayed varying degrees of moderate intestinal a-glucosidase inhibitory activity. Pongamol (6) displayed potent intestinal a-glucosidase inhibition.

Yoshikawa *et al*., (2009) found a-glucosidase inhibitory (a-GI) effect of metal ions and their complexes which showed the high blood glucose lowering effect in diabetic model animals. The Cu(II) ion and its complexes showed strong a-GI activity greater than clinically used acarbose in *in vitro* studies. Furthermore, in *in vivo* experiments, a-GI action was newly discovered in normal ddy mice. These results suggested that one of action mechanisms of the antidiabetic metal ions and complexes is related to the a-GI effects.

Shinde, *et al.*, (2008) evaluated *Syzygiumcumini* seed kernel extracts for the inhibition of a-glucosidase from mammalian (rat intestine), bacterial (*Bacillus stearothermophilus*), and yeast (*Saccharomyces cerevisiae*, baker's yeast). *In vitro* studies using the mammalian a-glucosidase from rat intestine showed the extracts to be more effective in inhibiting maltase when compared to the acarbose control. Since acarbose is inactive against both the bacterial and the yeast enzymes, the extracts were compared to 1-deoxynojirimycin. We found all extracts to be more potent against a-glucosidase derived from *B. stearothermophilus* than that against the enzymes from either baker's yeast or rat intestine. In an *in vivo* study using Goto–Kakizaki (GK) rats, the acetone extract was found to be a potent inhibitor of a-glucosidase hydrolysis of maltose when compared to untreated control animals. Therefore, these results point to the inhibition of a-glucosidase as a possible mechanism by which this herb acts as an antidiabetic agent.

Andrade-Cetto *et al.*, (2007), tested the butanolic extracts of four Mexican plants with respect to their a-glucosidase inhibition activity, without excluding other possible mechanisms of action. The plants *Cecropia obtusifolia* Bertol., *Equisetum myriochaetum* Schlecht Cham, *Acosmium panamense* (Benth.) Yacolev and *Malmea depressa* (Baill) R.E. Fries are used in traditional medicine to treat type 2 diabetes. In previous studies, we have demonstrated these plants hypoglycemic activity and determined the phytochemical composition of their extracts. The results in

n-STZ diabetic rats loaded with maltose showed that *Malmea* and *Acosmium* extracts decreased plasma glucose significantly from 30 min on resembling the effect of acarbose. *Cecropia* extract produced the highest reduction of plasma glucose, and at 90 min, the glucose level was lower than the fasting level, which suggests another mechanism of action. *Equisetum* did not exert any effect. *In vitro* assays of a-glucosidase activity showed an IC50 of 14 mg/ml for *Cecropia*, 21 mg/ml for *Malmea*, and 109 mg/ml for *Acosmium*, which were lower than that of acarbose (128 mg/ml). *Equisetum* did not show any significant effect on this assay, either. These results contribute to understand the mechanism of action of these plants on glucose metabolism.

Ortiz-Andrade *et al.*, (2006), investigated a-glucosidase inhibitory activity of *Tournefortia hartwegiana* (METh) and established one of the possible modes of action of METh to induce antidiabetic activity. METh (310 mg/kg) effect on a-glucosidase activity was investigated. METh intragastric administration was conducted to determine oral glucose tolerance test (OGTT), using different substrates: glucose, sucrose and maltose. The increase in plasma glucose level was significantly suppressed ($P < 0.05$) by the extract after substrates administration. On the other hand, METh inhibited a-glucosidase activity *in vitro*, in a concentration dependent manner (IC50 of 3.16 mg/mL). These results suggest that METh might exert its antidiabetic effect by suppressing carbohydrate absorption

from intestine and thereby reducing the post-prandial increase of blood glucose.

Schäfer *et al.*, (2006) found out that the standardized maritime pine bark extract (Pycnogenol) was reported to exert clinical antidiabetic effects after peroral intake. However, an increased insulin secretion was not observed after administration of the extract to patients. The aim was to elucidate whether the described clinical effects of Pycnogenol are related to inhibition of a-glucosidase. Therefore, the inhibitory activity of Pycnogenol, green tea extract and acarbose towards a-glucosidase was analyzed. Furthermore, he explored different fractions of Pycnogenol containing compounds of diverse molecular masses from polyphenolic monomers, dimers and higher oligomers to uncover which components exhibited the most pronounced inhibitory activity and found that Pycnogenol exhibited the most potent inhibition (IC50 about 5 µg/mL) on a-glucosidase compared to green tea extract (IC50 about 20 µg/mL) and acarbose (IC50 about 1 mg/mL). The inhibitory action of Pycnogenol was stronger in extract fractions containing higher procyanidin oligomers. The results obtained assign a novel, local effect to oligomeric procyanidins and contribute to the explanation of glucose-lowering effects of Pycnogenol observed in clinical trials with diabetic patients.

Babu *et al.*, (2004) reported that methanolic extract of rhizome of Himalayan rhubarb *Rheum emodi* displayed mild yeast as well as mammalian intestinal a-glucosidase inhibitory activity. However, further

fractionation of active extract led to the isolation of several potent molecules in excellent yields, displaying varying degrees of inhibition on two test models of a-glucosidase. Rhapontigenin, desoxyrhapontigenin, chrysophanol-8-O–β-d-glucopyranoside, torachrysone-8-O-β-d-glucopyranoside displayed potent yeast a-glucosidase inhibition. However, chrysophanol-8-O–β-d-glucopyranoside, desoxyrhaponticin and torachrysone-8-O–β-d-glucopyranoside displayed potent to moderate mammalian a-glucosidase inhibitory activity. Other compounds displayed mild activity on both the tests. Except desoxyrhapontigenin and rhapontigenin that increased Vmax, other compounds including crude extract decreased the Vmax significantly ($p<0.02$) in yeast a-glucosidase test. Further kinetic analysis on mammalian a-glucosidase inhibition showed that chrysophanol-8-O–β-d-glucopyranoside, desoxyrhaponticin and torachrysone-8-O-β-d-glucopyranoside may be classified as mixed-noncompetitive inhibitors. However, desoxyrhapontigenin and rhapontigenin may be classified as modulators of enzyme activity. Presence and position of glycoside moiety in compounds appear important for better inhibition of mammalian a-glucosidase. This is the first report assigning particularly, mammalian intestinal a-glucosidase inhibitory activity to these compounds. Chrysophanol-8-O–β-d-glucopyranoside, desoxyrhaponticin, desoxyrhapontigenin and rhapontigenin have been isolated in substantial yields from *R. emodi* for the first time. Therefore, these compounds may have value in the treatment and prevention of hyperglycemia associated diabetes mellitus.

