Exploring the Therapeutic Synergistic Intervention of *Ceriops Decendra* with enrich protein of *Cyamopsis Tetragonoloba* against STZ, Nicotinamide induced type-2 Diabetes.

This Thesis is Submitted for the partial fulfilment of Degree

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2023

CERTIFICATE

This is to certify that Md Mustahedin Hoque has carried out the research on the Project entitled "**Exploring the Therapeutic Synergistic Intervention of *Ceriops Decendra* with enrich protein of *Cyamopsis Tetragonoloba* against STZ, Nicotinamide induced type-2 Diabetes**" Under my supervision, In the Division of Pharmacology and Toxicology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata- 700032.

He has incorporated his findings into this thesis of the same title being submitted by his in partial fulfillment of the requirement for the award of **Degree of Master of Pharmaceutical Technology, Jadavpur University**. I am pleased that he has carried out his thesis work with proper care and confidence to my entire satisfaction.

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Declaration

I do hereby declare that the project entitled "**Exploring the Therapeutic Synergistic Intervention of *Ceriops Decendra* with enrich protein of *Cyamopsis Tetragonoloba* against STZ, Nicotinamide induced type-2 Diabetics**" has been carried out during my academics as a dissertation work. And it has been not submitted for the degree to any other university, all of the source and the quotation are acknowledged by exclusive reference.

Name of the Student Md. Mustaledin Hoque

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Place & Date Jadavpur, Kolkata, 22/7/23 .

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24/8/23

Date

Signature

Chapter-1
Introduction

1.1

A concerned lifestyle disease called diabetes mellitus has grown into a problem on a global scale. According to the 2015 Diabetes Atlas published by the International Diabetes Federation (IDF), there are 415 million individuals worldwide who have been identified as having diabetes, or 8.8% among individuals aged 20 to 79[1]. By 2040, it is expected that there will be more than 640 million adults worldwide who have diabetes. Since over 80% of all persons with diabetes who have not been diagnosed live in low- and middle-income countries, which account for 75% of the global population, the increase in numbers will be greatest there [1]. China and India will have the largest increases in the number of diabetics among developing nations. Urban locations in developing economies have a higher rate of diabetes prevalence [2]. Rapid socioeconomic shifts brought on by industrialisation and urbanisation are the primary causes of the global diabetes epidemic, which is also largely driven by population expansion, poor lifestyle choices, and a lack of vigorous physical activity. Diabetes is a silent disease that can cause deadly and severe complications as well as raise expenditures. Nearly every system in the body is impacted by diabetes' long-term problems, but the eyes, kidneys, heart, feet, and nerves are particularly vulnerable. Anatomical, structural, and functional alterations as a result of the micro- and macrovascular complications result in numerous organ dysfunction [3]. thereby, it is clear that diabetes has already emerged as a major global danger to public health. This diabetes-related burden can have an impact on an individual, a family, society as a whole and health systems [1].

INDIA'S DIABETES BURNOUT 1.2

India is undergoing a shift in the prevalence of diabetes from urban to rural areas, the affluent to the less privileged, and older to younger individuals. India ranks second after China in the world in terms of the diabetes epidemic. In both urban and rural parts of India, the prevalence of diabetes has been rapidly rising [4]. The most common form of diabetes is type 2, which is also the main cause of the diabetes epidemic in India. Type 1 diabetes is becoming more common in India as well. Type 2 diabetes prevalence increased gradually in the 1990s and significantly more quickly after 2000[5]. Diabetes now affects more people than it did in

2000, when there were 32.7 million cases [6]. compared to 35.5 million in 2003[7], in 2007 there were 40.9 million [8], 50.8 million people in 2010[9], 2013 saw 65.1 million [10],69.2 million people in 2015[1], The predicted number in 2040 is 1.23 billion [1]. The first study on the prevalence of diabetes was carried out in Calcutta (now Kolkata) in 1938. After verifying 96 300 medical records, it was discovered that 1% of the population had diabetes [11]. Between 1973 and 2015, the prevalence of diabetes ranged from 1.1% in urban Lucknow to 25.2% in New Delhi [12]. Few nationwide studies on the level of prevalence of this disorder have been conducted, and the majority of those studies used capillary fasting and a two-hour post glucose load to diagnose diabetes. The countrywide prevalence of diabetes has been reported to be 2.1% in the multi-centre study done between 1972 and 1975 by the Indian Council of Medical Research (ICMR) in six cities (Trivandrum, Calcutta, Cuttack, Delhi, Poona, and Ahmadabad) and nearby rural areas [13]. Phased implementation of the massive ICMR-INDIA diabetes (ICMR-INDIAB) research, a nationwide representative epidemiological investigation, is currently taking place in all of the Indian states and union territory regions.[14]. Using capillary fasting and 2-hour post-glucose load, the study will assess the prevalence of diabetes and prediabetes by state in India and compare urban and rural disparities nationwide. 14 000 people participated in Phase I of the ICMR-INDIAB project, which found that the prevalence of diabetes was 10.4%, 8.4%, 5.3%, and 13.6% in 2011, respectively, in Tamil Nadu, Maharashtra, Jharkhand, and Chandigarh [2]

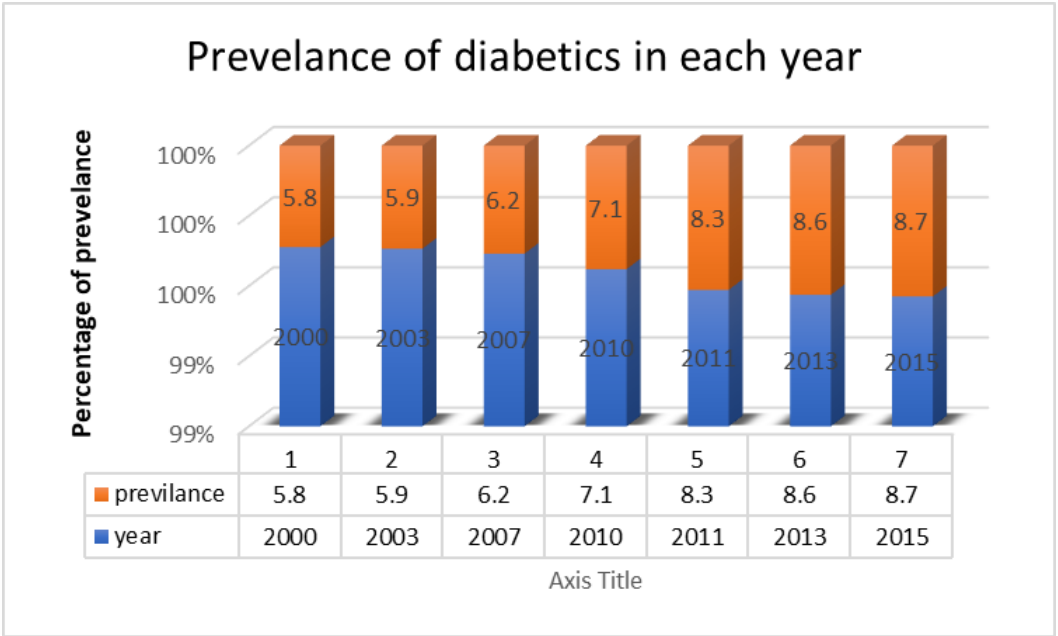


Figure: Diabetes prevalence increased in India between 2000 and 2015 among people (aged 20 to 79) [1,6-11].

In Jharkhand, the incidence in urban regions (13.5%) was four times greater than that in rural areas (3%), while in Tamil Nadu, the prevalence in urban areas (13.7%) was double that in rural areas (7.8%). Similar patterns were seen in Maharashtra (urban: 10.9 vs. rural: 6.5%) and Chandigarh (urban: 14.2 vs. rural: 8.3%). According to this data, 62.4 million persons in India were predicted to have diabetes overall in 2011[2]. The 2010 China non-communicable illness surveillance revealed that 11.6% of the population had diabetes, which is higher than the prevalence in other Asian nations [16]. Adults in Bangladesh were observed to have a prevalence of 11.0% for diabetes [17].

MORBILISM RELATED TO DIABETES 1.3

The majority of the morbidity and mortality brought on by diabetes are due to its complications. Despite the fact that uncontrolled diabetes and the length of the condition appear to be the main risk factors. Diabetic retinopathy (DR), diabetic nephropathy (DN), and diabetic neuropathy are terms for microvascular problems that affect the inner section of the eye, specifically the retina, kidney, and peripheral nerves. The macrovascular problems, which are also known as cardiovascular disease, cerebrovascular disease, and peripheral vascular disease (PVD), respectively, damage the arteries that supply blood to the heart, brain, and extremities [18].

Global Economic burden and Epidemiology of Diabetics 1.4

By 2030, the absolute cost of the global economy will rise from US$1.3 trillion (95% CI 1.3-1.4) in 2015 to $2.2 trillion (2.2-2.3) in the baseline scenario, $2.5 trillion (2.4-2.6) in the previous trend's scenario, and $2.1 trillion (2.1-2.2) in the goal scenario. This amounts to costs rising from 1.8% (1.7-1.9) of global GDP in 2015 to a maximum of 2.2% (2.1-2.2) [19]. Diabetes-related expenditures include increased healthcare utilisation, productivity loss, and disability brought on by vascular problems. Diabetes and its consequences come with a lifetime cost that places a heavy financial load on individuals, families, society, national

healthcare systems, and even nations. The type of sickness, the frequency and severity of disease complications, and the demographics of the patient population are only a few of the many factors that affect an illness's cost. The IDF estimates that in India in 2012, the mean diabetes-related expenditure per diabetic was 67.98 US$ (Rs. 4146/-, using an exchange rate of Rs. 61/US$) [20]. Numerous research has been conducted in India to determine the financial impact of diabetes. Few research has analysed direct and indirect costs; the majority of studies have exclusively evaluated direct costs. In 1999[21]. Indian rupees (INR) 15 460/- per patient was reported as the total annual cost (direct) for treatment, counselling, monitoring, tests, and medical services for the 611 diabetes patients in Bangalore, while INR 3572/- per patient was stated as the total annual cost (indirect) for patients [22].

Complication of Diabetics: 1.5

Diabetic Retinopathy (1.5.1)

The most common cause of new-onset blindness in adults in industrialised nations is DR, which is thought to be the most specific consequence of diabetes and is quickly becoming so in poor countries as well. In

The prevalence estimates of DR among people with known and newly diagnosed diabetes have exhibited significant diversity in India, according to a number of studies (clinic and demographic based). The average prevalence of DR was estimated to be 34.1% in a clinic-based study carried out in a tertiary diabetes care centre in south India, where diabetic individuals were examined using a combination of clinical examination and retinal imaging [23]. The prevalence of DR among type 2 diabetic subjects was found to be 37.9% in a different study that involved 226 228 patients enrolled in the diabetes electronic health record system in between 1991 and 2010 in the same tertiary diabetes care centre [24].

Diabetic-Nephropathy (1.5.2)

102 non-protein uric diabetic patients in north India were examined to determine the prevalence of microalbuminuria, and the frequency was found to be 26.6%.

Another study found that the average frequency of microalbuminuria was 25.5% among 670 patients who presented in an endocrine ambulatory clinic in north India in 2010[25].

Microalbuminuria was found to be more common than other problems in people with diabetes overall (26.9%), whereas overt nephropathy was found to be less common (2.2%), according to the Indian study CURES [26]. A recent study in metropolitan south India found that 41.5% of the type 2 diabetes group investigated had microalbuminuria [27].

Cardiovascular Complication (1.5.3)

According to research conducted in clinics, people with diabetes had a prevalence of CAD that ranged from 11.4 to 28.0%.[28] In the urban community-based Chennai Urban Population Study in Chennai, south India, CAD was found in 21.4% of participants with diabetes, while prediabetes and normal glucose tolerance had prevalence rates of 14.9% and 9.1%, respectively [29].

