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Antidiabetic, Antioxidant Activity and Development and Evaluation of Sugar Free Cookies Using Medicinal Plants Formulations – An *Invitro* Approach

By

Karthika S

21PBC006

II M.Sc. Biochemistry

Department of Biochemistry, Biotechnology and Bioinformatics

A thesis Submitted to Avinashilingam Institute for Home Science And Higher Education for Women, Coimbatore -641 043.

In partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE IN BIOCHEMISTRY

MAY 2023

Certificate

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Vasundhara

Signature of the Supervisor

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Signature of Head of the Department

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Introduction

1.0 INTRODUCTION

A chronic metabolic condition called diabetes mellitus (DM) is characterized by persistent hyperglycemia. It can be caused by decreased insulin production, insulin resistance or both. In patients with diabetes mellitus, chronic hyperglycemia can damage various organ systems in combination with other metabolic abnormalities, which can result in life-threatening and disabling health complications. The most common of these complications are microvascular (retinopathy, nephropathy, neuropathy, dermopathy, cardiomyopathy and cheiroarthropathy) and macrovascular complications (coronary artery disease, peripheral arterial disease and stroke) which increases cardiovascular disease risk by two to four times (Goyal *et al.*, 2022).

Diabetes mellitus (DM) is a severe, long-lasting and complicated metabolic condition with numerous aetiologies and dangerous acute and long-term effects. People in both affluent and developing countries are impacted by its problems, creating a significant socioeconomic problem. This disease is thought to afflict 25% of the world population. Genetic and environmental factors are the major contributors for the disease. When diabetes develops, the body cells are unable to adequately metabolize sugar because of inadequate insulin activity on target tissues as a result of insulin sensitivity or insulin deficiency. As a result, the body starts converting its own fat, protein and glycogen into sugar which leads to high levels of sugar in blood (Salehi *et al.,* 2019*).*

Diabetes is generally recognized as an epidemic that's currently continuing to spread and that affects practically every nation, population and economic system in the globe. The World Diabetes Federation estimates that 415 million people globally have diabetes in 2015 and that figure will rise to 640 million by the year 2040. As an estimated 50% of diabetic patients are unaware about their condition that they are more likely to experience diabetic complications. Apparently, the price of managing diabetes can be prohibitive in terms of resources used and lives lost. Although diabetes was blamed for around 5.0 million deaths in 2015, more than 12% of global health spending had been utilized towards treating the diabetic condition (Papatheodorou *et al.,* 2018*).*

Diabetes was exclusively divided into two categories: type 1 diabetes mellitus, which was first recognized in children and type 2 diabetes mellitus, which was recognized in adults. As a result, there are currently over 50 subcategories that are recognized, each of which is produced by a different pathogenic mechanism and may also accompany another disease or syndrome (Genuth *et al.,* 2018).

Type 1 diabetes mellitus (T1DM) is an autoimmune condition which results in the death of beta cells in the pancreas that produce insulin. Insulin is a crucial anabolic hormone with numerous impacts on growth, lipid, protein, glucose and mineral metabolism. Crucially, insulin promotes the uptake of amino acids, slows the rate of breakdown of fat in adipose tissue, permits glucose to enter muscle and adipose cells, stimulates the liver to store glucose as glycogen and produce fatty acids and promotes the uptake of potassium into cells. Type 1 Diabetes mellitus patients need ongoing insulin replacement therapy. Prolong diabetic mellitus leads to a life threatening disease called diabetic ketoacidosis which develops without insulin (Lucier *et al.,* 2023).

Type 2 Diabetes Mellitus, one of the most chronic metabolic illnesses, is caused on by a confluence of two main factors: improper insulin secretion by pancreatic beta-cells and improper insulin response in insulin-sensitive tissues. The molecular mechanisms involved in the production, release and detection of insulin, as well as their detection are closely regulated because these actions are crucial for maintaining glucose homeostasis. The development of the disease is attributed to a metabolic imbalance caused by defects in any of the mechanisms involved in these processes (Galicia- Garcia *et al.,* 2020).

Prediabetes is a complicated, multidimensional metabolic condition that affects more than just glucose regulation. According to recent research, the onset of glycemic dysregulation is often accompanied by a number of other conditions, including cancer, periodontal disease, cognitive dysfunction, blood pressure changes, obstructive sleep apnea, low testosterone levels, fatty liver disease, microvascular disease (neuropathy, nephropathy, retinopathy, dermopathy, cardiomyopathy and cheiroarthropathy) and macrovascular disease (stroke, coronary artery disease and peripheral vascular disease). The likelihood of getting type 2 diabetes increases three to ten times in people who have

prediabetes. To stop or delay the development of type 2 diabetes, it is essential to recognize and treat prediabetes (Wilson *et al.,* 2017).

Women who experience elevated blood glucose levels during pregnancy and who have not previously been diagnosed with diabetes are said to have gestational diabetes mellitus (GDM). The activation of human placental lactogen and prolactin during normal pregnancy leads to pancreatic B-cell hyperplasia, which raises insulin levels. Insulin resistance increases as a result of the maternal release of diabetogenic substances like growth hormone, corticotropin-releasing hormone, placental lactogen and progesterone. Gestational diabetes mellitus results from the inability to overcome the pregnancy-related insulin resistance despite B-cell hyperplasia. Increased risks associated with Gestational diabetes mellitus include hypertension, delivery weights more than 4000 grams and shoulder dystocia for both the mother and the newborn. Therefore, it is critical to recognize and treat Gestational diabetes mellitus (Mack *et al.,* 2017).

Almost all societies have employed medicinal plants as a source of medicine. In both industrialized and developing nations, assurance of the efficacy, safety and quality of medical plants and herbal products has recently taken center stage. For thousands of years, people have utilized medicinal plants to preserve and flavor food, treat illnesses and prevent diseases like epidemics. The biological characteristics of plant species used around the world for a variety of purposes, including the treatment of infectious diseases, are typically due to active chemicals created during secondary metabolism. Medicinal plants are widely utilized as raw materials to extract the active components that are then used to synthesize various medications (Singh, 2015).

The use of therapeutic plants for healing is as old as mankind itself. The knowledge of using medicinal plants came about as a result of man's long-standing battles with disease, which enabled to look for pharmaceuticals in the barks, seeds, fruit bodies and other parts of plants. Modern pharmacology today includes a variety of plant-based medications that have been used for millennia and were known to ancient cultures. Modern science has recognized their active effect. The ability of pharmacists and doctors to respond to difficulties that have occurred with the proliferation of professional services in the facilitation of man's life has increased as a result of their knowledge of the development of

ideas linked to the use of medicinal plants as well as the evolution of consciousness (Petrovska, 2012).

Free radicals are produced as a result of radiation exposure, environmental contaminants and drug metabolism byproducts. These free radicals are combated by substances that are naturally antioxidants. The elements that prevent oxidation are antioxidants. They are also known as "free radical scavengers" because they use radicals to create small reactive oxygen species. They can be divided into exogenous and endogenous antioxidants based on where they come from. An antioxidant lowers the risk of developing a variety of diseases, including nephrotoxicity, cataracts, cancer, diabetes, inflammation, liver disease and neurological illnesses. It is postulated that dietary antioxidants may be able to prevent diseases caused by oxidative stress (Neha *et al.*, 2019).

Over the course of human history, traditional therapies have been a highly regarded source of medicine. These are widely utilized globally, demonstrating that herbs are becoming an increasingly important component of contemporary and cutting-edge therapies. A total of 21,000 plants are registered by the World Health Organization (WHO) as being used as medicines globally. There are more than 400 plants among them that can be used to treat diabetes. Although there are many herbal medications for diabetes, only a few of these plants have been subjected to scientific and medical evaluation to determine their efficacy. The medicinal plants antidiabetic properties are caused by the presence of phenolic substances, flavonoids, terpenoids and coumarins (Kumar *et al.*, 2021).

Fenugreek (*Trigonella foenum graecum*) from leguminosae family is an annual plant. It is the well-known spices used in human cuisine. Fenugreek seeds and green leaves are utilized food and medicine, a long-standing custom in human history. It has been used to improve the flavour and colour of food ingredients and to change their texture. Fenugreek seeds contain medical qualities such as hypocholesterolemic, lactation aid, antimicrobial, stomach stimulant, anorexia, antidiabetic, galactogogue, hepatoprotective and anticancer. The intrinsic dietary fibre component of fenugreek, which has a promisive nutraceutical value, is primarily responsible for these favourable physiological effects, including the antidiabetic and hypocholesterolemic effects (Srinivasan, 2006).

Flax (*Linum usitassimum*), a member of the Lineaceae family, is an annual herb with blue flowers and small, flat seeds that range in colour from golden yellow to reddish brown. Flaxseed has a nutty flavour and a crunchy texture. Linseed is another name for flaxseed and these two names are frequently used interchangeably. Linseed designates when flax is utilized only for industrial purposes, while flaxseed are frequently referred to as flax when it is consumed by humans. Practically every component of the linseed plant is used in some way or the other. Oil found in seeds can be refined and utilized for cooking. The stem produces fibre with great strength and durability that is of good grade. Flaxseed has been consumed by people for a very long time. It has been grown for fibre as well as for nutritional and therapeutic benefits (Kajla *et al.,* 2014).

Swietenia macrophylla King (Meliaceae) can be found in tropical regions of the world. Due to the fruit's seeming pointing towards the direction of the sky, it is also known as "sky fruit." In many traditional and folkloric systems of medicine, various components of the *Swietenia macrophylla* have been used to cure a wide variety of illnesses. Particularly the seeds are said to be significant in terms of ethnomedicine for treating a variety of illness. According to reports, *Swietenia macrophylla* contains antiviral, antibacterial, antimalarial and hypoglycemic properties. Bioactive tetranorterpenoids, or limonoids of the phargmalin type, are abundant in *Swietenia macrophylla* (Balijepalli *et al.,* 2015*).*

Sweet basil (*Ocimum basilicum L*) is one of the aromatic and annual plant from lamiaceae family. The best and most useful culinary and fragrant herb is undoubtedly basil. This species is significant on a global scale as a decorative plant, a source of phytochemical preparations with demonstrated health advantages, a common folk remedy and commonly used in food as a garnish. The basil plant is a well-known source and has a wide range of bioactive substances, including phenol derivatives, terpenoids, flavonoids and phenylpropabnoids, with known biological, pharmacological and industrial uses (Corrado *et al.,* 2020).

The present study was undertaken to investigate the “Antidiabetic, Antioxidant Activity and Development of Sugar Free Cookies Using Medicinal Plant Formulations - An *Invitro* Approach” with the following objectives:

- To identify the active constituents present in the medicinal plant formulation by qualitative and quantitative analysis.
- To assess the antidiabetic, antioxidant, antiglycation and antimicrobial activities of the medicinal plant formulation.
- To develop and evaluate the cookies prepared using the medicinal plant formulation.

Review of Literature

2.0 REVIEW OF LITERATURE

2.1. Diabetes Mellitus

Diabetes mellitus is a syndrome that contains multiple aetiologies and is distinguished by a metabolic dysfunction with a degenerative potential that involves energetic sources as a result of changes in the production, secretion and inability of the insulin to exert its effects as necessary. It is a chronic ailment that necessitates ongoing self-management of the lifestyle and adaptation to the sickness by individuals living with the illness. Diabetes mellitus is frequently associated with poor medical care and is viewed as a silent illness. It is a major cause of morbidity and mortality, though these consequences are not due to its acute side effects instead related to the illness that develops as a result of chronic diabetes mellitus. These include diseases of macrovascular (coronary artery disease, stroke and peripheral artery disease) and microvascular diseases (retinopathy, nephropathy, neuropathy, dermopathy, cheiroarthropthy and cardiomyopathy) which results in life threatening health complications (Silva *et al*., 2018).

Diabetes mellitus is a collection of metabolic illnesses that are characterized by persistent hyperglycemia brought on by deficiencies in insulin secretion, insulin action or both. Insulin's role as an anabolic hormone contributes to metabolic irregularities in carbs, lipids, and proteins. These metabolic abnormalities are caused by inadequate insulin levels to produce an adequate response and/or insulin resistance of target tissues, primarily skeletal muscles, adipose tissue and to a lesser extent, liver, at the level of insulin receptors, signal transduction system and effector enzymes or genes. Diabetes type and duration both affect how severe the symptoms are. Others with high hyperglycemia and notably in children with absolute insulin insufficiency may experience polyuria, polydipsia, polyphagia, weight loss, and blurred vision. Some diabetes patients, particularly those with type 2 diabetes during the early years of the disease are asymptomatic. If untreated, uncontrolled diabetes can cause coma, stupor and in rare cases, death from nonketotic hyperosmolar syndrome or ketoacidosis (Kharroubi *et al*., 2015).

Hyperglycemia in postprandial or fasting stages is what is referred to as diabetes. In organs and tissues such the retina, kidney, nerves, heart and blood vessels, the chronic hyperglycemia of diabetes mellitus (DM) is linked to end organ damage, dysfunction, and failure (Alam *et al*., 2014). According to the International Diabetes Federation, there are approximately 415 million individuals (20-79 years old) who have diabetes mellitus globally. This projection is anticipated to increase to 642 million during the following 20 years. If nothing is done, the death rate associated with diabetes will keep rising over the ensuing decades (Simos *et al*., 2021).

2.2. Classification of Diabetes Mellitus

Diabetes Mellitus can be divided into several categories based on its aetiology: Type 1 diabetes mellitus, Type 2 diabetes mellitus, Prediabetes and Gestational Diabetes. From this, the two primary subtypes of DM are type 1 and type 2; each has a unique aetiology, presentation and therapy, although both can result in hyperglycemia.

