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STUDIES ON α-AMYLASE INHIBITORS AND ANTIOXIDANTS IN *Psidium guajava* LEAVES AND *Syzygium cumini* SEEDS

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BONAFIDE CERTIFICATE

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[N. DHAYANANTH]

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ABSTRACT

Inhibitors of carbohydrate -hydrolyzing enzyme play an important role to control post prandial blood glucose levels in diabetic patients. We investigated the effect of crude extract from *Psidium guajava* leaves and *Syzygium cumini* seeds on α-amylase . These crude extracts containing α-amylase inhibitors , strongly inhibit in porcine pancreatic amylase. α-Amylase inhibitory activity was determined by using starch as substrate in a colorimetric reaction using 3,5 – nitrosalicylic acid. The amylase inhibitory activity was tested separately and results were compared with standard amylase inhibitor, acarbose.. From the standard graph, IC_{50} value for acarbose,*Psidium guajava* leaves *Syzygium cumini* seeds were 0.625 mg/ml, 0.65 mg/ml ,0.80 mg/ml respectively. The effect of α-amylase inhibitors from *P.guajava* leaves and *S.cumini* seeds on blood gulcose of alloxan induced diabetic rats was determined.. The *in vivo* studies with rats treated with inhibition showed a reduction of blood glucose levels at variable interval of time (0 min – 180 min) when compared to the untreated diabetic rats. The siginificant difference between treatments and the time ($p<0.05$) was observed. The antioxidant activity, reducing power, free radical scavenging, and metal chelating activities were evaluated to determine the total antioxidant capacity of *P.guajava* leaves and *S.cumini* seeds extracts.The results suggest that these natural amylase inhibitors from food- grade sources offer an attractive therapeutic approach to the treatment of post prandial hyperglycemia by decreasing glucose release from starch and also in scavenging free radiacls diabetes mellitus.

INTRODUCTION

1.INTRODUCTION

Diabetes mellitus with all its fatal complications , is a major public health problem in the developed as well as developing countries The prevalence of diabetes is increasing worldwide due to changes in modern life styles. The disease occurs worldwide and its incidence is increasing rapidly in most parts of the world. People suffering from diabetes are not able to produce or properly use insulin in the body, so they have a high level of blood glucose. Hence diabetes is becoming the third 'killer' of mankind, after cancer and cardiovascular diseases, because of its high prevalence, morbidity and mortality .Approximately 4% population worldwide and is expected to increase by 5.4% in 2025 . The number of adults suffering from diabetes in India is expected to increase threefold, from 19.4 million in 1995 and 57.2 million in 2025. Recent studies on geographical and ethical influences have shown that people of Indian origin are highly prone to diabetes.

Diabetes mellitus [DM] is a metabolic disorder of the endocrine system. . It is a metabolic disease characterized by hyperglycaemia and glycosuria due to absolute or relative lack of insulin.Diabetes mellitus is further characterized by an inability to reabsorb water resulting in increased urination (polyuria), excessive thirst (poldipsia) and excessive eating (Polyphagia) . Changes in lipid concentration and consequent disorders of lipid metabolism have been observed in diabetes mellitus .

Diabetes mellitus is a group of diseases marked by high levels of blood glucose resulting from defects in insulin production, insulin action, or

both. Diabetes can lead to serious complications and premature death, but people with diabetes can take steps to control the disease and lower the risk of complications.

Type 1 Diabetes was previously called insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes. Type 1diabetes develops when the body's immune system destroys pancreatic beta cells, the only cells in the body that make the hormone insulin that regulates blood glucose. To survive ,people with type 1 diabetes must have insulin delivered by injection or a pump. This form of diabetes usually strikes children and young adults, although disease onset can occur at any age. Type 1 diabetes accounts for 5 percent to 10 percent of all diagnosed cases of diabetes. Risk factors for type1 diabetes may be autoimmune, genetic, or environmental. There is no known way to prevent type 1 diabetes.

Type 2 diabetes was previously called non-insulin dependent diabetes mellitus (NIDDM) or adult-onset diabetes.Type 2 diabetes accounts for about 90 percent to 95 percent of all diagnosed cases of diabetes. It usually begins as insulin resistance, a disorder in which the cells do not use insulin properly. As the need for insulin rises, the pancreas gradually loses its ability to produce it. Type 2 diabetes is associated with older age, obesity,a family history of diabetes.

Type 2 diabetes is caused by insulin resistance, which is defined as defective insulin signaling and a decreased insulin efficiency to induce glucose transport from the blood into key target cells such as muscle and fat (adipocyte) cells . In general, obesity leads to hyperglycemia, which in turn

leads to and exacerbates insulin resistance. Insulin resistance, if not treated, results in hyperinsulinemia and eventually leads to full blown type 2 diabetes . Obesity or excessive adiposity,particularly visceral adiposity, contributes to and worsens insulin resistance . Most antidiabetic drugs are hypoglycemic or anti-hyperglycemic (blood glucose level reducing). However, most of these drugs are, to different extents, weight gain promoting (adipogenic) . Thus, these drugs treat one of the key symptoms of type 2 diabetes, hyperglycemia, but exacerbate the condition ofbeing overweight or obese, one of the leading causes of type 2 diabetes. Therefore, while these drugs are beneficial over the short term, they are not optimal for long term health of type 2 diabetic patient. The most desirable situation would be the development of new types of antidiabetic drugs that are either hypoglycaemic or anti-hyperglycemic without the side effect of promoting weight gain (adiposity). Herbal medicines known to be useful in diabetes treatment may be able to lead to compounds with such a combination of ideal therapeutic properties .

Nearly 85-95% of the diabetic patients suffer from type 2 diabetes. Currently available pharmacological agents for type 2 diabetes have a number of limitations, such as adverse side effects and high rates of secondary failure. Due to these factors, diabetic patients and healthcare professionals are increasingly considering complementary and alternative approaches, such as the use of medicinal herbs with anti-hyperglycemic activities. It is estimated that more than 200 species of plants exhibit hypoglycemic properties.

DM is a metabolic disorder affecting carbohydrate, fat, and protein metabolism. The worldwide survey reported that the DM is affecting nearly 10% of the population . The treatment of DM is based on oral hypoglycaemic agents and insulin. However, DM is also treated in Indian traditional medicine using anti-diabetic medicinal plants . The oral hypoglycaemic agents currently used in clinical practice have characteristic profiles of serious side effects .Hence, there is a need to search for newer anti-diabetic agents that retain therapeutic efficacy and are devoid of side effects that could be important sources of such agents.

Psidium guajava, is an important food crop and medicinal plant in tropical and subtropical countries is widely used like food and in folk medicine around of the world. This aims a comprehensive of the chemical constituents, pharmacological, and clinical uses. Different pharmacological experiments in a number of *in vitro* and *in vivo* models have been carried out. Also have been identified the medicinally important phyto-constituents. A number of metabolites in good yield and some have been shown to possess useful biological activities belonging mainly to phenolic, flavonoid, carotenoid, terpenoid and triterpene. Extracts and metabolites of this plant, particularly those from leaves and fruits possess useful pharmacological activities. A survey of the literature shows *P. guajava* is mainly known for its antispasmodic and antimicrobial properties in the treatment of diarrhoea and dysentery. Has also been used extensively as a hypoglycaemic agent. Many pharmacological studies have demonstrated the ability of this plant to exhibit antioxidant, hepatoprotection, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, antiinflamatory and antinociceptive activities,

supporting its traditional uses. Suggest a wide range of clinical applications for the treatment of infantile rotaviral enteritis, diarrhoea and diabetes.

Psidium guajava (Guava), a member of the Myrtaceae family, have been described by historical Mexican herbal sources dating back 500 years. Different parts of the plant are used in various indigenous systems of medicine, its leaves, roots and fruits have been used for prevention and treatment of diabetes. The aqueous extract of *Psidium guajava* leaves has a good effect to lower blood glucose. The effect of the leaf extract of *P.guajava* on α-glucosidase activity in small intestine of diabetic mouse was studied by Wang. Hence we ventured to identify the presence of α-amylase inhibitor in *P. guajava* to test verify the possible mechanism of hypoglycemic action in diabetic rats. . Almost the entire guava plant (inclusive of stem) showed significant α-amylase inhibition. The organic solvent extracts showed comparatively negligible amount of inhibition. Since, the water extract of the guava leaf powder at room temperature having the maximum α-amylase inhibition.

The effect of *Psidium guajava* bark, leaves and fruit as antidiabetic agents has been studied by several authors. They evaluated anti-hyperglycaemic activity of the ethanol extract obtained from the stem bark of *Psidium guajava* on blood glucose levels of normal, alloxan-induced hyperglycaemic rats and normal glucose loaded rats. The results showed that ethanol stem bark extract exhibited statistically significant hypoglycaemic activity in alloxan-induced, hyperglycaemic rats but was devoid of significant hypoglycaemic effect in normal and normal glucose loaded rats.

Many herbal remedies individually or in combination have been recommended in various medical treatises for the cure of different diseases. The therapeutic value of *Eugenia jambolana* Lam. commonly known as 'Jamun' has been recognized in different system of traditional medication for the treatment of different diseases and ailments of human beings. It contains several phytoconstituents belonging to category alkaloids, glucosides, flavonoides and volatile oil. It has been reported as digestive, astringent to the bowels, anthelmintic, sore throat, bronchitis, asthma, thirst, biliousness, dysentery, blood purifier, ulcers and diabetes. Several studies using modern techniques have authenticated its use in diabetes and its complication (nephropathy, cataract, insulin resistance), as antibacterial, analgesic, anti-inflammatory, antioxidant, as well as gastro protective agents. Most importantly, the studies have shown that it protects against the radiation-induced DNA damage and it has significantly decreased the fertilizing capacity of the male albino rats. There are few reports available on clinical uses of *Eugenia jambolana* in diabetes that have shown promising results.

Syzygium cumini ,a tree belong to family of mytraceae distributed in Asia (India,China,Malaysia).*Syzygium cumini* seeds are used for the treatment of diabetes before insulin therapy was available(Karthic et al., 2008). The therapeutic value in different stem of traditional medication for the treatment of different diseases and ailments of human beings. It contains several phytoconstituents belonging to category alkaloids, glucosides, flavonoides and volatile oil. It has been reported as digestive, astringent to the bowels, anthelmintic, sore throat,bronchitis, asthma, thirst, biliousness, dysentery, blood purifier, ulcers and diabetes. Several studies using modern

techniques have authenticated its use in diabetes and its complication (nephropathy, cataract, insulin resistance),as antibacterial, analgesic, anti-inflammatory, antioxidant, as well as gastro protective agents.

The seeds of *Syzygium cumini* are used by the Ayurvedic physicians (and also in Indian folklore) in the treatment of diabetes mellitus. The hypoglycaemic activity of the seeds has been studied by several workers in animal models.. In alloxan diabetic rats, S.*cumini* seed extract led to a decrease in the levels of blood glucose, urea and serum triglyceride levels.