Factor affecting for Diabetics (1.6)

Epigenetic factor

One of the primary underlying causes in the onset of disease is thought to be mitochondrial malfunction brought on by change and damage to mtDNA. [30]. The effects of xenobiotic agents on the levels of mtDNA methylation, in particular, 5mC and 5hmC levels, have also been investigated in light of the recognition that epigenetic changes like methylation play a role in mtDNA regulation. Exposure to a variety of external variables, including as air pollutants, metals, cigarette smoke, dietary oils, food supplements, and therapeutic medications, can have an impact on mtDNA methylation [30]. Patterns of mtDNA methylation can be changed by environmental metal ions like chromium and arsenic that build up as a result of occupational exposure or contaminated drinking water.

The MT-TF and MT-RMR1 genes were shown to be hypomethylated in chrome plating workers after exposure to chromium [31]. Similarly, in an Indian community exposed to high levels of arsenic in the water they consumed, arsenic exposure was linked to hypomethylation of the D-loop and MT-ND6[32]. Baby's mitochondrial OXPHOS performance can be modulated by the maternal diet, which may have long-term effects on energy balance. This happens by changing the babies' mtDNA methylation levels. Betaine supplementation has been linked to a decreased methyl in the D-loop area and upregulation of mitochondrial encoded OXPHOS genes in newborn piglets, for instance, and protein shortage during

pregnancy affects mtDNA methylation levels in a sex-specific manner [33] As seen in giant yellow croaker fish, meal lipid content can also influence mtDNA methylation. A high fat diet enhances D-loop methylation, but an inadequate lipid diet decreases methylation in MT-RMR1[34]

Pharmaceutical Drug

By modifying DNMT/TET enzyme activities and resulting changes in mt-DNA methylation levels, pharmaceutical drugs can lead to mitochondrial malfunction. Because of mitochondrial malfunction, the anticonvulsant and mood stabiliser valproic acid (VPA), which is frequently used to treat epilepsy, may cause liver damage [35]. Seven mitochondrial genes were found to be hypomethylated in a separate study using primary human hepatocytes after exposure to VPA, and in the same study, two nuclear genes necessary for DNA methylation, DNMT and MAT (methionine adenosyltransferase), were discovered to be hypermethylated, leading to decreased levels of the DNMT enzymes and Sadenosyl methionine (SAM). This may affect mtDNA methylation downstream, which indicates nucleus-mitochondrial communication following VPA exposure [36]. B-cell insufficiency can result from pathological changes to the b-cell epigenome that make it difficult for these cells to maintain their differentiated, functioning condition [37] In the islets of T2DM patients, the Paired box 4 (PAX4) gene, which is essential for the development of mature b-cells, is hypermethylated and silent [38]

Type of diabetics: Main classification

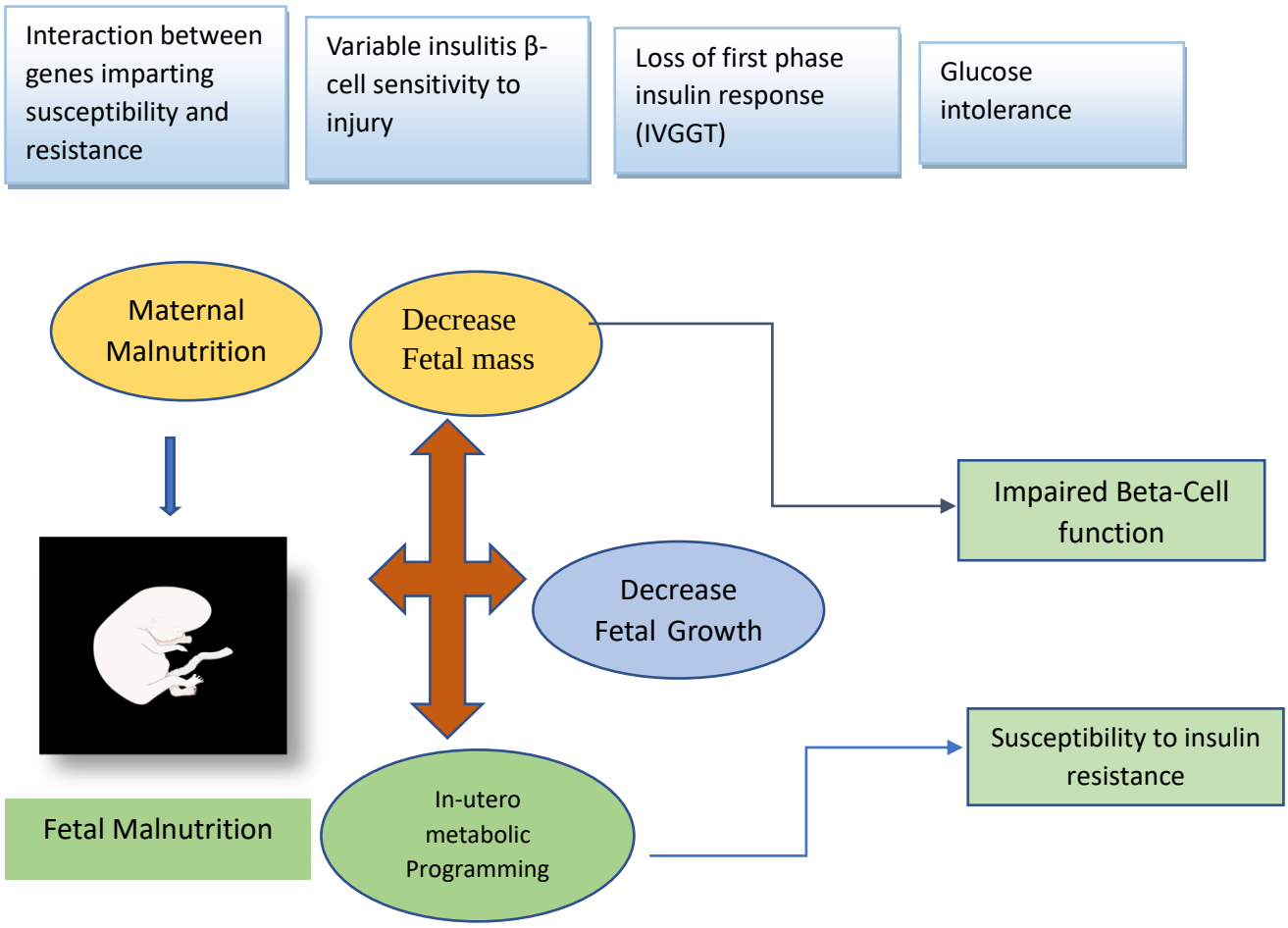
Insulin-Dependent (Type-1)

The human leukocytic genes (HLA) at the (MHC) loci and some non-HLA loci are among the numerous susceptibility genes that influence illness risk. The sequence of maternal nutrition and its effects on the foetus is depicted in Flowchart 1.0, even if the actual cause of T1DM is still unknown.

interplay between genetic factors and a variety of environmental stresses (viruses, cow milk protein, etc. When a genetically vulnerable person is subjected to an environmental insult like a protein in their diet or a virus (such as coxsackie, CMV, rubella, or the mumps), a series of

autoimmune processes are set off, finally resulting in the selective destruction of pancreatic beta-cells.

Even before the clinical manifestation of the disease, autoantibodies to the enzymes glutamic acid decarboxylase-65 (GAD65), insulinoma antigen 2 (IA-2) and insulin autoantibody (IAA) can be found in the serum of people as a response to an underlying destructive process (and do not contribute to disease pathogenesis). Following this, monocytes, macrophages, and T cells infiltrate the pancreas to start the immune system's destruction process. Insulitis is the defining feature of immune infiltration at this stage. A stage of absolute insulin insufficiency is caused by chronic death of the insulin-producing cells that occurs later, and by the time of clinical presentation, around 60–70% of the pancreatic beta-cells have been killed [39].



Flow chart 1.0: *Possible interactive finding which leads to type-1 diabetics*

Non-insulin dependent (type-2)

The most significant underlying disease is an imbalance between energy intake and expenditure, which is controlled by a complicated interplay between numerous genes and environmental factors.

The most common theory for the disease's cause is that there are flaws in both insulin resistance and insulin secretion. T2DM has a high heritability (estimated to be >50%), which supports the hereditary risk for the disease. However, because the majority of these loci show relatively low effect sizes, only 10% of the heritability may be attributed to them. A total of 30 loci have been shown to be connected to features relevant to diabetes. The greatest susceptibility locus linked to -cell dysfunction among these is TCF7L2. Non-synonymous variations in the PPARG and KCNJ11 genes are two additional loci that have a strong association. Table 2.3 provides a brief summary of significant genetic variations and their alleged functions. Studies on the risk variations of type 2 diabetes in the general population have shown that these variants function through disruption of insulin production rather than action of insulin [40]

Epigenetic pathways that underlie the immunological basis of diabetes and inflammation have recently come into greater focus, according to mounting research.

High glucose levels have been shown to alter the methylation and acetylation of the p65 promoter, which upregulates its expression and activates the pro-inflammatory pathway of NF-kB [41]

The activation of the NF-kB pathway causes the synthesis of various cytokines, such as TNF-a, IL-1, and IL-6, as well as adhesion molecules, such vascular cell adhesion molecule-1 (VCAM-1), in endothelial cells, macrophages, and monocytes/macrophages [42] Epigenetic alterations, inflammation, and diabetes have all been linked in human studies. A recent study on the adipose tissue of monozygotic twins with T2DM (diabetics vs. non-diabetics) found elevated expression of inflammatory genes, including secreted phosphoprotein (SPP)1 (also known as osteopontin, OPN), chemokine (C-C motif) ligand (CCL)18, and IL1RN, in conjunction with variations of global and localised DNA methylation [43]

Diagnosis:

Plasma glucose levels are used to diagnose diabetes. There are three techniques to diagnose diabetes, and each one needs to be verified the next day if there isn't clear evidence of hyperglycaemia. When used to diagnose diabetes, the 75 g oral glucose tolerance test (OGTT) is less repeatable but somewhat more sensitive and specific than fasting plasma glucose (FPG). Measuring FPG is the primary diagnostic test due to its simplicity, patient acceptability, and reduced cost. Because of the absence of worldwide standardisation and the lack of clarity surrounding diagnostic thresholds, the use of haemoglobin A1c (also known as glycosylated haemoglobin or HbA1c) for the diagnosis of diabetes was previously not advised.

HbA1c normal range and interpretation:

• 4.5–5.8% is the normal non-diabetic range.

• Diabetic range: > 6.5%

• Serious risk of hypoglycaemia less than 4.5

• 5.8 to 6.5% of adults are prediabetic.

HbA1C %	*Mean Plasma glucose Mg/dl*
5	**97**
6	**126**
7	**154**
8	**183**
9	**212**
10	**240**

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Chapter -2
Rational and Objective of the Study

Role of medicinal plant and their Phytochemicals:

Since ancient times, natural medicinal plants have served as the main source of treatments for a wide range of illnesses and disorders. These plants have a wide range of bioactive substances with therapeutic benefits that have been used extensively in traditional medical practises in several cultures all over the world. Since many of these plants have demonstrated promising outcomes in the treatment and management of diseases, the use of natural medicinal plants is still relevant today.

Role of Phenol and Flavonoids

Physical, environmental, metabolic, chemical, and genetic elements all have a direct or indirect impact in the development and progression of tumours. Cancer is a multi-step illness. A diet heavy in antioxidant-rich fruits and vegetables lowers the incidence of many different cancer types, strongly indicating that these antioxidants may be useful cancer-inhibiting agents [1]. Numerous studies showed that the direct suppression of early processes in inflammation is the cause of quercetin's considerable anti-inflammatory effect [2]. It has also been proven that quercetin has strong anticancer properties. For instance, investigations revealed that it has anticancer qualities, such as the ability to suppress the proliferation and migration of cancer cells [3].

Natural medicinal plants varied chemical make-up is one of their main benefits. Alkaloids, flavonoids, terpenoids, and phenolic compounds are just a few of the diverse secondary metabolites that plants create. These molecules have a variety of biological actions. These substances may also have actions that are analgesic, antibacterial, anti-inflammatory, antioxidant, anti-cancer, and others. Natural plants have the ability to effectively treat ailments by utilising their therapeutic characteristics.

