

ANALYSING INSULIN MIMETIC ACTIVITY OF MEDICINAL PLANTS FOR ANTI-DIABETIC PROPERTY

Submitted in partial fulfilment of the requirements for the award of
Master of Technology degree in Biotechnology

By

PRAVEENA DEVI M S (41810013)



**PROJECT PHASE II-REPORT
DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of PRAVEENA DEVI M S (41810013), who carried out the Project entitled "**ANALYSING INSULIN MIMETIC ACTIVITY OF MEDICINAL PLANTS FOR ANTI-DIABETIC PROPERTY**" under our supervision from JAN 2023 to MAY 2023.

Internal Guide

Dr. M. Masilamani Selvam, Ph.D.

Head of the Department

DR. V. RAMESH KUMAR

Submitted for Viva voce Examination held on 05/05/2023

Internal Examiner

External Examiner

DECLARATION

I, PRAVEENA DEVI MS hereby declare that the Project Report entitled “ANALYSING INSULIN MIMETIC ACTIVITY OF MEDICINAL PLANTS FOR ANTI-DIABETIC PROPERTY” done by me under the guidance of Dr.M. Masilamani Selvam, Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfilment of the requirements for the award of Master of Technology degree in Biotechnology.

DATE: 5/5/2023

PLACE: Chennai

A handwritten signature in black ink, appearing to be 'P. Praveena Devi', written over a horizontal line.

SIGNATURE OF THE CANDIDATE

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ABSTRACT

Diabetes mellitus is one of the metabolic problems procuring around 9.3% of the world's populace and is expected to cross 10.9% probably in 2045. Since long back natural drugs have been the exceptionally regarded medication. subsequently, they have turned into a developing piece of current and super advanced medication. Considering the above factors the experiment gives the information about the plants with hypoglycemic properties, accessible through the source from different data set with legitimate information as per the utility, method of decrease in blood glucose (insulin mimetic or insulin secretagogues action) and dynamic phytoconstituents having insulin mimetics action. From the review it was noted that some species have enormous capability of being an effective medicine for diabetes, its role and activity is keenly studied and beneficial results have been obtained. The outstanding plant species showing clear and flexible results have taken and reviewed that is completely based on its performance and on analysable reactions on testing.

Keywords: *Diabetes mellitus, hypoglycemic, insulin mimetic, blood glucose, phytoconstituents, secretagogues actions.*

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LIST OF ABBRIVATIONS

STZ	Streptozotocin
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
DM	Diabetes Mellitus

CHAPTER 1- INTRODUCTION

Due to microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart attack, stroke, and peripheral vascular disease) -consequences, Diabetes mellitus, one of the most prevalent endocrine metabolic illnesses, has significantly increased morbidity and mortality [Sharma S., etal.2015]. Reactive oxygen species are the cause of many degenerative diseases, including diabetes, but human bodies have both enzymatic and non-enzymatic antioxidative mechanisms that reduce their production [Mbodj NA., etal.2010]. This disease is spreading quickly around the world and is now present everywhere. People with diabetes have high blood glucose levels because of an inadequate supply of insulin. The majority (90–95%) of cases of inadequate insulin production or improper insulin use are caused by type 2 diabetes, also known as noninsulin-dependent diabetes mellitus [kootara y., etal.2008]. By 2045, there may be 300 million or more diabetics worldwide, according to the World Health Organization.

Type 1 diabetes (T1DM) & type 2 diabetes (T2DM) were the two most often mentioned kinds of the disease. Diabetes that is insulin-dependent is another name for T1DM. It is characterized by a lack of insulin synthesis in the body and is mostly induced by loss of pancreatic islet beta cells. Individuals with T1DM are vulnerable to ketoacidosis and require constant insulin treatment to keep their blood glucose levels in check. T1DM primarily affects children and teenagers. T2DM, or non-insulin-dependent diabetes, on the other hand, affects the majority of persons with diabetes worldwide and is caused by the body's inefficient usage of insulin and hyperglycemia. Type 2 diabetes, which affects 90% of people with diabetes, typically affects adults almost exclusively but is now increasingly harming youngsters. Since 1980, the adult population's overall incidence of diabetes has increased from 4.7% to 8.5%. Furthermore, it has been discovered that the diabetes rate has been rising gradually for the past three decades, and that it has increased more quickly in lower- and middle-income nations than in high-income nations. Along with a rise in related risk factors including being overweight or obese, diabetes prevalence is rising. Diabetes can have long-term effects that have a major negative influence on one's quality of life, including renal dysfunction, lower leg amputation, blindness, and other long-term problems. several groups of oral hypoglycemic medications, including sulfonylureas, biguanides, glucosidase inhibitor, thiazolidinediones, and non-sulfonylurea secretagogues, have

anti-diabetic effects by various methods. Blood sugar levels are lowered with oral sulfonylureas such as glimepiride and glyburide, mostly via increasing islet of Langerhans insulin secretion. This is accomplished by attaching to the sulfonylurea receptor on cells, which closes potassium channels that are adenosine triphosphate-dependent. The cell membrane then depolarizes, which causes a calcium influx and the release of existing insulin from secretory vesicles inside the cells. Only when there is insulin present does this mechanism function.

Insulin and several oral antidiabetic medications such as sulfonylureas, biguanides, and glinides are being used to treat diabetes. One of the key areas of research is the hunt for more powerful and secure hypoglycemic agents because many of them have a lot of dangerous side effects [Cissé A., et al. 2014]. The polyol pathway's essential enzyme, aldose reductase, catalyzes the conversion of glucose to sorbitol. As sorbitol builds up in the body, it can lead to a number of problems, including cataract, neuropathy, and nephropathy [Khalid N., et al. 2002]. Numerous plants utilized as antidiabetic medicines have been shown to have a hypoglycemic impact, and researchers are currently looking into how these plants work. Compounds with possible antidiabetic effects that function either as insulin mimetic or secretagogues additionally.

The value of conventional therapeutics and natural diabetes treatments derived from conventional medicinal plants. The development of new antidiabetic medications has a lot of potential thanks to traditional medicines made from easily accessible medicinal plants [Abesundara KJ., et al. 2010]. Recently certain medicinal plants have been utilized empirically in anti-diabetic and anti-hyperlipidemic therapies and have been reported to be helpful in treating diabetes globally. The primary mechanisms through which plants exhibit antihyperglycemic activity are their capacity to boost insulin secretion, restrict glucose absorption from the intestine, or promote the activity of metabolites in insulin-dependent activities. There are more than 400 plant species with hypoglycemic properties.

Natural substances, especially those with a plant origin, are the key source for finding good lead candidates and are crucial to the next drug development programmes. Plant-based treatments are the dominant player among all accessible medications, especially in rural regions, because they are simple to get, inexpensive, and have little side effects.[Saeedi P.,etal.2019] In addition, many plants offer a plentiful supply of bioactive compounds, which have potent pharmacological benefits without any negative side effects. Many of the pharmaceuticals that are currently available were either directly or indirectly derived from plants, which have traditionally been a prime source of medication.

Numerous plants have long been viewed as the main source of effective anti-diabetic medications. Particularly in underdeveloped nations, medicinal plants are utilized to cure diabetes in order to alleviate the financial strain that the expense of conventional medications places on the populace.[Shammah.,etal.2016] These days, it is advised to use medicinal plants to treat disorders like diabetes since they include diverse phytoconstituents such flavonoid, terpenoid, saponin, carotenoid, alkaloid, & glycoside that may have antidiabetic properties. The possible beneficial properties of each plant matrix result from the combined effect of biologically active compounds (such as polyphenolic compounds, carotenes, lignans, coumarins, glucosinolates, etc.), and this can serve as a starting point for acknowledging their bioactivities and beneficial properties[Brownlee M.,etal.2005].