Ali *et al.*, (2006) studied extracts of six selected Malaysian plants with a reputation of usefulness in treating diabetes were examined for a-amylase inhibition using an *in vitro* model. Inhibitory activity studied by two different protocols (with and without pre-incubation) showed that *Phyllanthus amarus* hexane extract had a-amylase inhibitory properties. Hexane and dichloromethane extracts of *Anacardium occidentale*, *Lagerstroemia speciosa*, *Averrhoa bilimbi*, *Pithecellobium jiringa* and *Parkia speciosa* were not active when tested without pre-incubation. Extraction and fractionation of *Phyllanthus amarus* hexane extract led to the isolation of dotriacontanyl docosanoate, triacontanol and a mixture of oleanolic acid and ursolic acid. Dotriacontanyl docosanoate and the mixture of oleanolic acid and ursolic acid are reported from this plant species for the first time. All compounds were tested in the a-amylase inhibition assay and the results revealed that the oleanolic acid and ursolic acid (2:1) mixture was a potent a-amylase inhibitor with IC50 = 2.01 mg/ml (4.41mM) and that it contributes significantly to the a-amylase inhibition activity of the extract. Three pure pentacyclic triterpenoids, oleanolic acid, ursolic acid and lupeol were shown to inhibit a-amylase.

Kotowaroo *et al.*, (2005) investigated seven exotic/indigenous medicinal plants of Mauritius, namely *Coix lacryma-jobi* (Poaceae), *Aegle marmelos* (Rutaceae), *Artocarpus heterophyllus* (Moraceae), *Vangueria madagascariensis* (Rubiaceae), *Azadirachta indica* (Meliaceae), *Eriobotrya japonica* (Rosaceae) and *Syzigium cumini* (Myrtaceae) for

possible effects on starch breakdown by alpha-amylase *in vitro*. The results showed that only *Artocarpus heterophyllus* significantly ($p < 0.05$) inhibited alpha-amylase activity *in vitro*. To confirm the observed effects, a further biochemical assay was undertaken to investigate the effects of *Artocarpus heterophyllus* on alpha-amylase activity using rat plasma *in vitro*. It was found that the aqueous leaf extract significantly ($p < 0.05$) inhibited alpha-amylase activity in rat plasma. The highest inhibitory activity (27.20 +/- 5.00%) was observed at a concentration of 1000 µg/mL. However, in both cases dose dependency was not observed. Enzyme kinetic studies using the Michaelis-Menten and Lineweaver-Burk equations were performed to establish the type of inhibition involved. In the presence of the plant extract the maximal velocity (Vmax) remained constant (1/150 g / L/s) whereas the Michaelis-Menten constant (Km) increased by 5.79 g/L, indicating that the aqueous leaf extract of *Artocarpus heterophyllus* behaved as a competitive inhibitor. Results from the present study tend to indicate that *Artocarpus heterophyllus* could act as a 'starch blocker' thereby reducing post-prandial glucose peaks.

Hubert *et al.*, (2005) reported novel acaricidal compounds with inhibitory effects on the digestive enzymes of arthropods are a safe alternative to the traditional neurotoxic pesticides used for control of the stored-product pests. He explored the properties of acarbose, the low molecular weight inhibitor of a--amylases (AI), as a novel acaricide candidate for protection of the stored products from infestation by *Acarus*

siro (Acari: Acaridae). *In vitro* analysis revealed that AI blocked efficiently the enzymatic activity of digestive amylases of *A. siro* and decreased the physiological capacity of mite's gut in utilizing a starch component of grain flour. *In vivo* experiments showed that AI suppressed the population growth of *A. siro*. The mites were kept for three weeks on experimental diet enriched by AI in concentration range of 0.005 to 0.25%. Population growth of *A. siro* was negatively correlated with the content of AI in the treated diet with a half-population dose of 0.125%.

Tormo *et al.*, (2004) investigated alpha-amylase inhibitory activity of extract of white kidney beans *(Phaseolus vulgaris).* The acute oral administration of the inhibitor (50 mg/kg body weight) to adult Wistar rats together with a starch load (2 g/kg body weight suspended in NaCl (9 g/l)) reduced the increase in glycaemia ov0065r the basal value (NaCl, 222 (SEM 49); inhibitor, 145 (SEM 16) mmol/l £ 180 min; P,0·05) without modifying the insulin response. On administering the inhibitor orally (50 mg/kg body weight dissolved in NaCl (9 g/l)) for 21 d to rats fed on a standard diet, a decline was observed in the glycaemia values on day 0 (NaCl, 5·53 (SEM 0·12); inhibitor, 5·25 (SEM 0·16) mmol/l) relative to those obtained on days 10 (NaCl, 5·00 (SEM 0·14); inhibitor, 4·60 (SEM 0·08) mmol/l; P,0·05) and 21 (NaCl, 5·22 (SEM 0·22); inhibitor, 4·50 (SEM 0·12) mmol/l; P,0·01) of treatment, without modifying the plasma concentration of insulin. There was found to be a significant anorexigenic action of the inhibitor; there was reduced food intake (NaCl,23·07 (SEM 0·31); inhibitor,

19·50 (SEM 0·49) g/d; P,0·01), a reduced weight gain (NaCl, 52 (SEM 3); inhibitor, 21·33 (SEM 8·9) g/21 d; P,0·01), as well as changes in the activity of some intestinal enzymes such as maltase (NaCl, 87 (SEM 7); inhibitor, 127 (SEM 11) U/g proteins; P,0·05). The present study has shown,for the first time, that the prolonged administration of an alpha-amylase inhibitor reduces blood glucose levels and body-weight gain in Wistar rats.

Svensson *et al.*, (2003) reported proteins that inhibit a-amylases have been isolated from plants and microorganisms. These inhibitors can have natural roles in the control of endogenous a-amylase activity or in defense against pathogens and pests; certain inhibitors are reported to be antinutritional factors. The a-amylase inhibitors belong to seven different protein structural families, most of which also contain evolutionary related proteins without inhibitory activity. Two families include bifunctional inhibitors acting both on a-amylases and proteases. High-resolution structures are available of target a-amylases in complex with inhibitors from five families. These structures indicate major diversity but also some similarity in the structural basis of a-amylase inhibition. Mutational analysis of the mechanism of inhibition was performed in a few cases and various protein engineering and biotechnological approaches have been outlined for exploitation of the inhibitory function.

Umamaheswari *et al.*, (2007) evaluated the antiulcer and antioxidant activities of ethanolic extract of leaves of *Jasminum*

grandiflorum L (JGLE). Antiulcerogenic activities of JGLE was evaluated employing aspirin + pylorus ligation (APL) and alcohol (AL) induced acute gastric ulcer models ulcer-healing activity using acetic acid-induced chronic ulcer model in rats. The antioxidant activity of JGLE has been assayed by using *in vitro* methods like DPPH assay, reductive ability, superoxide anion scavenging activity, nitric oxide scavenging activity and total phenolic content. The reduction in gastric fluid volume, total acidity and an increase in the pH of gastric fluid in APL rats proved the antisecretory activity of JGLE. These results suggest that leaves of *Jasminum grandiflorum* possess potential antiulcer activity, which may be attributed to its antioxidant mechanism of action.