Significance of protein in the management of Diabetics:

Protein consumption induces the release of glucagon, a hormone that aids in controlling blood sugar levels. In contrast to insulin, glucagon encourages the liver's stored glucose to be

released, keeping blood sugar levels from falling too low. This system promotes glucose homeostasis and guards against hypoglycaemia.

Production and Secretion of Insulin:

Consuming protein activates the hormone the insulin's release, which aids in the uptake and utilization of glucose by cells. For those with diabetes, especially type 2 diabetes patients who may have resistant to insulin, a sufficient protein intake maintains sufficient hormone synthesis and secretion.

Gluconeogenesis is a process through which the liver makes glucose from non-carbohydrate sources, like amino acids. By supplying a source of amino acids that can be used to produce glucose, when necessary, protein consumption assists in controlling gluconeogenesis. This mechanism contributes to steady blood sugar levels by limiting the generation of too much glucose.

Protein is crucial for preserving and gaining muscle mass:

It also helps the body use glucose. Due to its importance as a location for insulin-mediated glucose elimination, muscle tissue plays a crucial role in the uptake and utilisation of glucose. Consuming enough protein promotes muscle health, which enhances the uptake and utilisation of glucose by muscles. This process may help with better insulin sensitivity and glycaemic management.

Thermogenesis and Energy Expenditure: The thermic effect of food refers to the increase in energy expenditure that occurs during digestion and processing of different nutrients. Protein has a higher thermic effect compared to carbohydrates and fats, meaning that it requires more energy to digest and absorb. This increased energy expenditure associated with protein digestion can contribute to overall calorie expenditure and potentially aid in weight management.

Objective Of the Study:

Evaluation of in-vitro synergistic effect alpha amylase- and alpha Glucosidase enzyme inhibition assay.

Evaluation of Anti-diabetic activity of the selected sample through in-vivo assay.

Evaluation of in-vitro anti-oxidant and in-vivo tissue anti-oxidant parameter.

Plan of work:

- Collection and Authentication of Ceriops Decendra plant stem and bark.
- Hydro-Alcoholic extraction of the selected sample by the Soxhlet Extraction
- Phytochemical screening
- In-Vitro enzyme inhibitory assay α-Amylase & α-Glucosidase (Synergistic)
- Anti-Oxidant free Radical scavenging
- Evaluation of In-vivo Anti-Diabetics potential
- OGTT test
- Tissue Anti-Oxidant Parameter
- Serum Biochemical Parameter
- Histopathology

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Chapter-3
Literature review

Plane Taxonomy:

Plant name: Ceriops Decendra (Griff.)

- Kingdom- Plantae
- Phylum-Tracheophyta
- Class - Magnoliopsida
- Order- Malpighiales
- Family -Rhizophoraceae
- Genus - *Ceriops*
- Species - *Ceriops decandra*

(Source: *https://www.marinespecies.org/aphia.php?p=taxdetails&id=235087*)

Plant Name: Cyamopsis Tetragonoloba

- Kingdom: Plantae
- Clade- Tracheophytes
- Order – Fabales
- Family – Fabaceae
- Genus- Cyamopsis
- Species – C. Tetragonoloba

(Source: *https://en.wikipedia.org/wiki/Guar*)

Plant Detail:

Ceriops Decendra

Synonyms: *Bruguiera decandra* Griff.

Local name: Malatangal, Chiru kandal (Tamil), Gatharu (Telegu), Kadol (Singhalese**)** jhamti Goran **(**Bengali)

Parts use for medicinal value: Leaf, stem, bark, root.

Geographical Distribution: C. Decandra is a widespread species in India and may be found in fragmented inner tidal wetlands and sporadically in outer mangrove habitats, but C. tagal is either rare or extremely uncommon on both the east and west coasts. C. Tagal, however, has substantial

The number of people living in the mangrove forests of the Sundarbans and the Andaman & Nicobar Islands. While C. Decandra flowers all year long and bears fruit, C. tagal flowers only sometimes, from November to February. Some C. tagal individuals blossom late and display flowers as late as June [3].

Plant Morphology: The petals of C. Decandra are slightly interlocked by basal short hairs, which results in a small corolla tube capped by a series of clavate filamentous appendages. Each stamen of C. Decandra is longer than the stigma; anthers are longer than filaments. The stamen ring's disc is fully grown, and the base of the thick filaments is enclosed by the anther lobes. In C. Decandra, ripe buds open between 04:30 and 1100 hours. In C. Decandra, the pollen grains are ovate, triangular in shape light yellow, and smooth on the exine. In C. Decandra, a bloom yields 12,810 + 30,87 pollen grains. In C. decandra, very little nectar is produced, however there are 5.65 + 1.0 l of nectar per flower. Fruits of C. decandra are light green, ovoid, conical, and blunt at the apex**.**

Floral event	Characteristic (s) C. Decandra
Anthesis (h)	0430-1100
production of pollen/flower	12,810
ovule-to-pollen ratio	2,135:1
Fruit	In 45-55 days
Hypocotyl	In 85-90 days
Abortion rate for buds (%)	41.66
Rate of natural fruit set (%)	15

Phytochemical Content: Locals have a high demand for the wood and bark of C. Decandra. The bark has a high tannin content, ranging from 68 to 75%, and when dyed with

this tannin, it turns brown [4]. This species' bark served as a significant source of superior tannin. This tree's bark sap serves as the source of a black dye for the batik industry.

Haemorrhages are treated using a decoction of the bark [5]. whereas the leaves, fruits, and bark are used to cure ulcers and hepatitis [6]. Additionally, C. decandra is used to treat pain, ulcers, and hepatitis and has been claimed to have astringent and anti-haemorrhagic qualities [7].

Leaves	**Bark**	**Wood**
lupenone	decandrins A-K	3β,13β-dihydroxy-8-abietaen-7-one and 3β-hydroxy-8,13-abietadien-7-one
lupeol	propanoic acid (3β-hydroxylupan-29- oic acid	ceriopsin A-D
betulinaldehyde	procyanidin	ceriopsin F and G
3β-E-coumaroyllupeol,	d-catechin, leucoanthocyanidins	ceriopsin E
3β-Z-coumaroyllupeol	(-)-syringaresinol, (-)-pinoresinol, β-sitosterol, stigmasterol, palmitic acid, and 3,4-dihydroxybenzoic acid Et ester	
betulin		
3-epi-betulinic acid		
betulinic acid		
3β-E-feruloylbetulin		
3β-E-caffeoyllupeol		
3β-hydroxylupan-29-oic acid		
Oleanolicacid		
3β,20-dihydroxylupane		
3β-Z-feruloyllupeol		
3β-E-feruloyllupeol a		
ursolic acid		
α-amyrin, β-amyrin		

(Source: *DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i11.19461*)

Biological Activities:

Larvicidal activity- Lab-raised S. litura (Fab.) larvae in their third and fourth instars were used for bioassays. A L16:D8 photoperiod was used to raise insects on castor (Ricinus communis) leaves at room temperature (24e28 C). Topical application to early third and fourth instar larvae of S. litura allowed researchers to assess the crude extracts of C. Decandra leaves' larvicidal activity (measured as mortality after 24 h [9]. Different quantities of the crude extracts (100–5000 mg/mL) were used to determine lethality. Each dosage was tested on ten larvae in triplicate. The LD50 and LD90 concentrations were determined using a probit analysis [10].

Antifungal activity: P. Aphanidermatum, a phytopathogenic fungus, was purchased from the Horticultural Research Station in Ambajipeta, Andhra Pradesh. Test organisms included R. solani (MTCC 4633), P. oryzae (MTCC 1477), Curvularia oryzae (MTCC 2605) and F. oxysporum (MTCC 287), all of which were obtained from the Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. On Potato Dextrose Agar (PDA), the strains were kept and tested.

By determining the zone of inhibition diameter (IZD) using the Agar cup technique, the antifungal activity of crude extracts of leaves of C. decandra Griff. Ding Hou was ascertained at doses of 100 mg/mL, 250 mg/mL, and 500 mg/mL [11].

Antinociceptive: Mice's frequency of acetic acid-induced writhing was considerably and dose-dependently decreased when the ethanol extract of C. decandra leaf and pneumatophore was administered orally at dosages of 250 and 500 mg/kg. There was no antinociceptive action in the bark's ethanolic extract. The observed outcome appears to be in favour of the conventional usage of C. decandra leaves in the treatment of pain [12].

Anti-Bacterial: Using LB-agar, YPD-agar, and PD-agar plates, the disc diffusion technique (Bauer et al., 1966) was used to assess the antibacterial and antifungal activities. 30 ml of media was added to a clean, 90 mm Petri dish to create the assay plates, which were then given time to set up. A sterile cotton brush dipped into the experimental microbial slurry was used to disseminate the produced inoculum uniformly across agar plates. Then, 5.5 mm-diameter paper discs (prepared from Whatman No. 1 filter paper) with 8 L of 250 mg/ml

extract dissolved in dimethylsulphoxide (DMSO) were put on the agar surface. Each disc received 2 mg of the extract. The plates were then incubated for 18 hours (for bacteria) or up to 36 hours (for viruses) at the appropriate ambient temperatures. LB-agar, YPD-agar, and (for fungus) were used to test the antibacterial and antifungal activities using the disc diffusion technique (Bauer et al., 1966), and the diameter of the inhibitory zone surrounding each disc was measured in millimetres. To ascertain the sensitivity of the microorganisms under test, 3 L of common antibiotics, including ampicillin (125 and 500 g/ml), chloramphenicol (10 mg/ml), and commercial fluconazole (10 mg/ml), were utilised. DMSO was used as a negative control [13]

Cyamopsis Tetragonoloba:

Synonyms: *Cyamopsis psoralioides* (Lam) DC.

Local name: Guar

Medicinal value: seed, Folk medicine as a digestive enhancer, a cooling agent, an ulcer preventative, a secretion inhibitor, an anti-hyperglycemic, and a cathartic

Biological Importance:

Anti-Cancer (in-vitro)- A 96-well plate with 8 104 seeds per well was used to seed the cells. Cells were exposed to various dosages of extracts made from C. tetragonoloba seeds and pods after being incubated at 37 C and 5% CO2 for an entire night. One milligramme of dried powder was dissolved in thirty millilitres of the organic solvent DMSO to create the plant extracts, and one millilitre of phosphate buffer saline was used to bring the final volume to one millilitre. Reaching the cells at the right concentration of DMSO was safe [14]. For 48 hours, the extracts were treated. Following the addition of MTT [3- (4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide] (SRL) in equal amounts to each well, the assay was incubated for 4 hours before the formazan crystals produced by the mitochondria of living cells were dissolved in DMSO. Using a Multiskan Micro plate Spectrophotometer from Thermo Scientific, absorbance was measured at wavelength 570 nm with a differential filter of 630 nm [14].

Anti-Hyperglycaemic- Cyamopsis tetragonoloba (C. tetragonoloba), sometimes known as Cluster Bean or Guar, is a member of the Fabaceae family. Due to the presence of flavonoids

and other phenolic compounds in the plant, it had a negligible antihyperglycemic impact on blood glucose level in normal fasting rats, but the blood glucose-lowering effect was significant in alloxan-induced hyperglycaemic rats [15].

Other studies have revealed that this herb can boost insulin release while reducing HbA1c levels. Beans from C. tetragonoloba had polyphenols that lowered blood sugar levels and protected -cells. As a consequence, the potential for using this plant to treat type 2 diabetes may be explored [16].

Geographical distribution: According to reports, the Cyamopsis genus originated in Africa. Cyamopsis tetragonoloba (L.) Taub., often known as the cluster bean or guar, is a commonly produced summer feed crop, especially in several Asian nations where dry and semiarid environments are common. Despite being predominantly planted for fodder, it has a variety of other possible applications, including the pods as fresh vegetables and the entire plant as green manure [17]. Guar is generally grown in semi-arid and arid climates, such as North West India and South East Pakistan. Due to its abundance in vitamins, minerals, fibre, and proteins, guar pods are eaten as vegetables all over the world [18].