Creating an effective antidiabetic composition which is plant derived helps in saving us from the harmful effect of chemically derived antidiabetics which happens on long term usage. The perfect and tested composition said to give beneficial solution to a largely infesting disease that affects the livelihood of many people even for young adults and worst scenario even in kids. Gestational diabetes is also one case that happens during pregnancies that develops a condition of creating insulin secretory blocker and blood sugar level get heightened due to the improper take up of sugar from blood with the help of insulin [kumar.V.,etal.2012] .the side effects of diabetes linked to blood sugar level is also a considerable issue which makes it hard to do daily activities and hinders in a disease-free lifestyle.

Recently animal models have been used widely for the molecular study of the treatment methodology for many diseases. zebra fish is the greatest study model due to its lesser complicated survival rate and physiological similarity and co-ordinance with human genetic system. [Alia tejacal.I.,etal.2022] The chronic induction of diabetic state (hyperglycemic) easier via high glucose diet and the exhibitable ability of zebra fish is also high which makes it a most desirable model for metabolism and functional studies. The hypoglycemic activity of plant species is analysed and determined through *in vitro* and *in vivo* methods in this experiment with the motive of justifying the antidiabetic activity of plant species which can be consumed for a longer period without any harmful side effects.

CHAPTER 2- LITERATURE SURVEY

2) PLANTS FOR TREATING DIABETES

Many of the pharmaceuticals that are now available have been produced from plants, which have historically been a highly good source of medications from them, either directly or indirectly. According to ethnobotanical data, 800 different plants may have anti-diabetic properties; among these, *Momordica charantia*, *Pterocarpus marsupium*, and *Trigonella foenum greacum* have all been shown to be effective in treating type 2 diabetes [Malviya S., et al.2010]. Several of these plants have demonstrated antidiabetic efficacy when tested using various experimental methodologies [Lee HS., et al.2002]. Numerous plant-derived active ingredients have been shown to have biological effects, including the treatment of diabetes. These include alkaloids, glycosides, galactomannan, polysaccharides, peptidoglycans, hypoglycans, guanidine, carbohydrates, glycopeptides, terpenoids, amino acids, and inorganic ions.

The prevalence of diabetes today has indeed been rising, raising worries among the public and the medical profession. Despite the availability of antidiabetic medications on the market, medicinal herbs are frequently effective in treating diabetes. Worldwide, important therapeutic solutions for the diabetes treatment include herbal medications and plant elements with negligible toxicity and no adverse effects. The majority of studies have shown the advantages of medicinal herbs with hypoglycemic characteristics in the treatment of diabetes. described the mechanisms of natural products as antidiabetic agents and the medicinal plants used to treat diabetes and its comorbidities, paying special attention to compounds of high interest like fukugetin, palmatine, berberine, honokiol, amorfrutins, trigonelline, gymnemic acids, gurmardin, and phlorizin. The antihyperglycemic effects of plant therapy are typically linked to their capacity to enhance pancreatic tissue function, which is accomplished by raising insulin outputs or decreasing intestinal glucose absorption.

One of the main pathogenic pathways of diabetes mellitus involves the production of oxidative stress, an increase in free radical production, a compromised cellular antioxidant system, and a simultaneous instability of the oxidant/antioxidant status. The beginning and progression of diabetes problems have been found to be prevented by inhibiting this sequence of oxidative activities. Anti-diabetic herbs have been shown to quench electrically excited molecules, scavenge free radicals, and limit the generation of hydroperoxide. More specifically, plant phytonutrients like polyphenols are known to improve the function of the body's own antioxidant system, maintain a healthy balance of oxidants and antioxidants, prevent oxidative damage, mitigate oxidative stress, increase plasma antioxidant capacity, and stimulate antioxidant enzymes like glutathione peroxidase, catalase, and superoxide dismutase.

While synthetic pharmaceuticals do not permanently cure ailments, herbal medicines permanently heal the patient and treat the illness. Its formulas include natural extracts of herbs, fruits, and vegetables that are helpful in treating a variety of disorders without causing any negative side effects. While the majority of medicines are made from chemically and chemically altered natural ingredients, herbal formulations are made from natural products. The shift in research toward traditionally used treatments is due to the rise in resilience and numbers of individuals at some risk, as well as the limited number of commercially accessible therapies for diabetes that still have many side effects and other issues such as undesirable hypoglycemia effect.

2.1 JAMUN FRUIT (*Syzygium cumini*)



Fig:2.1: **Jamun fruit**

A food or component of food that has exhibited therapeutic properties against many diseases, in addition to the prevention and treatment of illness, is referred to as a nutraceutical [Lee.W.J., et al.2004]. Worldwide, a large number of herbal medications have been identified as having hypoglycemic effects. One of the fruits used to treat diabetes in the past was jamun, which is being used today by doctors. Similar to this [Nayak B., et al.2010] discovered that jamun seeds have antidiabetic properties. *Syzygium cumini*, Because of its impact on the pancreas, its fruit is generally regarded to be of exceptionally high quality for its curative role, particularly against diabetes [Nayak B., et al.2010]. it has blood-pressure-regulating effects. Jamun has antihypercholesterolemic properties and helps in regulating the blood cholesterol profile. Studies done in the last twenty years have shown that jamun have a good amount of naturally present antioxidant compounds. Studies have shown that jamun fruit and seed have a lot of anti-oxidants in them like phenolic acids, flavonoids and anthocyanins. These compounds help prevent different syndromes.

[Li WL., et al.2004] The antihyperglycemic properties of jamun fruit and seed were evaluated in the present research. After injecting alloxan into male Wistar rats to cause diabetes, three dosages of jamun seed extracts—50, 100, and 200 mg/kg daily—were administered to the animals. Then three weeks were spent receiving treatment. When given with jamun extract, diabetic rats' blood glucose levels significantly decreased. The results and the outcome are consistent. The results showed that the alcoholic jamun seed extract significantly lowered the blood glucose level in diabetic rats, Ethyl acetate and a methanolic extract of jamun were found to have an impact on normal and streptozotocin-induced diabetic rats. In comparison to glibenclamide, jamun seed extract was administered to rats at two distinct doses (200 & 400 mg/kg) for 15 days, Jamun seed extract considerably decreased serum glucose, it was found. The findings of the current study are in agreement with publications [Prasad SK., et al.2002] that showed a 65% drop in glucose levels in rats fed jamun seed. They concluded that jamun had a beneficial effect on the pancreatic b-cells' ability to secrete insulin, which lowers blood glucose levels. In conclusion, jamun fruit and seed provide nutritional value for treating many diet-related problems, particularly hyperglycaemia. In the current study, extracts from jamun seeds and fruits were effective in controlling insulin and blood sugar levels. By giving out jamun seed extracts, hyperglycaemia and hyperinsulinemia were also controlled. According to the findings [Liu.X., et al.2005] fruit

has less ability than seed to reduce the blood sugar levels of diabetic rats. Jamun has the potential to be a source of naturally occurring bioactive components that can regulate blood glucose levels and be utilised to treat diabetes.

2.2 GUAVA (*Psidium guajava*)



Fig:2.2: **Guava**

Psidium guajava, is a representative medicinal plant with antidiabetic properties, and interest in guava has grown recently. Numerous polyphenol substances, including polyphenol [Loyax., etal.1994], terpenoids, flavonoids, and tannins, are present in guava leaves. Additionally, it has been shown that guava leaves have both direct and indirect effects on blood sugar levels via regulating glucose levels and the activity of carbohydrate hydrolyzing enzymes. In diabetic rats, the insulin concentration was 8% lower than in normal control rats, and the glucose concentration was 500 mg/dl or higher. In addition, diabetic control rats had lipid concentrations that were 2 to 4 times greater than those of normal control rats, demonstrating the symptoms of Type 1 DM (hyperglycaemia, hyperlipidemia, and hypoinsulinemia). However, ingesting guava leaf extracts decreased diabetic rats' blood glucose levels by 19% or more and raised their insulin release by more than two times. These extracts demonstrated anti-diabetic effects by lowering hyperlipidemia that had occurred and restoring the Langerhans islets' damaged beta cells. There were differences between the anti-diabetic impacts of the extracts used in this experiment. However, as guava extract consumption was more successful than extract consumption in increasing insulin secretion, lowering glucose levels, improving hyperlipidemia, and recovering the beta cell of the Langerhans islet. Therefore, different flavonoids, glycosides, and terpenoids can cause the antidiabetic actions of medicinal plants. Depending on the technique of

extraction and how much ethanol is used, the proportion of polyphenol may change. The anti-diabetic actions of guava water extracts include lowering plasma sugar levels and reducing carbohydrate hydrolyzing enzyme activity. In conclusion, when contrasted to extracts that include 1% corosolic acid, guava water extracts demonstrated greater antidiabetic benefits in STZ-induced rats.