2.2.1. Type 1 Diabetes Mellitus (IDDM)

A chronic autoimmune condition called type 1 diabetes is distinguished by a lack of insulin and the ensuing hyperglycemia. Over the past 25 years, there has been a tremendous improvement in our understanding of type 1 diabetes, leading to a wide comprehension of many facets of the disease, including its genetics, epidemiology, immunological, cell phenotypes and disease burden. Despite the fact that type 1 diabetes has known genetic roots, the majority of those who are diagnosed with it do not have a family member who also have the condition. Even while there is still no cure for type 1 diabetes, patient health and survival have significantly improved, especially in the last 25 years. Further, despite technological advancements, the majority of persons with type 1 diabetes do not have optimal glycemic control, and many cannot afford even the most basic medical care, making it difficult for them to receive new medications (DiMeglio *et al*., 2018).

Type 1 diabetes, also known as juvenile-onset diabetes mellitus and insulin-dependent diabetes mellitus, is brought on by a complete lack of the hormone insulin as a result of the death of the pancreatic beta cells that produce the hormone. It is the most common form of diabetes mellitus in children. Although it can appear at any age from infancy to adulthood,

type 1 diabetes commonly manifests in childhood. Environmental factors that cause an autoimmune response against pancreatic beta cells in a genetically predisposed person are thought to be the cause of type 1 diabetes. The major histocompatibility complex on chromosome 6 contains the protective DR2DQ6 allele as well as the risk-increasing DR3-DQ2 and DR4-DQ8 alleles, which together make up the biggest genetic component of diabetes risk. Relatives of people with diabetes are more likely to develop the condition due to the genetic component of the disease's risk. Despite this familial risk, only 10% to 20% of those with type 1 diabetes have a family relative who also has the disease. There have been several chemicals, nutritional elements, and viral infections suggested as potential environmental variables influencing the aetiology of type 1 diabetes mellitus (Cooke *et al*., 2008).

The significant morbidity and mortality in Type 1 diabetes mellitus are caused by diabetes-related complications, both microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular (cardiovascular disease, peripheral artery disease). Although the length and intensity of hyperglycemia cause diabetes related problems. The molecular mechanisms by which too much glucose causes a particular organ to malfunction are still poorly understood and probably unique to the organ system in question. Diabetes related complications are likely multifactorial and involve cellular pathways like advanced glycation end products, sphingolipid metabolism (neuropathy), cytokines, multiple growth factors (VEGF in retinopathy) and oxidative stress. They may also be caused by genetic susceptibility to glucose exposure, epigenetic changes brought on by hyperglycemia, associated dyslipidemia and associated dyslipidemia (powers, 2021).

2.2.2. Type 2 Diabetes Mellitus

A chronic metabolic condition with a rapidly rising prevalence worldwide is type 2 diabetes mellitus (DM). Due to this pattern, it is quickly turning into an epidemic in some parts of the world, with the number of affected individuals expected to double in the next ten years due to an ageing population, adding to the burden already placed on healthcare providers, particularly in developing nations. People with type 2 diabetes mellitus are more prone to a variety of short and long term problems, which frequently result in early death. Patients with type 2 diabetes likely to have higher morbidity and mortality rates due to the

condition's prevalence, undetectable onset, and delayed diagnosis, especially in resource poor developing nations. Most people with type 2 diabetes are obese, with central visceral adiposity. As a result, the pathophysiology of type 2 diabetes mellitus depends greatly on adipose tissue. Most people with type 2 diabetes are obese, with central visceral adiposity. As a result, the pathophysiology of type 2 diabetes mellitus depends greatly on adipose tissue (Olokoba *et al.*, 2012).

Type 2 diabetes mellitus (T2DM), which can be caused by either decreased insulin production, insulin resistance or both, is characterised by dysregulation of carbohydrate, lipid and protein metabolism. Type 2 diabetes mellitus is the most prevalent of the three primary forms of diabetes, accounting for more than 90% of all cases, more frequent than type 1 diabetes mellitus (T1DM) or gestational diabetes. Our knowledge of the onset and evolution of T2DM has quickly advanced during the last few decades. The primary contributing factor is the pancreatic cell which results in increasing the impairment of insulin secretion, which typically occurs against a background of pre-existing insulin resistance in the skeletal muscle, liver and adipose tissue. T2DM has grown to be a significant global public health issue. According to the International Diabetes Federation, 382 million persons between the age of 20 and 70 had T2DM in 2013, with 80% of those affected residing in low and middle-income nations. By 2035, this number is anticipated to reach 592 million (DeFronzo *et al.*, 2015).

2.2.3. Gestational Diabetes

GDM-affected pregnancies carry a risk for both the mother and the unborn child due to the increased risk of macrosomia, shoulder dystocia, neonatal hypoglycemia and hyperbilirubinemia. Women with a history of GDM are more likely to develop type 2 diabetes mellitus in the years after giving birth, and their offspring are more likely to grow up obese and be diagnosed with type 2 diabetes mellitus. Approximately 5% of pregnancies result in gestational diabetes mellitus, though statistics can vary greatly depending on the criteria utilized and the demographics of the population. As the obesity pandemic persists, the prevalence is anticipated to rise. The two primary sites for whole-body glucose disposal are skeletal muscle and adipose tissue. In a healthy pregnancy, the body's ability to eliminate glucose through the action of insulin drops by 50%, necessitating a 200%–250%

increase in insulin secretion from the mother in order to maintain euglycemia. When a pregnant woman is unable to produce enough insulin to counteract this natural insulin resistance, gestational diabetes mellitus (GDM) develops (Kampmann *et al.,* 2015).

Additionally, type 2 diabetes mellitus (T2DM) and cardiovascular disease are more likely to strike women with a history of gestational diabetes mellitus. Babies born to gestational diabetes mellitus women are likely to be macrosomic, have more congenital disorders and are likely to later have neonatal hypoglycemia and type 2 diabetes mellitus. As a result, it is crucial for healthcare policy makers to comprehend the impact of gestational diabetes mellitus in order to facilitate early detection and subsequent intervention. The oral glucose tolerance test (OGTT) is precise and accurate enzymatic approach that measures the glucose levels in venous plasma to diagnose gestational diabetes mellitus. Malformations of the circulatory, genitourinary, musculoskeletal and central neurological systems were referred to as congenital anomalies in neonates (Lee *et al*., 2018).

Due to epidemiological factors such as the rising maternal age and background rates of obesity in women of reproductive age, as well as the adoption of the updated International Association of the Diabetes and Pregnancy Study Groups criteria and diagnostic procedures for gestational diabetes mellitus, the prevalence of gestational diabetes mellitus continues to rise globally. Given that gestational diabetes mellitus is currently one of the most prevalent pregnancy problems. The lack of international consensus for the diagnosis of gestational diabetes mellitus reflects their condition, complicated historical history and practical antenatal resource constraints. However, the longer-term prognosis of gestational diabetes mellitus should also be considered while developing a modern clinical strategy for treating it. Recent research shows the long-term negative effects of maternal hyperglycemia on a child's and adolescent metabolism as well as the effect of early in utero exposure to maternal hyperglycemia, with evidence for fetal overgrowth present before the usual diagnosis of gestational diabetes mellitus from 24 weeks' gestation. Given that gestational diabetes mellitus accounts for a large portion of the global epidemic of transgenerational cardiometabolic disease, it is critical to recognize gestational diabetes mellitus as an early risk factor for type 2 diabetes and cardiovascular

disease and to broaden the current clinical Approach to address longer-term complications for both the mother and the child after a diagnosis of gestational diabetes mellitus (Sweeting *et al*., 2022).

2.2.4. Prediabetes

Blood glucose levels that are over the upper threshold levels are regarded as normal but below the threshold for a diagnosis of diabetes are referred to as prediabetes, which is a chronic metabolic disorder. Importantly, when evaluating and summarizing prevalence and incidence data, caution must be given because the diagnostic criteria and terminology associated with prediabetes differ significantly between organizations. The World Health Organization (WHO) and American Diabetes Association (ADA) both offer recommendations on prediabetes screening based on evaluation of impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) levels. About 25% of subjects with prediabetes develop overt type 2 diabetes within 3-5 years and up to 70% of people with prediabetes develop overt diabetes over the course of their lifespan (Hostelek *et al*., 2019).

Women with prediabetes have a higher risk of having gestational diabetes mellitus in future. Through weight loss, a nutritious diet and regular exercise, lifestyle measures are strongly helpful for controlling prediabetes and postponing the onset of type 2 diabetes. Lifestyle modifications may be helpful for quitting smoking, getting better sleep and reducing stress. For persons with prediabetes, lifestyle adjustments are therefore advised (Bell *et al*., 2020).

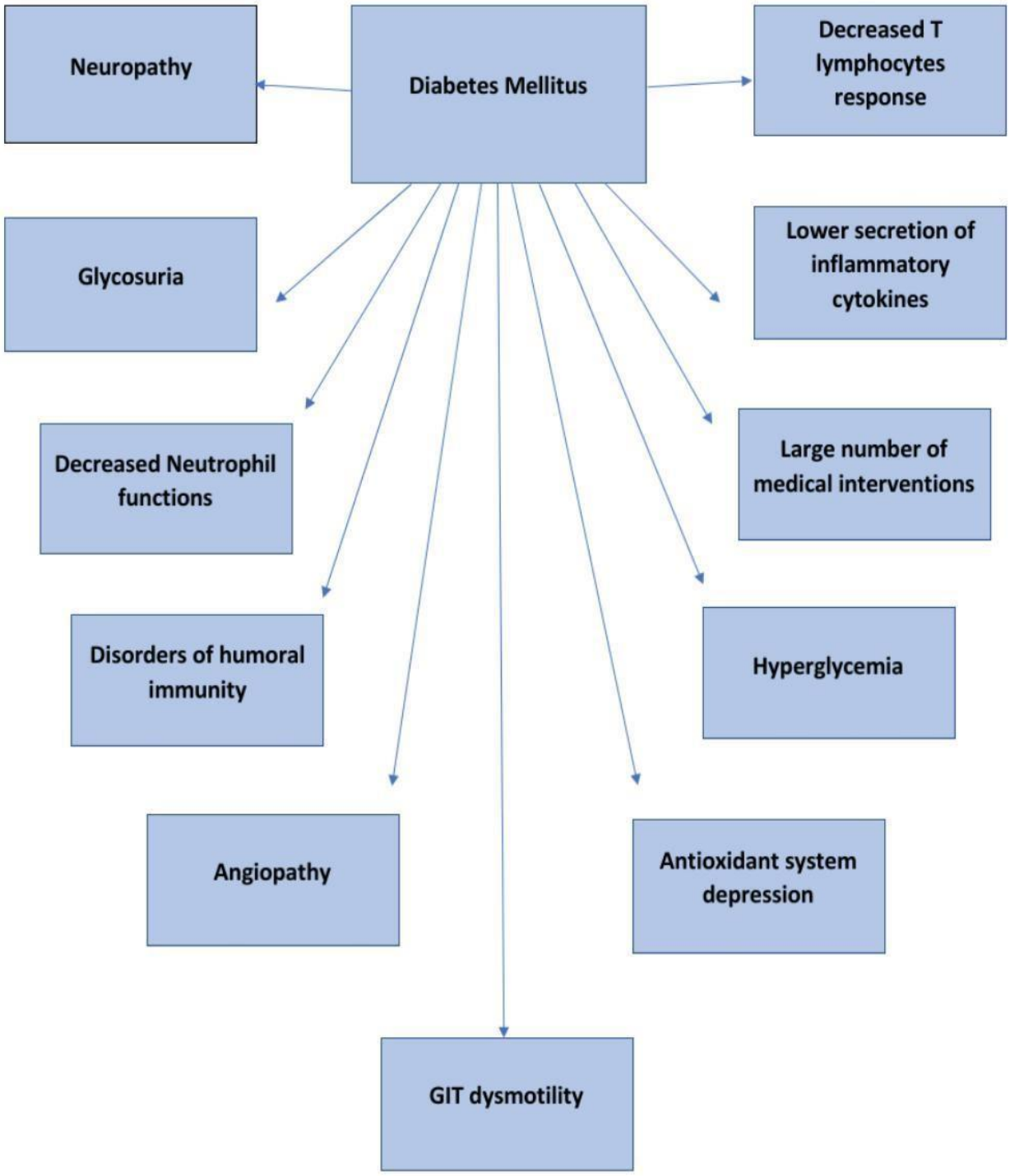


Figure 1: Pathophysiology of infections associated with Diabetes mellitus

2.3. Signs and Symptoms of Diabetes Mellitus

Due to the chronic nature of the condition, many people ignore the signs and symptoms of diabetes. Because the effects of hyperglycemia take time to appear, unlike many other diseases, many do not view this as a severe issue. People are unaware that damage can begin years before symptoms are visibly present. This is problematic because identifying early signs might aid in preventing vascular problems and quickly bringing the condition under control. The typical signs of diabetes, such as polyuria, polydypsia and polyphagia, are frequently present in both type 2 and type 1 diabetes, which have extremely high levels of hyperglycemia and type 1 diabetes that develops rapidly with severe hyperglycemia. Only type 1 diabetes or long-term type 2 diabetes are associated with severe weight loss, undiagnosed diabetes is also frequently characterised by unexplained weight loss, exhaustion, restlessness and physical pain. It is also possible for minor or slowly developing symptoms to go disregarded (Ramachandran, 2014).

2.4. Complications of Diabetes Mellitus

Long-term harm and systemic failure of numerous organs are linked to diabetes and its consequences. Diabetes alters the microvasculature, resulting in capillary basement membrane thickening and extracellular matrix protein synthesis, which are the pathognomic characteristics of diabetic microangiopathy. Macrovascular problems can result from these changes in combination with advanced glycation end products, oxidative stress, low grade inflammation and neovascularization of the vasa vasorum. Although it also appears to be a significant factor in the pathogenesis of macrovasculopathy, hyperglycemia is the main factor in microvasculopathy. Although micro and macro vascular issues are assumed to intersect, the two diseases appear to be closely related, with micro vascular diseases promoting atherosclerosis through mechanisms like hypoxia and alterations in the vasa vasorum (Chawla *et al*., 2016).