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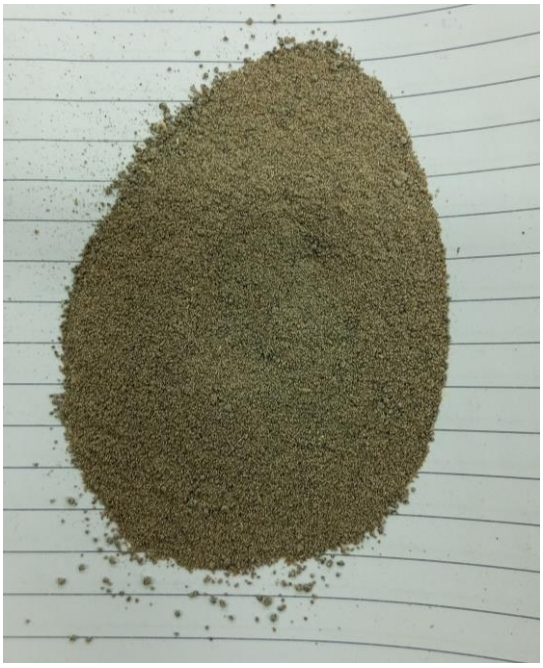
Chapter-4
Collection, Identification, and Extraction & Phytochemical Screening of plant substance.

The plant was collected from the Sundarbans area, and identified by the botanist ----------. The whole stem and the bark were washed at the beginning then it was break into small pieces then dry it in a room temperature for few days then it dried in a drier at 45* C. Afterall it makes as a coarse Particle for the final Extraction process. On the other hand, the Cyamopsis tetragonoloba raw Guar powder was collected from the Natural Product Manufacturing Company M/S Alona Hydrabad.

Ceriops Decendra Stem Grinded



Cyamopsis Tetragonoloba Enrich Protein Powder

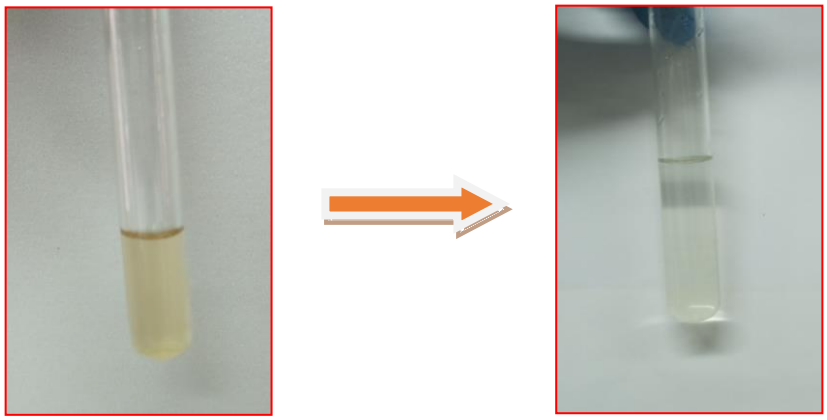
Extraction from *Ceriops Decendra* Stem: This broken stem sample 250 Gram was extracted with the Hydro-alcoholic Extraction Ethanol-Water (70:30) in a Soxhlet apparatus at a temperature (60- 70*C). Then the solvent Extract was then filtered in a member filter (Wartman Paper). To exclude the solvent from the filtrate material a solvent are then transfer into a rotary vacuum evaporator at a reduced pressure at 55*C. then concentrated sample

were taken to a dryer for 24 Hour at 40*C then the extract was subjected to transfer in a lyophilise machine for 48 Hours. The final yield was 13%.

Phytochemical screening: first a sample extract stock solution was prepared 10 mg/ml in 10 ml of distilled water and kept aside in a small beaker for the testing of Phytochemical.

Detection of flavonoids:

Alkaline Reagent test: from the stock solution 1 ml of plant sample solvent are taken in a test tube. On the other side 2% of NaOH solution are prepared in 10 ml of distilled in a calculative measurement 20 mg NaOH dissolved in 10 ml of distilled water. in a same way 1 ml of concentrate HCL (95%) are taken in a different small beaker then add 10 ml of water to make it as a dilute HCL. Now the sample contain test tube kept in a test tube holder and eventually add 2 ml of 2% NaOH stock solution by adding 2% NaOH solution the sample become intense yellow in colour, then added few drops of dilute HCL and kept it for few min. after all the whole sample system become colour less [1].

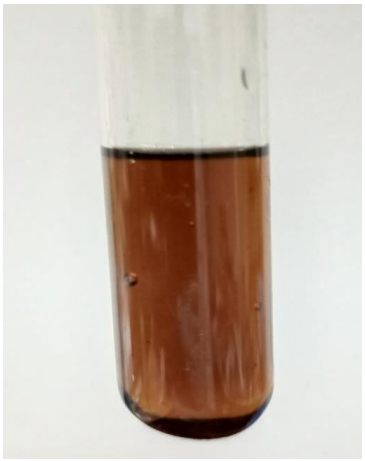


1 ml of sample +2ml of 2% NaOH solution fig-4.0

1 ml sample+2ml 25 NaOH + few drops of dilute HCL (5-7drops) fig-5.0

Lead acetate test: 1 ml of sample solution taken in a test tube. on the other hand, 10% lead acetate solution are prepared by dissolving 1.25 gram of lead acetate in 12.5 ml of distilled water. After all, in sample containing test tube added few drops of lead acetated solution. And kept it for few min. there is significance result seen in terms of yellow ppt which is supposed to show by this method [2].

Lead acetate test – no significance test result shows fig-6.0



1 ml of sample +0.1 gram of Zn + 8 ml of H2SO4 (red colour)

Pew's test:

In a test tube from the sample stock solution 1 ml sample solution are taken then 0.1 gram of metallic zinc are measured and kept it aside after that conc. Sulphuric acid are taken in a small beaker for the. Now in the sample contain test tube 0.1 gram of metallic zinc are added slowly metallic zinc sedimented in the sample test tube there is no changes show at all. then conc.H2SO4 added through the wall of the test tube slowly 8 ml followed by 1 ml each time and the reaction between the acid and the zinc metal start, and the whole system start from start to form a red colour at the end of addition of acid complete system become completed red in colour as the concentration increases the colour formation become deeper in red [3].

Test for the phenolic component:

Iodine test:

In a test tube 1 ml of sample solution are taken then Kept it aside and prepare 1% Iodine stock solution in 12.5 ml of distilled water by adding 0.12 gram of iodine powder eventually its stair by the glass rod for the completed dissolution in the water it take about 3-5 min to for to complete dissolved in the water by stair. Now the sample containing test tube are taken in a stand and then add few drops of dilute iodine solution slowly by the micro-pipet slowly the

colour formation appears after adding the iodine solution. A stable transient red colour formed in the whole system [4].

2 ml of sample + few dops of dilute iodine solution added (transient red color) fig-8.0

Sample+5%glacial acetic acid +5% sodium nitrite solution, no significant changes fig-9.0

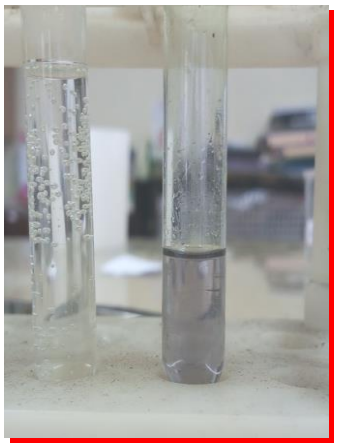
Ellagic acid test:

1 ml of sample solution taken in a test tube and kept it in test tube stand. Then in a different baker 5% glacial acetic acid prepared in a same way 5% sodium nitrite solution prepared by measuring 1.25 gram of sodium nitrite and added it into 25 ml of distilled water. After all sample containing test tube taken and added 1 ml of glacial acetic acid followed by 1 ml of sodium nitrite solution. There were no significance changes in the system no muddiness or brown ppt shown. Test shows absent of Ellagic acid [5].

Screening of Protein Cyamopsis Tetragonoloba:

*million's test***:** In a test tube 2 ml of stock solution of sample are taken then few drop of million's reagent are added. Initially there are was no such kind of changes happen with the adding of million's reagent. subsequently fewer drop of million's reagent is added then kept it for some time. After a while a white powder like appearance are seen in the down side of the test tube. Due to the white in colour its quite difficult to see the appearance of the test result [6].

A white PPT appear on the test tube down side fig-10 (Million's Test)



Here the observation it shows a light purple color fig-11. (Biuret Test)

Biuret test: At the beginning 2 ml of test sample solution are taken in a test tube from the stocks solution then added 2-3 drop of 2% copper sulphate solution. Then eventually 1 ml of 95% ethanol are added to the same test tube slowly with the wall of the test tube as the colour formation of the test result is suppose to the added ethanol. Hence precaution should be taken so that the entire process doesn't get hamper. Then one KOH pellet added and shake the test tube gently so the pellet gets dissolved eventually another one KOH pellet added to the test tube solution as the pellet started to dissolved formation of colour started to begun. After the complete dissolved of the KOH pellet a stable pink colour are formed [7].

Sl No-	Test- Name	Ceriops Decendra	Cyamopsis Tetragonoloba
1.	Flavonoids	++	-
2.	Alkaloids	++	+
3.	Phenol	++	-
4.	Saponin	-	-
5.	Tannin	+	-
6.	Glycoside	+	+
7.	Protein	-	+++
8.	Terpenoids	++	--

(*Preliminary Phytochemical test of Ceriops.Decendra and Cyamopsis tetragonoloba [+] presence [-] Absent*)

Conclusion of Phytochemical Screening:

On the screening test of the Phytochemical, we have found the presence of several phytochemical are present. Like alkaloids, flavonoids, and phenolic component which are responsible for the therapeutic activity, majorly the flavonoids improved the pathological condition and it help to restore other disorder as well. So, we have found a desirable presence of Phytochemical component which can be used in the evaluation of therapeutic activity [8].

Reference:

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Chapter-5

De-fatty nation and removal of Phenolic and flavonoids of the Guar powder prior to enrichment

Solution preparation for enrichment

NaOH solution (0.01 N) - here for to make (0.01 N) solution of NaOH 0.4 Gram of NaOH are dissolved in 1000 ml of distilled water.

HCL solution preparation (0.1N) – take a 100 ml of purified water or water in 1000 ml volumetric flask. Add 8.2 ml of concentrated HCL carefully. Add 700 ml of water allow solution to cool down to room, temperature and make up the volume 1000 ml [1].

Molar mass of the HCL is =36.461 g/mol

HCL specific gravity is =1.189

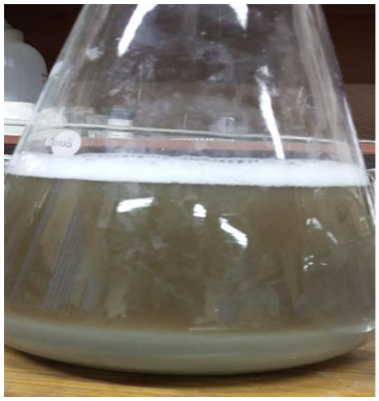
About 50 Gram of row sample was taken. Then 350 ml of NaOH solution (0.01) N was added. And it grinds for 1 min .it become fine mist wet powder and then additional 200 ml of water added to the mist fine slurry. It means about 550 ml of water added at the very beginning.

After adding additional 200 ml of NaOH solvent froth formation occur in the upper part of the sample beaker after keeping in the water bath. The slurry was kept in the water bath for 30 min to get the extract which is come in the solvent by the heat. Then filter the material in a different glassware. Then the process is continued two time and keep the filtrate in a different in glassware all combined.