This is so that less hydrophilic polyphenol molecules, which have stronger antidiabetic effects, can be used instead of the corosolic acid present in other extracts, which has modest anti-diabetic action. Therefore, more research should be done to determine which hydrophilic polyphenol provides the strongest anti-diabetic effects. [Loyax., etal1994]

2.3 BETEL NUT (*Areca catechu*)



Fig:2.3: *Areca nut*

Sometimes called Areca nut, betel nut, or Supari in Bengali, is a tall perennial palm that grows in sandy-clay soil all over the Indian subcontinent and other South East Asian nations. [Gokhale S.B., etal.2002] It is grown commercially in several locations around India for its masticatory seeds. In veterinary medicine, its seeds are utilised as an anthelmintic since they have sialagogue characteristics. the anti-diabetic effects of several *A. catechu* leaf extracts in STZ-induced diabetic rats. According to the study's findings, oral administration of pet ether, chloroform, and methanol extract at doses of each 200 mg/kg body weight effectively reduced blood sugar levels in STZ-induced diabetic rats and returned their body weight to a healthy level. [Jung M., etal.2018] Antibiotic streptozotocin (STZ) is made from *Streptomyces achromogenes*. By entering the pancreatic cells through the glucose transporter GLUT2, STZ damages

the pancreas by alkylating the DNA. Nitric oxide and reactive oxygen species work together synergistically to cause DNA fragmentation, and this is what gives it its hazardous qualities. Streptozotocin pancreatic cells are killed by necrosis as a result of STZ activity. STZ harms hepatocytes, nephrons, and cardiomyocytes in addition to pancreatic cells.

Upon 3 days of STZ induction, rats showed signs of hyperglycaemia. In STZ-induced diabetic rats, treatment with pet. ether, chloroform, and methanol extracts reduced fasting blood glucose levels after 5 days and resulted in full normoglycemia after 15 days. It was discovered that the anti-diabetic effects of pet. ether, chloroform, and methanol extract respectively at a dose of 200 mg/kg were comparable to those produced by the reference medication, glibenclamide at a dose of 0.5 mg/kg. [Jung M., et al.2018] In comparison to the corresponding control rats, the oral dosing of pet ether, chloroform, and methanol extracts for 15 days showed a significant reduction and regulation of increased blood sugar levels, i.e., an anti hyperglycemic or antidiabetic effect. The extracts made from methanol, chloroform, and petroleum ether were the most effective. It can be inferred from this that *Areca catechu* leaf extracts have a remarkable amount of anti-diabetic activity in the face of streptozotocin-induced diabetes on Wistar rats. The results of the current trial are positive enough to call for additional research in the search for a novel oral hypoglycemic medication.

2.4 BITTER GOURD (*Momordica charantia*)



Fig:2.4:*Bitter gourd*

commonly referred to as bitter melon or bitter gourd, is a well-known herb that is used to treat diabetes-related problems. *M. charantia* can be used as an adjuvant with

allopathic treatments of medicine for treating diabetes and to prevent the late consequences of diabetes due to its considerable antidiabetic and hypolipidemic activity. A key biological function of insulin is to promote the intake of glucose by various types of body cells for the creation of energy. It is crucial to comprehend whether *M. charantia* may directly cause a drop in blood glucose levels because *M. charantia* and its numerous extracts and components are known to exert hypoglycemic effects [Lee HS., et al.2000]. its actions on skeletal muscle that resemble insulin daily oral consumption of its fruit juice the level of Na⁺ was dramatically lowered during the course of 10 weeks.

Rat jejunum absorbs 14 C-D-glucose in a K⁺-dependent manner vesicles as opposed to brush boundary membrane vesicles acquired from diabetic rats induced with STZ. When combined, these outcomes made it quite evident that *M. charantia* extracts could effectively control blood sugar through two mechanisms. Firstly, it can control the amount of glucose that is after a meal, is absorbed by the intestines into the blood, Additionally, it can promote skeletal muscle glucose absorption like insulin, muscle cells. [Lee HS., et al.2000] Additionally, it appears to exert the same intracellular signalling pathways as to exert its effects Insulin controls how the body uses glucose. Blood sugar levels were significantly reduced by alcoholic extract of *M. charantia*, and islet histology also indicated improvement. Even when extract feeding was stopped for 15 days, the reduced blood sugar and enhancement in islet histology persisted. The anti-hyperglycaemic efficacy with this plant on diabetic animals and people was confirmed by the acetone extract of fruit powder of *M. charantia*, which decreased blood glucose from 13.3% to 50.0% following 8 to 30-day administration in alloxan diabetic albino rats. Only charantin, insulin-like peptide, and alkaloid-like extracts have hypoglycemic characteristics in connection to diabetes that are comparable toward the plant extracts. These manage and treat diabetes through a number of methods with positive consequences [Kim SK., et al.2011].

2.5 TURKEY BERRY (*Solanum torvum*)



Fig:2.5: *Turkey berry*

To control diabetes, hypertension, dental caries, and reproductive issues, traditional medical systems around the world use the medicinal plant Swartz, which is a member of the Solanaceae family. Swartz is a great therapeutic plant that is widely dispersed around the world. [Gobinath R., et al.2012] In recent years, it has become clear that a balanced diet and the use of the right herbs can help lower the occurrence of diabetes (9). A small portion of the population occasionally consumes *S. torvum* fruit as a vegetable. It is clear from the literature that it is used to treat a variety of ailments because of the abundance of phytochemicals it contains. Additionally, little research has been done on its potential as an antidiabetic, especially with the *S. torvum* available regionally in Malaysia. [Ahmed M., et al.2000] According to OECD423 requirements, an acute toxicity investigation was conducted. Streptozotocin (55 mg/kg) was injected intraperitoneally into male Sprague-Dawley rats to cause diabetes. Six groups of experimental diabetic rats were created: normal, diabetic control, and glibenclamide of 6 mg/kg body weight. The other three animal groups received oral administration of an ethanolic extract of *S. torvum* fruit at doses of 120, 160, & 200 mg/kg for quite a period of 28 days. By using RT-PCR, the effect of an ethanolic fruit extract of *S. torvum* on body weight, blood sugar, lipid profile, liver enzymes, histopathology of the pancreas, and the expression of the enzymes phosphoenolpyruvate carboxykinase (PCK1) and glucose transporter 2 (slc2a2) was assessed. According to tests on acute toxicity, the ethanolic extract of *S. torvum* fruit has an LD50 of 1600 mg/kg body mass. In rats given *S. torvum* fruit (200 mg/kg), blood sugar, total cholesterol, triglycerides, very low-density lipoproteins, sera alanine aminotransferase, & aspartate aminotransferase were dramatically decreased, and

high-density lipoproteins were considerably increased. [Jung M., etal.2006] A pancreatic histopathological analysis revealed that the islets of Langerhans' -cell count, size, and regeneration had increased. When compared with diabetic control animals, gene expression analyses on treated animals showed lower levels of slc2a2 and PCK1. In streptozocin-induced diabetic rats, an ethanol extract of *S. torvum* fruits demonstrated hypoglycemic, hypolipidemic, and hepatoprotective activity. Regeneration of cells in the islets of Langerhans was discovered through histopathological research. Studies on gene expression revealed that slc2a2 and PCK1 were expressed at lower levels in treated mice compared to diabetic study participants, suggesting that the treated animals preferred the gluconeogenesis pathway. [Jung M., etal.2006]

2.6 CASSIA (*Senna auriculata*)



Fig:2.6: *Cassia* flower

Also known as *Cassia auriculata* and belonging to the Fabaceae family, is a medicinal herb used extensively in the Indian medical systems of Ayurveda and Siddha to cure a variety of illnesses. The medicinal properties of almost all plant components, including the flowers, leaves, seeds, barks, and roots, have been documented. [Abesundara, K. J., etal.2004] It has traditionally been used to treat metabolic problems, asthma, and diabetes. Alkaloids, anthraquinones, flavone glycosides, sugars, saponins, phenols, terpenoids, flavonoids, tannins, steroids, palmitic, resorcinol, -tocopherol—D-mannosidase, epicatechin, and proanthocyanidin B1 are the main phytonutrients in *Senna auriculata*.