Devastating microvascular and macrovascular effects of diabetes increases mortality, cause blindness, kidney failure and generally lower quality of life in people with diabetes. Numerous genetic studies have shown that both diabetes and its consequences clearly have

a hereditary component, contradicting the notion that clinical risk factors and glycemic control alone may predict the development of vascular problems (Cole and Florez, 2020).

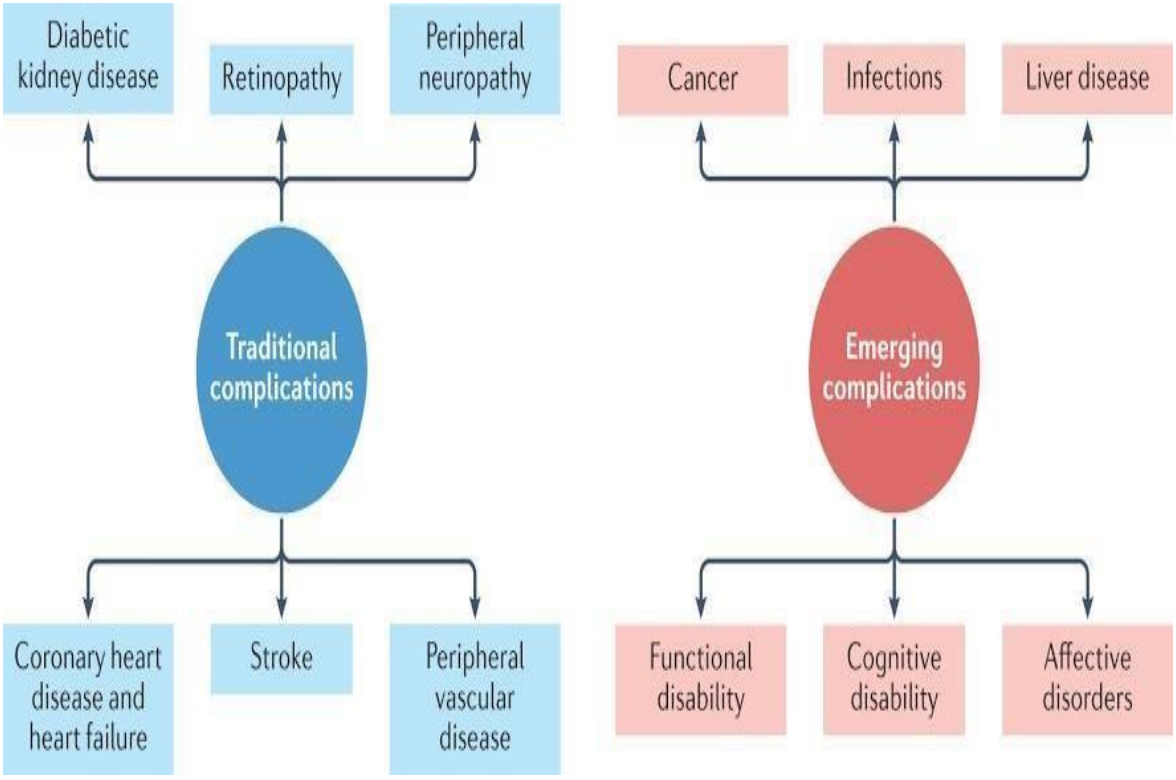


Figure 2: Major traditional complications and emerging complications of diabetes mellitus (Tomic *et al*., 2022)

2.5. Free Radicals

Free radicals are organic and inorganic compounds with a high reactivity and short half-life that are produced during regular physiological metabolism in living systems. Free radicals come in four different varieties: those with an oxygen, carbon, nitrogen or sulphur centre. As oxygen is used in both photosynthesis and aerobic respiration, oxygen radicals are the most prevalent free radicals in biological systems. During typical physiological processes, mitochondria creates 90% or more of the intracellular ROS. In the lysosomes, peroxisomes, cytosol, endoplasmic reticulum and nucleus, oxidative metabolism results in the production of ROS and RNS (Zaric *et al*., 2023).

The key role that free radicals and other oxidants play in many physiological situations as well as their involvement in a wide range of disorders have given them increased significance in the study of biology. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are two types of free radicals that can come from both endogenous (such as mitochondria, peroxisomes, endoplasmic reticulum, phagocytic cells, etc.) and exogenous (such as pollution, alcohol, tobacco smoke, heavy metals, transition metals, industrial solvents, pesticides, certain medications like halothane and paracetamol) sources. Free radicals have the potential to negatively impact a number of significant types of biological components, including proteins, lipids and nucleic acids, changing their normal redox state and increasing oxidative stress. The oxidative stress caused by free radicals has been implicated in the development of cataracts, rheumatoid arthritis, diabetes mellitus, neurodegenerative disorders (Parkinson's disease, Alzheimer's disease, and Multiple sclerosis), cardiovascular diseases (atherosclerosis and hypertension), respiratory diseases (asthma) and various cancers (colorectal, prostate, breast, lung and bladder cancers) (Phaniendra *et al*., 2015).

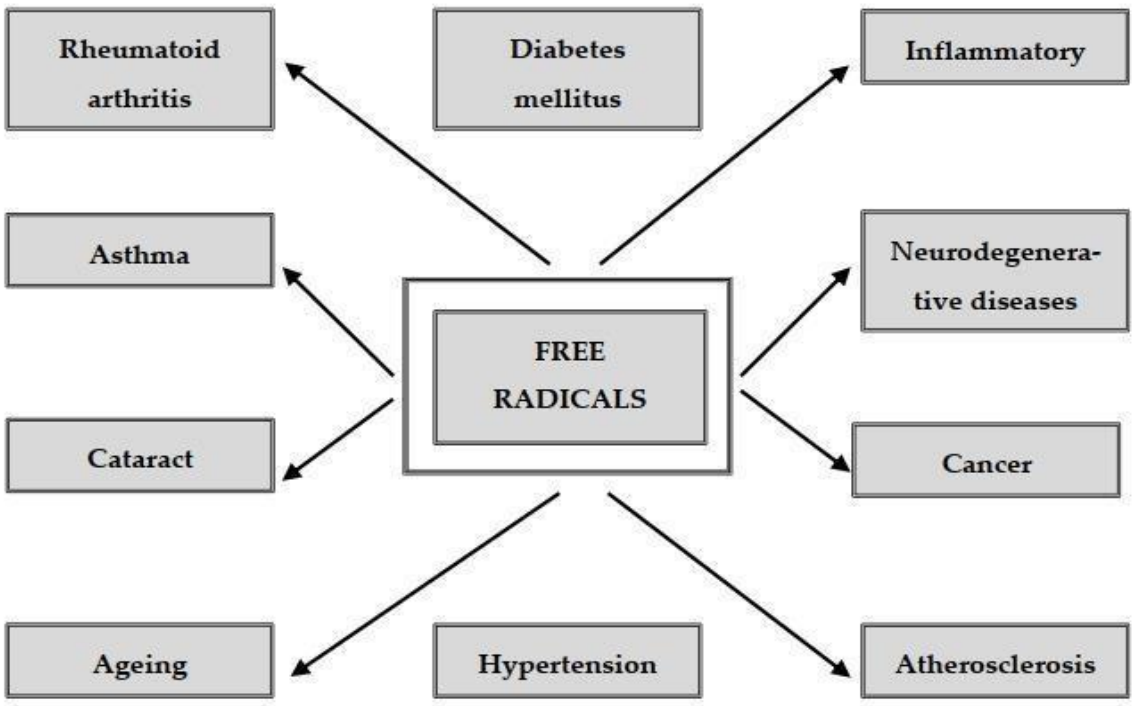


Figure 3: Free radicals and diseases (Martemucci *et al*., 2022)

2.5.1. Sources of Free Radicals

Exogenous or endogenous sources can produce free radicals. Before being released into the environment, they are created endogenously, intracellularly and act within the cell. Free radicals are produced by several external sources, but ionising radiation is one of the most significant. By transmitting energy to it, ionising particles ionise the water molecule. Free radicals are created when a molecule of ionised water dissociates. Endogenous sources include biological molecules such as thiols, flavins, catecholamines, ferredoxins, haemoglobin and metal ions such as Cu, Fe, Cd, As, Hg, Cr, Al and Ni that undergo auto-oxidation processes. Other examples of endogenous sources include enzymes and transport molecules like xanthine oxidase and aldehyde oxidase. Examples of external sources (both active and passive) include car exhaust, medications (especially anticancer drugs), ionising radiation, chemical pollutants such as pesticides, viruses, bacteria, parasites and smoking. Stressful conditions and excessive alcohol use can both produce free radicals (Sharma *et al*., 2018).

2.5.2. Formation of Free Radicals

Free radicals are generally formed by

1. By hemolytic cleavage of a covalent bond of an ordinary biomolecule
2. Through the removal of an electron from a regular biomolecule
3. Through the connection of electrons with normal biomolecules.

Excessive UV exposure, chronic stress, strenuous exercise, poor diet, and stimulant usage all lead to an increase in the creation of free radicals. Free radical production and elimination from the body are in equilibrium under physiological circumstances (Jakubczyk *et al*., 2020).

2.6. Oxidative Stress and Diabetes Mellitus

Free radicals (ROS/RNS), which are produced as a result of the constant exposure of the human body to various stimuli, promote the oxidation of cellular components by the transfer of their free unpaired electron. The body contains endogenous antioxidant systems or it gets exogenous antioxidants from the diet, which neutralize such species and maintain the body's homeostasis in order to deal with the harmful effects of such species. Any imbalance between RS and antioxidants creates "oxidative stress," a state that leads to the emergence of pathological conditions, one of which is diabetes. The majority of research suggest that oxidative stress plays a role in the aetiology of diabetes through changes in enzymatic systems, lipid peroxidation, poor glutathione metabolism and decreased levels of vitamin C. Different biomarkers of oxidative stress in diabetes mellitus include lipids, proteins, DNA damage, glutathione, catalane and superoxide dismutase. Diabetes problems caused on by oxidative stress can include stroke, neuropathy, retinopathy and nephropathy (Asmat *et al*., 2016).

Cellular damage caused by hyperglycemia is significantly influenced by oxidative stress. Free radical generation can be stimulated by high glucose levels. A situation of imbalance between ROS and their protection occurs as a result of the body's weak defence system's inability to counteract the increased ROS creation, which results in the dominance of the condition of oxidative stress. Since oxidative stress and ROS have a variety of regulatory functions in cells, they are required in small amounts for normal metabolic activities. In order to get rid of antigens, neutrophils and macrophages create ROS during the respiratory burst process. Additionally, they act as signals that promote the activity of a number of genes that code for transcription factors, differentiation and development in addition to promoting cell-cell adhesion, cell signalling, participation in vasoregulation and fibroblast proliferation and increased expression of antioxidant enzymes. Oxidative stress has a role in the development of insulin resistance, glucose intolerance and the onset of diabetes mellitus. These conditions support the emergence of atherosclerotic problems and the rise in both microvascular and macrovascular consequences (Tiwari *et al*., 2013).

Chronic hyperglycemia in diabetes mellitus (DM) causes a variety of impairments to develop, especially in tissues where insulin-independent glucose uptake predominates. Increased intracellular glucose levels also cause an excess production of reactive oxygen species (ROS), primarily through the mitochondrial electron transport chain. Additionally, the development of oxidative stress in DM has been facilitated by the decreased activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). Although it is well known that ROS harm biomolecules, they are also the starting point for a number of intracellular pathways that are triggered by hyperglycemia, including the polyol pathway, the production of advanced glycation end products (AGEs), the activation of protein kinase C (PKC) isoforms and an increase in flux through the hexosamine pathway; The development of DM's longterm problems is greatly influenced by all of these processes (Arcaro *et al.*, 2014).

2.7. Role of Antioxidants

Free radicals and reactive oxygen species (ROS) are squelched by several chemical molecules known as antioxidants. One of the most intriguing characteristics in the scientific community nowadays is the antioxidant activity in food and drink. Antioxidants shield the body from free radical damage and plays a significant role in the development of numerous chronic diseases, including cardiovascular disorders, ageing, heart disease, anaemia, cancer and inflammation. Natural antioxidants are compounds found in food that stop the process like disruption, sourness and colour change. Natural antioxidants are often produced from plant sources and the activity of these compounds depends on the species, diversity, extraction and processing techniques and environmental factors that affect the plant growth. (Zehiroglu *et al*., 2019).

The notion that oxidative/nitrosative stress plays a role in the aetiology of many human diseases has attracted a remarkable amount of attention. Exogenous or endogenous substances known as antioxidants can lessen the effects of oxidative/nitrosative stress in any way. They may work by directly removing free radicals or by boosting antioxidant defences. Decreased antioxidant intake, increased antioxidant utilization or the production of endogenous enzymes can all lead to antioxidant deficits. To maintain optimal

physiological function, antioxidant supplementation is a strategy that is growing in acceptance. However, under some circumstances, antioxidants can also have pro-oxidant action. Their dosage and the redox environment inside the cell are particularly significant. According to recent research, antioxidants may be useful in the prevention and treatment of diseases when administered to the correct subject at the right time for the right duration of time (Kurutas, 2016).

Antioxidants are chemicals that considerably reduce the role of oxidation of the targets even when present in very small doses. Numerous protective antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx), glutathione reductase (GSHRx), glutathione-S-transferase (GST) and nonenzymatic antioxidants, have been involved in dealing with toxic species because constitutive metabolic pathways continuously produce partially reduced forms of oxygen. An oxidizing agent gains electrons from the substance being oxidized in a chemical reaction known as oxidation. Free radicals are a byproduct of oxidation reactions and can trigger a cascade of events that harms cells. Antioxidants break these chains of events by removing the free radical intermediaries and they also stop other oxidation processes by becoming oxidized. Thiols, ascorbic acid and polyphenols, which are reducing agents, are frequent examples of antioxidants (Rasheed *et al*., 2019).