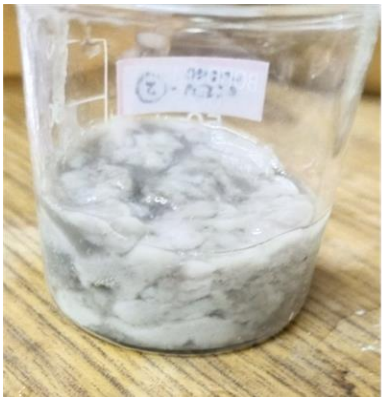
At the last 0.1N HCL solution was added to the filtrate sample solely to maintain the PH and to get the ppt started to form when it come around **the PH between 3-4** then it kept aside for the 1 hour to get complete the ppt formation. Then the solvent material is then decanted in the different glassware and the ppt left in a conical flux with little solvent are then transfer to a baker then the ppt material are centrifuged. here in centrifuged tube at the bottom side powder are get ppt. after all the ppt are then transfer to a Petridis and kept in a leaf dryer for the dry substance are black in colour. Then it has grind &kept in a sample holder [2].

Here we have kept PH 3.08

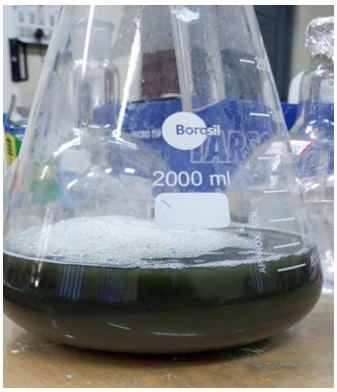
Total HCL solution was added 500+240 ml = 740 ml for the complete ppt formation.



Guar filtrate get ppt after adding HCL solution (0.1 N) here in down side ppt are formed



Fatty substance isolated from the filtrate by adding acetone to the solvent.



Guar filtrate with (0.01 N) NaOH solution.

Total filtrate gets combine in three cycle = 750 ml

Total we get =1490 ml. of solvent.

After that the filtrate ppt are kept in a different beaker.

And the first decant filtrate are then added acetone by adding acetone the fatty substance are separated. And floating over the solvent. And total weight of the fatty substance is 13 grams. filtrate ppt 12 gram and dry powder 10 gram. The filtrate colour was look like mat black in colour after heating in the water bath(slurry). Then the filtrate material is taken for **(total 1490 ml)** in a different conical flux ad after all add acetone solvent 500 ml to get the additional ppt form the filtrate. And then it keeps aside for the whole night. On next day it shows that white fatty like substance is floating over the solvent. Then the floating substance are separated from solvent…. In a different small beaker. It has seen that after adding the acetone to the solvent there was no ppt formation in the down side. It was considered that if we add the acetone in the solvent some material would ha e some probability to get ppt but there was no such kind of event was seen.

Total amount of sample taken	**Filtrate ppt**	**Dry powder gets**	**Fatty substance**
50 grams	12 grams	10 grams	13 grams

Here we can see that a large portion of fatty substances are present in solvent in the filtrate it means in acid salt alkaline method fatty substance are not get ppt by adding HCL solution which is quite good news for our experimental procedure. This fatty material is then can be removed by just adding acetone to the filtrate material in the different glassware which was then again discarded in small beaker.

In this this method I have lost some sample during procedure, it is a productivity lost it should be minimised as much as possible. 50 Gram of sample are taken are very first time. There is a separate sample was taken for the to repeat this process here after complete the procedure yield sample then analysed, how much it gets purified due to enrichment here we get desire result for the enrichment protein. The enrichment was shows about 84%. It means the protein component in the sample was purified up to 84%.

We have to repeat this procedure in another sample to make the robustness of the procedure here we need to repeat the procedure subsequent three time to make it robust.

Removal of Phenolic and Flavonoid component:

In a beaker with constant stirring for 30 minutes, dried Cyamopsis tetragonoloba seed powder was supplemented with 250 ml of cooled 0.01 M sodium hydroxide solution pH -3. After centrifugation at 4,000 rpm for 30 minutes at 4 °C, the crude preparation was recovered. This crude powder received 1% w/v of polyvinylpolypyrrolidone (PVPP) and was stirred for 3 hours. The purpose of PVPP is to eliminate phytochemicals by removing phenolic and flavonoid components from seeds sample. The powder was once more centrifuged for ten minutes at 4,000 RPM.The recovered supernatant was then further processed using acetone precipitation [3].

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Chapter -6
Gel Electrophoresis

Preparing the reagents for the stacking gel:

25 ml of 0.5 M Tris-HCL:

Tris-HCL is weighed out at 1.5 grammes, transferred to a beaker, and then mixed with 25 millilitres of distilled water to keep the solution's pH at 6.8.

10 ml of 1.5 M Tris-HCL solution:

10 cc of distilled water is used to dissolve 1.2 grammes of tris.

10 ml of distilled water with 10% SDS:

One gramme of SDS was measured and then dissolved in ten millilitres of distilled water.

Making a 10% APS solution in 10 ml of distilled water:

One gramme of APS was measured out and then dissolved in ten millilitres of distilled water.

Solution with Acrylamide 30% and Diarylamines 0.8

To create a 30% acrylamide solution in 10 ml of distilled water for the % W/V calculation, 3 grammes of acrylamide and 80 mg of bis-acrylamide are measured and dissolved in the tube along with 10 ml of distilled water [1].

Quantity and concentration of each molecule needed to prepare the stacking gel reagent

Unit	Chemical Name	Quantity of chemical	Quantity of dissolving solvent (DW)
0.5 Molar	Tris-HCL	1.5 Gram	25 ml
10%	SDS	1 Gram	10 ml
30% /0.8%	Acrylamide & Bisacrylamide	3 gram & 80 mg	10 ml
10%	Ammonium persulphate	1 Gram	10 ml

Chemical component for the manufacture of 5 cc of stacking gel:

Chemical composition and concentration	Quantity	Unit
ml of water	2.97	ml
1.5 M Tris-HCL (PH-8.8)	1.25	ml
10% SDS	50	µl
10% Acrylamide	0.67	ml
10% APS	50	µl
TEMED	10	µl

Chemical component for the manufacture of 5 cc of stacking gel:

Chemical Composition and concentration	Quantity	Unit
ml of water	3.8	ml
Acrylamide / Bis-acrylamide	3.5	ml
1.5 M Tris	2.6	ml
10% SDS	100	µl
10% APS	100	µl
TEMED	100	µl

Note- After adding APS and TEMED, the polymerization reaction is initiated; thus, it is important to note that these two chemical components are added to the sample reagent last. The entire reagent system is then stirred with a tiny glass rod to ensure that it is spread evenly in the small beaker.

preparing the loading buffer for Sample 10 ml:

10% SDS - 10 ml of distilled water are added once one gramme of SDS has been measured in a measuring device**.**

20% v/v- Glycerol is required here; 2.5 ml of glycerol must be added to the 10 ml loading buffer.

0.2 M Tris-HCL- 3.94 grammes of Tris-HCL are measured out and placed in a beaker

0.1 % 10 mg of bromophenol blue are administered in the same beaker

14 mM Beta-There are 14 mM of beta-marcaptoethanol and 14 µl of it in the beaker**.**

Once all the chemicals have been measured and transferred to a beaker, 10 cc of water must be added.

Preparation of Staining solution:

500 ml of staining solution are made using 0.5 grammes of Coomassie brilliant blue. 150 ml of methanol is measured in a measuring cylinder, transferred to a 2-liter beaker, and then 325 ml of water are added. The solution is then allowed to sit for a few minutes before being stirred with a glass rod for five minutes. The staining solution is then maintained for subsequent use at room temperature after being subjected to the addition of a fresh reagent, 25 ml of acetic acid, after all chemicals have been combined [2].

Method: Gel Casting

Two glass plates are taken and two casting frames are first set up for the gel casting. The glass plates are then cleaned with ethanol and let to dry for a short while. The spacer, which is 1mm in size and is used to divide the white and clear glass plates, is then carefully fastened with a screw. In order to check for water leakage from the casting frame, distilled water is first poured over the plate. Once it has been confirmed that there have been no leaks, it is

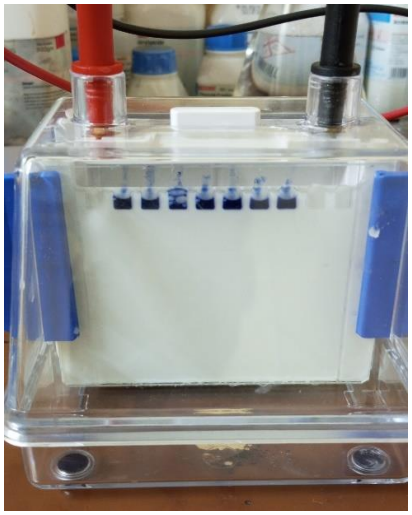
discarded. Regent mixture, which is prepared for the separating gel, is slowly poured into the casting frame so that bubbles do not form in the casting frame. The gel is then cast, and the casting frame is kept at 25°C for 25–30 minutes. Isopropyl alcohol is then added on top of the layer. so that the environment doesn't pollute it. When the resolving gel is ready, the top layer of IPA is scraped off and the stacking gel is poured in using the comb. After waiting for 25 minutes for the gel to completely cast, the comb is carefully removed from the casting frame so as not to affect the gel's outward look [3].

Gel Run:

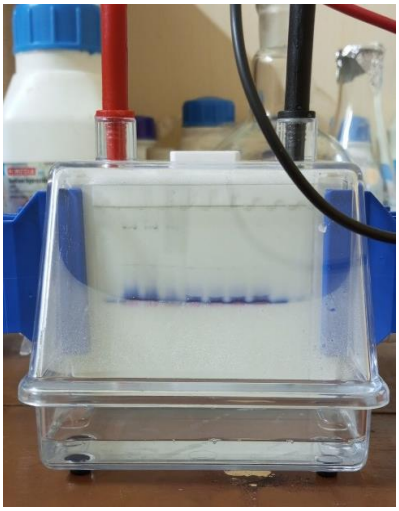
The casting gel is then transferred into the gel chamber on a glass plate, fastened with a clump, and 1x buffer is progressively poured into the chamber. After this, a glass pipette loads 15 l of the sample into each comb slot. Albumin from bovine serum is employed here as a standard component. The voltage was then set to 120 V and 200 W for one hour.

Staining of gel & de-staining.

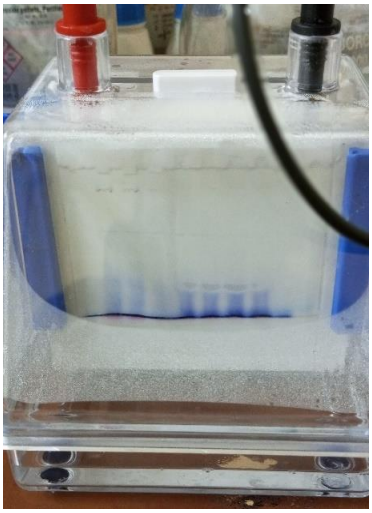
The gel is removed from the glass plate after an hour and maintained in the staining solution for 30 minutes. The gel is then left in the distilled water overnight while being gently shaken to remove the discoloration. The next day, the used water is removed once more, and new distilled water is put to the gel tray and left there for a few hours until a clear region forms in the gel. In the end, it continued to use the UV chamber to identify the sample's protein band [4].



Sample loaded before



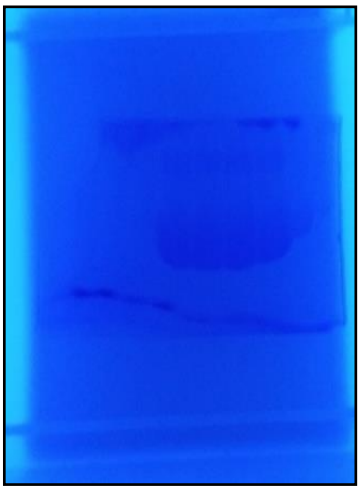
After 30 min



Final Run End point

Gel is de-staining with the distilled water

Band of protein molecule at 254 nm of UV radiation



Identification of band at 366 nm of UV radiation

Reference:

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[4] Ladner-Keay, C. L., Turner, R. J., & Edwards, R. A. (2018). Fluorescent Protein Visualization Immediately After Gel Electrophoresis Using an In-Gel Trichloroethanol Photoreaction with Tryptophan. *Methods in Molecular Biology*, 179–190. https://doi.org/10.1007/978-1-4939-8745-0_22

Chapter -7
In-vitro Antioxidant and Comparative alpha amylase and Glucosidase inhibitory assay.