A wide range of pharmacological activities, including anti-diabetic, antioxidant, anti-inflammatory, anti-hyperlipidemic, anticarcinogenic, nephroprotective, cardioprotective, anticancer, and anti-melanogenesis, are possessed by the extracts from its various parts and their isolated compounds. [Mbodj NA., etal.2015]

The ethanolic root extract interrupted in acacia up to a dose of 3,000 mg/kg body mass per oral was used in a study of acute toxicity on albino Wistar male rats, and it was observed that no major changes in the involuntary muscle or behavioural changes, including death, were produced during the observation time. *Senna auriculata* roots were used in an acute toxicity research (OECD 423 guideline) with dose levels of 5, 50, 300, & 2,000 mg/kg. The rats didn't exhibit any harmful side effects or notable behavioural changes. *Senna auriculata* exhibits safe pharmacological activities on numerous pathways implicated in the aetiology of diabetes and various other disorders, in addition to its ethnomedicinal benefits for diabetes mellitus [Saxena A., etal.2005]. Additionally, comprehensive animal experiments using various extracts from *Senna auriculata* botanical components have shed insight on the plant's traditional applications as a remedy for a variety of diseases. ITS metabolites 's offer the best opportunity for the scientific community to accept it and learn more about it. Its cultivation and safety pharmacological elements will boost the national economy and its medical uses will help to lessen the burden of NCDs.

CHAPTER 3 - AIM AND SCOPE

AIM

To create a natural remedy for diabetes, check for its action on blood PH level and blood glucose/sugar level and test for antidiabetic activity on zebra fish model.

SCOPE

1. Create an effective composition that is naturally derived
2. A plant-based medication that does not cause any harmful effects even on prolonged usage
3. Antidiabetics that can be multifunctional and nutritiously beneficial.

CHAPTER 4 - MATERIALS AND METHODOLOGY

The methodology involves 2 different types of testing *in vitro* and *in vivo* animal testing in order to prove the antidiabetic effect of the plant species . after through study regarding each step and analysing researches done in past , the experimental procedure has been set accordingly and the methods involved are :

Initially the plants have been selected that is enriched with antidiabetic and insulin mimetic property. then the extraction of the sample plays the part according to the need the sample is extracted as crude extract , alcohol extract or straining after boiling in water bath if the sample is in dried form. The PH value of the extracted sample should be estimated and then the glucose content of the sample is tested after that the glucose content analysis is done via *in vitro* lab testing and the absorption level is checked for the reduction property. The methodology is then shifted to *in vivo* testing on Zebra fish model, once the diabetic condition is established in the testing model then our plant sample (drug) is administered and blood glucose level of fish model is tested.

4.1 SELECTION OF PLANT SPECIES

After considering necessary factors like regional condition, availability, hypoglycemic action and previous research, the plant species that has been taken for the study of antidiabetics are:

1. Fenugreek
2. Areca nut
3. Cassia flower
4. Green tea
5. Turkey berry
6. Bitter melon

4.2 SAMPLE EXTRACTION

The aqueous extract of plant samples has been taken for invitro analysis achieved by extracting from crude samples and in the case of dried samples the extract is collected by boiling the sample at water bath with evenly distributed heat for 30 minutes. The ethanolic extract have been taken for *in vivo* testing to get the phenolic compounds and flavonoids done by 1:1 ethanolic water extraction method and after evaporation it is stored under -4°C for further use [sharabi K., etal.2017]

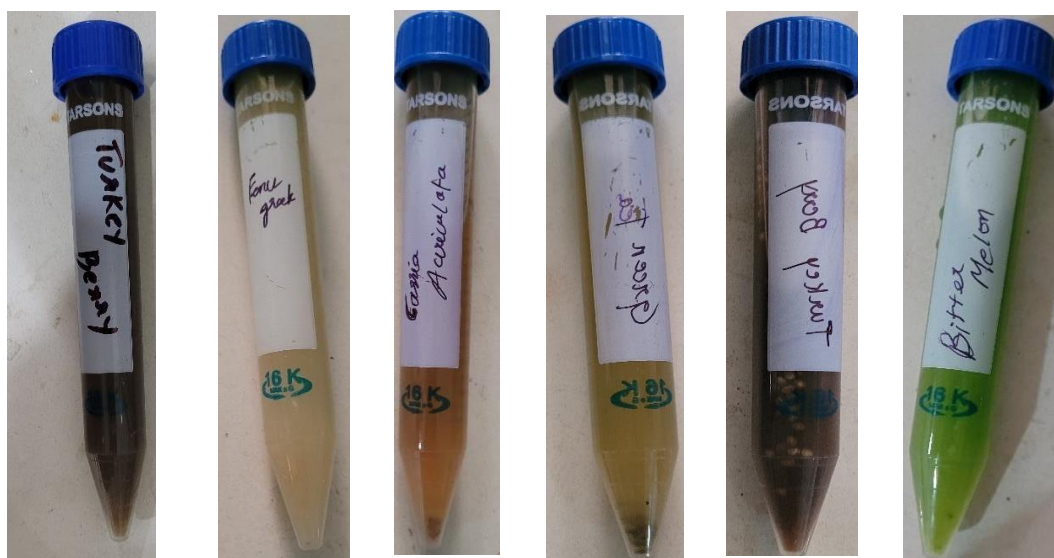


Fig : 4.2: *extracts of the plant samples*

4.3 PH ANALYSIS

Table:4.3: **PH VALUE OF THE PLANT SAMPLES**

SAMPLE	PH VALUE
TURKEY BERRY	5.38
FENUGREEK	4.94
CASSIA FLOWER	5.6
GREEN TEA	6.25
BITTER MELON	4.21
ARECA NUT	6.15

The *in vitro* testing involves many steps for the testing of antidiabetic property, initially the PH content of our samples are measured using PH meter.

And then the presence of glucose content available in the samples are tested by simpler BENEDICT'S method using benedict's reagent, the change in colour actually indicates the presence of glucose content .



Fig : 4.3: ***presence of glucose using benedict's reagent***

4.4 GLUCOSE CONTENT ANALYSIS

Analysing the Extracts action on glucose content by determining the adsorption capacity [Halaas J.,etal.2017]

Initially the aqueous plant extracts (1%) were added to 25 ml of glucose solution of increasing concentration in different batches , Then the mixture was stirred well and incubated in water bath at 37°C for 6 h and is Centrifuged at 4800 r/min for 20 min and the glucose content is determined from the supernatant.