Velickovic *et al.*, (2007) performed the extraction of flavonoids from garden (*Salvia officinalis* L.) and glutinous (*Salvia glutinosa* L.) sage by ultrasonic and classical maceration. In that study flavonoids were analysed in the extracts of garden (*Salvia officinalis* L.) and glutinous (*Salvia glutinosa* L.) sage. Ultrasonic extraction (20 minutes at 40°C) and classical maceration (6 h at room temperature) of the extractable substances from dried herbs and dried residual plant materials from which the essential oil had previously been removed by hydrodistillation were performed with petroleum ether, 70 % aqueous solution of ethanol and water. It was found that the extracts from both plants contained flavonoids, apigenin and its derivatives (*e.g.*, apigenin 4'-methyl ether), scutellarein 6-methyl ether, isoscutellarein 8-methyl ether, luteolin and 6-

OH-luteolin-6-methyl ether where distinctive for *S. officinalis*. Apigenin, luteolin, 6-OH-luteolin-6-methyl ether, kaempherol 3-methyl ether, kaempherol 3,7-dimethylether, quercetin 3,7,3'-trimethyl ether and quercetin 3,7,3',4'-tetramethyl ether were distinctive for *S. glutinosa*.

Hsu (2006) reported the antioxidant activities of *Polygonum aviculare* L extract for its free radical scavenging, superoxide radical scavenging, lipid peroxidation and hydroxyl radical induced DNA strand scission assays. All the methods employed in their study proved the antioxidant activity.

Conforti *et al.*, (2005) investigated the biological properties , antioxidant properties and antidiabetic activity of two varieties of *Amaranthus caudatus* seeds, the phenolic contents, oil, squalene were determined. The IC_{50} values were determined for the various extracts and were found to be not significantly different from each other and a amylase inhibitory activity was found to be significant.

Li *et al.*, (2005) investigated the effect and action mechanism of a methanolic extract from the flowering part of *Punica granatum* Linn. (Punicaceae) (PGF) on hyperglycemia *in vivo* and *in vitro*. When orally administered, the PGF extract markedly lowered plasma glucose levels in non-fasted Zucker diabetic fatty rats (a genetic model of obesity and type 2 diabetes), whereas it had little effect in the fasted animals, suggesting it affected postprandial hyperglycemia in type 2 diabetes. Also the extract was found to markedly inhibit the increase of plasma glucose

levels after sucrose loading, but not after glucose loading in mice, and it had no effect on glucose levels in normal mice. *In vitro*, PGF extract demonstrated a potent inhibitory effect on a glucosidase activity. The inhibition is dependent on the concentration of enzyme and substrate, as well as on the length of pretreatment with the enzyme. Hence PGF extract improves postprandial hyperglycemia in type 2 diabetes and obesity, at least in part, by inhibiting intestinal a glucosidase activity.

Mensor *et al.*, (2001) studied the Brazilian plant extracts belonging to 16 species of 5 different families. They were tested against the stable DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) free-radical. The ability to scavenge DPPH radical was measured by the discoloration of the solution. Based on the results, they concluded that ethanol extracts of plants showed lower EC_{50} values than the other plant extracts.

Gomes *et al.*, (2001) tested two natural products *Polypodium leucotomos* extract (PL) and kojic acid (KA) for their ability to scavenge reactive oxygen species. Hydroxy radicals were generated by the Fenton reaction, and the rate constant of scavenging were $1.6x10^9$ $M^{-1}s^{-1}$ for KA and $1.0x10^9$ M^{-1} s^{-1} for PL, similar to that of ethanol ($1.4x10^9$ M^{-1} s^{-1}). With superoxide anion generated by the xanthine/hypoxanthine system, KA and PL (0.2-1.0 mg/ml) inhibited $^{\cdot}O_2$ dependent reduction of nitroblue tetrazolium by up to 30 and 31%, respectively. The present study demonstrates that PL showed an antioxidant effect, scavenging three of four reactive oxygen species tested.

Guzman *et al.*, (2001) studied the anti-inflammatory, analgesic and free radical scavenging activities of hydro soluble and liposoluble extracts of the marine microalgae *Chlorella stigmatophora* and *Phaeodactylum tricornutum*. The hydro soluble components of both species showed significant anti inflammatory, analgesic and free radical scavenging activity. These activities were not detected in the liposoluble fractions.

3. OBJECTIVE AND PLAN OF WORK

Diabetes is probably the most common disease in this century. It has been estimated that over 150 million people worldwide have diabetes and that this will increase to 220 million by 2010 and 300 million by 2025 (Li *et al.*, 2005). These metabolic disturbances result in acute and long term diabetic complications. Free radicals and oxidative stress may act as a common pathway to diabetes as well for later complications. The increased oxidative stress in diabetes includes the auto-oxidation of glucose and nonenzymatic glycation and also changes in antioxidant defense system. Though there are many synthetic drugs available for the treatment of diabetes they all have side effects associated with their uses.

Herbal formulations are considered to be less toxic and more effective and also free from side effects than synthetic ones. Numerous plants are claimed to possess antidiabetic phytoconstituents in folk medicines; however, one among them is *Benincasa hispida* (Thunb) Cogn fruit and seed. Since there is no specific scientific report regarding its use as a hypoglycemic agent, the plant was selected for the study and from the literatures reviewed, it was found that the fruit and seeds have been reported for its mast cell stabilizing effect, diuretic, antiulcer (Rachchh *et al.*, 2008) nootropic, antidepressant and nephroprotective activity against mercury poisoning in rats (Kumar *et al.*, 2004). The seeds have a higher capacity on anti-oxidation and inhibition of angiotensin-converting enzyme (ACE) activity than the fresh fruit (Huang *et al.*, 2004). It may be

due to its high phenolic contents and superoxide dismutase activity (Rachchh *et al.*, 2008).

Review of literatures also urges that, the hypoglycemic activity of the fruit extract has to be evaluated as the plant tissue is constituted by secondary metabolites like flavonoids, terpenes, C-glycosides and sterols which have antioxidant effects (Bhalodia *et al.*, 2009). Review of literatures for the seed extract confirms the presence of active saponins, urea, citrulline, linoleic acid, oleic acid and fatty acids.

The main objective of the present study is to evaluate the hypoglycaemic activity of *Benincasa hispida* (Thunb). Cogn fruits and seeds using various *in vitro* models like a-amylase and a-glucosidase inhibitory effects and antioxidant activity.

The scope of the present study is attributed in exploring the potential of the bioactive compounds from the medicinal plants and in revealing their safety & efficacy, there by realizing the promising ethno botanical herbs, towards the development of phytomedicine.

PLAN OF WORK

I. Collection, authentification, preparation & preliminary phytochemical screening of aqueous extracts of the fruit and seeds of *Benincasa hispida* (Thunb) Cogn.

II. To evaluate the hypoglycaemic activity of *Benincasa hispida* (Thunb) Cogn using *in vitro* models such as,

1. a glucosidase inhibition assay.
2. a amylase inhibition assay.