2.8. Medicinal plants

It is believed that medicinal plants are abundant in phytochemical elements, which are crucial in the creation of new pharmaceuticals. There are numerous recommended medical procedures that use medicinal herbs. The majority of plants have carotenoids, flavonoids, terpenoids, alkaloids and glycosides, which frequently have anti-diabetic properties. The anti-hyperglycemic benefits of plant therapy are frequently brought on by their capacity to enhance the function of pancreatic tissue, which is done by raising insulin secretion or lowering intestinal glucose absorption. The prevalence of diabetes has been rising, causing concerns among the public and the medical profession. Numerous medicinal plants are included in conventional diabetic treatments. Many currently used medicines are derived from these plants, which have no negative side effects (Kooti *et al*., 2016).

2.9. Medicinal plants selected for the study

2.9.1. Sweet basil

Ocimum basilicum L, an aromatic plant, is used in food for honeybees, as a decorative plant and as a raw material in a variety of industries. Among the economically significant species of basil, sweet basil is the one that is most extensively cultivated because of its great economic value, popularity and demand. Sweet basil is commonly grown in India right now and is found all across subtropical and tropical areas. Additionally, it is widely employed in the food, aromatherapy, cosmetics and pharmaceutical industries. The production of honey and its usage as a decorative plant are two more uses for sweet basil. Malaria, colic vomiting, the common cold, cough, headaches, diarrhoea, inflammation, pain, skin conditions and other conditions are treated medically using this herb (Egata *et al*., 2021).

2.9.2. Sky Fruit

Mahogany, often referred to as *Swietenia macrophylla* King, is a member of the Meliaceae family and is found throughout much of the Amazon, as well as Western India, Southern China, Malaysia, Mexico, Central and Tropical South America and Mexico. The evaluation of the conservation status of plants places *Swietenia macrophylla* in the critically endangered conservation category. The leaves and Seeds are known to be used in various diseases such as hypertension, diabetes mellitus, leishmaniasis and showing different biological properties including antimutagenicity, antitumor, antioxidant and anti-inflammatory (Coello *et al*., 2020).

2.9.3. Flax Seed

The lignan secoisolariciresinol diglucoside, alpha linolenic acid, omega-3 fatty acids and fibre are all abundant in flaxseed. Through their anti-inflammatory action, anti-oxidative capability and lipid regulating capabilities, these substances offer bioactivity that is beneficial to the health of both humans and animals. The research literature supporting the use of dietary flaxseed in a range of health issues is impressive and constantly expanding. Alpha-linolenic acid (ALA), lignans and fibre are the primary bioactive components of flaxseed. Diabetes, a serious condition with an increasing prevalence

worldwide, is impacted by flaxseed as well. Supplementing with flaxseed decreased type 2 diabetic individuals' blood sugar levels and prediabetic subjects' blood sugar levels (Parikh *et al*., 2019).

2.9.4. Fenugreek

One of the oldest herbs used for medicine, fenugreek has a remarkable therapeutic and nutritional profile. The seeds of fenugreek are a rich source of phospholipids, glycolipids, oleic acid, linolenic acid, linoleic acid, choline, vitamins A, B1, B2, C, nicotinic acid, niacin and numerous other useful substances. It can thrive in a broad variety of environments and can even be cultivated profitably on marginal grounds because it is relatively resistant of salinity and drought. Fenugreek fibre has the potential to control human glucose metabolism medically. Mucilage, tannins, pectin and hemicellulose also prevent bile salt absorption in the colon, which helps lower blood levels of low density lipoprotein cholesterol (LDL). It traps food toxins and, inadvertently, guards intestinal epithelial barrier against the development of cancer. Additionally, it aids in sugar level regulation and reduced blood glucose absorption, which makes insulin action easier (Ahmad *et al*., 2015).

Materials and Methods

3.0 Experimental Procedure

Diabetes, a long-term physiological disorder that affects people of all ages. It significantly disrupts people regular lives globally and is one of the major contributor. Despite the availability of insulin preparations and a number of synthetic oral antidiabetic medications, there is a critical need for the discovery and development of innovative antidiabetic medications due to the emergence of drug resistance and the negative side effects of allopathic medications when used for an extended period of time. In contrast, scientists, researchers and pharmaceutical companies are increasingly turning to plants or herbal sources to find potential bioactive compounds for the discovery and development of targeted novel antidiabetic drugs that might control diabetes with the fewest side effects compared to conventional antidiabetic drugs (Alam *et al*., 2022).

The primary cause of type 1 diabetes is the autoimmune-mediated death of pancreatic beta cells, which results in insulin insufficiency. This is typically followed by changes in lipid metabolism, increased oxidative stress brought on by hyperglycemia, endothelial cell failure and apoptosis (Ndisang *et al.*, 2017). Type ll diabetes mellitus is a complicated metabolic condition caused by interactions between several environmental and genetic susceptibilities. Although hereditary factors undoubtedly contribute to the pathophysiology of T2DM, environmental and epigenetic variables appear to be the main causes of the recent increase in T2DM prevalence (Javeed *et al.*, 2018).

Plants are known to naturally have curative powers for a variety of diseases from centuries and is found to have contributed significantly to the development of modern medicine. Understanding the various usage of numerous medicinal plants in various cultures around the world and the potencies of these plants to cure ailments have made positive result to generations worth of information (Salleh *et al*., 2021). Due to their lower toxicity and mild side effects, secondary metabolites in natural products are the main source of lead compounds for the optimization of pharmacological activities. Plants produce phytochemicals, also known as secondary metabolites, through a number of different chemical routes. A wide range of biological actions, including antioxidant, antibacterial, anti-inflammatory, anticancer, antidiabetic and other activities are exhibited by the metabolites, such as flavonoids, alkaloids and polyphenols (Sapkota *et al.*, 2022).

3.1. Collection of plant sample

Ocimum basilicum, Linum usitatissimum, Trigonella foenum graecum and *Swietenia macrophylla* are used in combination for the study. The plant samples were collected from the local herbal shop in kanuvai area, Coimbatore.

(a) Sky Fruit

(b) Fenugreek

(c) Flax Seed

(d) Sweet Basil

Figure 4: Medicinal plants used for the study

3.2. Preparation of aqueous and ethanolic extract of medicinal plants

The plants were collected from the local areas in coimbatore, the leaves were washed thoroughly with water. The washed leaves were dried at room temperature for 10 days. The dried samples were powdered and stored in air tight container for further use. A known, weight of the medicinal plant samples such as 8g of sweet basil, 4g of fenugreek, 2.7g of flax seed and 0.3g of sky fruit were taken and mixed together based on the previous studies. To 15g of the total powdered sample 150ml of ethanol and water were added separately. Then the mixtures were kept in the mild shaker for about seven days. It was then filtered by using Whatmann no.1 filter paper and the crude extracts were obtained using rotary evaporator, the extracts obtained was further dried to completely remove the solvents and were used for further studies.

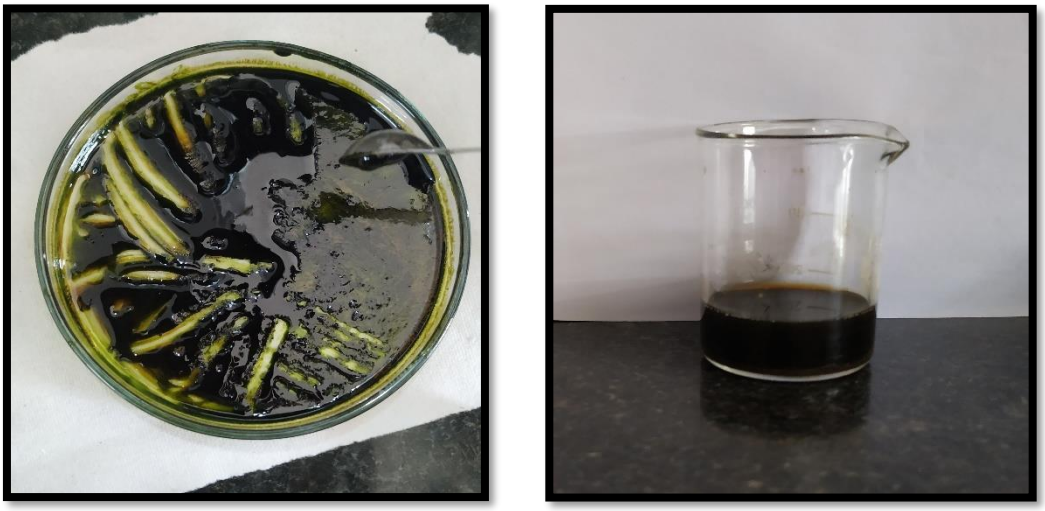


Figure 5: Aqueous and ethanolic extract of medicinal plant formulation

3.3. Phytochemical screening of aqueous and ethanolic extract of medicinal plants

3.3.1. Qualitative analysis of phytochemical screening of aqueous and ethanolic extract of medicinal plants

Phytochemical screening of the freshly prepared crude extract was qualitatively tested for the presence of chemical constituents and it was performed using the following reagents and chemicals. The phytochemicals analysed include alkaloids, steroids, phenolic

compounds, flavonoids, saponins, tannins, anthraquinones, glycosides, carbohydrates, proteins and terpenoids. They were analyzed using the established techniques (Khandelwal, 2002).

3.3.1.1. Test for alkaloids

1mg of extract is dissolved in 1ml of dilute HCl (10%) and it is filtered. The filtrate is used for detection of alkaloids.

Mayer's test

0.5ml of mayer's reagent (mercuric chloride in potassium iodide) is added to 0.5ml of the filtrate. The formation of white or yellow creamy precipitate confirms the presence of alkaloids.

Wagner's test

To 0.5ml of filtrate, added 0.5ml of Wagner's reagent (iodine in potassium iodide). A brown or reddish brown precipitate confirms the presence of alkaloids.

3.3.1.2. Test for flavonoids

Lead acetate test

Added a few drops of lead acetate solution to 0.5ml of extract. The formation of yellow colour precipitate confirms the presence of flavonoids.

Sulphuric acid test

To 0.5ml of extract, added a few drops of sulphuric acid which produces orange colour indicating the presence of flavonoids.

3.3.1.3. Test for steroids

To 1ml of extract, added 0.5ml of sulphuric acid and 0.5ml of acetic anhydride. The colour change from violet to blue or green colour indicates the presence of steroids.

3.3.1.4. Test for terpenoids

Salkowski's test

Added 0.5ml of chloroform to 1mg of the extract. To this added 0.5ml of concentrated sulphuric acid (along the sides of the test tube). The appearance of reddish brown colour in the interphase indicates the presence of terpenoids.

3.3.1.5. Test for anthraquinones

Borntrager's test

To 1mg of extract, added 0.5ml of 10% HCl and boiled it for a few minutes, filtered the extract and then cooled it. To this added 0.5ml of chloroform and a few drops of 10% HCl and boiled it. The appearance of pink colour indicates the presence of terpenoids.

3.3.1.6. Test for phenols

$FeCl_3$ test

To 0.5ml of extract, added a few drops of 5% ferric chloride solution. The appearance of bluish black or dark green colour indicates the presence of phenols.

Lead acetate test

To 0.5ml of extract, added a few drops of 10% lead acetate solution. The formation of yellow or white coloured precipitate indicates the presence of phenols.

3.3.1.7. Test for saponins

2.0 ml of distilled water was added to 1mg of the extract and it was shaken well. The appearance of foam indicates the presence of saponins.

3.3.1.8. Test for tannins

1ml of water was added to 1mg of the extract and it was heated in a boiling water bath and then filtered. To the filtrate, added a few drops of 5% ferric chloride solution. The appearance of dark green colour indicates the presence of tannins.

3.3.1.9. Test for carbohydrates

1mg of plant extract was dissolved in 1ml of water and filtered.

Molisch's test

To 1ml of filtrate, added 2 drops of alcoholic solution of alpha naphthol. Mixed well and added 0.5ml of concentrated sulphuric acid along the sides of the tube. The formation of violet ring indicates the presence of carbohydrates.

3.3.1.10. Test for proteins and amino acids

Biuret test

To 0.5ml of extract, added 0.5ml of 40% sodium hydroxide solution and 2 drops of 1% calcium sulphate solution. The appearance of violet colour indicates the presence of proteins and amino acids.

Ninhydrin test

To 0.5ml of extract, added 2 drops of 0.2% ninhydrin. It was heated and the appearance of pink or purple colour indicates the presence of proteins and amino acids

3.3.1.11. Identification of glycosides (Raaman, 2006)

For detection of glycosides, 50 mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hours on water bath, filtered and the hydrolysate was subjected to Borntrager's test. To 2.0 ml of filtered hydrolysate, 3.0 ml of chloroform was added and shaken, chloroform layer was separated and 10 per cent ammonia solution was added to it. Pink colour indicated the presence of glycosides.

3.3.2. Quantitative analysis of phytochemicals

3.3.2.1. Determination of total flavonoid content (Shankhalkar *et al*., 2016)

The total flavonoid content (mg/mL) was determined by using aluminum chloride ($AlCl_3$) method. The assay mixture consisting of 0.5 mL of the plant extract, 0.5 mL distilled water and 0.3 mL of 5% $NaNO_2$ was incubated for 5 min at 25°C. This was

followed by addition of 0.3 mL of 10% $AlCl_3$ immediately. 2 mL of 1 M NaOH was then added to the reaction mixture, and the absorbance was measured at 510 nm. Quercetin was used as a standard.

3.3.2.2. Determination of total phenol content (Shankhalkar *et al*., 2016)

Total phenolic content was determined using Folin-Ciocalteu (FC) reagent. The plant extract (0.5 mL) was mixed with 0.5 mL of FC reagent (1:1 diluted with distilled water) and incubated for 5 min at 22°C followed by the addition of 2 mL of 20% Na_2CO_3. The mixture was then incubated further at 22°C for 90 min and the absorbance was measured at 650 nm. The total phenolic content (mg/mL) was calculated using gallic acid as standard.

3.4. *In vitro* antidiabetic activity of the aqueous and ethanolic extract of medicinal plants

3.4.1. *In vitro* alpha amylase inhibitory activity of the aqueous and ethanolic extract (Subramanian *et al*., 2008)

In vitro alpha amylase inhibitory activity of aqueous and ethanolic extract was done by the method explained by Subramanian *et al*., 2008.