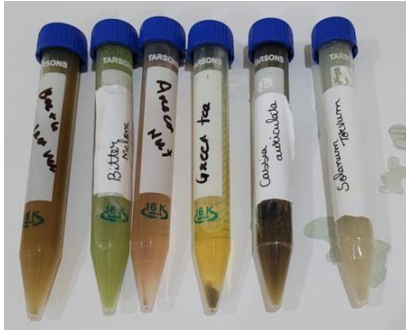


Fig : 4.4.1 : ***Aq extract of plant species***

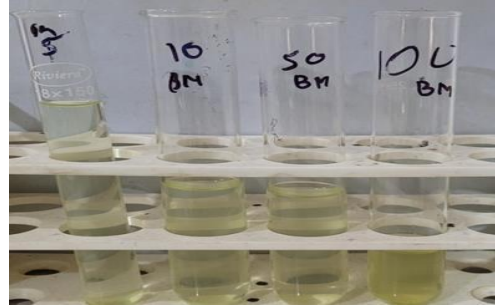


Fig: 4.4.2 : ***Plant extract with glucose solution in increasing concentration (G1)***

Centrifuged at 4800 r/min for 20 mins



Fig : 4.4.3: ***Incubated in water bath for 6h***



Fig : 4.4.4 : ***centrifuged plant samples after incubation***

Glucose content in supernatant determined

The absorbance value of the samples is noted and then the difference in the glucose concentration before and after the incubation of sugar solution with the samples is determined through comparing the values by plotting the absorbance value against the glucose concentration .

4.4.1 REDUCTION IN GLUCOSE CONTENT

Table :4.4.1: **BITTER MELON** (*MOMORDICA CHARANTIA*)

CONCENTRATION (mmol/L)	G1	G6
5	0.612	0.044
10	0.096	0.033
50	0.112	0.027
100	0.254	0.017

Table : 4.4.2: **TURKEY BERRY** (*SOLANUM TORVUM*)

CONCENTRATION (mmol/L)	G1	G6
5	0.302	0.216
10	0.321	0.213
50	0.338	0.178
100	0.494	0.160

Table :4.4.3: **CASSIA AURICULATA** (CASSIA FLOWER)

CONCENTRATION (mmol/L)	G1	G6
5	0.029	0.019
10	0.031	0.017
50	0.038	0.023
100	0.042	0.031

Table :4.4.4: **GREEN TEA** (*CAMELLIA SINENSIS*)

CONCENTRATION (mmol/L)	G1	G6
5	0.045	0.038
10	0.044	0.031
50	0.065	0.020
100	0.107	0.012

Table :4.4.5: **ARECA NUT** (*ARECA CATECHU*)

CONCENTRATION (mmol/L)	G1	G6
5	0.299	0.069
10	0.268	0.148
50	0.365	0.158
100	0.370	0.124

Table : 4.4.6: **FENUGREEK** (*Trigonella foenum- graecum*)

CONCENTRATION (mmol/L)	G1	G6
5	0.203	0.069
10	0.213	0.119
50	0.256	0.076
100	0.210	0.034

4.5 *In vivo* TESTING

Out of all the samples, ARECA NUT showed the highest reduction in glucose level, Therefore Areca nut sample is carried out for the *in vivo* testing on zebra fish model for the Antidiabetic activity. Initially the Antidiabetic property of plant samples have been confirmed by *in vitro* lab testing and then out of all the samples high result yielding plant species is selected and then tested on Zebra Fish model with chronic induction of diabetes and after Administration the diabetic level of Fish model will be tested and further research will be carried out according to the result.

(*Danio rerio*) The zebra fish is a great model for Biomedical studies and is been widely used as a model for diabetes, cardiovascular disease, immune system studies and cancer. It has approximately 75% physiological and genetic similarity with humans and promising results have been acquired in many previous researches [Amor A J., etal.2020].

14th day after chronic diabetic induction in zebra fish model using D glucose 111 mM, plant sample will be administered and the glucose level will be checked. [Gilmore R M., etal.2016] The ethanolic extract of Areca nut (90 mg /L - 96 hours) [Zupanc k., etal.2005] is tested for toxicity on the zebra fish model and no sign of troubled mobility or reactivity that disrupts the normal wellbeing of zebra fish is shown

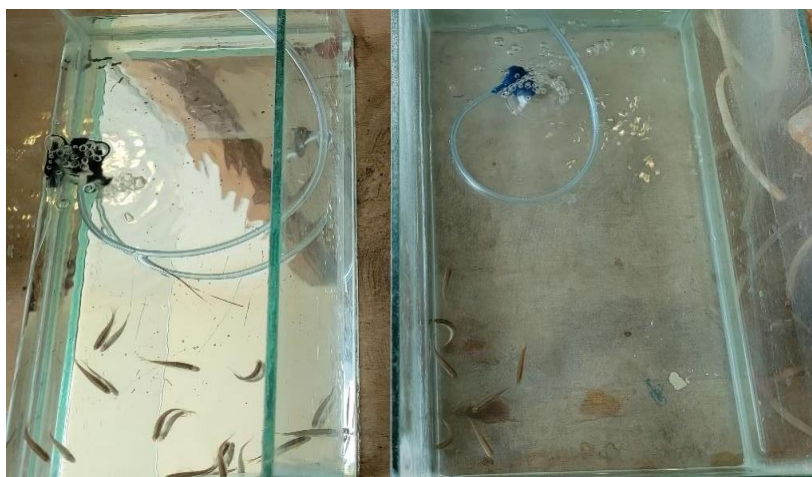


Fig :4.5: **Adult zebra fish (2 batches – control and test)**

Major steps involved in the *in vivo* testing is:

1. Chronic Diabetic induction
2. Administration of drug in Fish model
3. Glucose level Testing after administration
4. Result interpretation and Further Researches

The blood glucose level of zebra fish is measured using glucometer, initially the zebra fish is covered with ice for hypothermic condition in order to reach anesthetic stage and after making sure its mobility and reactivity and then blood is drawn when eye socket is removed and the eye cavity is filled with blood and the it is transferred to testing strip then measured in glucometer (Accu-chek ®) [Muniz-Ramirez. A., etal.2021]

CHAPTER-5 RESULT AND DISCUSSION

5.1 RESULTS

We found that the plant extracts analysed showed anti-diabetic properties. This might be due to the presence of phenolic compounds that have anti-diabetic effects

The reduction of glucose content in invitro testing shown the desirable results and there is major drop in the glucose level comparing G1 (Plant extract with glucose solution in increasing concentration) and G6 (Glucose content in supernatant after incubation).