III. To evaluate the antioxidant activity of *Benincasa hispida* (Thunb) Cogn using *in vitro* models such as,

1. DPPH radical scavenging assay (Hydrogen donating ability)
2. Deoxy ribose degradation assay (Hydroxyl radical scavenging activity)
3. NBT reduction assay (Superoxide radical scavenging activity)
4. Reducing power ability

IV. Statistical analysis.

V. Writing up and submission of dissertation.

4. MATERIALS AND METHODS

4.1. DRUGS AND CHEMICALS

Acarbose (Biocon Ltd), a-amylase & a-glucosidase (Sisco Research Laborotaries Ltd Mumbai), Glucose assay kits (Agappe diagnostics, Kerala), 2,2-diphenyl-1-picryl hydrazyl were purchased from HiMedia Laboratories, Mumbai, ascorbic acid, 2-deoxy-2-ribose, xanthine oxidase, quercetin, kaempferol, hesperidine, rutin, xanthine oxidase, hypoxanthine, pyrocatechol were purchased from Sisco Research Lab, Mumbai and butylated hydroxy toluene from Loba Cheme. Thiobarbituric acid, trichloroacetic acid, and potato starch were purchased from SD Fine Chemicals Ltd. All other chemicals used in the study were of analytical grade purchased from respective suppliers.

4.1.1. INSTRUMENTS USED

Rotary Evaporator (Heidolph WB 2000, Mumbai), Cooling centrifuge (Remi Instruments, Chennai), Ultrasonic cleaner (Vibronics Ltd, Mumbai), Digital Balance (Sartorius Ltd., USA), Shimadzu-Jasco V-530 UV/Vis spectrophotometer, ELCO L1/27 pH meter.

4.2. PLANT MATERIAL

4.2.1. Collection and authentication

The fruits of *Benincasa hispida* (Thunb) *Cogn.* was collected from the local market during the month of June and July 2009. The plant specimen was identified and authenticated by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Coimbatore-3, Tamil Nadu and the specimen has been preserved in the institute Herbarium with voucher specimen code No. BSI/SC/5/23/09-10/Tech – 456 dated July 24, 2009.

4.2.2. Preparation of the *Benincasa hispida* (Thunb) Fruit Extract

Seeds were removed from the fruit and the fruit pulp is made into a juice. The pulp juice is evaporated at 70°C. The juice was reduced to gummy state and then it was dried in vacuum desiccators to obtain the *Benincasa hispida (Thunb) Cogn.* fruits powder (BHFP) and stored in an airtight container.

4.2.3. Preparation of the *Benincasa hispida* (Thunb) Seed Extract

The seeds were dried in shade and finely powdered. The powdered seeds were macerated with water and stirred continuously in a mechanical shaker for 4 hours. The preparation was kept aside for 24 h. It was again stirred in the mechanical shaker for 4 h and kept aside for 12 h. These contents were taken and filtered through a muslin cloth and the filtrate was distilled to get a dark gummy material. This is then dried, to

obtain the *Benincasa hispida* (Thunb) *Cogn.* seed powder (BHSP) and stored in an airtight container.

4.2.4. Preliminary phytochemical screening of *Benincasa hispida* fruit and seed powders (BHFP and BHSP)

BHFP and BHSP were subjected to qualitative phytochemical tests to determine the presence of various phytoconstituents (Trease and Evans, 2002; Sanni *et al.*, 2008) like tannins, phenolics, saponins, flavonoids, terpenoids, alkaloids, proteins and glycosides.

a. Test for tannins and phenolics

To the solution of the extract, a few drops of 0.1% ferric chloride, 1% gelatin solution, 10% lead acetate was added and observed for brownish green or a blue-black color.

b. Test for saponins

About 10 ml of the extract was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent-froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously and then observed for the formation of emulsion. When mixed with dilute sulphuric acid and boiled with 90% ethanol, if the initial frothing disappears it confirms the presence of higher concentration of saponins.

c. Test for flavonoids

1. To a portion of the extract concentrated H_2SO_4 was added. A yellow colouration indicates the presence of flavonoids. The yellow colour disappears on standing.
2. Few drops of 1% $AlCl_3$ solution was added to a portion of extract. A yellow colouration indicates the presence of flavonoids.
3. A portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration indicates a positive test for flavonoids.

d. Test for terpenoids

About 5 ml of the extract was treated with 2 ml of chloroform and about 3 ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

e. Test for alkaloids

A small portion of the extract was stirred with few drops of dil. HCl and filtered.

1. To the filtrate, Dragendorff's reagent (potassium bismuth iodide solution) was added and an orange brown precipitate indicates the presence of alkaloids.
2. To the filtrate, Mayer's reagent was added and a cream precipitate indicates the presence of alkaloids.

f. Test for proteins

A portion of the extract was mixed with few drops of water and added Millon's test and Biuret reagents. A yellowish brown precipitate indicates the presence of proteins.

g. Test for glycosides

A portion of the extract was mixed with few drops of Fehling's solution A & B and heated gently. A brick red precipitate indicates the presence of glycosides.

4.3. *IN VITRO* HYPOGLYCAEMIC STUDIES

Glucose can be readily absorbed from the G.I.T. by the presence of enzyme α-amylase and α-glucosidases. Inhibition of these enzymes reduces the postprandial blood glucose levels. Hence *in vitro* α-amylase & α-glucosidase inhibition models were carried out to screen BHFP and BHSP and evaluate its potential hypoglycaemic activity.

4.3.1. Inhibition of a amylase *in vitro*

PROCEDURE

A 1% starch solution was prepared in 25 ml of 20 mM sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9 at 65°C for 15 minutes. The a amylase enzyme was obtained from porcine pancreas and its solution was prepared by mixing 1 mg of a amylase in 250 ml of cold deionised water. The calorimetric reagent was prepared by mixing sodium

potassium tartarate (12 g in 8 ml of 2 mM sodium hydroxide and 96 mM of 3, 5-dinitrosalicylic acid solution. BHFP and BHSP extracts were dissolved in 5% DMSO (Dimethylsulphoxide) to give a final concentration of 1 mg/ml.

One ml of starch solution was mixed with 1 ml of increasing concentration of the BHFP and BHSP (100-1000 µg/ml) and mixed by swirling and equilibrated to 20°C. Then added one ml of a amylase solution and incubated at 20°C for 5 minutes to undergo the reaction with the starch. To the above solution add 1 ml of the colorimetric reagent solution and heated in a water bath for 15 minutes. The reduction of 3, 5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid corresponds to the maltose generation with the colour change from yellowish orange to wine red. Then it is cooled and added 9 ml of deionised water to make a final volume of 13 ml and then the absorbance was recorded at 540 nm for both test and blank using a suitable spectrophotometer (Sigma Aldrich, 1997; Thalapaneni *et al.*, 2008)

Assay condition

T = 37°C, pH = 6.9, $A_{540nm,}$ Light path =1 cm, Calorimetric method

Unit definition

One unit will liberate 1mg of maltose from starch in 5 minutes at pH 6.9 at 20°C and pH 6.9 under specified conditions.

The a amylase inhibition was expressed as percentage of inhibition and the IC_{50} values determined by linear regression of plots with varying

concentration of BHFP and BHSP against the percentage inhibition from three separate tests.