Antioxidants act as "free radical scavengers" and hence prevent and repair damage done by these free radicals. Health problems such as heart disease, macular degeneration, diabetes, cancer etc are all contributed by oxidative damage.Antioxidants are substances or nutrients in our foods which can prevent or slow the oxidative damage to our body. When our body cells use oxygen, they naturally produce free radicals (byproducts) which can cause damage. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells.

Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols or polyphenols. Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of

multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells.

Diet and exercise therapy are key factors for preventing and treating .maintenance of healthy blood glucose levels is of particular importance and is greatly affected by dietary polysaccharides such as starch and glycogen. These polysaccharides are broken down by digestive enzymes such as α-amylase a key enzyme in polysaccharides digestion. control of α-amylase activity is important in order to inhibit excess energy supply and to regulate blood glucose levels.

To regulate the blood glucose levels and also to find the free radicals scavenging when there is damage to the system with therapeutic values from the food sources such *Psidium guajava* leaves and *Syzygium cumini* seeds having potent inhibition on amylase enzyme to control the anti hyperglycaemic levels and its antioxidant .

OBJECTIVE

- To study the potential of α-amylase inhibitor from *P.guajava leaves, S.cumini seeds* in inhibiting porcine pancreatic α -amylase

- Comparative studies with crude inhibitor of *P.guajava leaves* and *S.cumini seeds* and to with standard amylase inhibitor acarbose.

- To test the potential of amylase inhibitor in Alloxan induced diabetic rats with the extracts from *Psidium guajava* leaves and *Syzygium cumini* seeds.

- To investigate *in vitro* antioxidant properties of *P. guajava* leaves and *S. cumini* seeds.

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1 *Psidium guajava*

Psidium guajava, is an important food crop and medicinal plant in tropical and subtropical countries is widely used like food and in folk medicine around of the world. This aims a comprehensive of the chemical constituents, pharmacological, and clinical uses. Different pharmacological experiments in a number of *in vitro* and *in vivo* models have been carried out and medicinally important phyto-constituents have been reported.It was used extensively as a hypoglycaemic agent. Many pharmacological studies have demonstrated the ability of this plant to exhibit antioxidant, hepatoprotection, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, antiinflamatory and antinociceptive activities, supporting its traditional uses. Suggest a wide range of clinical applications for the treatment of infantile rotaviral enteritis, diarrhoea and diabetes.(Rosa Martha et ,al 2008) .

The leaves of *Psidium guajava* according to the folkfore in Chinese medicine, have been used in the treatment of diabetes mellitus. The acute treatment of guava juice have produced a marked hypoglycaemic action in normal and alloxan- treated diabetic mice . (Ojewole 2005)

Treatment with aqueous extract of unripe peel fruit (400 mg/kg) showed a significant fall in the blood glucose levels (BGLs) in the normal rats and diabetic induced rats The significant fall observed in FBG,

postprandial glucose and urine sugar levels of severely diabetic rats (Rai et.al,2007)

The leaf of Psidium guajava (family, Myrtaceae) is used traditionally in African folk medicine to manage, control, and treat a plethora of human a ilments, including diabetes mellitus and hypertension. Acute oral administr ations of the leaf extract (PGE; 50-800 mg/kg, p.o.) caused dose-related, sig nificant hypoglycemia in normal (normoglycemic) and STZ-treated, diabetic rats.Tannins, polyphenolic compounds,flavonoids, pentacyclic triterpenoids, guiajaverin, quercetin, and other chemical compounds present in the plant are speculated to account for the observed hypoglycemic and hypotensive effects of the plants leaf extract. (Ojewole, . .,2005)

The antioxidant related compounds have been isolated from methanol extracts of the leaves of Guava (*Psidium guajava* L.) . The isolated compounds possessed siginificant antioxidant activity that was reavealed by a DPPH free radical scavenging assay.which provides a scientific basis for the use of this plant as traditional medicine. The isolated compound was found to be quercetin ,quercetin-3-*O*-glucopyranoside and morin. (Suganya Tachakittirungrod et.al., 2008)

The antioxidant activities of guava fruit contains relatively high antioxidant activity than other citrus fruits because of the presence of polyphenolics and ascorbic acid. It also has high primary, but low secondary antioxidant potential. Storage at 4◦C has the effect of increasing ascorbic acid content, and the non-peeled fruit has higher total phenol and ascorbic

acid contents compared to the peeled fruit. The length and width of the seeds guava were also monitored over a period of 17 weeks to define specific stages of fruit ripening.(Lim Yau Yan et.al., 2006)

2.2 Syzygium Cumini

Many herbal remedies individually or in combination have been recommended in various medical treatments for the cure of different diseases. The therapeutic value of *Eugenia jambolana* Lam. commonly known as 'Jamun' has been recognized in different system of traditional medication for the treatment of different diseases and ailments of human beings. It contains several phytoconstituents belonging to category alkaloids, glucosides, flavonoids and volatile oil. It has been reported as digestive, astringent to the bowels, anthelmintic, sore throat, bronchitis, asthma, thirst, biliousness, dysentery, blood purifier, ulcers and diabetes.(Sagrawat and Khanya., 2006)

Syzygium cumini is a plant that has been used in popular medicine for the treatment of insulin dependent diabetes mellitus (DMID). Studies have proved the effect of *Syzygium cumini* upon the regeneration of insulin producing cells in the pancreatic duct wall. The aqueous *Syzygium cumini* bark extract have proved to stimulates development of insulin positive cells from the pancreatic duct epithelial cells.(Deila Rosély ,et.al.,2004)

The α-amylase inhibitors from *S.cumini* seeds extract separated by preparative thin layer chromatography showed maximum inhibitor activity,. The compounds identified from the seed extract of *S.cumini* were betulinic acid and 3,5,7,4`-tetrahydroxy flavanone, which were reported earlier from

S.formosanum and other plants. Dixon plot showed that the inhibition was non-competitive in nature. (Karthic et.,al 2008)

Zhi ping ruan had determined the antioxidant activity of *Syzygium cumini* leaf extracts using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging and ferric-reducing antioxidant power (FRAP) assays. The methanolic extract and its four water, ethyl acetate, chloroform, and *n*-hexane fractions were prepared and subjected to antioxidant evaluation. Their results showed that the ethyl acetate fraction had stronger antioxidant activity than the other ones. HPLC data indicated that *S. cumini* leaf extracts contained phenolic compounds, such as ferulic acid and catechin, responsible for their antioxidant activity. A significant linear relationship between antioxidant potency, free radical-scavenging ability and the content of phenolic compounds of leaf extracts supported this observation.(Zhi Ping Ruan et.al., 2008)

Alloxan and streptozotocin are widely used to induce experimental diabetes in animals. The mechanism of their action in B cells of the pancreas has been intensively investigated and now is quite well understood. The cytotoxic action of both these diabetogenic agents is mediated by reactive oxygen species, however, the source of their generation is different in the case of alloxan and streptozotocin. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide.Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous

massive increase in cytosolic calcium concentration causes rapid destruction of B cells.

Streptozotocin enters the B cell *via* a glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenicity of streptozotocin than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD+ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage.As a result of the streptozotocin action, B cells undergo the destruction by necrosis.(Szkudelski, 2005)

Annie et al., reported *Annona squamosa* L.(Annonaceae) had antidiabetic properties. Diabetes mellitus was induced with streptozotocin–nicotinamide and graded doses of the aqueous leaf extracts were then administered in drinking water to normal and experimental diabetic rats for 12 days. Fasting plasma glucose levels, serum insulin levels, serum lipid profiles and changes in body weight were evaluated in normal rats while liver glycogen levels and pancreatic TBARS levels were evaluated additionally in diabetic rats. The diabetic groups treated with the aqueous leaf extract were compared with standard , glibenclamide. The findings of the study support the antidiabetic claims of *Annona squamosa*.(Annie Shirwaikar et.al 2004).

Rammohan studied the effect of black/bitter cumin seeds *Centratherum anthelminticum* (L.) Kuntze extract (CA) containing mixture of polyphenolic compounds was tested on rat intestinal α-glucosidases, human salivary α-amylase activity and postprandial hyperglycemia in rats. Polyphenolic components of *C. anthelminticum* seeds (CA) dose dependently inhibited rat intestinal disaccharidases. CA also inhibited human salivary α-amylase activity with IC_{50} value of 185.5 ± 4.9 μg. The inhibitory effect of CA was found to be 8–32 fold more potent than DL-catechin but less effective than acarbose on rat intestinal disaccharidases and salivary α-amylase. The in vitro inhibition of glucosidases was further confirmed by in vivo maltose tolerance test in rats. Feeding of CA at 50–200 mg/kg body weight (b.wt) to maltose (2.0 g/kg b.wt), loaded rats significantly reduced the postprandial plasma glucose levels compared with acarbose. They reported that CA exerts antihyperglycemic effect by decreasing postprandial glucose in rats by modulating α- amylase and glucosidases (sucrase and maltase) activity and thus may be useful for the management of diabetes mellitus (Rammohan et al., 2008) .

The antioxidant activities and the inhibition of pancreatic α-amylase of the aqueous, ethanolic and hydroethanolic extracts of *Laportea ovalifolia*, *Luffa aegytiaca*, and *Cola nitida*. The phytochemical screening of these extract was also carried out revealing the presence of flavonoids, tannins and saponins. The highest total phenol content was reported in the ethanolic extract of *C. nitida and* the aqueous extract of the same plant and the hydroethanolic extract of *L. aegyptiaca* exhibited the best antioxidant activity in FRAP and in DPPH methods, respectively. The presence of active phytochemical substances with antioxidant properties may provide a

substantial basis for the use of these plants in ethnomedicine for the treatment of diabetes.

Rapid gastrointestinal absorption of refined carbohydrates (CHO) is linked to perturbed glucose-insulin metabolism .They assessed the ability of various natural substances, commonly referred to as "CHO blockers," to influence starch and sucrose absorption *in vivo* in ninety-six rats . These natural enzyme inhibitors of amylase lessen breakdown of starches in the gastrointestinal tract, limiting their absorption. To estimated absorption, groups of nine SD rats were gavaged with water or water plus rice starch and circulating glucose was measured at timed intervals .In rats, glucose elevations above baseline over four hours internal control after ingesting bean extract, hibiscus extract, and l-arabinose respectively in addition to the rice starch. The former two were significantly different from control. L-Arabinose virtually eliminated the rising circulating glucose levels after sucrose challenge, whereas hibiscus and bean extracts were associated with lesser decreases than l-arabinose that were still significantly lower than control. The glucose elevations above baseline over four hours in rats receiving sucrose (AUC) were 51%, 43% and 2% of control for bean extract, hibiscus extract, and L-arabinose, respectively. Evidence for dose-response of bean and hibiscus extracts is reported. Giving the natural substances minus CHO challenge caused no significant changes in circulating glucose concentrations, indicating no major effects on overall metabolism. A formula combining these natural products significantly decreased both starch and sucrose absorption, even when the CHO were given simultaneously. These results support the hypothesis that the enzyme inhibitors examined

here at reasonable doses can safely lower the glycemic loads starch . (Harry,et.al.,2007).