5.1.1 BITTER MELON

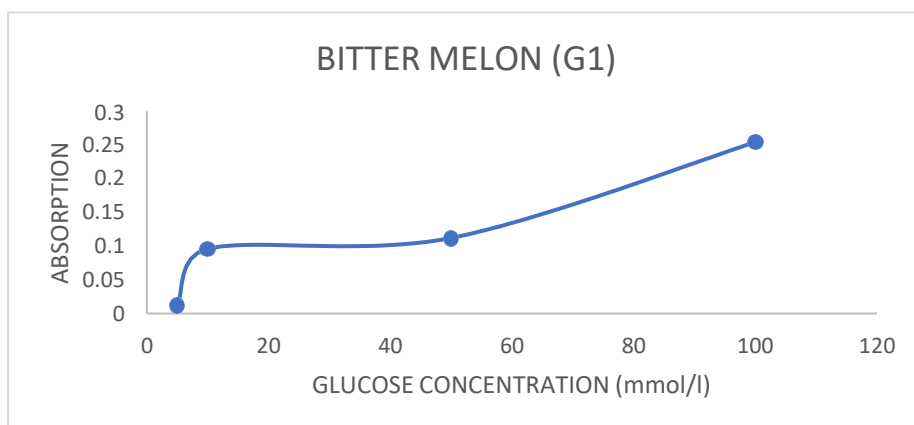


Fig:5.1.1.a: **Glucose content (G1) of the bitter melon sample**

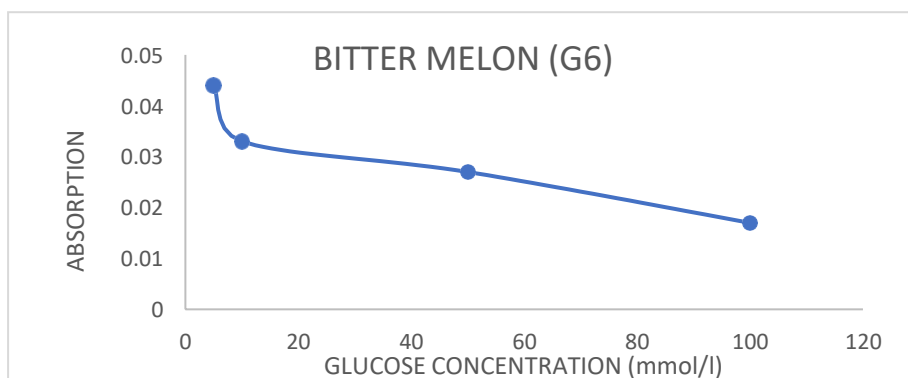


Fig: 5.1.1.b: **Glucose content (G6) in the bitter melon sample**

5.1.2 TURKEY BERRY

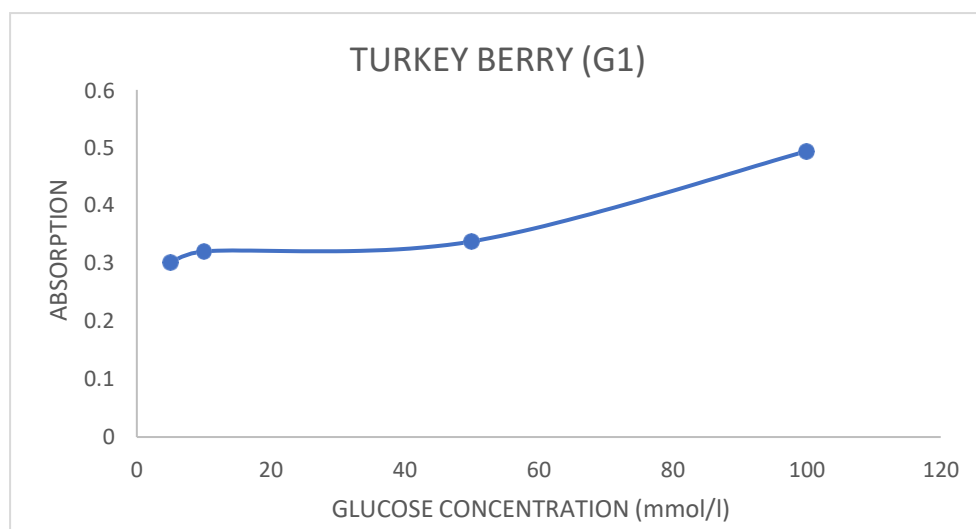


Fig:5.1.2.a: **Glucose content (G1) in turkey berry sample**

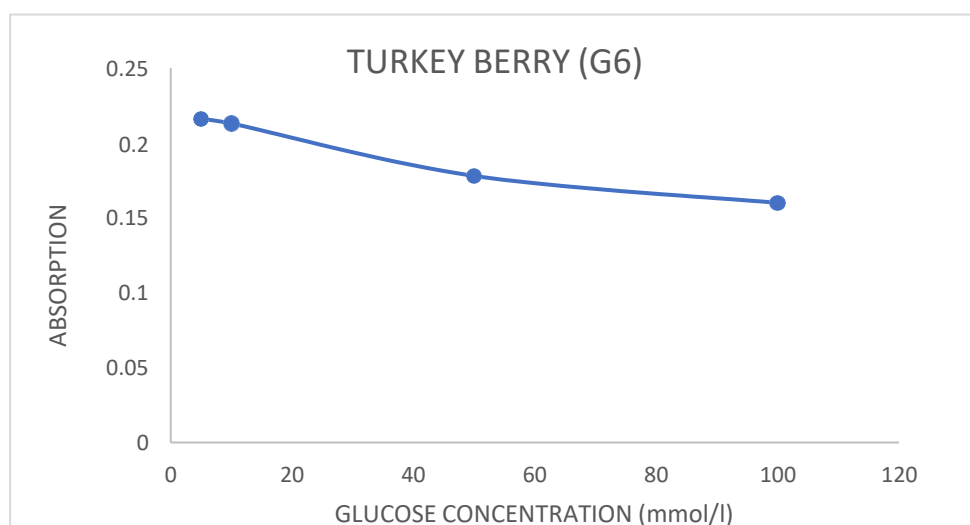


Fig :5.1.2.b: **Glucose content (G6) turkey berry sample**

5.1.3 CASSIA AURICULATA

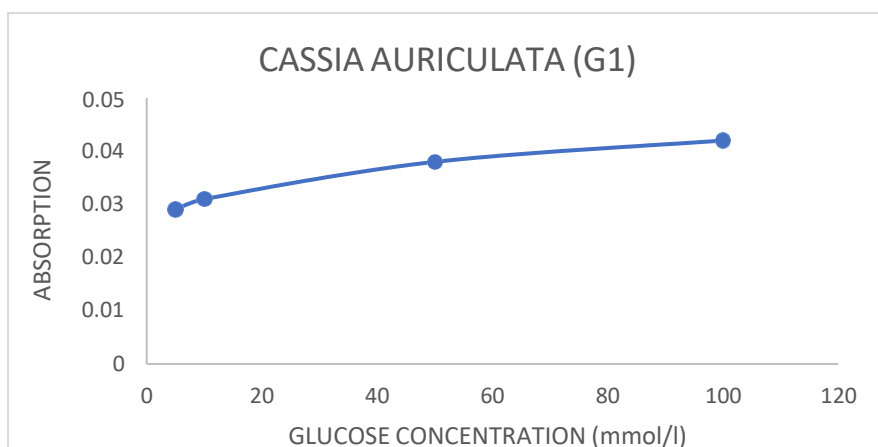


Fig : 5.1.3.a. **Glucose content (G1) cassia flower sample**

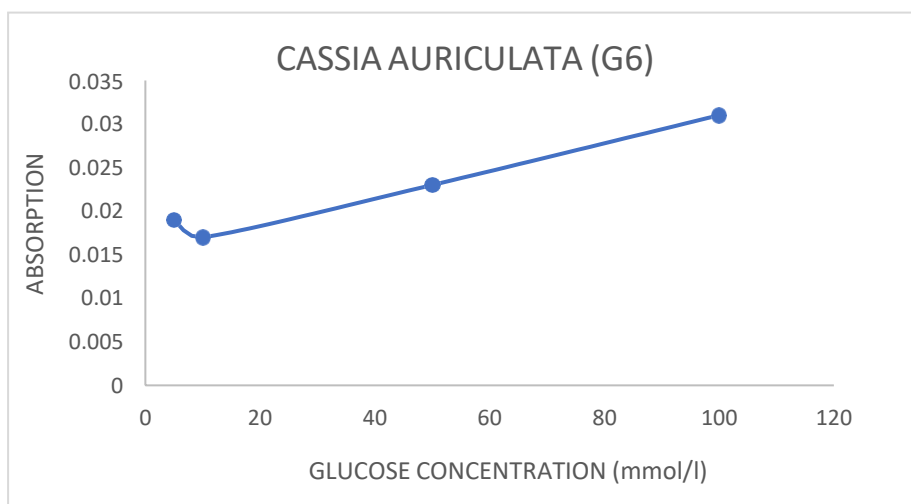


Fig:5.1.3.b: **Glucose content (G6) in cassia flower sample**

5.1.4 GREEN TEA

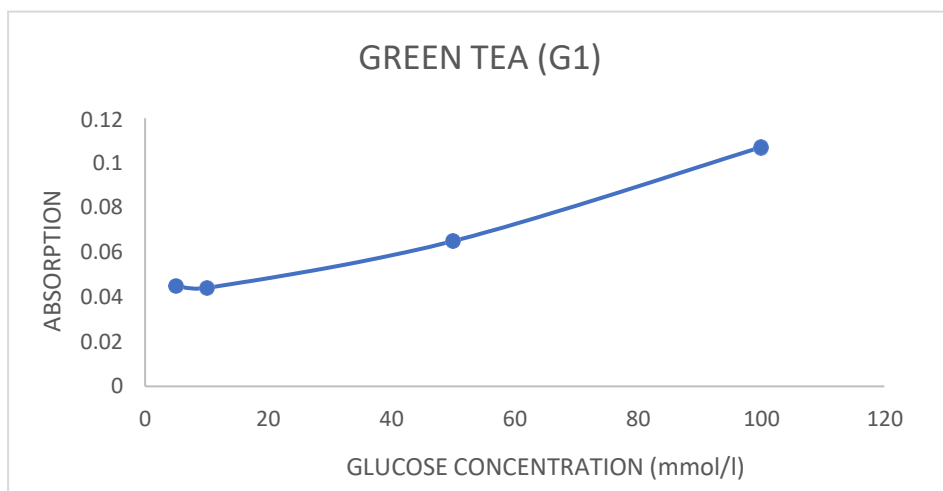


Fig:5.1.4.a: **Glucose content (G1) in green tea sample**

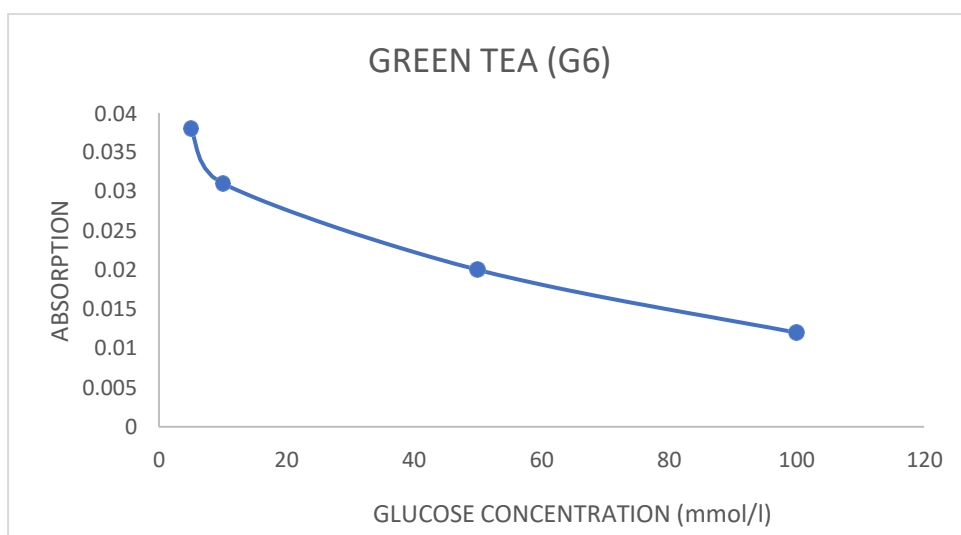


Fig :5.1.4.b : **Glucose content (G6) in green tea sample**

5.1.5 ARECA NUT

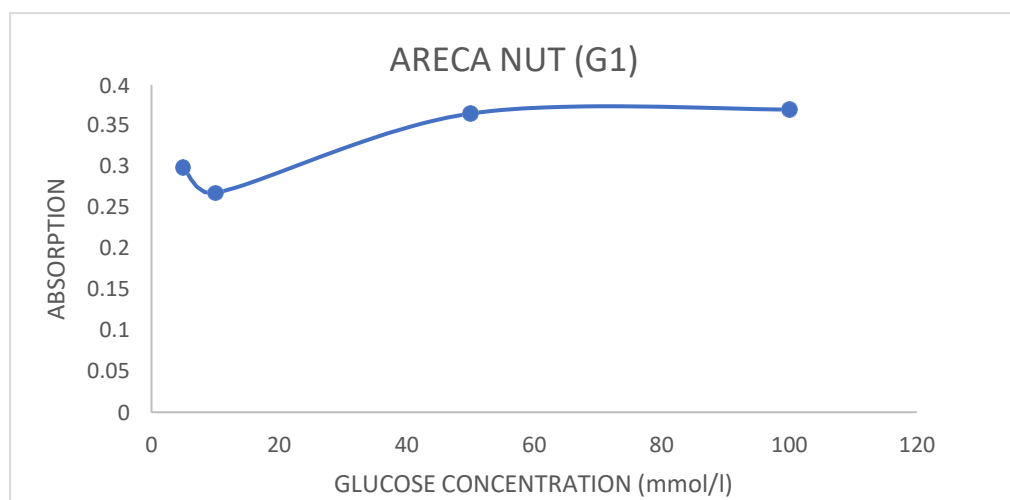


Fig :5.1.5.a : **Glucose content (G1) in Areca nut sample**

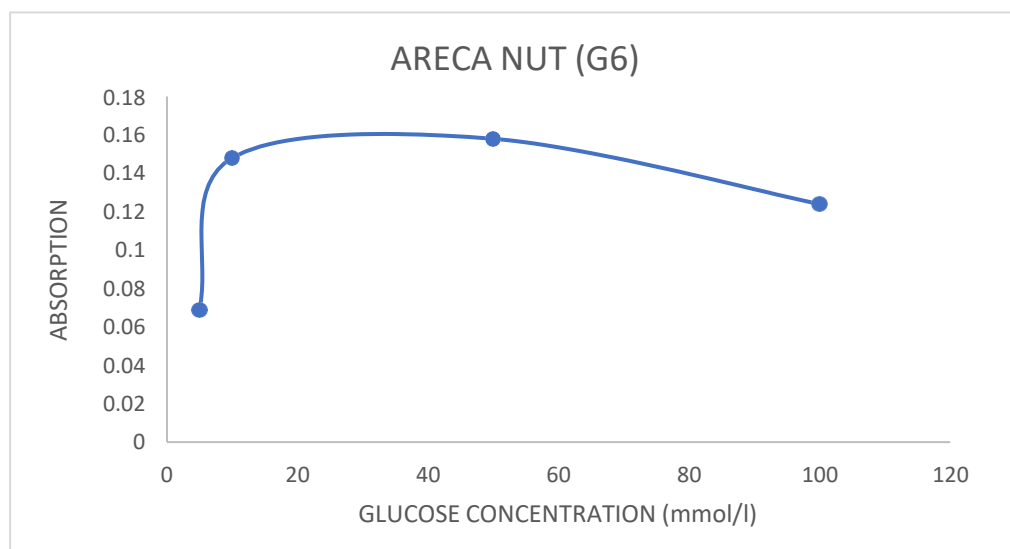


Fig:5.1.5.b: **Glucose content (G6) in areca nut sample**

5.1.6 FENUGREEK

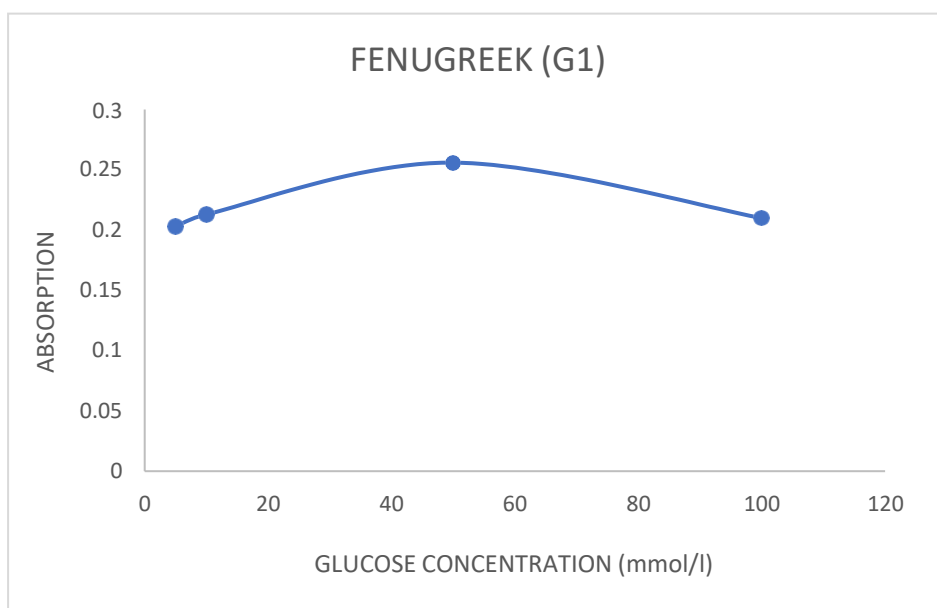


Fig:5.1.6.a: **Glucose content (G1) in fenugreek sample**

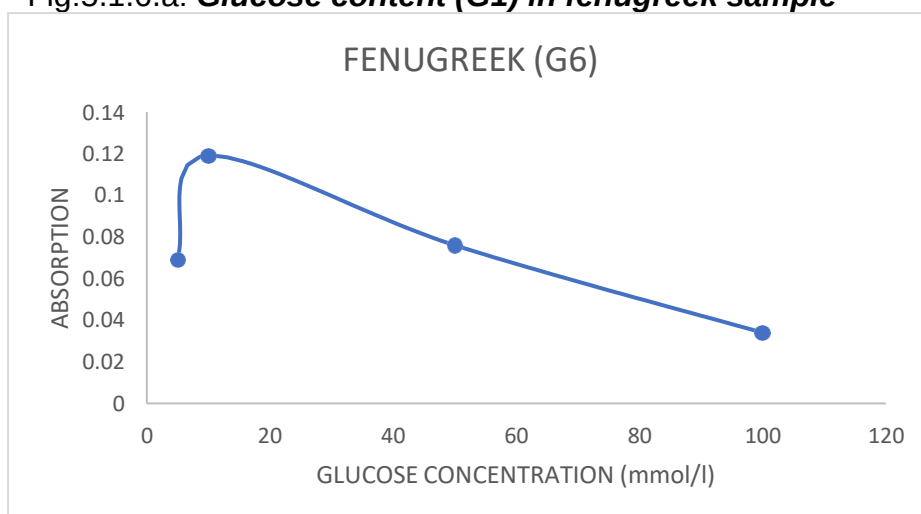


Fig:5.1.6.b: **Glucose content (G6) in fenugreek sample**

The Adult zebra fish is taken for Antidiabetic testing goes through Glucose induction and then the glucose level is measured then subjected to the plant sample, finally the blood glucose level for estimating antidiabetic activity is measured by glucometer .

Table 5.1: **Blood glucose level of the zebra fish**

S.NO	STAGES OF ZEBRA FISH	BLOOD GLUCOSE LEVEL (mg/dl)
1	Normoglycemic	81
2	Diabetic induced	102
3	Sample Administered	94

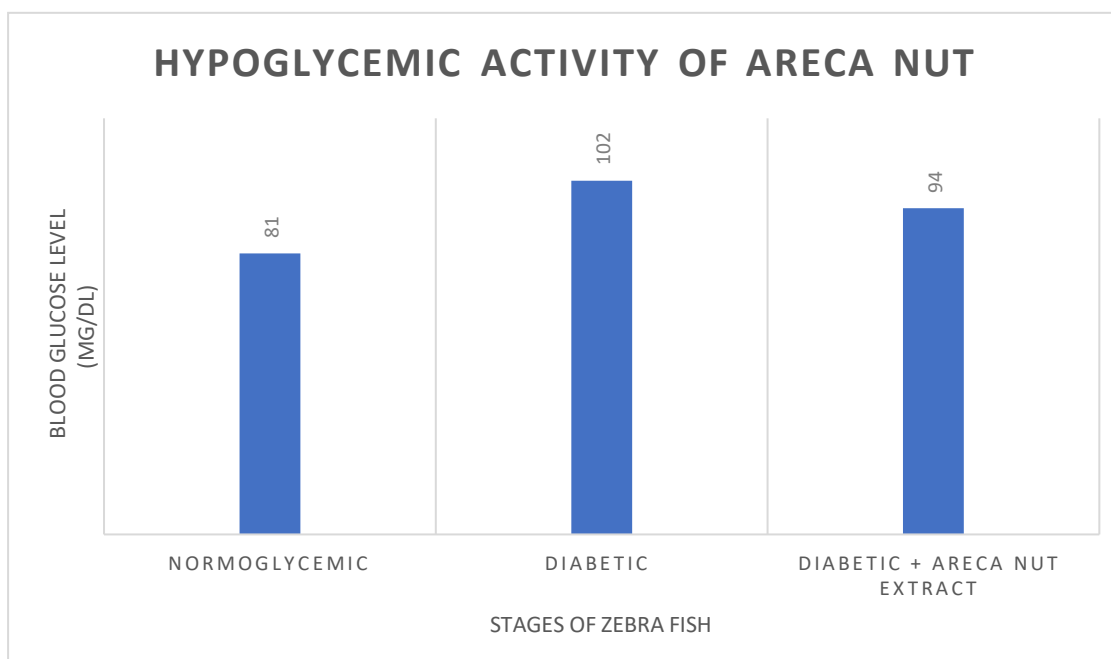


Fig:5.1.7: ***Effect of areca nut sample on blood glucose level***