4.3.2. Inhibition of a glucosidase *in vitro*

a glucosidase enzyme obtained from yeast as lyophilised powder was used as the target protein source for the study of the enzyme inhibition using maltose as the substrate. Acarbose is used as positive control and the plant extract is prepared at the concentration of 1 mg/ml with 5% v/v DMSO (Dimethyl sulphoxide).The enzyme and the substrate were dissolved in 0.2 M Tris buffer at pH 8.

PROCEDURE

The enzymatic assay mixture consists of 1 ml of glucosidase enzyme (1U/ml), 1 ml of 37 mM of maltose substrate, 1 ml each of BHFP and BHSP & acarbose at varying concentration (10µg - 100µg) in 5% v/v DMSO (Dimethyl sulphoxide) which is incubated at 37°C for 30 min. After incubating for 30 min, 0.2 ml of the assay mixture is mixed with 1 ml of the kit reagent. Glucose released in the assay mixture is quantified with commercial glucose oxidase assay kit (GOD-POD Kit, Agappe Diagnostics, Kerala). The enzymatic activity was measured by the amount of glucose released, which was detected spectrophotometrically at 505 nm. The rate of carbohydrate breakdown was determined by calculating the amount of glucose obtained when carbohydrate was completely digested. The enzyme inhibitory activity was determined as the

percentage inhibition and the assays were carried out in triplicate & the rate of prevention was calculated. The IC_{50} of the BHFP and BHSP required to inhibit the activity of the enzyme by 50% was determined by linear regression of the plots with varying concentration of BHFP and BHSP Vs percentage inhibition from the three separate tests (Subramanian *et al.*, 2008; Thalapaneni *et al.*, 2008)

Assay condition

T = 37°C, pH = 6.9, $A_{540nm,}$ Light path =1 cm, Calorimetric method

Unit definition

One unit will liberate 1mg of maltose from starch in 5 minutes at pH 6.9 at 20°C and pH 6.9 under specified conditions.

The a amylase inhibition was expressed as percentage of inhibition and the IC_{50} values determined by linear regression of plots with varying concentration of BHFP and BHSP against the percentage inhibition from three separate tests.

4.4.2. Inhibition of a glucosidase *in vitro*

a glucosidase enzyme obtained from yeast as lyophilised powder was used as the target protein source for the study of the enzyme inhibition using maltose as the substrate. Acarbose is used as positive control and the plant extract is prepared at the concentration of 1 mg/ml with 5% v/v DMSO (Dimethyl sulphoxide).The enzyme and the substrate were dissolved in 0.2 M Tris buffer at pH 8.

PROCEDURE

The enzymatic assay mixture consists of 1 ml of glucosidase enzyme (1U/ml), 1 ml of 37 mM of maltose substrate, 1 ml each of BHFP and BHSP & acarbose at varying concentration (10µg - 100µg) in 5% v/v DMSO (Dimethyl sulphoxide) which is incubated at 37°C for 30 min. After incubating for 30 min, 0.2 ml of the assay mixture is mixed with 1 ml of the kit reagent. Glucose released in the assay mixture is quantified with commercial glucose oxidase assay kit (GOD-POD Kit, Agappe Diagnostics, Kerala). The enzymatic activity was measured by the amount of glucose released, which was detected spectrophotometrically at 505 nm. The rate of carbohydrate breakdown was determined by calculating the amount of glucose obtained when carbohydrate was completely digested. The enzyme inhibitory activity was determined as the percentage inhibition and the assays were carried out in triplicate & the rate of prevention was calculated. The IC_{50} of the BHFP and BHSP required to inhibit the activity of the enzyme by 50% was determined by linear regression of the plots with varying concentration of BHFP and BHSP Vs percentage inhibition from the three separate tests (Subramanian *et al.*, 2008; Thalapaneni *et al.*, 2008)

STATISTICAL ANALYSIS:

P values are expressed as mean±standard error mean (SEM) and analyzed using GraphPad InStat software.

4.5. *IN VITRO* ANTIOXIDANT STUDIES

4.5.1. DPPH radical scavenging assay (hydrogen donating ability)

The hydrogen donating ability of BHFP and BHSP were examined in the presence of DPPH stable radical. One millilitre of 0.3 mM DPPH ethanol solution was added to 2.5 ml of BHFP and BHSP solutions of different concentrations in ethanol and allowed to react at room temperature. After 30 min the absorbance values were measured at 518 nm. 1.0 ml ethanol plus 2.5 ml of BHFP and BHSP solutions were used as a blank. DPPH solution (1.0 ml; 0.3 mM) plus ethanol (2.5 ml) was used as negative control. The positive controls were those using the standard (Ascorbic acid) solutions. Percentage inhibition of DPPH scavenging effect was calculated and the IC_{50} values were determined by linear regression of plots with varying concentration of BHFP and BHSP against the percentage inhibition from three separate tests (Mensor *et al.*, 2001).

4.5.2. Deoxyribose degradation assay (Hydroxyl radical scavenging activity)

The decomposing effect of BHFP and BHSP on hydroxyl radicals was determined by the assay of malondialdehyde chromogen formation due to 2-deoxy 2-ribose degradation. The assay mixture contained in a final volume of 1ml: 100 μl of 28 mM 2-deoxy 2-ribose dissolved in phosphate buffer, pH 7.4, 500 μl of the BHFP and BHSP of various concentrations in

buffer, 200 µl of 200 mM ferric chloride (1:1 v/v) and 1.04 mM EDTA and 100 µl of 1.0 mM hydrogen peroxide and 100 µl of 1.0 µM ascorbic acid. After incubation of the test sample at 37°C for 60 min (Fentons system-generation of hydroxyl radicals). The extent of deoxy ribose degradation by the formed hydroxyl radicals was measured directly using thiobarbituric acid (TBA) test; 1.0 ml TBA (1%) in 0.05M NaoH) and 1.0 ml 2.8% (w/v) trichloroacetic acid were added to the test tubes and heated at 100 °C for 15 min ,cooled and the absorbance was measured at 532 nm against the blank containing deoxyribose and buffer solution. The positive controls were those using the standard (Quercetin) solutions. Percentage inhibition of deoxyribose degradation was calculated and the IC_{50} values were determined by linear regression of plots with varying concentration of BHFP and BHSP against the percentage inhibition from three separate tests (Gomes *et al.*, 2001)

4.5.3. NBT reduction assay (Superoxide radical scavenging activity)

The capacity of the plant extracts to scavenge the superoxide anion was assayed by using NBT reduction assay. The superoxide anion radical was generated *in vitro* with hypoxanthine and xanthine oxidase. A reaction mixture with a final volume of 3 ml per tube was prepared with 1.4 ml of 50 mM KH_2PO_4-KOH pH 7.4 containing 1 mM EDTA, 0.5 ml of 100 µm hypoxanthine, 0.5 ml of 100 µM nitro blue tetrazolium (NBT). The reaction was started by adding 0.066 units per tube of xanthine oxidase freshly diluted in 100 µl of phosphate buffer and 0.5 ml of BHFP and BHSP in

saline. The xanthine oxidase was added last. The subsequent rate of NBT reduction (measure of superoxide scavenging activity) was determined on the basis of spectrophotometric determinations of absorbance at 560 nm. Ascorbic acid was used as standard. The results are expressed as the percentage inhibition of NBT reduction rate with respect to the reaction mixture without test compound (Guzman *et al.*, 2001; Gulcin., 2003).