The enzyme solution was prepared by dissolving alpha – amylase in 20mM phosphate buffer (6.9) at the concentration of 0.5mg/ml. 1ml of the extract of various concentrations (250, 500, 750 and 1000 µg/ml) and 1ml of enzyme solutions was mixed together and incubated at 25ºC for 10min. After incubation, 1ml of starch (0.5%) solution was added to the mixture and further incubated at 25ºC for 10min. The reaction was then stopped by adding 2ml of dinitrosalicylic acid (DNS, color reagent), heating the reaction mixture in a boiling water bath for 5min. After cooling, the absorbance was measured colorimetrically at 565nm. The inhibition percentage was calculated using the given formula,

$$\% \text{ Scavenging activity } = \frac{\text{A (Control)} - \text{A(Sample)}}{\text{A (Control)}} \times 100$$

Where Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample.

3.5. Free radical scavenging activity of aqueous and ethanolic extract of medicinal plants

3.5.1. DPPH radical scavenging activity (Mensor *et al*., 2001)

In vitro 2,2'-Diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity of aqueous and ethanolic extract was carried out by the method proposed by Mensor *et al.*, 2001.

Principle

DPPH is a stable free radical containing an odd electron in its structure, which is responsible for the absorbance at 515-517nm. In the presence of antioxidants, DPPH radical can accept an electron donated by antioxidants thereby converting it to diphenyl picryl hydrazine. The degree of discoloration from deep violet to light yellow was measured at 515nm, which indicates the scavenging effect of the extracts.

Reagents

1. DPPH – (2, 2-diphenyl-2-picryl hydrazyl hydrate) (0.3mM in methanol)

2. Ethanol

Procedure

The Aqueous and Ethanolic extract of different concentrations were added with 0.5ml of 0.3mM DPPH and made up to 1.0 ml with ethanol. The mixture was allowed to react at room temperature for 30 minutes. DPPH solution with ethanol was used as positive control and methanol alone served as blank. After 30 minutes of incubation, the discoloration from deep violet to yellow color was measured at 515nm.

The per cent scavenging activity was calculated by the following formula,

$$\% \text{ Scavenging activity } = \frac{\text{A (Control)} - \text{A(Sample)}}{\text{A (Control)}} \times 100$$

3.5.2. FRAP radical scavenging activity (Vijayalakshmi and Ruckmani., 2018)

In vitro ferrous reducing antioxidant power (FRAP) radical scavenging activity of aqueous and ethanolic extract was carried out by the method proposed by Vijayalakshmi and Ruckmani., 2018.

Principle

The total antioxidant activity can be measured by the ferric reducing antioxidant power assay (FRAP). This method is based on the principle of increase in the absorbance of the reaction mixtures, the absorbance increases the antioxidant activity. The antioxidant compound present in the samples forms a coloured complex with potassium ferricyanide, trichloroacetic acid and ferric chloride which is measured at 595nm by colorimeter.

Procedure

Different concentrations of the aqueous and ethanolic extracts and its various fractions (20-100 μg/mL) was added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [K3Fe(CN)6] solution. The reaction mixture was vortexed well and then incubated at 50°C for 20 min using vortex shaker. At the end of the incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3,000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of deionised water and 0.5 mL of 0.1% ferric chloride. The colored solution was read at 595 nm against the blank with reference to standard using colorimeter. Here, ascorbic acid was used as a reference standard, the reducing power of the samples were comparable with the reference standard.

3.6. BSA-glucose antiglycation activity of aqueous and ethanolic extract of medicinal plants

In vitro Antiglycation activity of aqueous and ethanolic extract was carried out by the method proposed by Sultana *et al*., 2009.

The inhibition efficacy of extract against glycation was analysed by using BSA-Glucose antiglycation assay. Each tube contains 1.5ml of reaction mixtures (500 µL BSA (10 mg/mL+400 µL of glucose anhydrous (360 mg/mL)+100-500µL test sample), 100µL of DNPS as colouring reagent). Glycated control contain 500 µL BSA+400 µL glucose+500 µL sodium phosphate buffer (pH 7.4, 67 mM)+ 100µL DNPS. While blank contains 500 µL BSA and 400 µL sodium phosphate buffer+ 100µL DNPS. Reaction mixture was incubated at 37°C for 7-days. After incubation, 1200µL (100%) of TCA was added in each tubes and centrifuged (5,000 rpm) for 12 minutes at 4°C. After centrifugation, the pellets were washed with 1200 µL (10%) of TCA. The supernatant containing glucose, inhibitor and interfering substance was removed and pellet contains AGE-BSA were dissolved in 12 ml PBS. The absorbance was measured in colorimeter at 565nm.

$$\% \text{ Scavenging activity } = \frac{\text{A (Control)} - \text{A(Sample)}}{\text{A (Control)}} \times 100$$

3.7. *In vitro* antimicrobial activity of aqueous and ethanolic extract of medicinal plants (Kirby- Bauer method)

In vitro Antibacterial activity of aqueous and ethanolic extract was carried out by the Kirby- Bauer method.

Inoculum Preparation

The growth method was prepared as follows

1. Three to five, well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is

transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as Nutrient broth.

2. The broth culture is incubated at 35°C until it achieves or exceeds the turbidity (usually 2 to 6 hours)

3. The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain a turbidity. This results in a suspension containing approximately 1 to 2 x 108 CFU/ml for *Pseudomonas aeruginosa*

Inoculation of Test Plates

1. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.

2. The dried surface of a Nutrient agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60 (each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed.

3. The lid may be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

4. The media was punctured by making a well of 6 mm in diameter and filled with 20 µl of a sample. Further the petriplates were placed inversely for complete diffusion and inhibition zones were examined by measuring the diameter (mm) formed around the well after 24 hrs incubation at 37°C. The zones were measured by using standard (Hi-Media) scale.

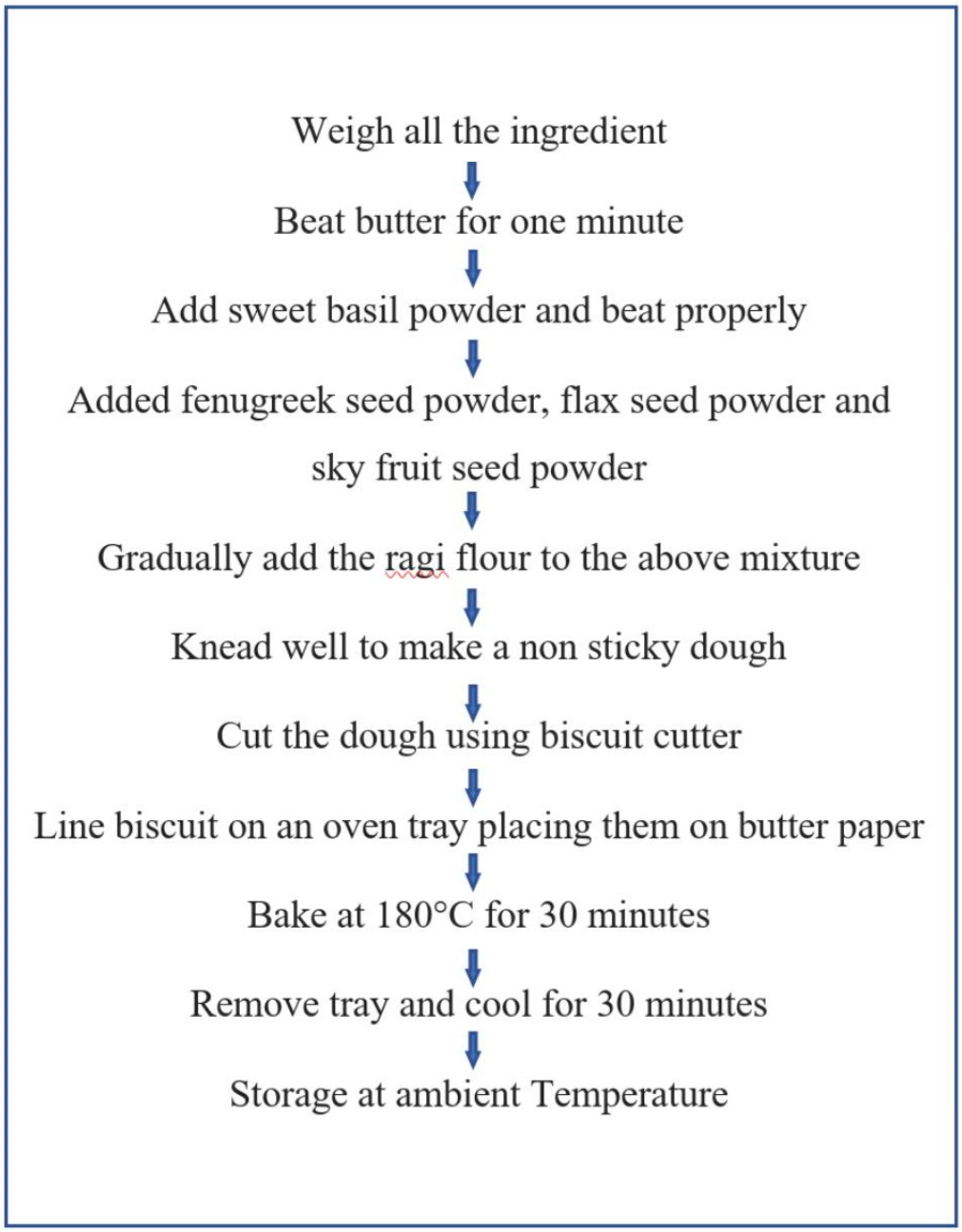
3.8. Preparation and assessment of cookies made with medicinal plants

A bigger and more demanding market for the management of health conditions is focusing on popular foods since they are efficient carriers for nutrient absorption. Among these foods, cookies have the potential to be a better option for preventing diet related

illnesses or meeting dietary needs. The management of human nutrition related problems can be accomplished in a number of ways through cookies. A lot of people consume them as snacks or go with other cuisines. Cookies come in a variety of shapes and flavors, they are convenient and have lengthy shelf life. Cookies are one of the top-selling items in the fast moving consumer goods category (Goubgou *et al.*, 2021). The cookies family of baked goods includes a wide range of options, including high and low fat, high and low sugar and more or less combinations. These can be produced in a variety of forms, dimensions and textures (Singh and yadav., 2018).

3.8.1. Preparation of Cookies

The medicinal plants *Ocimum basilicum, Linum usitatissimum, Trigonella foenum graecum* and *Swietenia macrophylla* were used as ingredients in the development of cookies along with ragi (*Eleusine coracana)* flour. The ingredients were dried, powdered and sieved. A known weight of the medicinal plants were taken for the preparation of cookies. Using electric beater, 50 grams of butter was beated about a minute and about 10 grams of sweet basil powder was added and beating was continued till it attained light fluffy consistency. To this added about 1 gram of fenugreek powder, 5 grams of flax seed powder, 0.3 grams of sky fruit seed powder, 8 grams of SMP (Skimmed milk powder) and 0.5 gram of baking powder. The beating was done continuously for 30 seconds until a smooth wet mixture was obtained. About 100 grams of ragi flour was sieved and weighed. The ragi flour was added slowly to the wet mixture and kneaded gently to make a non sticky dough. The dough was then cut into equal round shapes with uniform thickness using a biscuit cutter and placed in a preheated oven to bake at 180°C for 30 minutes. After baking the biscuit were taken out of the oven and allowed to cool.



Flow diagram for preparation of cookies

Table 1: Quantity of ingredients used for cookies preparation

INGREDIENTS	QUANTITY
RAGI FLOUR	100 g
SWEET BASIL POWDER	10 g
FENUGREEK POWDER	1 g
FLAX SEED POWDER	5 g
SKY FRUIT SEED POWDER	0.3 g
BUTTER	50 g
SKIMMED MILK POWDER	8 g
BAKING POWDER	0.5 g

3.8.2. Proximate analysis of cookies

The nutrient content of the product plays an important role in consumer acceptability. Nutrients like carbohydrates, fat, protein, dietary fiber along with energy were analyzed in the product.

3.8.2.1. Moisture

The moisture was estimated by drying the known weight (5 g) of the sample in an oven at 100 ºC followed by cooling in a desiccator. The process of heating and cooling was repeated till a constant weight was achieved (AOAC, 2006).

3.8.2.2. Ash

Ash content was determined in a muffle furnace at 600 ˚C for 4 to 6 hours. After cooling, the weight of the sample was noted (AOAC, 2006).

3.8.2.3. Protein

The Protein content was estimated by the Kjeldahl nitrogen distillation method. The method consists of heating a substance with sulphuric acid, which decomposes the organic substance by oxidation to liberate the reduced nitrogen as ammonium sulphate. In this step, potassium sulphate is added to increase the boiling point of the medium (from 337 ˚C to 373 ˚C). The chemical decomposition of the sample is complete when the initial very dark-colored medium has become clear and colorless. This nitrogenous substance is converted into ammonium borate by absorbing 4 % boric acid and is titrated against N/70 H_2SO_4. The volume of acid required to bring the test sample to the colour of blank gives the acid equivalent to ammonia. The amount of protein present was obtained by multiplying the nitrogen value by 6.25. The result obtained was expressed as grams of protein per 100 grams of the sample (AOAC, 2006).

3.8.2.4. Energy

The energy was calculated using a food composition table based on the calorific value of nutrients such as carbohydrates, protein and fat providing 4, 4 and 9 Kcal per gram respectively (Gopalan *et al*., 2011).

3.8.2.5. Carbohydrate

The carbohydrate content was determined by subtracting the sum of the percentage of moisture, ash, dietary fiber, protein and fat contents from 100 % according to the AOAC method (2006).