Many plant polysaccharides exhibit hypoglycaemic effect. Though the fruit of *Psidium guajava* is known to contain free sugars, the fruit extract showed hypoglycaemic effect in alloxan treated mice and human subjects. The study was aimed to determine the glycaemic potential of *P. guajava* fruit peel extract on blood glucose level (BGL) of normal and streptozotocin induced sub-diabetic rats during fasting blood glucose (FBG) and glucose tolerance test (GTT).Female albino Wistar rats (n=42) were divided into seven equal groups, and were given different doses of fruit peel extract. Diabetes was induced by streptozotocin injection (ip) at a dose of 45 mg/kg body weight. Blood glucose levels were measured after collecting the blood from tail veins.The diabetic and sub-diabetic models showed hyperglycaemic effect from a single oral administration of variable doses of *P. guajava* fruit peel extract. The maximum rise of 26.51 per cent was observed in BGL from a dose of 400 mg/kg bw exactly after 8 h of administration in normal rats whereas the maximum rise of 90.7 per cent was observed with the same dose of 400 mg/kg bw after 2 h of glucose administration in sub-diabetic rats. The hyperglycaemic effect of *P. guajava* fruit peel suggests that the diabetic patients should peel off the guava fruits before consuming. Their results can be useful in controlling hypoglycaemia occasionally caused due to excess of insulin and other hypoglycaemic drugs.(Rai et.al.,2007).

To investigate the effect of low dietary zinc intake and experimental diabetes (IDDM) on the zinc and carbohydrate metabolism, 8-week-old male

wealing normal albino (Wistar) rats were fed diets containing either adequate (54mg/kg) or low zinc (1mg/kg) quantities for one week. Ten rats from each group (n=20) were then intraperitoneally injected with alloxan to induce diabetes. The rats were sacrificed after a further three weeks. Body weight gain and food intake were recorded regularly. On day 28, after an overnight fast, the animals were sacrificed and blood glucose, serum insulin, serum cholesterol concentrations, liver glycogen contents, and femur and pancreatic zinc concentrations were determined. Diabetic rats fed a low zinc or control diet had a low body weight gain, high total food intake (hyperphagia), low serum insulin, low liver glycogen contents and high serum cholesterol concentrations compared to normal rats. The consumption of the low zinc diet had only a minimal effect on the zinc status of rats as indicated by the growth rate, food intake and femur and pancreatic zinc concentrations. However, both diabetic and non-diabetic rats fed a low zinc diet had higher blood glucose than their control counterparts. Liver glycogen was also found to be higher in the low zinc non-diabetic rats than in their controls. Serum insulin and serum cholesterol concentrations were unaffected by dietary regimen. To conclude, the present study demonstrates that a reduced zinc intake had an effect on glucose utilization in both diabetic and non-diabetic rats and on glycogen deposition in non-diabetic rats. However, there were negligible changes in zinc status. Therefore, it appears that abnormalities in the carbohydrate metabolism may occur before tissue zinc depletion becomes apparent.
(Zine Kechard et.al., 2002).

Mary sujin study reveals the anti-diabetic effect in stomach of albino wistar rats using *Gymnema sylvestre* (Retz) herbal powder. The

histopatholoical and biochemical assays were carried out in organs and blood serum of stomach. The different concentration of *G. sylvestre* treated as 5, 10, 15, 20/gms/25 days. The effect of crude drugs in rats reduced the body weight and the diabetics was assessed in stomach by measuring the levels of selected blood parameters of protein, glucose, cholesterol, insulin and triglycerides and the effect of histopathology. Treatment with *G. sylvestre* reduced the stomach weight of animals and reduced significant level of insulin, protein, triglycerides, cholesterol and glucose. The data was analyzed using mathematical calculation values and were expressed as significant. (Mary Sujin.A et.al.,2008).

The effect of aqueous leaf extract of *Vernonia amygdalina* on blood glucose of alloxan induced diabetic albino wistar rats was evaluated. Animals received daily oral administration of aqueous leaf extract of *Vernonia amygdalina* for 14 days. The effect of 200mg/kg dose was studied during the treatment period. There was a significant reduction in blood glucose concentration ($P < 0.05$) with the mean blood glucose of the different groups having 5.3 ± 0.33 for normal control, 7.0 ± 0.40 for diabetic control and 4.5 ± 0.24 for diabetic treated. There was also 15.38 percent reduction in body weight of the rats. Based on these results, it was suggested that aqueous leaf extract of *Vernonia amygdalina* has hypoglycaemic effect. Further researches are therefore needed to identify all the hypoglycaemic inducing compounds in *Vernonia amygdalina*.(Chike et.al.,2006)

Zhang studied the hypoglycaemic activity of a new polysaccharide extracted from *Artemisia sphaerocephala* Krasch seed in alloxan-induced

diabetic rats. The *Artemisia* seed polysaccharide (ASP) was administered orally for 4 weeks and the blood glucose changes were determined in fasted rats. Plasma insulin, cholesterol and triglycerides levels were also determined. The ASP at a dose of 200 mg/kg body weight (bw) produced a significant decrease in blood glucose levels in diabetic rats (P <0.01). In the other hand, the effect of the ASP on the plasma cholesterol were also significant in diabetic rats (P <0.05). Furthermore, there was a significant effect of ASP on plasma triglycerides in both normal and diabetic groups. In order to characterise the active principle(s), which could be responsible for the therapeutic effect, a preliminary phytochemical analysis of the ASP was performed.(Zhang , et.al., 2006).

2.3 INDUCTION OF DIABETES IN RATS

The induction of experimental diabetes in the rat using chemicals which selectively destroy pancreatic B cells is very convenient and simple to use. The most usual substances to induce diabetes in the rat are alloxan and streptozotocin. The understanding of changes in B cells of the pancreas as well as in the whole organism after alloxan or streptozotocin treatment is essential for using these compounds as diabetogenic agents. The metabolic disturbances in alloxan- and streptozotocintreated rats were described recently by Szkudelski [illegible] (2005). This review focuses on the elucidation of the mechanism of cytotoxic action of alloxan and streptozotocin in B cells of the rat pancreas.

2.3.1 The mechanism of alloxan action

Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil) was first described by Brugnatelli in 1818. Wöhler and Liebig used the name "alloxan" and described its synthesis by uric acid oxidation . The diabetogenic properties of this drug were reported many years later by Dunn, Sheehan and McLethie , who studied the effect of its administration in rabbits and reported a specific necrosis of pancreatic islets. Since then, alloxan diabetes has been commonly utilized as an animal model of insulindependent diabetes mellitus (IDDM).

Alloxan exerts its diabetogenic action when it is administered parenterally: intravenously, intraperitoneally or subcutaneously. The dose of alloxan required for inducing diabetes depends on the animal species, route of administration and nutritional status. Human islets are considerably more resistant to alloxan than those of the rat and mouse . The most frequently used intravenous dose of this drug to induce diabetes in rats is 65 mg/kg . When alloxan is given intraperitonealy or subcutaneously its effective dose must be 2-3 times higher. The intraperitoneal dose below 150 mg/kg b.w. may be insufficient for inducing diabetes in the rat . Fasted animals are more susceptible to alloxan (Szkudelski . 2005), whereas increased blood glucose provides partial protection (Szkudelski . 2005). The mechanism of alloxan action has been intensively studied, predominantly *in vitro*, and is now characterized quite well. Using isolated islets and perfused rat pancreas it was demonstrated that alloxan evokes a sudden rise in insulin secretion in the presence or absence of glucose. This phenomenon appeared just after alloxan treatment and was not observed after repetitive exposure of islets to this diabetogenic agent . The sudden rise in blood insulin concentration was

also observed *in vivo* just after alloxan injection to rats (Szkudelski ., .2005). Alloxan-induced insulin release is, however, of short duration and is followed by complete suppression of the islet response to glucose, even when high concentrations (16.6 mM) of this sugar were used .

Alloxan is a hydrophilic and unstable substance. Its half-life at neutral pH and 37 °C is about 1.5 min and is longer at lower temperatures . On the other hand, when a diabetogenic dose is used, the time of alloxan decomposition is sufficient to allow it to reach the pancreas in amounts that are deleterious.

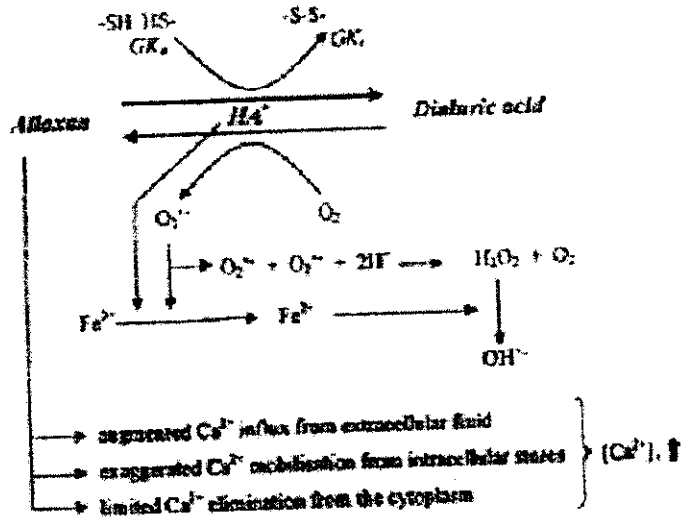
The action of alloxan in the pancreas is preceded by its rapid uptake by the B cells Rapid uptake by insulin-secreting cells has been proposed to be one of the important features determining alloxan diabetogenicity. Another aspect concerns the formation of reactive oxygen species. A similar uptake of alloxan also takes place in the liver. However, the liver and other tissues are more resistant to reactive oxygen species in comparison to pancreatic B cells and this resistance protects them against alloxan toxicity. The formation of reactive oxygen species is preceded by alloxan reduction. In B cells of the pancreas its reduction occurs in the presence of different reducing agents. Since alloxan exhibits a high affinity to the SH-containing cellular compounds, reduced glutathione (GSH), cysteine and protein-bound sulfhydryl groups (including SHcontaining enzymes) are very susceptible to its action .However, other reducing agents such as ascorbate may also participate in this reduction .they proposed that one of the SH-containing compounds essential for proper glucose-induced insulin secretion is glucokinase (EC 2.7.1.2), being very vulnerable to alloxan. Alloxan reacts with two -SH groups in the sugarbinding side of glucokinase resulting in the

formation of the disulfide bond and inactivation of the enzyme. Glucose can protect glucokinase against the inactivation hindering the access of alloxan to the -SH groups of the enzyme .