DPPH Antioxidant assay:

Introduction

A persistent free radical called DPPH (2,2-diphenyl-1-picrylhydrazyl) rapidly takes an electron or hydrogen atom from an antioxidant substance. Reduction is the method through which DPPH takes an electron.

DPPH is extremely reactive because it has an unpaired electron in its chemical structure. DPPH is unstable due to its unpaired electron, which also gives it its distinctive deep purple hue. The unpaired electron from DPPH is transferred to the antioxidant molecule when it comes into contact with an antioxidant chemical.

Principal

The radical scavenging or antioxidant activity of DPPH is determined by its capacity to receive an electron. Strong antioxidants will rapidly transfer an electron to DPPH, significantly reducing the amount of purple that DPPH exhibits. The amount of colour change is related to the tested compound's antioxidant strength.

Procedure:

Prepare a stock solution with a 0.1 mM suggested concentration by dissolving the DPPH powder in a suitable solvent (such as methanol or ethanol). Weight 1.97 gram of DPPH in 50 ml of methanol. Molecular weight of DPPH 394 gram /mol. Make careful to thoroughly mix the DPPH powder until it dissolves.

Cover the container with aluminium foil or store the DPPH solution in an amber bottle to shield it from light.

One well should be designated as a blank control that solely contains the solvent and DPPH solution. This well will assist in making any background absorbance corrections.

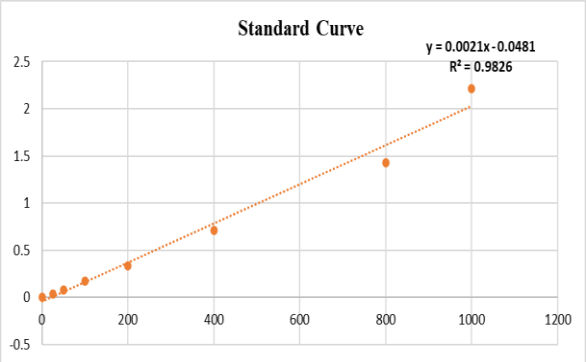
100 L of each sample or antioxidant standard should be added to the appropriate microplate wells. Add 100 µL of the DPPH solution to each well containing the samples or standards

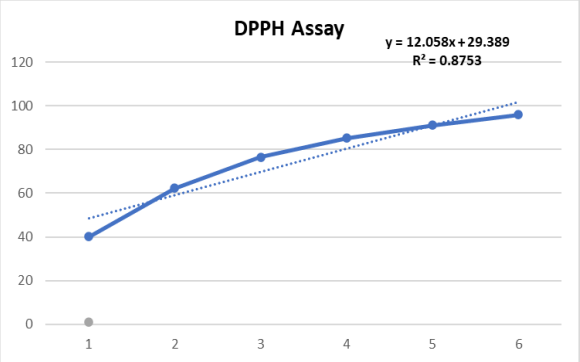
Set the timer for 30 minutes and allow the plate or cuvette to incubate there in the dark and at room temperature. During this period, the samples' antioxidant chemicals will interact with the DPPH radical.

Utilising a spectrophotometer, determine each well's absorbance following the incubation time at a certain wavelength, commonly 517 nm.

Utilise the blank control effectively to clean the spectrophotometer.

Graphical Result





Concentration µg/ml	Control	Sample	%RSA	IC50 Value
50	0.521	0.312	40.11516	1.709
100	0.51	0.192	62.35294	5.855
150	0.521	0.122	76.58349	10.002
200	0.452	0.067	85.17699	14.149
250	0.512	0.045	91.21094	18.295
300	0.511	0.021	95.88235	22.442

Alpha Amylase & Alpha Glucsidase inhibitory assay:

Introduction:

The alpha-amylase inhibitory test offers a quantitative evaluation of a compound's capacity to obstruct alpha-amylase's enzymatic activity. This test is very useful for identifying natural items or medicinal compounds that might have anti-diabetic or anti-obesity characteristics. Researchers may find novel medication candidates or functional food components with therapeutic advantages by discovering chemicals that efficiently block alpha-amylase.[1]

The test usually entails mixing the target substance with alpha-amylase and a substrate, such starch. Smaller pieces of the starch that are produced as a result of the enzyme's action can be measured using colorimetric or fluorometric techniques. The inhibitory impact can be determined by contrasting the enzymatic activity when the substance is present and when it isn't.

Principal:

Alpha-amylase is an enzyme involved in the digestion of carbohydrates, and the alpha-amylase inhibitory test measures a substance's capacity to block its activity.[2]

Chemical reagent preparation:

20 mM sodium Phosphate buffer:

Di- Sodium Hydrogen Phosphate = 3.576 Gram
Sodium Hydrogen Phosphate = 1.609 Gram
Take 200 ml of distilled water and dissolved above chemical reagent adjust PH and the volume PH (6.9)

Preparation of enzyme solution:

1mg of enzyme solution = 30 unit of enzyme

For the preparation of 1 unit/ ml of enzyme solution take 1 mg of enzyme and dissolved it in the 30 ml of buffer. And then store it in -20 Degree Celsius for the further use of the enzyme.

Preparation of starch substrate:

1% W/V 100 mg of starch dissolved in 10 ml of buffer solution and shake it gently and dissolved it in a hot water contact and shake the system over and over again.

Preparation of 5.3 M Potassium sodium tetrahydrate solution:

Dissolved 14.96 gram (PST) in 10 ml 2 M NaOH solution. And before that prepare 2 M NaOH solution by dissolving 1.6 Gram of NaOH are dissolved in 20 ml, of distilled water. [3]

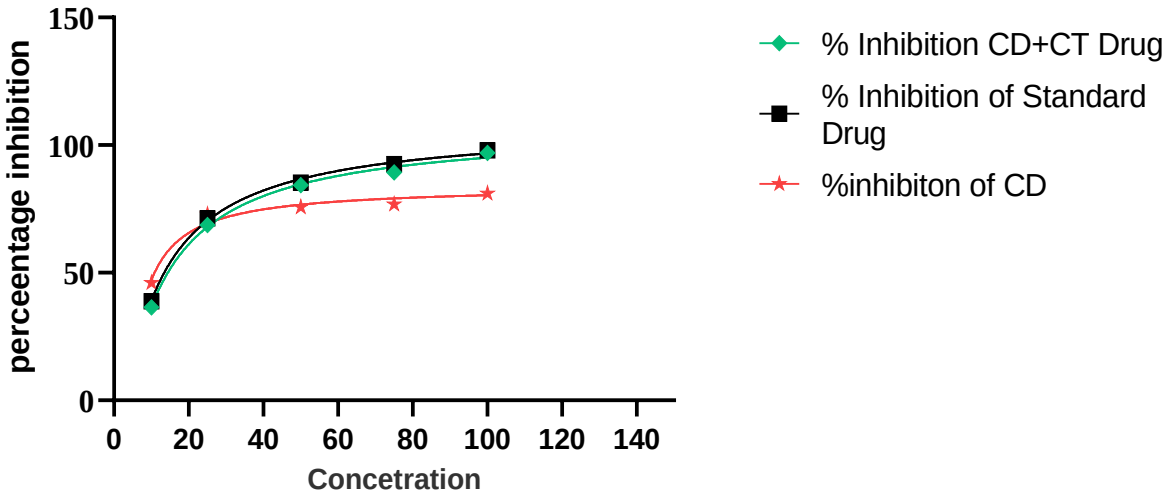
Prepare of 96 mM DNSA solution: 21.9 mg/ml 438 mg of DNSA in 40 ml of buffer solution.

Sample preparation: prepare the stock solution of test sample and in different concentration right from to lower to upper conc. 10,20,40,60,80, & 100 µg/ ml

Procedure:

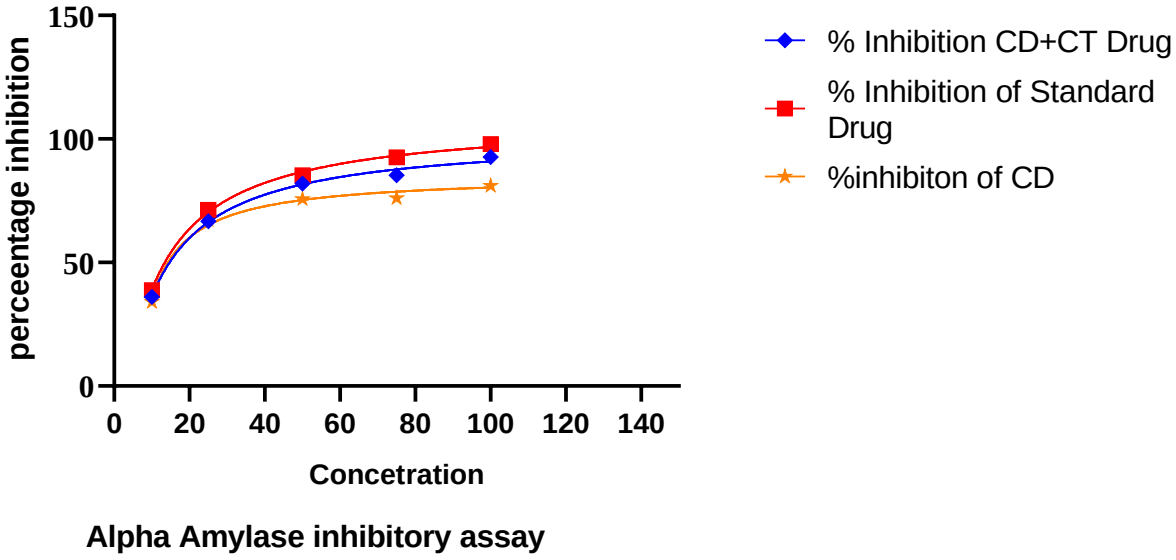
30 µl of phosphate buffer or plant extracts Acarbose was used as a positive control after 30 l of -amylase enzyme solution was combined with it and pre-incubated at 37°C for 10 minutes. Following this, 40 l of starch solution (the substrate) was added, and the reaction was started at 37 °C for 30 minutes. The stop reagent, 20 µl of DNSA solution, was then added and heated in a water bath for 5 minutes. The mixture was diluted with 80 µl of buffer and warmed to room temperature. At 540 nm, the mixture's absorbance was measured using a Spectramax ID3 spectrophotometer [4]

Graphical result:



Alpha Glucosidase inbibitory assay

IC_{50} value of CD+CT= 7.152 Std= 7.819 CD IC_{50} = 8.219



Alpha Amylase inhibitory assay

IC_{50} value of CD+CT= 7.554 & Standard= 8.186 And IC_{50} value of CD is = 9.12

Reference:

[1] 10.1016/S0271-7964(08)70107-1. (2000). *CrossRef Listing of Deleted DOIs*, *1*. https://doi.org/10.1016/s0271-7964(08)70107-1

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Chapter - 8

Total Phenol & Flavonoids content

Regent: Quercetin use as a standard component it dissolved in methanol 1mg/ml

Aluminium tri-chloride AlCl3 solution (10%) = 1 Gram of AlCl3 dissolved in 10 ml of distilled water

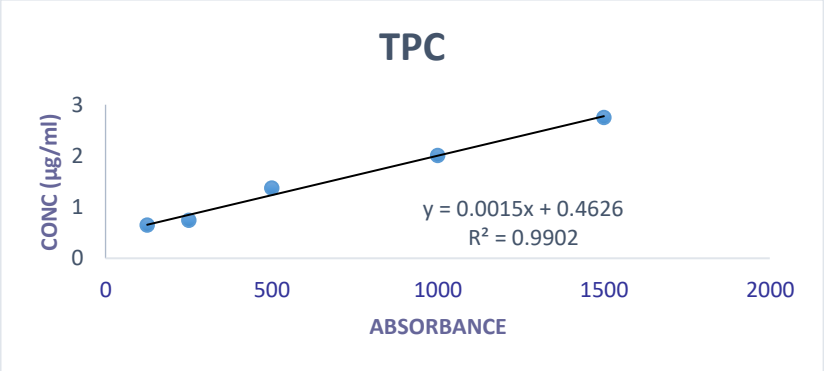
Sodium Nitrite NaNO2 (5%) = 0.5 gram of sodium Nitrite dissolved in 10ml of distilled water

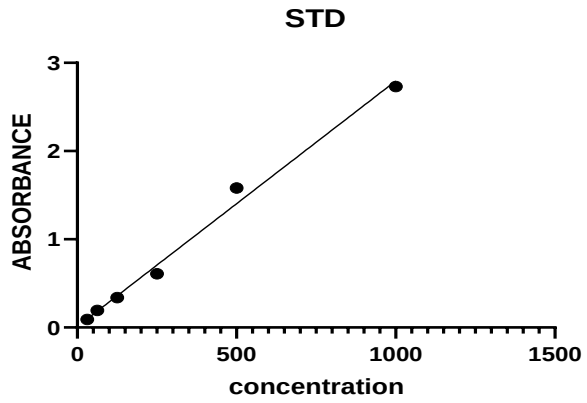
NaOH 1M solution = 10 gram of NaOH dissolved in 250 ml of distilled water

Procedure: 50 µl of sample or standard are placed in a microplate, followed by 100 µl of distilled water, then add 15 µl of NaNO2, then the microplate kept a side for 5 minutes at room temperature, then added 15 µl of AlCl3, wait for 1 minute after the addition of the solution, lastly add 100 µl of NaOH solution, and 10 minutes of darkness before the absorbance at 510 nm is measured.