4.5.4. Reducing power ability

Reducing power ability was measured by mixing 1.0 ml BHFP and BHSP of varying concentrations (50, 100, 200, 400, 800 µg/ml) in 1 ml of distilled water to 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide and incubated at 50°C for 30 min. Later 2.5 ml of trichloroacetic acid (10%) were added to the mixture and centrifuged at 3000 rpm for 10 min. Finally 2.5 ml from the supernatant were mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride. The absorbance was measured spectrophotometrically at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power. All experiments were done in triplicate using butylated hydroxyltoluene (BHT) as positive control (Yildrim *et al.*, 2001).

4.6. Determination of % Inhibition

Percentage antioxidant activity (%AA) was calculated using the formula,

$$\% \text{ Antioxidant Activity (\%AA)} = 100 - \left[\frac{A_0 - A_1}{A_0} \times 100 \right]$$

Where, A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

4.6.1. Determination of 50% Inhibitory Concentration (IC_{50})

The concentration (mg/ml) of the plant extracts required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the extract. The IC_{50} values were calculated using GraphPad Instat statistical software.

4.6.2. Statistical analysis

All determinations were carried out in triplicate and the values are expressed as the mean ± SEM.

5. RESULTS

5.1. Percentage yield

The percentage yield of the powder of the fruits of ***Benincasa hispida*** (Thunb) Cogn was 30% and that of seed powder was 17%.

5.2 Phytochemical screening

Preliminary phytochemical screening of the ***Benincasa hispida*** fruit and seed powders (BHFP and BHSP) are as follows: The BHFP showed the presence of triterpenoids, flavonoids, saponins, proteins, tannins and phenolics.

The preliminary phytochemical screening of BHSP showed the presence of flavonoids, alkaloids, saponins, proteins, tannins and phenolics.

5.3. Inhibition of α amylase *in vitro*

Benincasa hispida fruit powder (BHFP) showed α amylase inhibitory activity at the varying concentrations tested (50, 100, 200, 400 and 800µg/ml). There was a dose dependent increase in the percentage inhibition for all the concentrations tested (Table 3). BHFP at a concentration of 50 µg/ml showed a percentage inhibition of 8.59, for 100 µg/ml it was 20.42, for 400 µg/ml it was increased to 45.66, 800 µg/ml it was 59.68. The IC_{50} value was found to be 581.23 µg/ml.

Benincasa hispida seed powder (BHSP) showed significant α amylase inhibitory activity at the varying concentrations tested (50, 100, 200, 400 and 800µg/ml). There was a dose dependent increase in the percentage inhibition for all the concentrations tested (Table 3). BHSP at a concentration of 50 µg/ml showed a percentage inhibition of 16.68, for 100 µg/ml it was 30.70, for 400

µg/ml it was increased to 54.30, 800 µg/ml it was 71.23. The IC_{50} value was found to be 338.66 µg/ml.

Acarbose was used as the standard drug for the determination of α amylase inhibitory activity. The concentration of acarbose is varied (50, 100, 200, 400 and 800µg/ml). Acarbose at a concentration of 100µg/ml exhibited a percentage inhibition of 37.21% and for 800 it was found to be 94.20%. A graded increase in the percentage inhibition was observed for the increasing concentrations of the drug. The IC_{50} values of acarbose were found to be 170.80. An increase in the IC_{50} value was observed for the plant extract when compared with the standard drug acarbose (Table 3).

5.4. Inhibition of α glucosidase *in vitro*

The study revealed that BHFP and BHSP had significant α glucosidase inhibitory activity at the varying concentrations tested (50, 100, 200, 400, 800 µg/ml). There was a dose dependent increase in the percentage inhibition for all the concentrations tested (Table 4).

BHFP at a concentration of 50µg/ml showed a percentage inhibition of 7.71%, at 200µg/ml it was found to be 27.89% and at 800 µg/ml it increased to 53.42. The IC_{50} value was found to be 734 µg/ml.

BHSP at a concentration of 50µg/ml showed a percentage inhibition of 15.59%, at 200µg/ml it was found to be 36.48% and at 800 µg/ml it increased to 49.29. The IC_{50} value was found to be 429 µg/ml.

Acarbose was used as the standard drug for the determination of α glucosidase inhibitory activity. The concentration of acarbose is varied from 50,

100, 200, 400, 800 µg/ml. Acarbose at a concentration of 100µg/ml exhibited a percentage inhibition of 40.73% and for 800 µg/ml it was found to be 72.56%. A graded increase in the percentage inhibition was observed for the increasing concentrations of the drug. The IC_{50} values of acarbose were found to be 202.15µg/ml. An increase in the IC_{50} values was observed for the plant extract when compared with the standard drug acarbose (Table 4).

5.5. DPPH radical scavenging assay (hydrogen donating ability)

The extracts BHFP and BHSP at various concentrations demonstrated H-donor activity. The radical scavenging activity of BHFP and BHSP were determined from the reduction in the absorbance at 518 nm due to scavenging of the stable DPPH free radical. The DPPH scavenging potential for BHFP and BHSP varied at varying concentrations (10, 20, 40, 80, 160 µg/ml) and the results are shown in Table 5. BHFP and BHSP showed the graded increase in percentage of inhibition for all the doses tested and the percentage inhibition ranged from 9.57% to 57.91%. The IC_{50} values of BHFP and BHSP were found to be 148.16 and 130.06 µg/ml respectively.

Ascorbic acid was used as the reference standard and similar increase in the percentage of inhibition was observed for all the concentrations (10, 20, 40, 80, 160 µg/ml) tested. The DPPH scavenging effect for various extracts was less than that of standard compound, ascorbic acid. The IC_{50} value of standard was found to be 53.04µg/ml. DPPH was reduced with the addition of BHFP and BHSP in a concentration dependent manner**.**

5.6. Deoxyribose degradation assay (hydroxyl radical scavenging activity)

Hydroxyl radical scavenging activity was quantified by reaction with thiobarbituric acid and the results are shown in the (Table 6). The BHFP and BHSP showed 30.12% of activity at 20µg/ml and it was increased to 54.79 % at 80µg/ml. The IC_{50} value of BHFP and BHSP were found to be 64.8 and 27 µg/ml respectively. Quercetin was used as the reference standard and similar increase in the percentage of inhibition was observed for all the concentrations (5, 10, 20, 40, 80 µg/ml) tested. The IC_{50} value of quercetin was found to be 18.5 µg/ml. The degradation of deoxyribose by Fe^{3+}-ascorbate-EDTA-H_2O_2 system was markedly decreased by BHFP and BHSP tested at various concentrations indicating hydroxyl radical scavenging activity.