5.2 DISCUSSION

Diabetes is one of the critical metabolic disorders in which natural drugs are more preferred. The disadvantage of diabetes condition is well known since it is increasing in the evolving economy, the experiments start with the literature study regarding the plant derived antidiabetics so that there won't be any side effects even on long time usage compared to the chemically derived ones.[Li WL., etal.2004]

To create a cure for the metabolic disorder that is globally occurring in increasing number , the antidiabetic activity of plants and its phenolic compounds should be studied and the experimental methodology should be framed accordingly . In previous researches the glucose absorption test is been evaluated by *in vitro* testing and then the results have been established.[Narayanan DS., etal.2011] by keeping the results as a reference , our experiment is carried out in 4 different concentration, PH and the presence of glucose is being estimated then the *in vitro* testing process is executed and the methodology is comprised by revising many procedures in previous studies [Prabhakar PK., etal.2011] and then carefully formed considering the effectiveness, accuracy and time consumption .

Initially the plant species were selected by analysing the reactivity and availability and their metabolic activity of phenolic compounds and flavonoids are studied . these plant species are extracted as a form of aqueous extract for the initial *in vitro* testing. The PH value and the presence of sugar is checked , then the plant species ability to reduce the glucose content is checked using in vitro analysis .In the analysis all the species showed the promising results and the results are noted and drafted in graphical manner. Among all the plant sample, Areca nut showed the more prominent result therefore that sample is taken for further testing. After that the *in vivo* testing method is studied and the chronic induction method is framed for achieving the diabetic stage [Perez Guiterrez., etal.2021] .