Result

Book 1=TFC, R2 Value=0.9902, EQ: Y = 0.0015x + 0.4626.....Data EMF=TPC STD Curve, R^2 = 0.9914, EQ: Y = 0.002784*X + 0.01076, CD= 60.23±1.46 QE.





Total Phenolic content

Reagent:

- Folin -Coicalteau choline, FCR (10 time dilute)
- Sodium carbonate solution (Na2CO3) 7.5%
- Methanol
- Gallic acid (reagent grade) as a standard

Preparation of standard gallic acid solution preparation (standard)

1mg of gallic acid was dissolved in 1 ml of distilled water, the final concentration of the solution 1mg/ml or 1000 µg/ml. we use it as stocks solution and a serial dilution are performed in-order to prepare the solution in different concentration or the experiment.

Preparation of blank solution

Blank consist of 5 ml of Folin-Ciocalteau reagent with the addition of 1 ml of methanol then add 4 ml of sodium carbonate solution.

Experimental Procedure:

30µl of sample / Standard are added in the microplate wells then add 150µl of FCR reagent then kept it for the incubation for 5 min, after the incubation 120µl of sodium Carbonate solution were added into the wells and again kept it for 20 min at the room temperature after-all absorbance are taken at 760 nm of uv rays.

Result:

TPC: CD= 107.56±1.52 GAE

Chapter -9
In-Vivo Anti-Diabetic Study

Group of Animal for the experimental procedure:

The animal was feed with the normal diet for 5 Days in a animal house in acclimatize condition humidity 55% Temp- 22±5 *C.

For the experiment in each group 6 animal was selected which has average body weight more then = 170 Gram

Initially- 110 mg/kg *Nicotinamide* was injected then wait for 20 min after all 40 mg/kg *STZ* was injected. Then the animal was provided dextrose water for three days after three days blood glucose was measure.

No significant raise of blood glucose Average blood glucose was – 125 mg /dl. Then again animal was feed for five days with the normal diet when the animal become healthy then 35 mg/dl STZ was injected.

After three days blood glucose measure, average blood glucose of each group. Blood glucose was measure in a regular interval (5 days) interval

Fasting Blood Glucose measurement in a regular interval

Group- A Standard control (Metformin) 50 mg/kg

Group-B Diabetics control (Nicotinamide + STZ) Induced

Group -C Normal control (Vehicle control)

Group-D Test control Ceriops Decendra (Nicotinamide + STZ induced) CD extract 250 mg/kg)

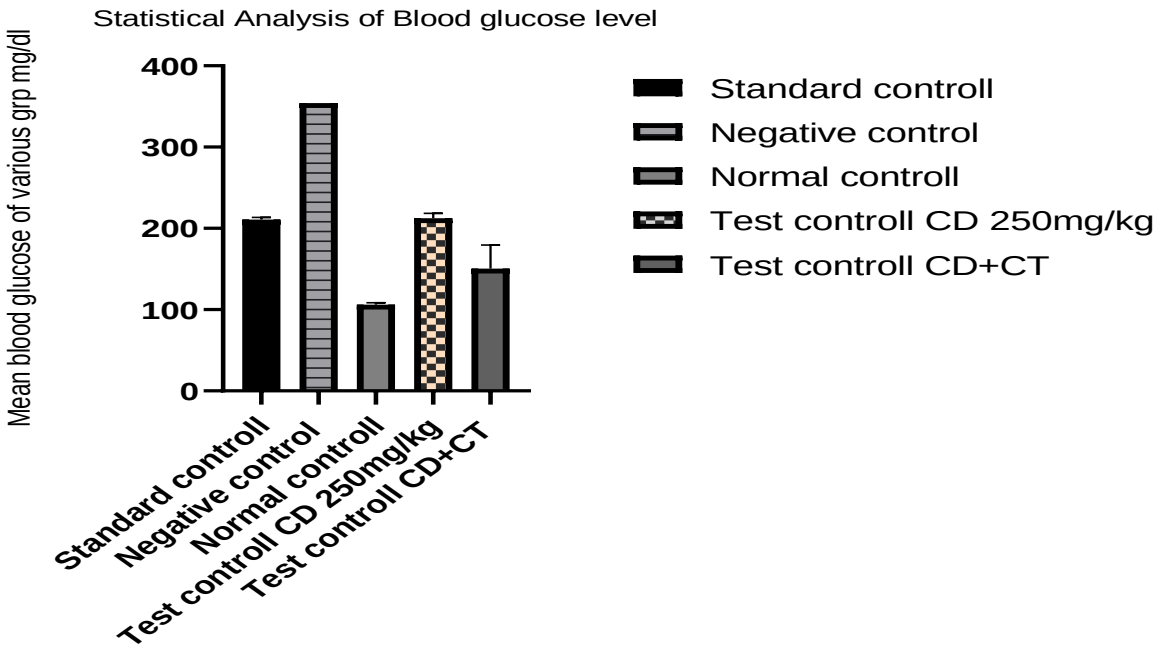
Group-E Test control CD+CT (Nicotinamide + STZ induced) CD 250 mg/kg +CT 0.9 gram /kg Enriched protein.

Fasting Blood Glucose (Group-A)	Fasting Blood Glucose (Group-B)	Fasting Blood Glucose (Group-C)	Fasting Blood Glucose (Group-D)	Fasting Blood Glucose (Group-E)
298 ±2 *mg/dl*	298±7 *mg/dl*	110±8 *mg/dl*	299±4 *mg/dl*	300±8 *mg/dl*
285±3 *mg/dl*	314±5 *mg/dl*	102 ±7 *mg/dl*	286±3 *mg/dl*	285 ±2 *mg/dl*
265 ±2 *mg/dl*	325±3 *mg/dl*	105 ±5 *mg/dl*	*268*±4 *mg/dl*	*272* ±4 *mg/dl*

191±4*mg/dl*	346±4 *mg/dl*	106±4 *mg/dl*	193 ±6 *mg/dl*	161±3 *mg/dl*
165±5 *mg/dl*	372±2 *mg/dl*	112±3 *mg/dl*	168±4 *mg/dl*	163±4 *mg/dl*
145±3 *mg/dl*	410±3 *mg/dl*	108 ±4 *mg/dl*	152 ±3 *mg/dl*	152 ±2 *mg/dl*
133±5 *mg/dl*	418±4 *mg/dl*	112±2 *mg/dl*	142 ±5 *mg/dl*	131 ±3 *mg/dl*

Statistical Analysis:

For the analysis of the mean blood glucose level of various group One-way ANOVA use for the measurement of mean difference between and within the group (Mean ± & SD). The analysis shows there is significant different between test control supplement with the extract group and standard group mean P*** value <0.008 compare to the Ceriops Decendra dose group and the p**<0.05 compare to the standard treatment group & Significant diff. among means (P < 0.05) with 95% CI. It means the average blood glucose of the combinational drug is much more effective compare to the drug extract along or the standard treatment.



Serum biochemical Parameter:

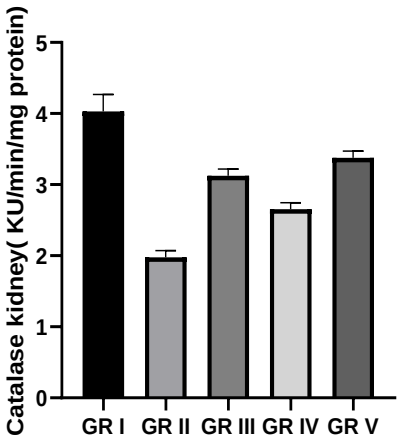
Serum biochemical parameter was analysed by the fully automated Auto-Analyser Selectra

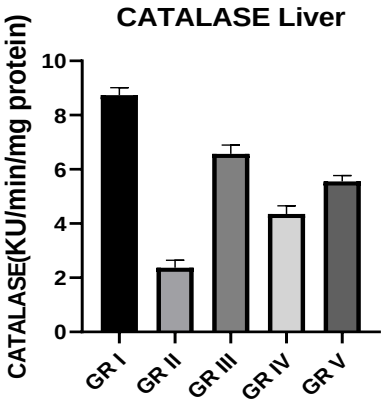
Test Name	Standard Control	Diabetics control	Normal control	Test control Ceriops Decendra	Test control CD+CT
Total cholesterol	50± 5mg/dl	68± 3mg/dl	48±2mg/dl	53±8mg/dl	42±6mg/dl
Total Protein	90±2 g/l	115±6 g/l	95±3 g/l	126± g/l	115±g/l
SGPT	38±2 U/l	72±8 U/l	35± u/l	52±6 u/l	41±u/l
SGOT	44±3 IU/l	83±3 IU/l	38±2 IU/l	63±3 IU/l	51±2 IU/l
ALT	162±2IU/l	182±3IU/l	150±5IU/l	175±3IU/l	164±3IU/l
AST	35±3IU/l	65±3IU/l	46±3IU/l	42±3IU/l	55±3IU/l
Bilirubin	4.8±0.5mumol/l	7.8±0.5mu mol/l	3.8±0.2mu mol/l	2.8±0.3mu mol/l	3.8±0.4mu mol/l
HbA_1C	7.2±0.5	9.2±0.3	6.2±0.4	7.2±0.2	6.8±0.3
HDL	35±2 mg/dl	25± mg/dl	38±3 mg/dl	34 ± 2 mg/dl	31±5mg/dl±
LDL-C	78±3 mg /dl	91±2 mg /dl	70±3 mg /dl	88±2 mg /dl	80±4mg /dl
Urea	78.70±8 mg/dl	112.50±3mg/dl	65.70±2 mg/dl	88±8 mg/dl	75.70±8 mg/dl

Tissue Anti-Oxidant Parameter :

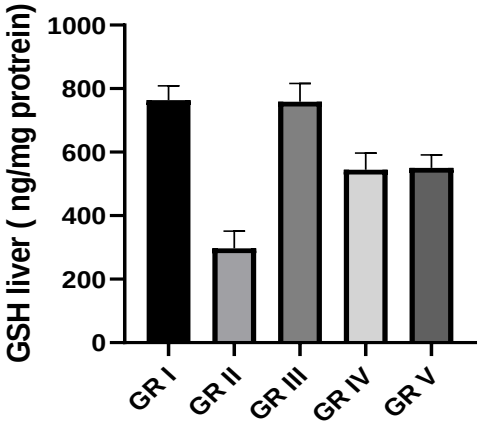
The tissue homogenate was produces to study the anti-oxidant property of various organ like liver, kidney. On the other hand the tissue anti-oxidant parameter shows their difference from each other group there are few tissue anti-oxidant parameter were measured after the cpmpletion of in-vivo study of dose treatment like-Catalase, GSH , & LPO. The statistical analysis shows that the anti-oxidant parameter of the combination supplimantory feeding group has significant and promising effect interms of GSH and Catalase activity p**< 0.0001 with the SE of Diff. 41.30 compare to the stadard drug treatment group, and Catalase on kidney has way more good efecct compare to the single extract group along p**<0.001. But the liver anti-oxidant catalse enzyme dose not show any statistical valueble effect on compare