Dialuric acid is formed as a result of alloxan reduction. . It is then re-oxidized back to alloxan establishing a redox cycle for the generation of superoxide radicals(Munday). The reaction between alloxan and dialuric acid is a process in which intermediate alloxan radicals (HA) and an unidentified "compound 305" (maximum absorption at 305 nm) is formed. The latter appears when alloxan is reduced by GSH

$$O_2^{\cdot-} + O_2^{\cdot-} + 2\,H^+ \rightarrow H_2O_2 + O_2$$

Superoxide radicals are able to liberate ferric ions from ferritin and reduce them to ferrous ions. Fe can also be reduced by alloxan radicals .Moreover, superoxide radicals undergo dismutation to hydrogen peroxide. This reaction may occur spontaneously or may be catalyzed by superoxide dismutase (EC 1.15.1.1)



The mechanism of alloxan-induced reactive oxygen species generation in B cells of rat pancreas. GKa, GKi – glucokinase active and inactive, respectively; HA – alloxan radicals; [Ca2+] – intracellular calcium concentration.

One of the targets of the reactive oxygen species is DNA of pancreatic islets. Its fragmentation takes place in B cells exposed to alloxan. DNA damage stimulates poly ADP-ribosylation, a process participating in DNA repair. Some inhibitors of poly ADP-ribosylation can partially restrict alloxan toxicity. This effect is, however, suggested to be due to their ability to scavenge free radicals rather than to a restriction of poly ADPribosylation initiated by alloxan .Superoxide dismutase, catalase (EC 1.11.1.6) and non-enzymatic scavengers of hydroxyl radicals were also found to protect against alloxan toxicity. Therefore, chemicals rendering anti-oxidative properties and inhibiting poly ADP-ribosylation can attenuate alloxan toxicity.

It has been argued that glucose counteracts alloxan cytotoxicity *in vitro* and *in vivo*. This ability, however, is not only the result of the protection of glucokinase. The protective effect of glucose against necrotic

death of B cells may be due to interaction of the sugar with the glucose transporter GLUT2 resulting in limited alloxan uptake. It has been previously proposed that the action of glucose is also related to its metabolism and to the increased generation of reducing equivalents (NADH and NADPH) accelerating the recirculation of glutathione. GSH is known to provide protection against free radicals. It may thus divert hydrogen peroxide from the pathway leading to the formation of hydroxyl radicals .

$$GSSG + 2\,NADPH \rightarrow 2\,GSH + 2\,NADP^{+}$$
$$H_2O_2 + 2\,GSH \rightarrow GSSG + 2\,H_2O$$

Moreover, observed that the *in vitro* generation of hydroxyl radicals in the presence of alloxan strongly depends on GSH concentration. GSH in low concentrations potentiated the formation of these radicals, whereas the oxygen consumption, autoxidation of dialuric acid and formation of hydroxyl radicals were significantly inhibited in higher concentrations. GSH at high concentrations can also inhibit HA• generation and directly neutralize hydroxyl radicals. Thiyl radicals (GS•) formed in this reaction are then converted to GSSG:

$$GSH + OH^{\cdot} \rightarrow GS^{\cdot} + H_2O$$
$$GS^{\cdot} + GS^{\cdot} \rightarrow GSSG$$

Indeed, in rat islets incubated with alloxan the GSH content and GSH/GSSG ratio were decreased, whereas glucose evoked the opposite effect.

In the *in vivo* experiment, glucose given to rats 20 min prior to alloxan partially restricted alloxaninduced increase in the activity of glutathione peroxidase (EC 1.11.1.9) and mitigated the drop of liver nonprotein -SH

groups (especially reduced glutathione) (Szkudelski . 2005). The protective action of this sugar is, however, strongly glucose and alloxan dose-dependent .

It has been proposed that disturbances in intracellular calcium homeostasis constitute an important step in the diabetogenic action of alloxan. This concept was confirmed by *in vitro* and *in vivo* experiments demonstrating that alloxan elevates cytosolic free Ca2+ concentration in pancreatic B cells This effect arises from several events: alloxan-induced calcium influx from extracellular fluid, exaggerated calcium mobilization from intracellular stores and its limited elimination from the cytoplasm. The calcium influx may result from the ability of alloxan to depolarize pancreatic B cells. Depolarization of the cell membrane opens voltagedependent calcium channels and enhances calcium entry into cells. Alloxan was also found to exert a stimulatory effect on mitochondrial Ca2+ efflux with simultaneous inhibitory action on Ca2+ uptake by mitochondria. The restriction of calcium removal from the cells due to alloxan-induced inhibition of liver plasma membrane Ca2+ -ATPase was also reported. The effect of alloxan on intracellular calcium concentration seems to be mediated, at least partially, by H2O since hydrogen peroxide itself exerts a similar effect on calcium concentration in B cells .

Thus, the previously mentioned sudden rise in insulin release from B cells treated with alloxan may be one of the effects of alloxan-induced augmentation in cytosolic Ca2+ concentration. The exaggerated concentration of this ion contributes to supraphysiological insulin release and, together with reactive oxygen species, causes damage of pancreatic B cells.

The results of experiments with calcium channel antagonists have confirmed the important role of cytosolic calcium in the cytotoxic action of alloxan. Pretreatment of rats with verapamil prevented the alloxan-induced increase in B cell Ca2+ concentration and abolished the stimulatory effect of alloxan on insulin release. The calcium channel antagonists (verapamil and diltiazem) also suppressed hyperglycemia and the onset of alloxan diabetes in rats.Summing up, the toxic action of alloxan on pancreatic B cells, described many years ago are the sum of several processes such as oxidation of essential -SH groups, inhibition of glucokinase, generation of free radicals and disturbances in intracellular calcium homeostasis.

Many investigators suggested that the selectivity of alloxan action is not quite satisfactory. Recent experiments confirmed this objection. The diabetogenic dose of alloxan was found to decrease -SH groups accompanied by a simultaneous rise in glutathione peroxidase activity in the rat liver two minutes after its administration (Szkudelski . 2005). At the same time, the blood insulin concentration rose dramatically. This exaggerated insulinemia did not evoke, however, any significant reduction of blood glucose suggesting impaired peripheral insulin sensitivity in the short time after alloxan treatment (Szkudelski 2005). It was also observed that alloxan intensified basal and epinephrine-induced lipolysis in isolated rat adipocytes and insulin failed to restrict this effect .

Thus, using alloxan to evoke diabetes, animals should be examined after proper period of time to minimize side effects of alloxan action. It should also be emphasized that the range of the diabetogenic dose of alloxan is quite narrow and even light overdosing may be generally toxic causing the loss of many animals. This loss is most likely due to kidney tubular cell

necrotic toxicity, in particular when too high doses of alloxan are administered .

2.4 LABORATORY ANIMAL

A laboratory rat is a rat of the species *Rattus norvegicus* which is bred and kept for scientific research. Laboratory rats have served as an important animal model for research in psychology, medicine, and other field.

Laboratory rats share origins with their cousins in domestication, the fancy rats. In 18th century Europe, wild Brown rats ran rampant and this infestation fueled the industry of rat-catching. Rat-catchers would not only make money by trapping the rodents, but also by turning around and selling them for food, or more importantly, for rat-baiting. Rat-baiting was a popular, but brutal bloodsport which involved filling a pit with rats and timing how long it took for a terrier to kill them all. Over time, breeding the rats for these contests produced variations in color, notably the albino and hooded varieties. The first time one of these albino mutants was brought into a laboratory for a study was in 1828, in an experiment on fasting. Over the next 30 years rats would be used for several more experiments and eventually the laboratory rat became the first animal domesticated for purely scientific reasons.

Over the years, rats have been used in many experimental studies, which have added to our understanding of genetics, diseases, the effects of drugs, and other topics in health and medicine. Laboratory rats have also proved valuable in psychological studies of learning and other mental processes. The historical importance of this species to scientific research is

reflected by the amount of literature on it, roughly 50% more than that on mice.

Domestic rats differ from wild rats in many ways. They are calmer and less likely to bite, they can tolerate greater crowding, they breed earlier and produce more offspring, and their brains, livers, kidneys, adrenal glands, and hearts are smaller.Scientists have bred many strains or "lines" of rats specifically for experimentation. Most are derived from the albino Wistar rat, which is still widely used. Other common strains are the Sprague Dawley, Fischer , Holtzman albino strains, the Long-Evans, and Lister black hooded rats. Inbred strains are also available but are not as commonly used as inbred mice.Rat strains are generally not transgenic, or genetically modified, because the gene knockout and embryonic stem cell techniques that work in mice are relatively difficult in rats. This has disadvantaged many investigators, who regard many aspects of behavior and physiology in rats as more relevant to humans and easier to observe than in mice and who wish to trace their observations to underlying genes. As a result, many have been forced to study questions in mice that might be better pursued in rats. In October 2003, however, researchers succeeded in cloning two laboratory rats by nuclear transfer. So rats may begin to see more use as genetic research subjects. Much of the genome of *Rattus norvegicus* has been sequenced.

2.4.1 Strains

A *strain*, in reference to rodents, is a group in which all members are genetically identical. In rats, this is accomplished through inbreeding. By having this kind of population, it is possible to conduct experiments on the

roles of genes, or conduct experiments that exclude variations in genetics as a factor. The opposite, *outbred* strains, are used when identical genotypes are unneccessary or a random population is required, and are more defined as *stocks* as opposed to *strains*.

Wistar rat

Wistar rats are an outbred strain of albino rats belonging to the species *Rattus norvegicus*. This strain was developed at the Wistar Institute in 1906 for use in biological and medical research, and is notably the first rat strain developed to serve as a model organism at a time when laboratories primarily used *Mus musculus*, or the common House mouse. More than half of all laboratory rat strains are descended from the original colony established by physiologist Henry Donaldson, scientific administrator Milton J. Greenman, and genetic researcher/embryologist Helen Dean King. The Wistar rat is currently one of the most popular rat strains used for laboratory research.

MATERIALS AND METHOD

3.MATERIALS AND METHODS

3.1 Plant material

The leaves –tender,young ,mature of *P.guajava* were collected from the orchard,Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu and authenticated by Dr.Balamohan. *Syzygium cumini* fruit were bought from the Coimbatore market.

3.2 Preparation of plant extract

The crude inhibitor was prepared in the powder form from the fresh leaves sample. The leaves of guava plant were taken and they are washed with distilled water three times untill the dust and other particles were removed. 50g of the leaves were weighed .Similarly seeds from *S.cumini* were taken removed all of the pulpy materials and washed with distilled water and taken about 50 g of seeds. The samples were taken and homogenized with 500ml of distilled water. Then the homogenate was filtered with cheese cloth and the samples were centrifuged at 10000 rpm for 10 minutes. The samples collected were allowed to concentrated with help of the rotatory vacuum evaporator maintained at 60oc under vacuum pressure. The concentrated samples of about 15-20 ml were lyophilized. Thus the crude inhibitor in powder form obtained. The crude inhibitor in the powder was taken for α-amylase inhibiting activity .