Carbohydrate = 100 - (moisture + protein + ash+ fat + dietary fibre)

3.8.2.6. Fat

The fat content was determined by the soxhlet extraction method (AOAC, 2006). The percentage of fat content was calculated by using the following formula

$$\text{Fat (\%)} = \frac{\text{Weight of fat in sample}}{\text{Weight of dry weight}} \times 100$$

3.8.2.7. Dietary fiber

The dietary fiber content of the cookies was estimated by the method described by AOAC (2006). Blank was run along with samples to measure any contribution from reagents to the residue. Defatting of samples was done with 25 ml of petroleum ether per gram of sample three times to remove fixed oil. Then, weighed 1 g of the sample. Phosphate buffer (pH 6.0, 50mL) was added to the sample. Adjust the pH 6.0 ± 0.2 by adding 0.3 N NaOH or 0.3N HCl. Enzyme hydrolysis of the sample was started by adding 0.1 mL α-amylase, incubating at 95 –100 ºC for 30 minutes in a water bath with continuous agitation. Cool to room temperature before adjusting to pH 7.5 ± 0.2 by adding 10 ml of 0.3 N NaOH. 5 mg of papain was added to the sample and was incubated at 60 ºC for another 30 minutes in a water bath with continuous agitation and cooled at room temperature. The pH was adjusted to 4.0 – 4.6 by adding 10 ml 0.3 N HCl. Amyloglucosidase 0.3 mL was added and incubated at 60 ºC for 30 minutes. Allowed to form a precipitate and filtered. The residue was weighed to measure the fiber content of the sample.

$$\text{Soluble dietary fiber (\%)} = \frac{\text{(residue (S1)-Protein ash-blank)}}{\text{Weight of test portion}} \times 100$$

$$\text{Insoluble dietary fibre (\%)} = \frac{\text{(residue (S2)-Protein ash-blank)}}{\text{Weight of test portion}} \times 100$$

$$\text{Total dietary fibre (\%)} = \text{Soluble dietary fibre} + \text{Insoluble dietary fiber}$$

Result and Discussion

4.0 RESULTS AND DISCUSSION

Diabetes mellitus, a chronic disorder which requires management for a long time where oxidative stress play a crucial role in progression of disease and development of secondary complications. There is still no known cure for diabetes or its dangerous consequences, despite all the study on the disease and recent developments in diabetic therapies. The anti-diabetic medications now in the market are successful in lowering blood sugar levels, they do have some negative effects when used over an extended period of time. As a result, medical professionals and people are increasingly turning to natural remedies in the form of medicinal foods and herbal substitutes that can be used on a long-term basis as a safer therapy with fewer severe side effects (Yimam *et al*., 2015).

Diabetes has become more common over time, and it is now considered one of the main causes of high death and morbidity rates (Tan *et al*., 2019). The pathophysiology of Type 1 diabetes mellitus and Type 2 diabetes mellitus is very diverse from one another, each type has a separate aetiology, presentation and course of treatment (Sapra and Bhandari, 2022). The formation of free radicals, which increases oxidative stress, a state that leads to the emergence of pathological conditions, which is intimately related to the difficulties associated with diabetes. As a result, using antioxidants has helped to lessen the severity of diabetic associated problems (Wickramaratne *et al*., 2016).

Medicinal plants, have drawn a lot of attention from the pharmaceutical research community in recent years. The richest and most accessible sources are medicinal herbs (Parasuraman, 2018). This trait of plants explains their ability to treat diseases and their advantages in conventional therapy. Plants synthesize these phytochemicals to defend themselves from internal stresses like free radicals and external stresses like insects and pests. These plant phytochemicals have been identified and a potential mechanism of action has been proposed (Odeyemi and Bradley., 2018).

In this context, The present study aims to investigate the anti-diabetic, anti-oxidant, anti-glycation, anti-microbial activities and phytochemicals screening of aqueous and ethanolic extracts of these selected medicinal plants such as *Ocimum basilicum, Linum usitatissimum, Trigonella foenum graecum* and *Swietenia macrophylla.*

4.1. Phytochemicals screening of aqueous and ethanolic extract of medicinal plants

4.1.1. Qualitative analysis of phytochemicals

Plants produce non-nutritive chemical substances known as phytochemicals through a number of chemical processes, often known as secondary metabolites. It is strongly recommended that eating foods high in phytochemicals can aid to improve health because numerous studies have shown the effects of these foods on health (Yoo *et al*., 2018).

Hence the qualitative phytochemical screening of the aqueous and ethanolic extract of the medicinal plant formulation was carried out for the presence of the bioactive compounds present and the results are tabulated in the table 2.

The phytochemical screening of the aqueous and ethanolic extracts of *Ocimum basilicum, Linum usitatissimum, Trigonella foenum graecum* and *Swietenia macrophylla* showed the presence of all the phytochemicals such as alkaloids, flavonoids, phenols, terpenoids, tannins, steroids, saponins and glycosides.

Table 2: Phytochemical analysis of aqueous and ethanolic extract of medicinal plant formulation

S.No	Phytochemical constituents	Ethanolic extract	Aqueous extract
1.	Alkaloids	+	++
2.	Flavonoids	++	++
3.	Phenols	++	++
4.	Terpenoids	++	+
5.	Tannins	+	+
6.	Steroids	++	++
7.	Saponins	+	+
8.	Glycosides	+	+
9.	Carbohydrates	++	++
10.	Proteins	+	+

(+ +) - Present in high intensity (+) - Present in low intensity

The phytochemical screening of various basil crude extracts contained both main and secondary plant compounds. Basil leaf aqueous extracts contains alkaloids, phenols, tannins, flavonoids, terpenoids, steroids and glycosides but no saponins. The presence of a wide variety of phytochemicals is confirmed by the crude extracts of O. *basilicum*, demonstrating the plants capacity to produce the anticipated and positive biological response (Nadeem *et al*., 2022).

Preliminary studies conducted by Methaq *et al*., 2017 using the aqueous and ethanolic extracts of fenugreek was found to show the presence of phenolic compounds, free amino acids, free flavonoids, steroids, carbohydrates, terpenes, tannins, saponins and glycosides.

Following the qualitative phytochemical analysis, the quantitative analysis for the total flavonoids and phenolic compounds were estimated.

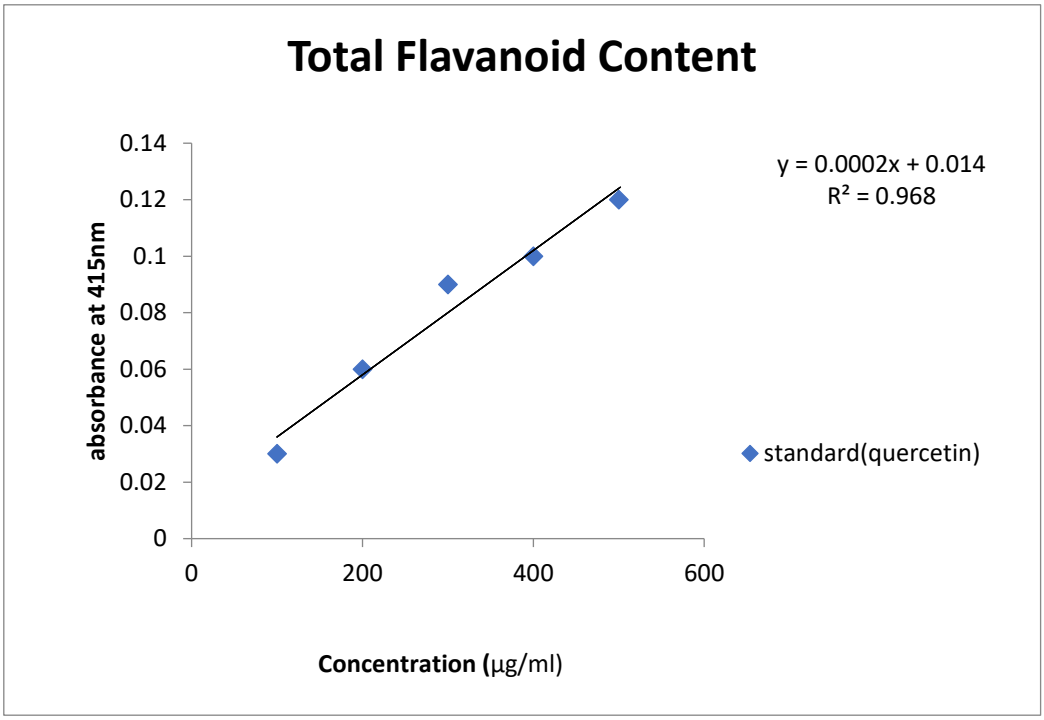
4.1.2. Quantitative analysis of phytochemicals

Natural diets high in phenolic and flavonoid compounds that have antioxidant action have stoked interest in nutrition and food science. Phenolic substances are effective electron donors because their hydroxyl groups can directly support antioxidant activity. Many works in the literature claim that phenolic compounds have the ability to suppress free radicals, degrade peroxide, inactivate metals or scavenge oxygen in biological systems, all of which reduce the burden of oxidative disease (Aryal *et al*., 2019).

4.1.2.1. Determination of total flavonoids contents

The total flavonoid content assay is used to determine the concentration of flavonoid compounds in the sample. The principle of this assay involves the reaction of flavonoids with a specific reagent that produces a colored product. The intensity of the color produced is directly proportional to the amount of flavonoid present in the sample.

Figure 6: Total Flavonoid Content



At a concentration of 500 µg/ml the standard quercetin showed a maximum absorbance of 0.12 the result showed that, increasing the concentration of the extract, absorbance also increased from 100-500 µg/ml.

The ethanolic extract of the medicinal plant was found to possess 0.03 optical density, Which corresponds to 80 µg of flavonoids. Hence 500 µg/ml of ethanolic extract of medicinal plant formulation contains 80 µg of flavonoids. Thus, the amount of flavonoid present in 1mg/ml of sample was found to be 160 µg.

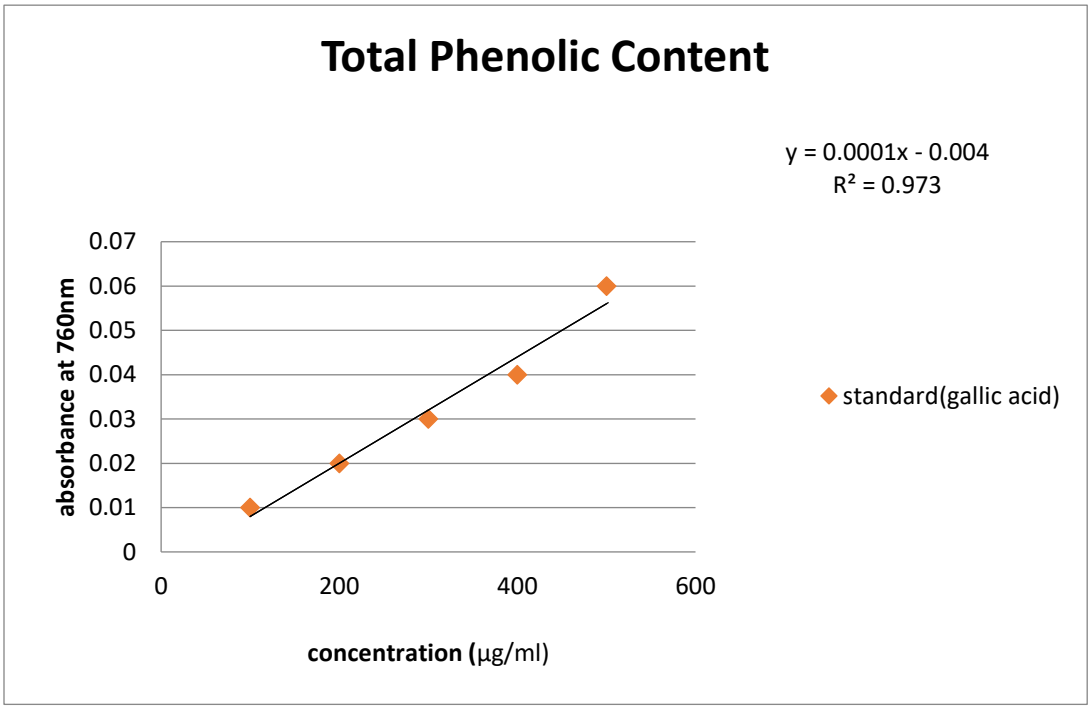
The aqueous extract of the medicinal plant was found to possess 0.06 optical density, Which corresponds to 230 µg of flavonoids. Hence 500 µg/ml of ethanolic extract of medicinal plant formulation contains 230 µg of flavonoids. Thus, the amount of flavonoid present in 1mg/ml of sample was found to be 460 µg.

Tran *et al*., 2020, reported that the highest flavonoid content was found in methanolic extract of *Phaseolus vulgaris L.* leaves 44.59 mg. Meanwhile, the methanolic extract of seeds and pods contained less flavonoid content 9.29 mg and 3.64 mg respectively.

4.1.2.2. Determination of total phenolic contents

The total phenolic content determines the ability of phenolic compounds to reduce certain oxidizing agents such as Folin-Ciocalteu reagent, resulting in a blue color complex. The intensity of the color produced is directly proportional to the amount of phenolic compounds present in the sample. The measurement is usually performed spectrophotometrically at 750 nm.

Figure 7: Total Phenolic Content



At a concentration of 500 µg/ml the standard gallic acid showed a maximum absorbance of 0.06. the result showed that increasing the concentration of the extract the absorbance was also found to increase from 100-500 µg/ml.

The ethanolic extract of the medicinal plant exerted an optical density of 0.84, Which corresponds to 844 µg of phenolic content. Hence 500 µg/ml of ethanolic extract of medicinal plant formulation contains 844 µg of phenol. Thus, the amount of phenolic content present in 1mg/ml of sample was found to be 16.88 mg.

The Optical density of aqueous extract of the medicinal plant was found to be 0.54, Which corresponds to 544 µg of phenolic content. Hence 500 µg/ml of ethanolic extract of medicinal plant formulation contains 544 µg of flavonoids. Thus, the amount of phenolic content present in 1mg/ml of sample was found to be 10.88 mg.