5.7. NBT reduction assay (superoxide radical scavenging activity)

The BHFP and BHSP at various concentrations were found to be a scavenger of superoxide anion generated in xanthine oxidase-NBT systems *in vitro* and their activity were comparable to that of ascorbic acid and the results were given in (Table 7). All the concentrations of BHFP and BHSP offered greater percentage of inhibition with increase in the concentrations. Ascorbic acid was used as the positive control. The IC_{50} value for BHFP and BHSP were 285 and 122 µg/ml respectively and that of standard was 39.26 µg/ml.

5.8. Reducing power ability

The reductive capabilities of BHFP and BHSP when compared to the standard BHT were given in Table 8. The reductive ability of the BHFP and BHSP serves as a significant indicator of its antioxidant activity. The reducing

power of BHFP and BHSP were dose dependent and found to increase with increasing concentrations. BHFP and BHSP increased the absorbance up to 0.563 and 0.394 at 800µg/ml respectively, when the absorbance of the standard at 800µg/ml was 1.397. All the concentrations of BHFP and BHSP offered higher absorbance values than the control.

Table 1: Preliminary phytochemical screening of BHFP

S.No	Phytochemical compounds	Presence or Absence
1	*Triterpenoids*	**Presence**
2	*Flavonoids*	**Presence**
3	*Glycosides*	**Absence**
4	*Alkaloids*	**Absence**
5	*Saponins*	**Presence**
6	*Carbohydrates*	**Absence**
7	*Proteins*	**Presence**
8	*Tannins and phenolics*	**Presence**

Table 2: Preliminary phytochemical screening of BHSP

S.No	Phytochemical compounds	Presence or Absence
1	*Triterpenoids*	**Absence**
2	*Flavonoids*	**Presence**
3	*Glycosides*	**Absence**
4	*Alkaloids*	**Presence**
5	*Saponins*	**Presence**
6	*Carbohydrates*	**Absence**
7	*Proteins*	**Presence**
8	*Tannins and phenolics*	**Presence**

Table 3: Alpha amylase inhibitory activity of BHFP and BHSP

Sample	Concentration (µg/ml)	% inhibition	IC $_{50}$ µg/ml
BHFP	50	8.59 ± 0.54	581.23 ± 1.557
	100	20.42 ± 0.53	
	200	30.30 ± 1.80	
	400	45.66 ± 1.11	
	800	59.68 ± 0.93	
BHSP	50	16.68 ± 1.40	338.66 ± 0.881
	100	30.70 ± 0.98	
	200	41.12 ± 0.46	
	400	54.30 ± 0.52	
	800	71.23 ± 0.97	
Acarbose (standard)	50	22.77 ± 0.21	170.80 ± 0.144
	100	37.21 ± 0.16	
	200	57.49 ± 0.17	
	400	77.98 ± 0.16	
	800	87.70 ± 0.16	

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM.

Table 4: Alpha glucosidase inhibitory activity of BHFP and BHSP

Sample	Concentration (µg/ml)	% inhibition	IC $_{50}$ µg/ml
BHFP	50	7.71 ± 0.20	734 ± 2.082
	100	14.89 ± 0.27	
	200	27.89 ± 0.62	
	400	36.40 ± 0.49	
	800	53.42 ± 0.27	
BHSP	50	15.59 ± 0.13	429 ± 1.155
	100	28.83 ± 0.14	
	200	36.48 ± 0.34	
	400	49.24 ± 0.35	
	800	61.07 ± 0.34	
Acarbose (standard)	50	22.77 ± 0.21	202.15 ± 1.021
	100	40.73 ± 1.39	
	200	49.34 ± 1.04	
	400	63.48 ± 0.91	
	800	72.56 ± 1.22	

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM.

Table 5: Hydrogen donating ability of BHFP and BHSP using

DPPH method

Sample	Concentration (µg/ml)	% inhibition	IC $_{50}$ µg/ml
BHFP	10	9.57 ± 0.25	148.16 ± 0.44
	20	13.93 ± 0.49	
	40	22.15 ± 0.56	
	80	32.49 ± 0.53	
	160	53.17 ± 0.61	
BHSP	10	15.54 ± 0.41	130.06 ± 0.096
	20	22.24 ± 0.19	
	40	30.63 ± 0.22	
	80	41.72 ± 0.30	
	160	57.91 ± 0.17	
Ascorbic acid (standard)	10	17.48 ± 0.38	53.04 ± 0.57
	20	32.87 ± 1.38	
	40	47.94 ± 0.30	
	80	68.99 ± 0.07	
	160	84.23 ± 0.21	

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM.

Table 6: Scavenging of hydroxyl radical activity by BHFP and BHSP using deoxyribose degradation method

Sample	Concentration (µg/ml)	% inhibition	IC $_{50}$ µg/ml
BHFP	5	10.25 ± 0.13	64.80 ± 0.07
	10	22.23 ± 0.06	
	20	30.12 ± 0.05	
	40	41.72 ± 0.13	
	80	54.79 ± 0.06	
BHSP	5	16.26 ± 0.09	27 ± 1.60
	10	29.13 ± 0.93	
	20	45.10 ± 0.07	
	40	55.61 ± 0.06	
	80	67.87 ± 0.10	
Quercetin (standard)	5	19.79 ± 0.39	18.5 ± 0.29
	10	35.16 ± 0.21	
	20	57.81 ± 0.28	
	40	73.75 ± 0.23	
	80	92.26 ± 0.21	

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM.

Table 7: Superoxide anion scavenging activity of BHFP AND BHSP using NBT reduction assay

Sample	Concentration (µg/ml)	% inhibition	IC_{50} µg/ml
BHFP	25	17.09 ± 0.17	285 ± 0.0247
	50	26.46 ± 0.23	
	100	33.35 ± 0.19	
	200	47.90 ± 0.29	
	400	61.21 ± 0.64	
BHSP	25	20.22 ± 0.12	122 ± 0.176
	50	33.66 ± 0.15	
	100	47.74 ± 0.09	
	200	61.67 ± 0.18	
	400	71.07 ± 0.10	
Ascorbic acid (standard)	25	33.33 ± 0.03	39.26 ± 0.54
	50	63.87 ± 0.45	
	100	76.78 ± 0.21	
	200	85.79 ± 0.17	
	400	91.82 ± 0.14	

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM.

Table 8: Reductive ability of BHFP and BHSP

Sample	Concentration (µg/ml)	Absorbance at 700 nm IC_{50} µg/ml
BHFP	50	0.133 ± 0.0002
	100	0.240 ± 0.0005
	200	0.318 ± 0.0005
	400	0.414 ± 0.0020
	800	0.563 ± 0.0015
BHSP	50	0.081 ± 0.0008
	100	0.125 ± 0.0023
	200	0.201 ± 0.0017
	400	0.279 ± 0.0029
	800	0.394 ± 0.0011
BHT (standard)	50	0.1647 ± 0.001
	100	0.3252 ± 0.001
	200	0.5862 ± 0.002
	400	0.7635 ± 0.003
	800	1.3972 ± 0.033

All determinations were carried out in triplicate manner and values are expressed as the mean ± SEM.