3.3 Materials required

Porcine pancreatic amylase

20mM phosphate buffer

DNS reagent

3.4 Flow procedure for Extraction of crude amylase inhibitor

50g of Fresh leaves

↓ 500 ml water

Homogenate & Filtrate

↓ 10,000 rpm for 10 min

Centrifuge

↓ 60° C

Crude extract in flash evaporator

↓

Lyophilization

↓

Crude extract in powder form

3.5 α-Amylase inhibitory assay

α-Amylase inhibitory activity of the extract was analyzed by the method of Bernfeld with a minor modification as described below. One hundred µl of the test extract was allowed to react with 200µl of the porcine pancreatic α-amylase (0.6 U) and 100µl of 20mM of phosphate buffer (pH 6.9) in a test tube. After incubating for 20min, 100µl of 1% starch solution was added. The same was performed for the control in another test tube where 200µl of the enzyme was replaced by the same quantity of buffer. After incubating for 5min, 500µl of dinitrosalicylic acid reagent was added to both control and tests. The tubes were then kept in boiling water bath for 5min.

Flow of procedure for testing α – amylase testing activity

100 µl of amylase +100 µl of extract

↓ 200 µl water

200 µl of 20mM phosphate buffer

↓ 20 min

200 µl 1% of starch solution

↓ 15 min

1000 µl of DNS reagent

↓ 10 min at 100° C

Absorbance at 540 nm

The absorbance was recorded at 540nm using a spectrophotometer and the percentage inhibition of α-amylase was calculated.

Inhibition % = control-test / control x 100.

The inhibition of a-amylase was tested with varying concentration of acarbose and crude leaf extracts and their IC50 values were then calculated

3.6 Method

3.6.1 Experimental animals:

All the experiment was carried out with healthy Female Albino Wistar rats between 2 and 3 months of age and weighed about 180–200 g animals included in the study. Housed individually in polypropylene cages, maintained under standard conditions (12 h light and 12 h dark cycle at 25±30◦C), the animals was fed with standard rat pellet diet and water *ad libitum*. They were divided into 6 groups of 6 animals in each .The research was carried out in accordance with the internationally accepted guidelines for laboratory animal use and care. The experiments report approved by Institutional ethical committee,PSG medical college,coimbatore.

3.6.2 Induction of diabetes

Diabetes was induced in the fasted rats for about 12 hours by intraperitoneal injection of 120 mg/kg of alloxan, fresly dissolved in 2% sodium citrate buffer ,0.05 M ,pH 4.5. six hours after induction animals

started to receive 5% glucose solution ad libitum to avoid hypoglycemic shock. The diabetic state was assessed by measuring the non fasting serum glucose concentration 72 h after alloxan treatment. Only animals with values above 180mg/dl were considered diabetic and used in the experiment

3.6.3 Oral carbohydrate challenge tests

The oral carbohydrate tolerance tests were carried out with starch separately in both normal and diabetic groups of rats and were equally divided into various treatment groups.

Treatment protocol:

The diabetic animals were divided into 6 groups of 6 animals each and treated as per the protocol given below. The animals were fasted overnight and had free access to water. Rats of experimental groups administered suspension of desired test samples orally with the help of intragastric tube.

Diabetic Control Group (G1) : The animals were treated orally with distilled water 4 ml/kg.

Treatment Group (G2) : Alloxan induced diabetic rats were given with *p.guajava* extract of 12mg/kg

Treatment Group (G3): Alloxan induced diabetic rats were given with *s.cumini* extract of 12mg/kg

Treatment Group (G4): Alloxan induced diabetic rats were given with standard drug Acarbose extract of 10mg/kg.

Acarbose was used as positive control at a dose of 10 mg/kg .

Diabetic rats were administrated with crude amylase inhibitors given as per dose fixed and comparative to that with standard drug acarbose. After 10 minutes , all rats were given with starch of 3g/kg body weight orally and tail was snipped for blood glucose estimation before (0 min) and at 30,60,90,120,150 and 180 min after starch administration. Blood glucose levels were determined at variable interval of time and peak blood glucose observed.

3.7.1 Glucose estimation

Blood samples were collected by end tail vein cutting method and blood glucose level was determined by glucometer using glucose strips.

3.8 FREE RADICAL SCAVENGING ACTIVITY OF EXTRACTS

3.8.1 Ferric Ion Reducing Antioxidant Power Assay (FRAP)

Principle

The antioxidants present in the sample reduced the oxidant probe and the respective product interacted with some coloring agents to forma colored complex. In this method, the antioxidant reduced the Fe^{3+} to Fe^{2+}. This ion then conjugated with the ferricyanide ion to form a Prussian blue colored product, which was spectophotometrically measured at 700 nm.

Fe^{3+} + antioxidant $\rightarrow$ Fe^{2+} + oxidized antioxidant,

$Fe^{2+} + Fe(CN)_6^{3-} \rightarrow Fe[Fe(CN)_6]^-$

Reagents

a, Sample preparation

In a clean dry conical flask, weighed 5 g of dried leaves (fresh leaves were air dried in the incubator at 37 ° C for two days and the same case is followed for bark. Fresh flowers were used for the experimental analysis) and added 50 ml of ethanol. Kept this in an orbital shaker for overnight. The contents were filtered with whatman filter paper and filtrate was collected. About 50 mg of the dried extracts was weighed in a 50 ml standard flask and made up to the mark with distilled water (1 ml = 1 mg). From this 1:20 dilution was performed i.e., 1:20. 2:20 upto 10:20 and 2ml of the extract corresponded to 100 µg to 1000 µg was used for the experimental analysis.

b, 0.2 M Phosphate buffer (pH= 6.6)

Weighed 35.6 g/ L of disodium hydrogen phosphate (A) and 31.2 g/L of sodium dihydrogen phosphate (B). Mixed 26.5 ml 0f (A) and 73.5 ml of (B) and made upto 200 ml with distilled water and adjusted the pH to 6.6.

c, 1% Potassium ferric cyanide

Dissolved 1 g of ferric cyanide in 100 ml of distilled water.

d, 10 % TCA

Dissolved 10 g of trichloro acetic acid in 100 ml of distilled water.

e, 0.1 % Ferric Chloride

Dissolve 0.1 g of ferric chloride in 100 ml of distilled water.

PROCEDURE

About 2 ml of the extract (100-1000 µg /ml), added 2.5 ml of Phosphate buffer (0.2 M, PH 6.6) and 2.5 ml of 1 % potassium ferric cyanide. The mixture was boiled in a water bath aat 50° C for 20 minutes, then rapidly cooled, mixed with 2.5 ml of 10 % trichloro acetic acid and centrifuged at 3000g for 10 minutes. From this, pipette out 2.5 ml of supernatant, 2.5 ml of distilled water and 0.5 ml of 0.1 % ferric chloride. Mixed well and allowed to stand for 10 minutes. The increase in the absorbance at 700 nm was used to measure the reducing power of the plant extract. Vitamin C,Vitamin E, Rutin, Quercetin was used as a positive control.

3.8.2 Ferrous Ion Chelating Ability Assay

Principle

Ferrozine [FZ, disodium salt of 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine] is a complexing agent which is highly specific to Fe^{2+} ions. The resulting complex Fe (II)-$(FZ)_3$ is a magenta colored product which is spectrophotometrically measured at 562 nm. In the presence of various antioxidants this complex formation is impeded and there by there a decreased color will be noticed.

Reagents

a, Sample preparation

In a clean dry conical flask, weighed 5 g of dried leaves (fresh leaves were air dried in the incubator at 37° C for two days and the same case is followed for bark. Fresh flowers were used for the experimental analysis) and added 50 ml of ethanol. Kept this in an orbital shaker for overnight. The contents were filtered with whatman filter paper and filtrate was collected. About 50 mg of the dried extracts was weighed in a 50 ml standard flask and made upto the mark with distilled water (1 ml = 1 mg). From this 1:20 dilution was performed i.e., 1:20. 2:20 upto 10:20 and 2ml of the extract corresponded to 100 µg to 1000 µg was used for the experimental analysis.

b, 2m M $FeCl_2$

Dissolved 19.9 mg of $FeCl_2$ in 50 ml of distilled water.

c, 5mM Ferrozine

Dissolved 49.2 mg of ferrozine in 20 ml of distilled water.

PROCEDURE

About 2 ml of the extract (100-1000 µg /ml)was mixed with 0.1 M of 2mM Fe Cl_2 and o.2 ml of 5mM ferrozine solutions and allowed to react for 10 minutes at room temperature. The absorbance at 562 nm of the resulting solutions were measured and recorded. The ferrous ion chelating was expressed in percentage [1- (test sample absorbance/ blank sample absorbance)] X 100 (%). The $Fecl_2$ and ferrozine acted as control solution. Rutin was used as a positive control.

3.8.3 DPPH Radical Scavenging Assay

Principle

The DPPH was reacted with absolute ethanol to yield a purple color DPPH radical (DPPH). The presence of antioxidants which included polyphenolics and flavonoids in the sample will scavenge the formed DPPH radical and there by a decreased color will be observed which is spectrophotometrically measured at 517 nm.

Reagents

a, Sample preparation

In a clean dry conical flask, weighed 5 g of dried leaves (fresh leaves were air dried in the incubator at 37° C for two days and the same case is followed for bark. Fresh flowers were used for the experimental analysis) and added 50 ml of ethanol. Kept this in an orbital shaker for overnight. The contents were filtered with whatman filter paper and filtrate was collected. About 50

mg of the dried extracts was weighed in a 50 ml standard flask and made upto the mark with distilled water (1 ml = 1 mg). From this 1:20 dilution was performed i.e., 1:20. 2:20 upto 10:20 and 2ml of the extract correspondsed to 100 µg to 1000 µg was used for the experimental analysis.

b, 0.1 MM DPPH (1,1 –Diphenyl-2-picryl-hydrazil) in 99.5 % ethanol.

PROCEDURE

To 0.5ml of DPPH solutions, added 2 ml of the extract (100-1000 µg /ml) and the reaction mixture was vortexed for 10 s and allowed to stand at room temperature for 30 minutes. The absorbance was recorded at 517 nm by using (Beckman DU-530) UV-Vis spectrophotometer and compared with the 75 % ethanol which acted as control solution. The percentage of $ABTS^{+}$ scavenging activity was expressed in percentage [1- (test sample absorbance/ blank sample absorbance)] X 100 (%).Vitamin C,Vitamin E, Rutin, Quercetin was used as reference antioxidant compound.

3.8.4 Cerium (IV) sulphate scavenging activity

Procedure

A method developed was adopted for the determination of reducing power. About 1 ml of the extract was made upto with 1 ml distilled water. To this added 1 ml of 0.002 M Ce(IV) sulphate solution. After shaking for a few minutes, the solution was let to stand for30 minutes at a room temperature. The absorbance of the reaction mixture was measured at 320 nm against a blank composed of distilled water. The decrease in the absorbance at 320 nm was used to measure the unreacted Ce(IV) ion in the extract sample, which indicates an increase in anti oxidant power of the sample.