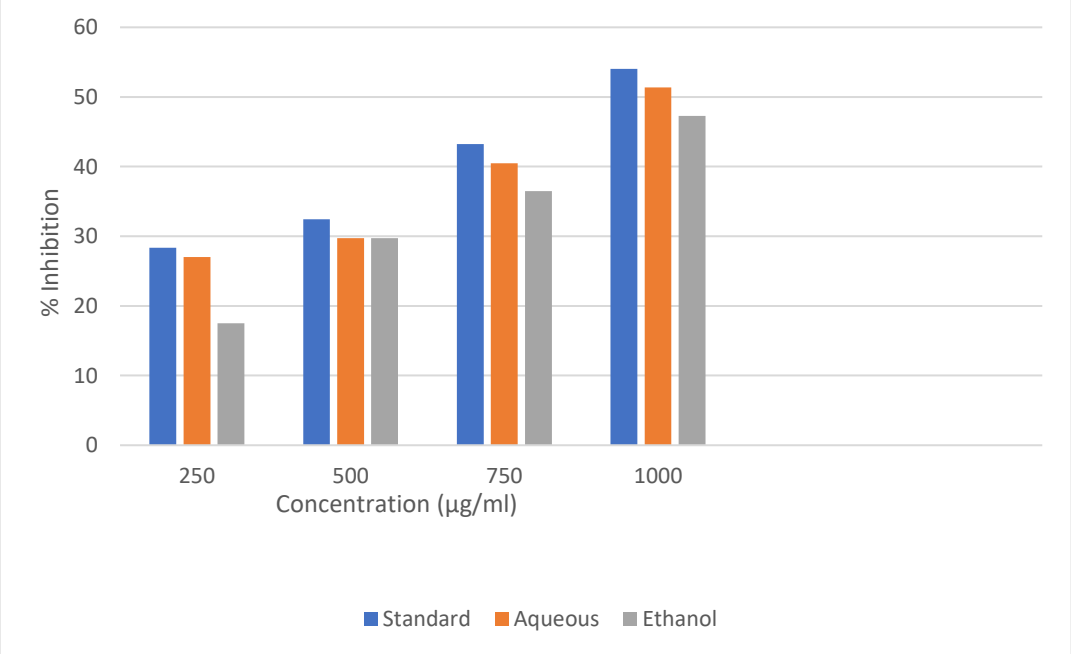
Johari and khong, 2019, reported that the methanolic extract of *Pereskia bleo* exhibited higher total phenolic content as compared to the chloroform and hexane extracts which are approximately about 40.82 mg for methanolic extract, 31.91 mg for chloroform extract and 25.2 mg for hexane extract.

4.2. *In vitro* anti-diabetic activity of aqueous and ethanolic extract of medicinal plants

4.2.1. *In vitro* alpha amylase inhibitory activity of aqueous and ethanolic extract of medicinal plants

One of the most important factors in the management of diabetes is the delay in starch digestion caused by the suppression of enzymes like amylase. Pancreatic amylase inhibitors slows the breakdown of carbohydrates, which in turn reduces the absorption of glucose and lowers post-prandial serum glucose levels (Sudha *et al*., 2011).

Figure 8: Alpha amylase inhibitory activity of aqueous and ethanolic extract of medicinal plants



The alpha amylase inhibitory activity of the aqueous and ethanolic extract of medicinal plant was found to be increased with increase in concentration from 250-1000µg/ml. At a concentration of 1000 µg/ml, the aqueous and ethanolic extract of medicinal plant showed a percentage inhibition of 51.35 and 47.25 respectively. The positive control of acarbose has exerted the highest potent inhibitory action against alpha amylase with 54.05 per cent. The inhibition of the aqueous extract was found to be maximum when compared to the ethanolic extract.

The chloroform extract of *Phyllanthus amarus* failed to inhibit α-amylase activity. However, the ethanol and hexane extracts of *Phyllanthus amarus* exhibited appreciable α-amylase inhibitory activity with the values 36.05 and 48.92 respectively, when compared with acarbose (Tamil *et al.,* 2018).

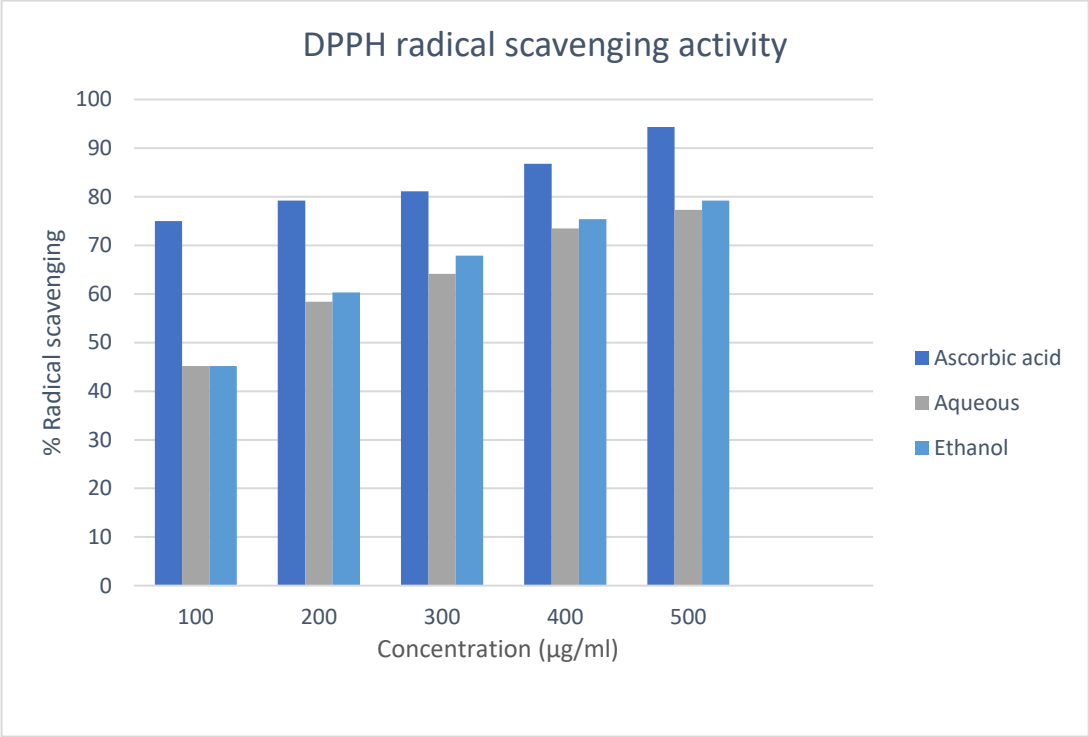
4.3. Free radical scavenging activity of aqueous and ethanolic extract of medicinal plants

Oxidative stress results when the body's defences against free radicals are overpowered. As a result, free radicals damage lipids, proteins and DNA and cause a variety of human disorders (Lobo *et al*., 2010). The present study was aimed to evaluate the free radical scavenging activity of aqueous and ethanolic extract of the medicinal plant and the results are discussed below.

4.3.1. DPPH radical scavenging activity of aqueous and ethanolic extract of medicinal plants

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method, uses free radicals to evaluate a substance capacity to act as a hydrogen source or a free-radical scavenger (FRS), is well-liked, rapid, simple and inexpensive for measuring antioxidant capabilities. The removal of DPPH, which would be a stabilised free radical, is connected to the DPPH testing technique. An odd electron combines with the free-radical DPPH to produce a significant absorbance at 517 nm, or a purple colour (Baliyan *et al*., 2022).

Figure 9: DPPH radical scavenging activity of aqueous and ethanolic extract of medicinal plants



The results of the study showed that, increasing the concentration of the extract, the scavenging activity was also found to increase from 100-500µg/ml for standard and sample. The scavenging activity of the solvent extract was found to be minimum when compared with the standard ascorbic acid. At the final concentration of 500 µg/ml, the standard ascorbic acid showed a maximum scavenging activity of 94.33%. The scavenging activity of the samples were found to be less when compared with the standard but they also showed a good scavenging activity. The aqueous and ethanolic extracts of medicinal plant formulation showed a strong antioxidant activity with scavenging rates of 77.3% and 79.2%. The percent inhibition of DPPH radical scavenging activity is presented in figure-10 and their IC50 value was found to be 127.23µg/ml and 112.27µg/ml respectively.

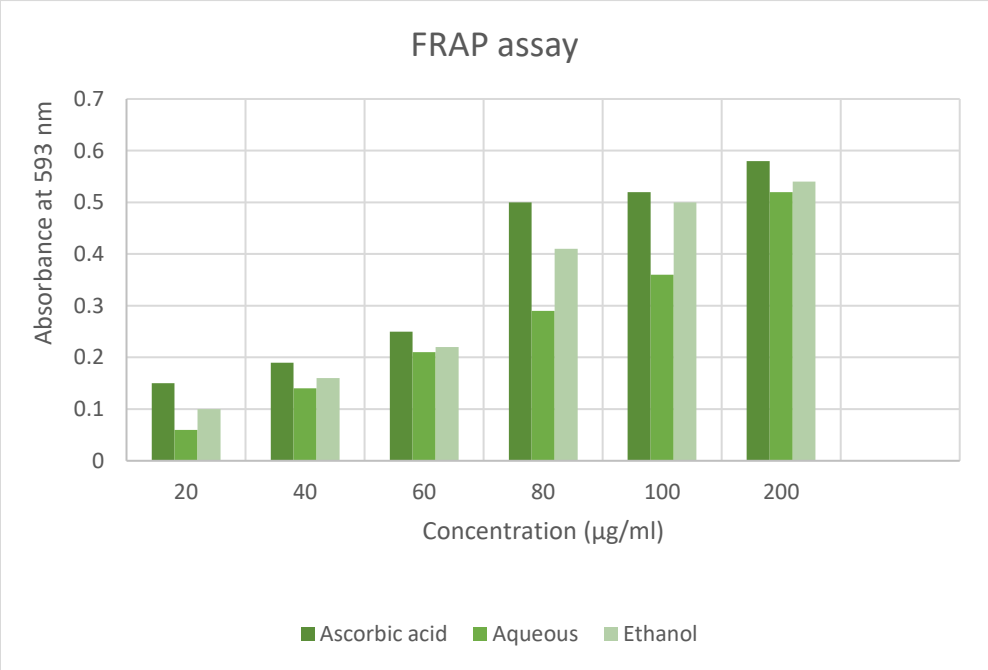
The chloroform, ethanol and aqueous extracts of *Schima wallichii* showed a concentration dependent rise in the scavenging of DPPH free radicals and a maximum

scavenging activity was recorded at a concentration of 160, 80 and 140 µg/ml chloroform, ethanol and aqueous extracts respectively (Lalhminghlui *et al.*, 2018).

4.3.2. FRAP radical scavenging activity of aqueous and ethanolic extract of medicinal plants

FRAP is based on the total quantity assignment of antioxidants by iron (III) reduction capacity. The method is only based on iron ions and not suitable for mechanic and physiologic antioxidant activities. However, it is simpler, faster and cheaper than the other methods. Solvent in different concentrations to search for iron reducing capacities of aqueous and ethanolic extract of medicinal plants were prepared. Iron (III) reduction capacity of aqueous and ethanolic extract of medicinal plants formulation were tested against a standard ascorbic acid.

Figure 10: FRAP radical scavenging activity of aqueous and ethanolic extract of medicinal plants



The results showed that, increasing the concentration of the extract, the antioxidant activity also increased from 20-200µg/ml for standard and sample. At the final

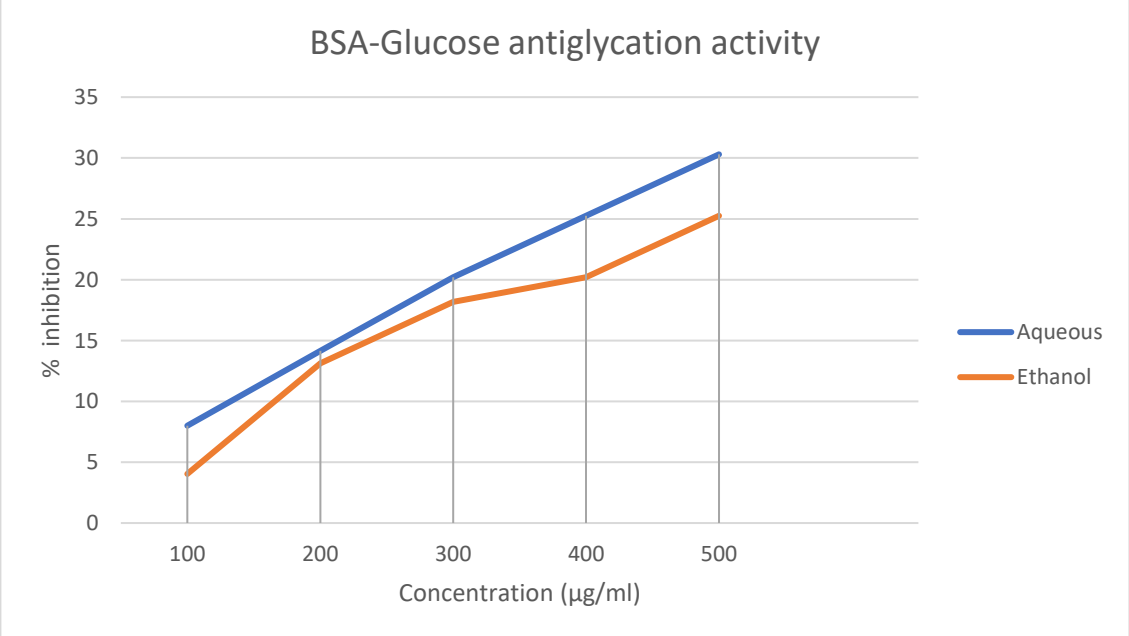
concentration of 200 µg/ml, the standard ascorbic acid showed a maximum absorbance of 0.58. When compared with that of the standard the aqueous and ethanolic extract of medicinal plants formulation showed a good antioxidant activity with the scavenging rates of 0.52 and 0.54 respectively.