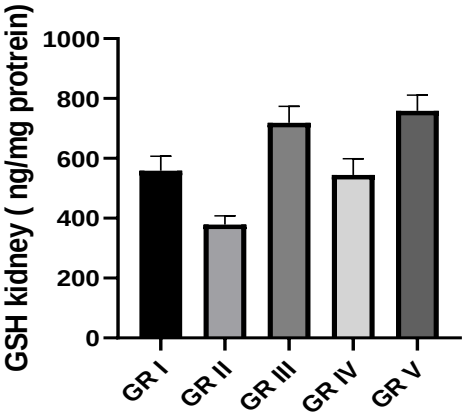
to standard treatment group. For the GSH we have used the liver and the kidney tissue for te measurement of the anti-oxidant parameter but the liver GSH anti-oxidant parameter shows quite similar type of potential activity of both group the only extract dose group and the protein supplimet gorup. On the other hand the LPO enzyme has significant fall in the group of suppliment redent animal tissue specially the liver tissue but for the kidney tisue its not so much as supplimentary group. Hence the higher the LPO cause the higher the grneration of the free-radical which causes harm to the cell membrae and increases the free radical generation in the tissue per-oxidation.



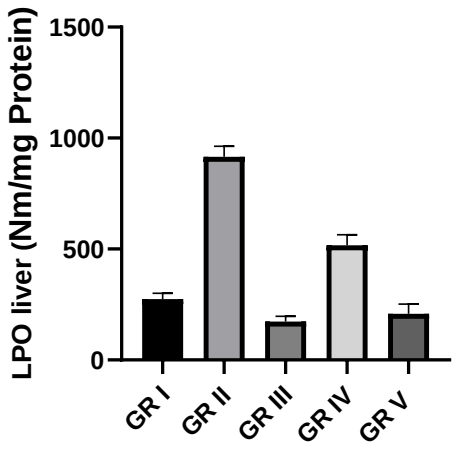


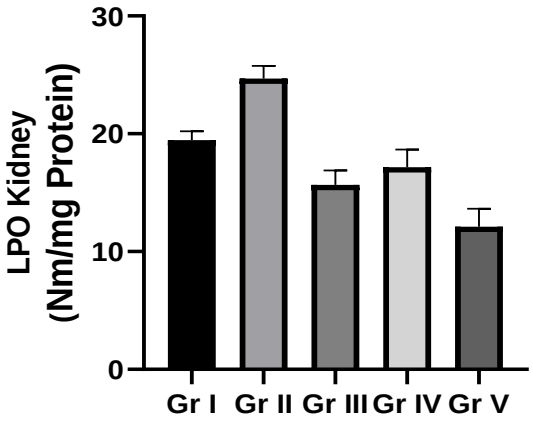
P-Value <0.001, with 95.00% CI of diff., Mean Diff. 2.052, SE of diff. 0.1109





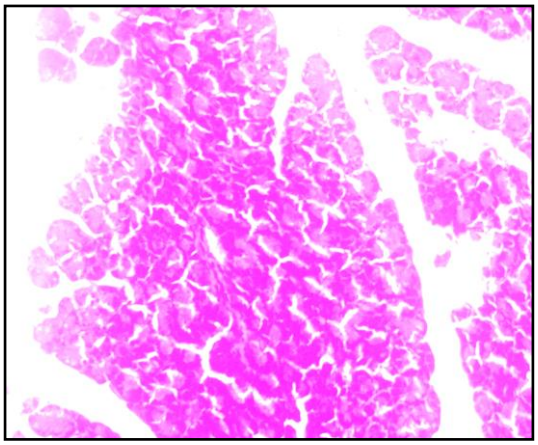
P-Value <0.0001, SE of diff. 41.30, Mean Diff. 466.6 Compare to negative controll and p** comapre to the standard and followed by the ceriops decendra treatment , CI of diff. 95.0%.



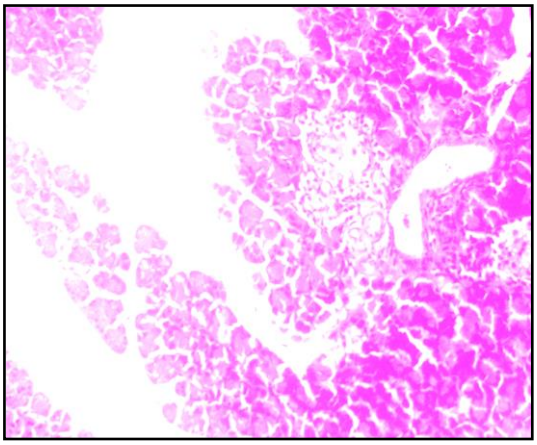


Adjusted P Value<0.0001***, 95.00% CI of diff., SE of diff. 1.018, Mean Diff. -5.221

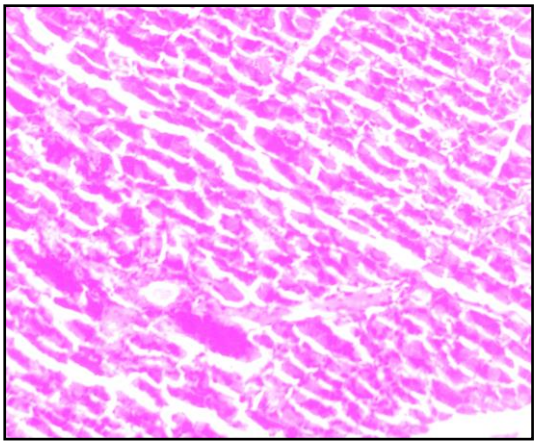
Tissue Histopathology:



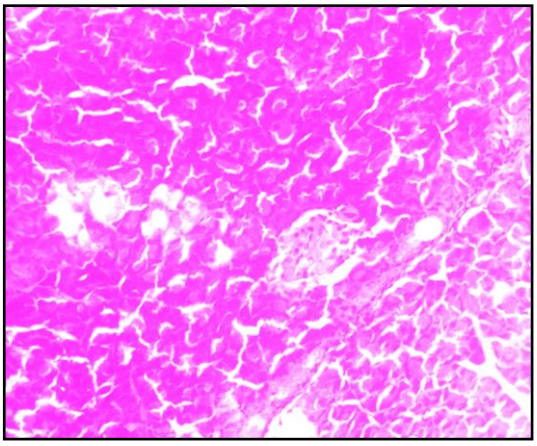
CD 250 mg/kg dose pancreatic beta cell the densensity of the beta-cell is less s comapre to standard or the supplimentary dose gorup.



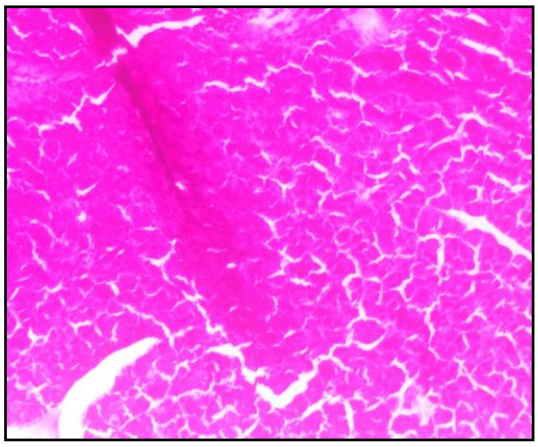
Negative controll Nicotinamde +STZ induced ; Here the number of beta-cell count decrease Gradually



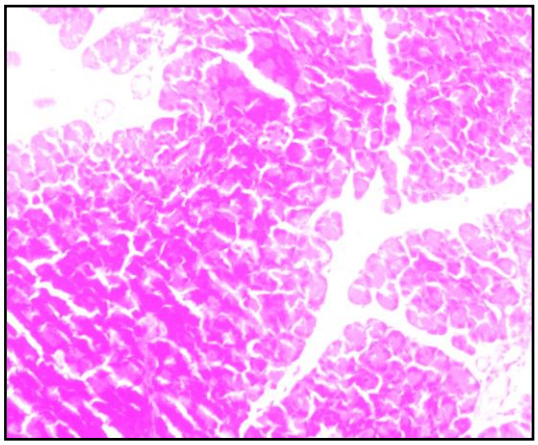
Standard control Metformine dose 50 mg/kg



Test controll Treatment CD + CT treatment the cluster beta beta cell- can be seen the test supplimentary group and cell are dence.



Normal controll Without diabeties here the density of the cell is normally distributed



Another picture of CD 250 mg/kg treatment pancreas the density of the cell is less compare to the standard and supplimentary dose group animal pancreas

Discussion and Conclusion:

On the above experiment the sole object tive was to identify the effect of protein componet over Pre-clinical study with the drug extract mangrove plant Ceriops Decendra. Manngrove plant has their several implication on thereapeutics in-terms of tradiotional use. Ceriops decendra used for the treatent of diaebtics traditionally and the protein component has their

significant role on the metabolism process[1]. Here in this study we have evaluate the effect of plant extract with the enriched protein Cyamopsis Tetragonoloba on pre-clinical stdy type-2 diabeties.

Right from the collection and identification of the sample here we have cualuate the comperative effectiveness and the enzyme-inhibitory assay. With solvent enrchment method purification of protein was carried out then it was suppose to quantify the enriched protein the purifification was about **83%** , analysis was carried out by the Micro-Kjhadal Apparatus[2]. After purification of the protein, we have assess the anti-oxidant and in-vitro alpha-amylae inhibitory asssay with combination of drug extract and the purified protein which show very promising result as compare to the drug extract along. In the in-vitro level we have analyse the combinational anti-oxidant activity DPPH assay and the comperative enzyme inhibitory assay[3]. The enzyme assay was quite surprising on the combination result its work as close to the standrard drug Acarbise. The IC_{50} value for the combination of enrich protein and the extract was 7.152 where as the standard drug IC_{50} value 7.819 and ceriops decendra is 8.219 which is quite differ from each other for the Alpha-Gluocsidase enzyme. On the other hand the enzyme inhibitory assay alpha – amylase has their own way of inhibitory potency the IC_{50} value of different drug and drug combination ar as follows IC_{50} value of CD+CT= 7.554 & Standard= 8.186 And IC_{50} value of CD is = 9.12 [4]. in In-vivo study there were five group in there after the 28 days dise treatment each of the group except the nornal controll and the negative controll group the mean blood gluse level after the 28 days which shiws significantly lower as comoare to the standard and the drug only etract treatment group. Nase on the statistical analysis the p- value is <0.001 witht the 95% confidance interval[5].

Overall the tissue anti-oxidant parameter are then use to analyse the effect of each and individual group of their effect on the anti-oxidant property. In every anti-oxidant parameter the combination supplimentory dose group shows good effect on GSH and catelase activity and and the statistical analysis ANOVA was conducted to determine the significance level of those individal group[6].

The experient has fewer limitation that the assessment of the proper protein band is quite imortant due improper estimation of the band can leads to wrong interpretation of the

molecular weight detection. During the experiment its was an little challnge to understant the protein band here after the gel electrophoresis this limitation need to assess again for to make the experiment robustness[7].As the protein plays a greater role on the the metabolic dysfunction a proper amoubt of protein specially essential and semi-essential has greater impact on the metabolic regiulation directly and indirectly Based on the above expermint here we need to make robustness of the data by the multiple time study and look the imporatce of the natural occuring plant supplent for the the treating the diabetes.

Referance:

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