6. DISCUSSION AND CONCLUSION

Diabetes mellitus is the world's largest growing metabolic disorder of carbohydrate, fat & protein metabolism. A relative or absolute deficiency of insulin secretion leads to impaired carbohydrate utilization by tissue, which is the characteristic feature of diabetes.

Alternate strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability of the modern therapies for many rural populations in developing countries.

Benincasa hispida (Thunb) Cogn. is used in the folk medicine and presumably it has no side effects reported. Since ancient times, people trusted on plants and herbs as medicine, as dietary supplements and as adjuvant with drugs for better control of diseases and symptoms.

The present study deals with the exploration of pharmacological and phytochemical screening of the selected Indian medicinal plant *Benincasa hispida* (Thunb) Cogn. belonging to the family Cucurbitaceae which is traditionally used by the local people and tribals in India for the treatment of asthma, cough, diabetes, haemoptysis, hemorrhages from internal organs, epilepsy, fever. The fruits and seeds are useful in diabetes (Indian Medicinal Plants, 1985).

Phytochemical screening of fruit and seed extracts revealed the presence of flavonoids and phenolics, accounting for its antidiabetic and

antioxidant properties. Flavonoids are a group of polyphenolic compounds which exhibit several biological effects such as anti-inflammatory, antihepatotoxic, antiulcer activities, antidiabetic, antithrombotic etc. They also inhibit enzymes such as aldose reductase and xanthine oxidase.

The present study was carried out to evaluate the *in vitro* antidiabetic and antioxidant activities of the fruits and seeds of ***Benincasa hispida*** (Thunb) Cogn.

The *in vitro* antidiabetic activity of BHFP and BHSP have been evaluated by measuring its a amylase and a glucosidase inhibitory activities and was compared with the standard drug acarbose, which is specific inhibitor of a glucosidase. Acarbose also possess inhibitory action of a amylase. The amount of glucose produced by the action of a glucosidase is estimated by using the enzyme glucosidase and peroxidase *in vitro*. The a amylase inhibitory activity of the BHFP is lesser when compared to BHSP. However both BHFP and BHSP has lesser inhibition percentage than that of the acarbose. However, the a glucosidase inhibition of the extract was much lesser than the inhibitory action shown by acarbose. The possible mechanism of action of a amylase may be due to the blocking of the starch binding site and a glucosidase inhibition may be due to blockade of the oligosaccharide binding site. Alpha amylase catalyses the hydrolysis of the internal a 1,4 glucosidic linkages in starch and other related polysaccharides which has been the targets for the

suppression of post prandial hyperglycemia. In the light of the result present study indicates that BHFP and BHSP both possess antidiabetic activity and to be specific the BHSP possess more antidiabetic activity than the BHFP.

Free radicals are known to play a definite role in the pathological manifestation of diabetes. Antioxidant fights against free radicals by protecting us from various diseases and scavenges the reactive oxygen radicals or protects the antioxidant defense mechanism. Reactive oxygen species (ROS) are capable of damaging biological macromolecules such as DNA, carbohydrates and proteins. ROS is a collective term, which includes not only oxygen radicals ($O_2^{\cdot}$ and $OH^{\cdot}$) but also some non-radical derivatives of oxygen like H_2O_2, HOCl, and ozone (O_3). In addition, antioxidant activity may be regarded as a fundamental property important for life.

The *in vitro* antioxidant activities of the BHFP and BHSP have been evaluated by measuring its scavenging activities by various methods such as DPPH radical scavenging assay, deoxyribose degradation assay, NBT reduction assay to study the superoxide scavenging activity and reducing power ability method to study the total antioxidant capacity.

DPPH is a stable free radical at room temperature, which produces a violet solution in ethanol DPPH is widely used to evaluate the free radical scavenging effect of natural antioxidant. DPPH shows a strong absorption band at 517 nm in visible spectrum (deep violet colour). As the electron

becomes paired in the presence of free radical scavenging, the absorption vanishes and the resulting discoloration stochiometrically coincides with the number of electrons taken up (Mensor *et al.*, 2001). The bleaching of DPPH absorption is representative of the capacity of the test drugs to scavenge free radicals independently. DPPH radical scavenging activities increased with increasing concentrations of BHFP and BHSP. Based on the mechanism of reduction of DPPH molecule described in the literature, it is correlated with the presence of hydroxyl groups on the antioxidant molecule. We can infer that the very good activity of BHFP and BHSP was probably due to the presence of substance with an available hydroxyl groups.

Hydroxyl radicals are the highly reactive radicals which are produced through the Fenton's reaction in living system. Hydroxyl radicals scavenging activity was quantified by measuring inhibition of the degradation of deoxyribose by free radicals (Guzman *et al.*, 2001). Deoxyribose levels were determined by reaction with thiobarbituric acid. BHFP and BHSP showed a good hydroxyl radical scavenging activity but lesser than standard, quercetin.

Superoxide anion radicals were generated enzymatically *in vitro* by the hypoxanthine and xanthine oxidase system was determined by using NBT reduction assay. Superoxide reduces NBT to form a blue coloured complex formazone which is measured spectrophotometrically. The decrease of absorbance at 560 nm with BHFP and BHSP indicates the

consumption of superoxide anion in the reaction mixture (Gulcin *et al.*, 2003). Determination of the mean rate of increase in absorbance over one minute period provides a measure of the extent to which the test fraction is capable of inhibiting NBT reduction by the superoxide anion radical and thus of superoxide scavenging activity.

The reductive capabilities of BHFP and BHSP were compared with BHT. For the measurements of the reductive ability, we investigated the Fe^{3+}-Fe^{2+} transformation in the presence of BHFP and BHSP. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the antioxidant activity of antioxidants have been attributed to various mechanism, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging antioxidant activity (Gulcin *et al.*, 2004; Amarowiz *et al.*, 2004). The reducing power of BHFP and BHSP increased with increasing amount of sample. Here the color change occurs from yellow to greenish blue depending upon the reducing power of BHFP and BHSP.

Thus from the present investigation, it can be said that the *Benincasa hispida* (Thunb) Cogn. exhibited remarkable antioxidant property in various i*n vitro* assay systems.

In conclusion, there has been a growing interest in the alternative medicine and the therapeutic properties of the natural products derived

from plants in the recent years. Based on the evaluation done using the various *in vitro* assay models it may be concluded that *Benincasa hispida* (Thunb) Cogn seed extract possess more a amylase and a glucosidase enzyme inhibitory actions than the fruit extract and thus retards glucose absorption and reduces post prandial hyperglycaemia. The antioxidant activity of the plant was proved using various *in vitro* assay systems. The preliminary chemical tests provided insights into the presence of polyphenolics compounds in the extract. Hence the above stated activities can be attributed due to the presence of these flavonoids. Further studies using *in vivo* models are necessary to confirm these activities and to explore the exact mechanism by which the plant constituents act.

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