Tanvir *et al.*, 2017, reported that the aqueous and ethanolic extract of *Curcuma longa* was found to have a higher scavenging rates such as 646.67 µM and 1972.6 µM.

4.4. BSA- Glucose antiglycation activity of aqueous and ethanolic extract of medicinal plants

BSA is glycated by glucose, resulting in the formation of advanced glycation end products (AGEs). The protein-DNPH complexes formed during the assay are indicative of the amount of AGEs present in the sample. The DNPH reacts with the carbonyl groups present in the AGEs, resulting in the formation of a yellow-orange chromophore, which can be measured using a microplate reader. The amount of protein-DNPH complexes formed is proportional to the amount of AGEs present in the sample. The standard curve is used to quantify the amount of AGEs in the samples.

Figure 11: BSA- Glucose antiglycation activity of aqueous and ethanolic extract of medicinal plants



The results showed that, increasing the concentration of the extract, the percentage of inhibition was found to increase from 100-500µg/ml for both aqueous and ethanolic sample. The inhibition of AGE formation of the aqueous extract was found to be maximum when compared to that of the ethanolic extract. At the final concentration of 500 µg/ml, the aqueous and ethanolic extract of medicinal plants formulation shows the inhibition percentage of 30.3 and 25.25 respectively. The percent inhibition of BSA- Glucose antiglycation activity is presented in figure-12 and their IC50 value was found to be 846.22µg/ml and 983.57µg/ml respectively.

Most promising effect against glycation was observed in the methanolic extract of *Fumaria officinalis* and in *Salvia hydrangea* least antiglycation activities were detected (Safari *et al*., 2018).

4.5. *In vitro* antimicrobial activity of aqueous and ethanolic extracts of medicinal plants

4.5.1. *In vitro* anti-bacterial activity

The antibacterial activity of aqueous and ethanolic extract of the medicinal plants formulation was investigated against gram negative strain (*Pseudomonas aeruginosa*) using well diffusion technique. The experiment involved measuring the zone of inhibition which indicates the excent to which the growth of the bacteria is inhibited by the test sample. The diameter of zone of inhibition is presented in Table 3

Table 3: Zone of inhibition (mm) of aqueous and ethanolic extract of medicinal plants

SAMPLE	ZONE OF INHIBITION (mm) (*Pseudomonas aeruginosa*)
Standard (Ampicillin)	14 mm
Negative control	0 mm
Aqueous sample	1 mm
Ethanol sample	10 mm

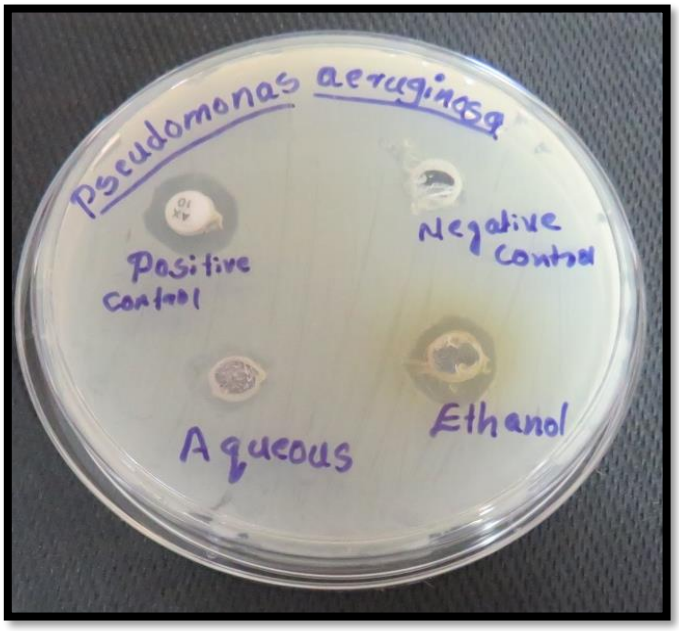


Figure 12: Antibacterial activity of aqueous and ethanolic extract of medicinal plants

The result were compared to a standard antibiotic (Ampicillin) and a negative control to assess the effectiveness of the samples. The standard ampicillin has a zone of inhibition (14 mm) against *Pseudomonas aeruginosa*. The negative control exhibited no zone of inhibition. The highest zone of inhibition was found in the ethanolic extract (10 mm) than the aqueous extract (1 mm). The result shows a good antibacterial activity for the ethanolic extract of medicinal plant formulation.

The chloroform and methanolic extract of *Ocimum basilicum* exhibited a strong antibacterial activity against *Pseudomonas aeruginosa* with a zone of inhibition 11mm and 13mm. The methanolic extract was found to have a highest zone of inhibition than the chloroform extract (Kaya *et al*., 2018).

4.6. Preparation and assessment of the cookies using medicinal plant formulation

4.6.1. Preparation of cookies using medicinal plant formulation

The selected medicinal plants such as *Ocimum basilicum, Linum usitatissimum, Trigonella foenum graecum* and *Swietenia macrophylla* were used as ingredients in the preparation of cookies using ragi (*Eleusine coracana)* flour. The cookies were prepared as per the formulation given in table 1

Figure 13: Cookies prepared using medicinal plant formulation

4.6.2. Proximate Analysis of Cookies

The cookies prepared using the medicinal plant formulation were evaluated for its proximate analysis and the results obtained were given in table 5

Table 5: Proximate analysis of cookies prepared using the medicinal plant formulation

S.NO	PARAMETER	UNIT	RESULTS
1	Moisture	%	2.92
2	Total Ash	%	3.28
3	Protein	%	6.19
4	Fat	%	15.29
5	Crude fibre	%	1.33
6	Carbohydrate	%	40.03
7	Energy	Kcal/g	613.1

Based on the information provided, it appears to be a nutritional analysis of the Cookies made using the medicinal plant formulation.

- The moisture content of the sample is 2.92%, indicating the amount of water content present in the sample.
- The total ash content of the sample is 3.28%, which represents the amount of minerals present in the sample.
- The protein content of the sample is 6.19%, which indicates that the sample has a significant protein source.
- Fat content is an important parameter in food analysis as it provides the body with energy, and it also affects the sensory properties of the food. The fat content in the cookies is relatively moderate at 15.29%.

- Fibre content is also an important parameter in food analysis as it promotes good digestion and can help prevent chronic diseases. The fiber content in the cookies is moderate at 1.33 %.
- The carbohydrate content of the sample is found to be 40.03%, indicating that the sample has a good source of carbohydrates.
- The energy value of the sample is 613.1 Kcal/g, indicating that the cookies prepared using medicinal plant formulation has a high energy density.

To summarise, the cookies made using the medicinal plant formulation was found to have a low moisture and ash content with moderate amount of protein and high amount of energy density.

The comparison of the proximate analysis of cookies prepared using medicinal plants and the cookies available in market is given in table 6

Table 6: Comparison of proximate analysis of cookies prepared using medicinal plants and cookies available in market

S.No	PARAMETER	COOKIES PREPARED USING MEDICINAL PLANTS	COOKIES AVAILABLE IN MARKET
1.	Protein	6.19%	8%
2.	Fat	15.29%	21%
3.	Crude Fibre	1.33%	6%
4.	Carbohydrate	40.03%	68%
5.	Energy	613.1 Kcal/g	493 Kcal/g

Summary and Conclusion

5.0 SUMMARY AND CONCLUSION

Diabetes mellitus is a chronic metabolic disorder that affects the body's ability to regulate blood sugar levels. It is characterized by high blood glucose levels either due to inadequate insulin production or the body's inability to effectively utilize insulin. There are different types of diabetes, type 1 diabetes typically occurring in childhood or adolescence and requiring insulin injections for survival. Type 2 diabetes, which is more prevalent, often develops in adulthood and can be managed with lifestyle modifications, medication or insulin therapy. Diabetes can lead to various complications, including cardiovascular disease, kidney problems, nerve damage and vision impairment. It exhibits a range of signs and symptoms that indicate an imbalance in blood sugar levels. It's important that the pathophysiology of diabetes mellitus is complex and involves a combination of genetic, environmental and lifestyle factors.

Free radicals are highly reactive molecules that contain an unpaired electron. They are formed as natural byproducts of various cellular processes such as metabolism and immune system activity or can be generated by external factors like pollution, radiation or certain chemicals. It can cause damage to cells and tissues by initiating a process called oxidative stress. Oxidative stress occurs when the production of free radicals exceeds the body's capacity to neutralize them with antioxidants. They are the substances that can neutralize free radicals, thus protecting cells from their harmful effects.

Medicinal plants also known as medicinal herbs or herbal medicines are plants that have been used for centuries in traditional medicine systems to treat various ailments and promote overall well-being. Medicinal plants are valued for their bioactive constituents, which include alkaloids, flavonoids, terpenoids, phenolic compounds and other chemical compounds. These natural compounds have different physiological effects on the human body and can possess various therapeutic properties such as antioxidants, antinflammatory agents, antimicrobials and immune boosting substances. There are several medicinal plants that have been traditionally used for managing diabetes mellitus.

The plants like *Ocimum basillicum* and *Swietenia macrophylla* have a positive impact on diabetes management. Both contains essential oils and phytochemicals that have been

found to possess antidiabetic properties including the ability to lower blood glucose levels. Some studies have indicated that the extracts of these plants may help to regulate insulin secretion and improve insulin sensitivity, which are crucial factors in diabetes control. *Swietenia macrophylla* may also have a protective effect on pancreatic beta cells, which are responsible for insulin production.

The present study aims to identify the phytochemicals present in the aqueous and ethanolic extract of *Ocimum basilicum, Linum usitatissimum, Trigonella foenum graecum* and *Swietenia macrophylla* and to investigate the antidiabetic, antioxidant, antiglycation and antimicrobial activities of the extracts. The small cookies were prepared using the medicinal plant formulation and assessing their nutritional contents.

The results of phytochemical screening showed the presence of several secondary metabolites including alkaloids, flavonoids, phenols, terpenoids, tannins, steroids, saponins and glycosides in the aqueous and ethanolic extract of the medicinal plant formulation. Some of these chemical compounds have been linked to its antioxidant properties. The flavonoids and phenolic compounds of aqueous and ethanolic extract of medicinal plant formulation were quantitatively analysed and showed a good result. The amount of phenolic content present in aqueous and ethanolic extract was found to be 10.88 mg and 16.88 mg. The amount of flavonoids present in aqueous and ethanolic extract was found to be 460 µg and 160 µg. The results showed that the phenolic compounds are present in higher amounts than flavonoids in the aqueous and ethanolic extract of the medicinal plants formulation.

The inhibitory activity of different concentrations (250 – 1000 µg/ml) of aqueous and ethanolic extract of medicinal plant formulation was investigated for its alpha amylase activity. The aqueous and ethanolic extract of medicinal plant formulation showed potent inhibitory activity against alpha amylase. The positive control of acarbose has exerted the highest potent inhibitory action against alpha amylase with 54.05 percent. The inhibition of aqueous extract was found to be maximum when compared to the ethanolic extract.

The free radical scavenging activity of different concentrations (100-500µg/ml) and (20- 200 µg/ml) of aqueous and ethanolic extract of medicinal plant formulation was

investigated for its DPPH and FRAP radical scavenging activities. The results of DPPH radical scavenging assay demonstrated that the aqueous and ethanolic extracts showed the maximum radical scavenging activity at a concentration of 500µg. A linear relationship was observed in the DPPH radical scavenging activity at various concentrations of the aqueous and ethanolic extract, while it showed IC50 value of 127.23 µg/ml and 112.27 µg/ml respectively. In FRAP radical scavenging assay, the results showed that the aqueous and ethanolic extract were able to scavenge the FRAP radical indicating the antioxidant potential of medicinal plant formulation.

The BSA-Glucose antiglycation activity of different concentrations (100-500 µg/ml) of the aqueous and ethanolic extract of medicinal plant formulation was analyzed. The result demonstrated that the aqueous and ethanolic extract of medicinal plant formulation showed a good antiglycation activity. At the final concentration of 500 µg/ml the aqueous and ethanolic extract of medicinal plants formulation shows the highest inhibition percentage. The IC50 value of the aqueous and ethanolic extract was found to be 846.22µg/ml and 983.57µg/ml. The results showed that increasing the concentration of the extract, the percentage of inhibition was also increased from 100-500µg/ml for both aqueous and ethanolic sample. The inhibitiom of AGE formation of the aqueous extract was found to be maximum when compared with the ethanolic extract.

The antibacterial activity of aqueous and ethanolic extract of medicinal plant formulation was investigated against of gram negative bacteria (*Pseudomonas aeruginosa*). The standard ampicillin has a zone of inhibition of 14mm against *Pseudomonas aeruginosa.* The highest zone of inhibition was found in ethanolic extract (10mm) than the aqueous extract (1mm). The result suggest that the ethanolic extract of medicinal plant formulation contains compounds or substance that possess antimicrobial properties, capable of inhibiting the growth of the bacteria.

The Cookies were prepared using the medicinal plant formulation and assessed for its nutritional contents. The analyzed cookies sample shows a moisture content of 2.92%, indicating a relatively less water content. The total ash content is 3.28%, representing the mineral content left after complete combustion. Protein content is 6.19%, indicating a good source of protein content in cookies. The fat content is 15.29 %, serving as a concentrated

energy source. Crude fiber content is 1.33%, contributing to a good digestive health. Carbohydrates make up 40.03% of the sample, serving as the good source of energy. With an energy value of 613.1 kcal/g, indicating that the cookies sample provides substantial energy and could be used as a source to lower the levels of blood glucose.

The result of the present study demonstrates that the ethanolic and aqueous extract of the medicinal plant formulation possess an effective antidiabetic and antioxidant property. Antibacterial and antiglycation activity was found to exhibit a moderate inhibition property. The cookies developed using the medicinal plant formulation found to have a good proximate values. Based on the results of the above study, it could be concluded that the medicinal plant formulation can be exploited further for its medicinal use in treating disease and associated disorders *in vitro* and *in vivo*. However, future studies has to be carried out in order to validate the medicinal plant formulation.

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