RESULTS AND DISCUSSION

4.RESULTS AND DISCUSSION

4.1 *In vitro* alpha amylase inhibition

The *in vitro* alpha amylase inhibitory studies demonstrated that both *P*sidium guajava leaves and *S*yzygium cumini seeds had inhibitory activity. A dose dependent inhibition was observed with α- amylase.Thus the highest concentration of 1250 µg/ml showed the maximum inhibition of nearly 70.8%, 73.9% from the extracts of *Psidium guajava* leaves and *Syzygium cumini* seeds respectively. The percentage inhibition varied as shown in the table 4.1.1

Table 4.1.1: Percentage inhibition on Porcine Pancreatic α- amylase by aqueous leaf extracts of *Psidium guajava* and Syzygium cumini seeds and standard Acarbose

Concentration (µg/ml)	% Inhibition	% Inhibition	% Inhibition
	Psidium guajava	*Syzygium cumini*	Acarbose
250	33.2	21.1	22.2
500	47.3	35.9	38.7
750	52.7	49.7	59.3
1000	65.8	58.7	78.9
1250	70.8	73.9	89.2

The extracts from the *P.guajava* leaves and *Syzygium cumini* seeds had the potent inhibition on the amylase .The values obtained from the *in vitro* studies showed that the both extract had more or less equal amount of inhibiting activity and they were compared with the standard drug acarbose. The extracts from the plants are comparatively less potent in alpha amylase inhibitory activity when compared to acarbose.

α- Amylase is more sensitive towards acarbose with the concentration required for 50% of inhibition i.e IC 50 being 0.625 mg/ml. The inhibitory concentrarions of *Psidium guajava* leaves and *Syzygium cumini* seeds were found to be 0.65mg/ml and 0.82 mg/ml .The IC 50 valuesof acarbose,*P.guajava* and *S.cumini* are presented in figures 4.1.1 to 4.1.4

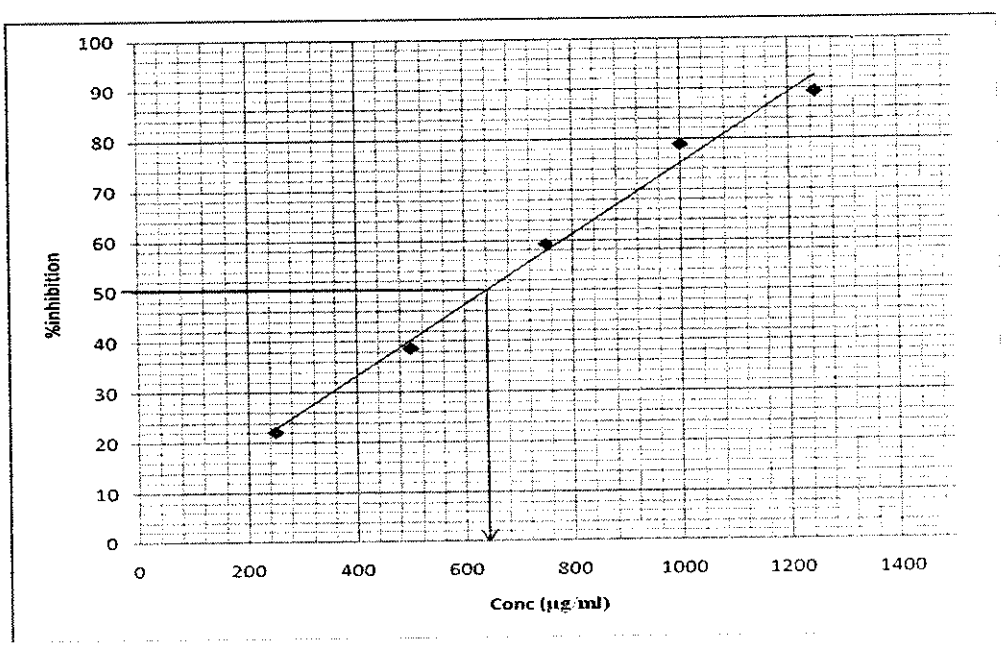


Figure 4.1.1 : Inhibition of porcine pancreatic amylase by acarbose

The graph showing inhibition of amylase at a various concentration. acarbose a standard drug used to inhibit the action of amylase on carbohydrates to regulate the blood glucose levels .

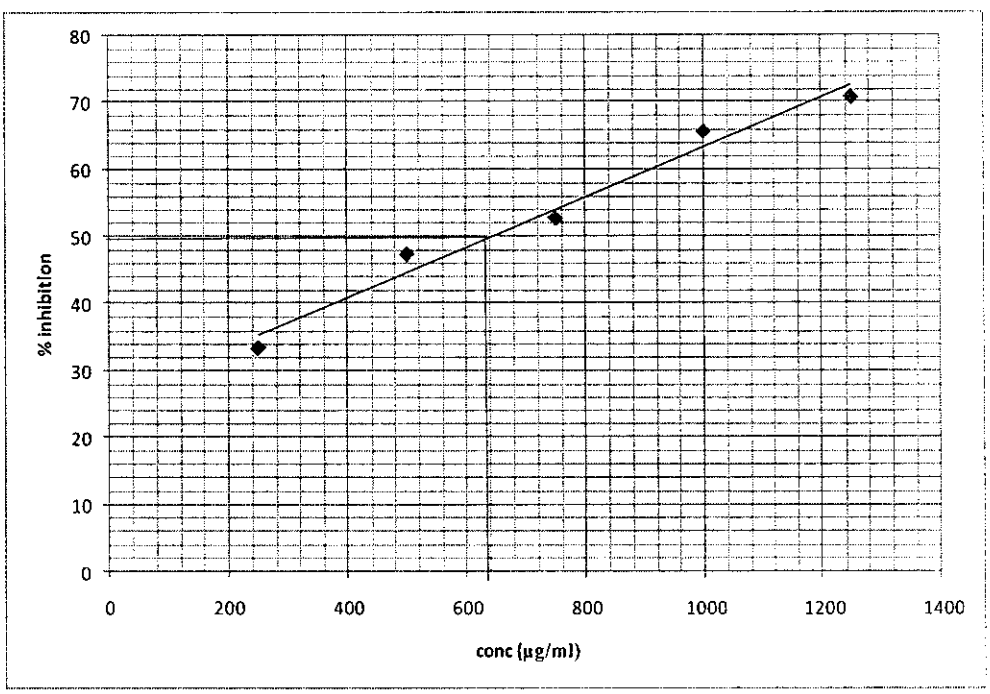


Figure 4.1. 2 : Inhibition of porcine pancreatic amylase by aqueous leaf extract of *Psidium guajava*

The graph showing inhibition of amylase at a various concentration .The extract from *Psidium guajava* leaves inhibit the action of amylase on carbohydrates to regulate the blood glucose levels .The inhibitory activity on porcine pancreatic amylase and the calculation of IC 50 values as shown.

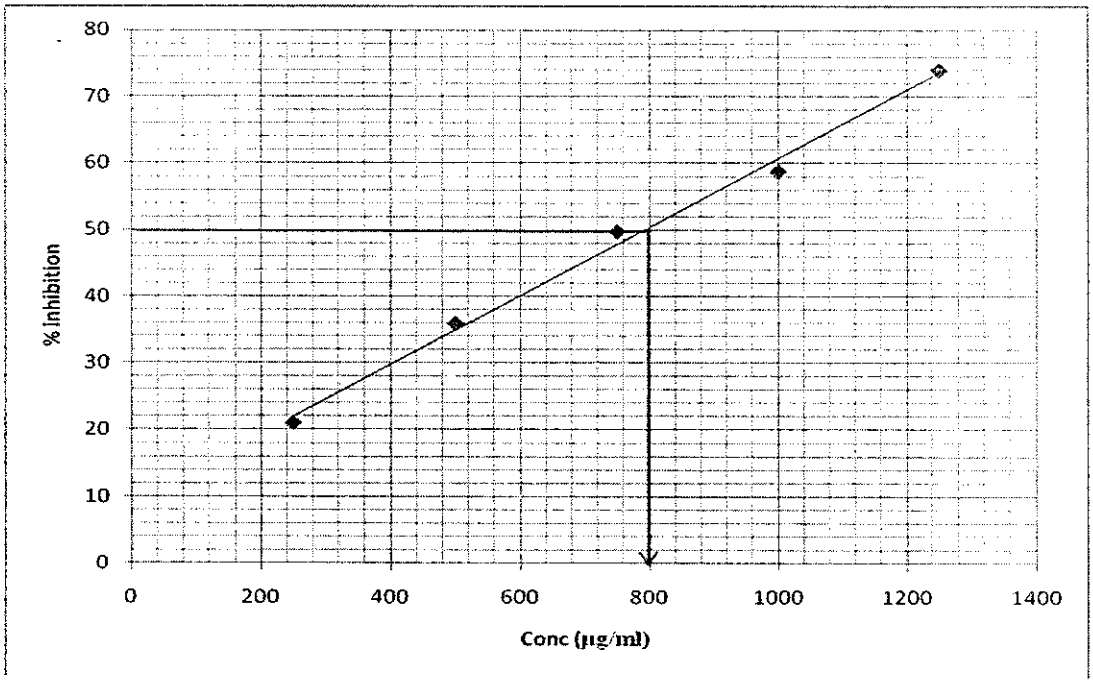


Figure 4.1.3 : Inhibition of porcine pancreatic amylase by aqueous seed extract of *Syzygium cumini*

The graph showing inhibition of amylase at a various concentration .The extract from *Syzygium cumini* seeds inhibit the action of amylase on carbohydrates to regulate the blood glucose levels .The inhibitory activity on porcine pancreatic amylase and the calculation of IC 50 values as shown

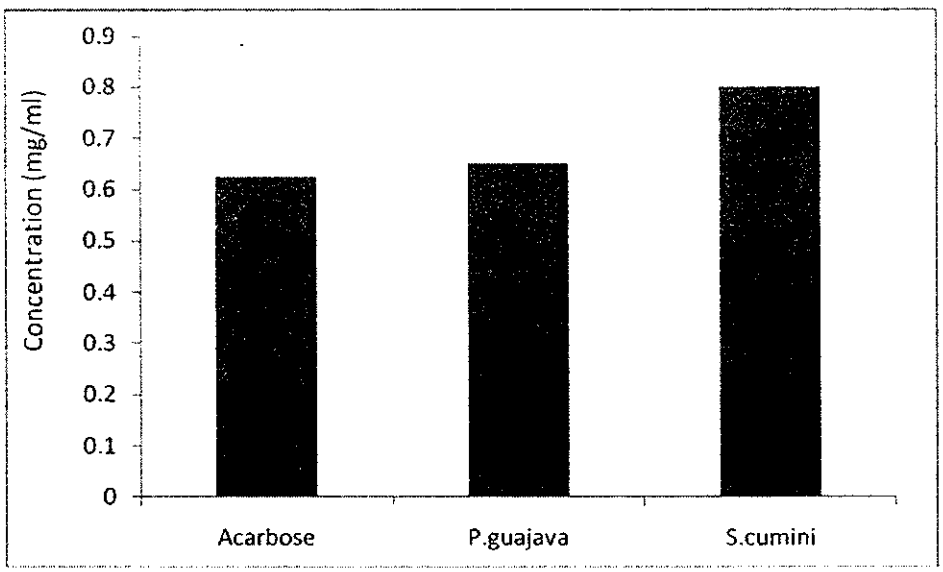


Figure 4.1.4 : Comparative IC 50 values of Acarbose, *Psidium guajava* , *Syzygium cumini* against porcine pancreatic α-amylase.