*In vivo* testing on zebra fish model is performed using the ethanolic extract of areca nut ,which does not show any toxicity towards the zebra fish model.

Zebra fish is one of the suitable experiment models for diabetic study as diabetes can be induced through a high glucose diet so that there won't be any resistivity development shown like when it is done enzymatically or through gene therapy. Initially diabetic condition is induced and the glucose level is measured using glucometer, after administering the extract (plant sample) there is reduction in blood glucose level of the zebra fish thus confirming the hypoglycemic effect of the plant extract which can act as a greater antidiabetic treatment.

Further researches are needed to study the insulin mimetic property of the flavonoids and phenolic compounds of the plant species to reveal the contribution of phytoconstituents towards studies anti-hyperglycemic (hypoglycemic) property .

CHAPTER-6 SUMMARY AND CONCLUSION

The in vitro analysis showed the glucose reduction property of samples when tested in increasing concentration , the most desirable result is achieved in 100 mmol/L in every sample and Areca nut showed the most prominent result in that concentration. 0.99 mg\L concentration of areca nut showed antidiabetic activity in zebra fish model by the reduction of blood glucose levels . the effect of extracts is good as the effect of common antidiabetic drug and it can be concluded that lower concentration of extract will also show antidiabetic effect after a longer treatment period . the active components that are individually responsible for this property can be identified by further research .

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