DM is a metabolic disorder affecting carbohydrate, fat, and protein metabolism. The worldwide survey reported that the DM is affecting nearly 10% of the population . The treatment of DM is based on oral hypoglycemic agents and insulin. DM is also treated with Indian traditional medicine using anti-diabetic medicinal plants . Hence, there is a need to search for newer anti-diabetic agents that retain therapeutic efficacy and are devoid of side effects that could be important sources of such agents. In this study the effect amylase inhibitors on starch digestion was observed which the regulate blood glucose levels over a period of time.

Many of amylase inhibitors are isolated from the plants, microbes and animals. Amylase inhibitors are roughly divided into proteinaceous and non-proteinaceous and the present amylase inhibitors from these plants are

not proteinaceous, because the inhibitory activity was observed at high temperature. When compared to solvent extraction the aqueous extract should more potent amylase inhibitory activity and they were determined from the *in vitro* study.

Psidium guajava, is an important food crop and medicinal plant in tropical and subtropical countries .Different parts of the plant are used in various indigenous systems of medicine, its leaves, roots and fruits have been used for prevention and treatment of diabetes(Rosa Martha *et al.*, 2008).

The seeds of *Syzygium cumini* are used by the Ayurvedic physicians (and also in Indian folklore) in the treatment of diabetes mellitus.

The present *in vitro* study indicated the crude inhibitor from *Psidium guajava* leaves and *Syzygium cumini* seeds had potent inhibitory activity of α- amylase .The percentage of inhibition varies with varying concentrations of crude inhibitor.

Similarly the standard α-amylase inhibitor was tested and to showed the maximum percentage of inhibition of 89.9% and these results were compared with crude inhibitors. IC50 values for Acarbose, *P.guajava* and *S,cumini* are 0.625mg/ml ,0.65 mg/ml,0.80 mg/ml respectively.

4.2 *In vivo* Study

In vivo studies were carried out with the female albino rats weighed between 180-200 g. The diabetic state was assessed by measuring the non fasting serum glucose concentration 72 h after alloxan treatment. Only animals with values above 180mg/dl were considered diabetic and used in the experiment.

The oral carbohydrate tolerance tests were carried out with starch separately in both normal and diabetic groups of rats and were equally divided into various treatment groups.

4.2.1 Oral starch tolerance test

Inhibitors of carbohydrate hydrolyzing enzyme plays a important role in post prandial blood glucose levels. Oral administration of starch (3g/kg) with amylase inhibitors to normal rats and with the diabetic rats significantly suppressed the increase of blood glucose levels after starch loading in a dose dependent manner. Blood glucose levels were determined at variable time intervel and peak blood glucose level observed. The results of blood glucose level in different experimental groups are presented in the table 4.2.1 and figure 4.2.1.

Table 4.2.1 : Blood glucose levels of diabetic control and Acarbose , *Psidium guajava* and Syzygium cumini treated rats

Time (min)	Diabetic control (mg/dl)	Acarbose (mg/dl)	*Syzygium cumini* (mg/dl)	*Psidium guajava* (mg/dl)
0	260	240	236.3	264.6
30	298.5	267.3	260.6	284.6
60	287	255.3	249.3	270
90	273.5	247.3	230.3	258.6
120	271.5	242	230.3	255
150	270.5	238.6	230	252
180	266	230	229.3	249.3

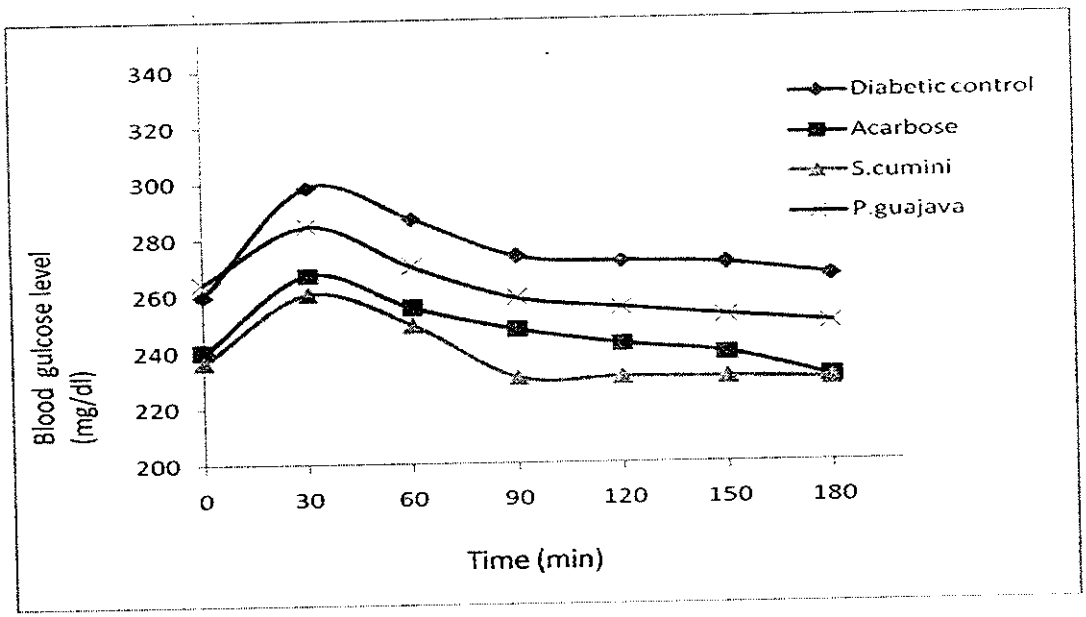


Figure 4.2.1 : Effect of blood glucose levels of diabetic control and Acarbose , *Psidium guajava* and Syzygium cumini treated rats

The *in vivo* studies with rats showed a reduction of blood glucose levels when compared to the control diabetic rats and the plants extrcts brought the blood glucose levels and not like diabetic rats. Oral starch test determine the reduction of blood glucose levels at various interval of time 0 , 30 ,60 ,90 ,120,150 ,180 minutes. The blood levels increases first and gradually reduces but as in the case of diabetic rats the increase to high and then decreases. When compared to treated groups there was increases in glucose levels but not like that of diabetic rats.

In the *in vivo* experiment , doses 12 mg/kg of both the aqueous extracts from *P.guajava* leaves and *Syzygium cumini* seeds and 10 mg/kg Acarbose reduced the blood glucose excursions and decreased the peak blood glucose in diabetic rats .The extract showed the reduction in blood glucose levels caused by starch loading between the 60 min to 120 min .The above results show a similarity to the effects of Acarbose which inhibit the amylase and showed the reduction in blood glucose levels .The aqueous extracts delay the quick digestion of starch and lengthen the duration of carbohydrate absorption over time and reducing the blood glucose levels. The siginificant difference between the treatments and time were found to be ($p<0.05$) which calculated in two anova table by using SPSS software. The difference between the diabetic control and treated rats with extracts and acarbose having significant at 5% level. Acarbose ,inhibit the amylase present in the system have demonstrated to decrease post prandial hyperglycemia. These medications are most useful for people who have blood glucose levels only slightly above the normal level .(Rammohan et al., 2008)

Therefore the retardation and the delay of carbohydrate absorption with a plant based amylase inhibitors offers a prospective therapeutic approach for the management of type 2 diabetes Although the extract seems to be promising in the treatment of type 2 diabetes mellitus by reducing post prandial hyperglycemia and recommended to use for humans.

4.3 FREE RADICAL SCAVENGING ACTIVITY OF EXTRACTS

4.3.1 FRAP (FERRIC REDUCING ANTIOXIDANT POWER ASSAY)

Fe3+/Fe2+ transformation was investigated in the presence of samples for the measurements of the reductive ability. The reducing power of *P.guajava* ranged from 0.505 to 1.887 Abs for 100 µg/ml to 500 µg/ml of extract (Figure 4.3.1) and (table 4.3.1) and *Syzygium cumini* ranged from 0.359 to 1.333 absorbance for 100 µg/ml to 500 µg/ml of extract (Figure 4.3.1). Okuda et al reported that the reducing power of tannins prevents liver injury by inhibiting the formation of lipid peroxides. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

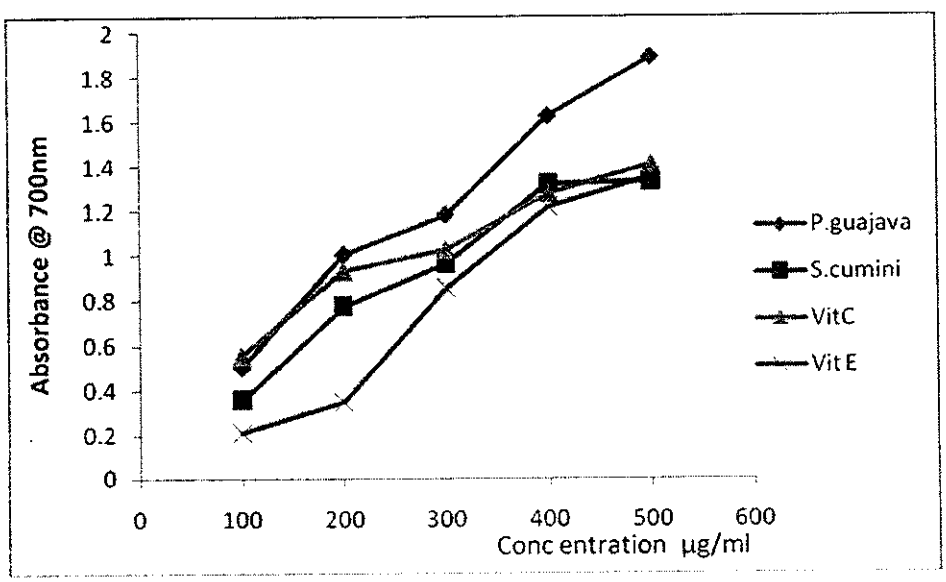


Figure 4.3.1: Ferric ion antioxidant/reducing power assay (FRAP) of *P,guajava* leaves, *S.cumini* seeds,Vitamin C, Vitamin E

Table 4.3.1 : Ferric ion antioxidant/reducing power assay (FRAP) of *P,guajava* leaves, *S.cumini* seeds,Vitamin C, Vitamin E

Concentration (µg/ml)	*P.guajava*	*S.cumini*	VitC	Vit E
100	0.505	0.359	0.555	0.212
200	1.006	0.776	0.931	0.348
300	1.184	0.965	1.027	0.856
400	1.624	1.32	1.279	1.214
500	1.887	1.333	1.411	1.354

4.3.2 Cerium IV sulphate reducing activity

The Ce (IV) sulphate reducing capacity of the sample is measured under carefully adjusted conditions of oxidants concentration and pH such that only antioxidants and not other organic compounds would be oxidized.The advantage of this method is its simplicity , applicability to conventional laboratories and to the possibility of measuring the fluorescence of the Ce (III) produced as a result of the redox reaction concerned besides absorptimetric measurement of the remaining Ce(IV) .a decreased absorbance as a function of increased volume noticed in the and indicated the extracts of *P.guajava* leaves and the *S.cumini* seeds having reducing potential .(Resat apak et al., 2007) .The ceriumIV sulphate reducing activity of *P.guajava* leaves and the *S.cumini* seeds are presented in the figure 4.3.2 and table 4.3.2

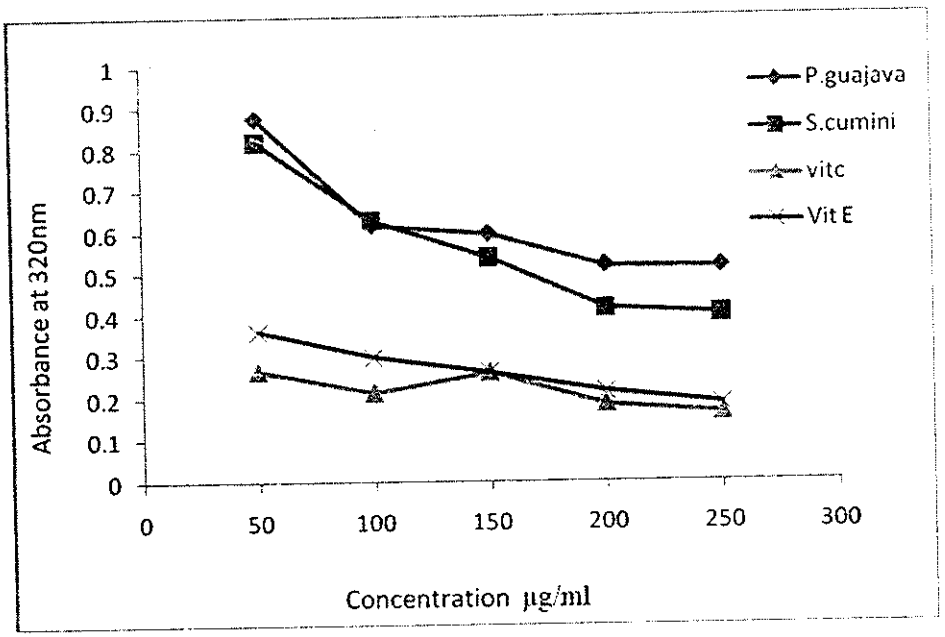


Figure 4.3.2 : Cerium IV sulphate reducing activity of *P,guajava* leaves. *S.cumini* seeds,Vitamin C,Vitamin E

Table 4.3.2 : Cerium IV sulphate of *P,guajava* leaves. *S.cumini* seeds,Vitamin C, Vitamin E

Concentration (µg/ml)	*P.guajava*	*S.cumini*	VitC	Vit E
50	0.878	0.821	0.267	0.362
100	0.619	0.631	0.214	0.3
150	0.597	0.54	0.262	0.262
200	0.52	0.417	0.186	0.216
250	0.517	0.403	0.164	0.186

4.3 Ferrous ion chelating activity of extracts

The chelation of Fe2+ ions was estimated by the method of Decker and Welch in which ferrozine quantitatively forms complexes with Fe2+. In the presence of chelating agents, the formation of this complex is disrupted, thereby impeding the formation of red color imparted by the complex as well. Measurement of this color change therefore allows for the estimation of the chelating activity of the coexisting chelator. As shown in Figure 4.3.3 and table 4.3.3 the formation of Fe2+-ferrozine complex is not complete in the presence of extracts,P.guajava showed 28.6 % inhibition with 500 µg/ml concentration and *S.cumini* showed 25.8 % inhibition 500 µg/ml . Metal chelating agents reduce the concentration of catalyzing transition metal in lipid peroxidation by forming sigma bonds with metals, reducing the redox potential, thereby stabilizing the oxidized form of the metal ion (Meir *et al.*, 1995)

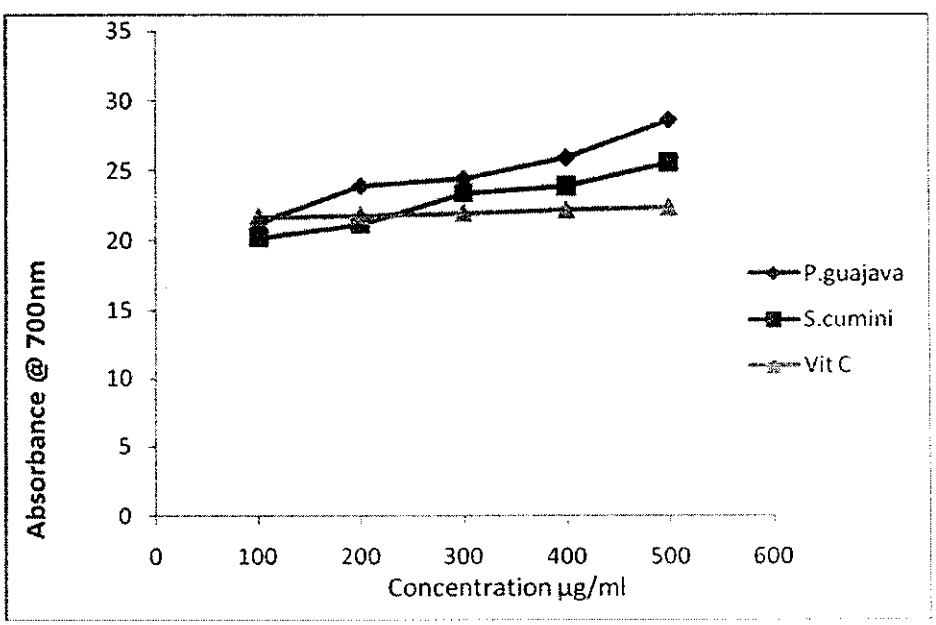


Figure 4.3.3: Ferrous ion chelating activity of of *P,guajava* leaves. *S.cumini* seeds, Rutin

Table 4.3.3: Ferrous ion chelating activity of of *P,guajava* leaves. *S.cumini* seeds,Rutin

Concentration (µg/ml)	*P.guajava*	*S.cumini*	Rutin
100	21.2	20.2	21.7
200	23.9	21.2	21.8
300	24.4	23.4	22
400	25.9	23.9	22.2
500	28.6	25.6	22.4

4.3 DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging activity

The stable DPPH radical model is a widely-used, relatively quick method for the evaluation of free radical scavenging activity. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability . DPPH· is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule . The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm, induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between

antioxidant molecules and radical, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in color from purple to yellow. Hence, DPPH· is usually used as a substrate to evaluate the antioxidative activity. Figure (4.3.4) illustrates a significant decrease in the concentration of DPPH radicals due to the scavenging ability of the both extracts and to the standards rutin and quercetin. Comparatively standards showed more inhibition than extracts Free radical scavenging activity also increased with increasing concentration.(Elmastaşa et al., 2006)

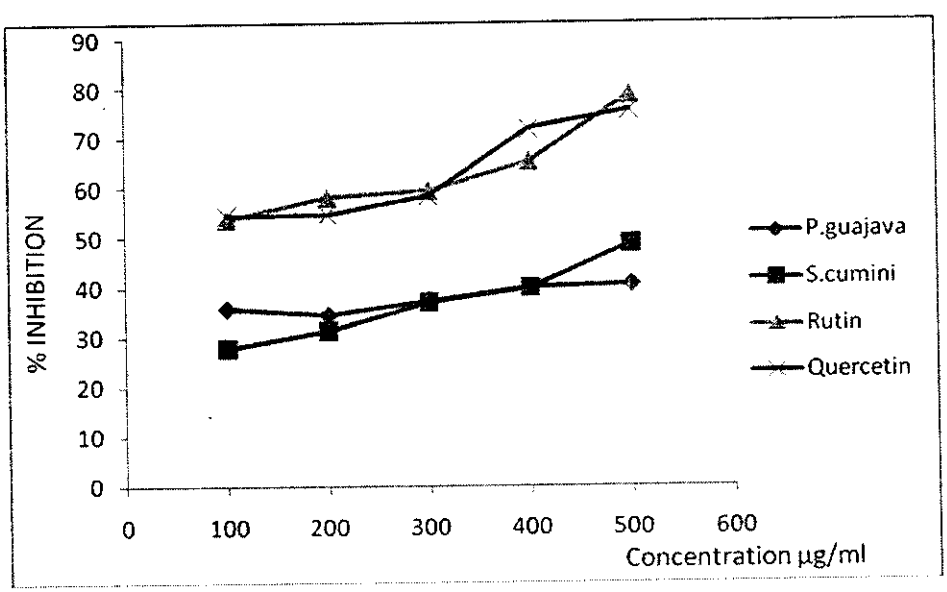


Figure 4.3.4: DPPH scavenging activity of *P,guajava* leaves. *S.cumini* seeds,Vitamin C, Vitamin E

Table 4.3.4:DPPH scavenging activity of *P,guajava* leaves. *S.cumini* seeds,Rutin , Querctin

Concentration (µg/ml)	*P.guajava*	*S.cumini*	Rutin	Quercetin
100	35.7	27.7	53.7	54.4
200	34.4	31.3	58.1	54.7
300	37.3	36.9	59.5	58.3
400	39.9	39.8	65.3	71.8
500	40.5	48.7	78.8	75.6

CONCLUSION

5.CONCLUSION

Inhibitors of carbohydrate -hydrolyzing enzyme play an important role to control post prandial blood glucose levels , in diabetic patients. We investigated the effect of crude extract from *Psidium guajava* leaves and *Syzygium cumini* seeds on α-amylase. There has been an enormous interest in the development of alternative medicines for diabetes, specifically screening for phytochemicals with the ability to delay or prevent glucose absorption. The antioxidant activity reducing power, free radical scavenging and metal chelating activities were determined from the extracts. The results suggest that these natural amylase inhibitors from food- grade sources offers an attractive therapeutic approach to the treatment of post prandial hyperglycemia by decreasing glucose release from starch and also in and scavenging of free radiacls may have potential for use in the treatment of diabetes mellitus and obesity.

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