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In-Vitro Evaluation of Anti-Glycation Potential of Bruguiera Sexangula

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Abstract: ***Diabetes mellitus (DM) is a metabolic disorder that affects people all over the world and causes hyperglycemia. Further, chronic hyperglycemia may lead to non-enzymatic protein glycation where the excess blood glucose reacts with free amino acids in proteins to form a labile Schiff's base. Then the base gets stabilized to form advanced glycation end products (AGEs), one of the hallmark compounds of glycation. Therefore, antiglycation therapy is considered as one of the important aspects of post diabetic treatment. Recently, many phytochemicals have been reported for their antiglycation potential. In the present study, Bruguiera sexangula an medicinal mangrove plant from the Eastern coastal regions of Odisha has been evaluated for its antiglycation potential using in vitro techniques. The anti-glycation activities of aqueous bark extract of this plant were determined by bovine serum albumin-fructose glycation model. The multistage glycation markers-fructosamines, thiols and β aggregation of albumin using amyloid-specific dyes–Congo red were evaluated. The studies were carried out for the 0th,1st, 2nd , 3rd and 4th consecutive weeks. The present study showed aqueous bark extract of Bruguiera sexangula at 1mg/ml concentration could inhibit amyloid cross beta aggregation in the 0th, first, third and the fourth week. However, no inhibition was observed in the second week. On the other hand, the standard anti-oxidant compound Quercetin could inhibit the amyloid beta aggregation in all the 4 weeks in the Congo red assay. And the extract could only show amadori product inhibition potential during the 1st and the 4th week of study. While, the compound Quercetin could inhibit the amadori product in 1st, 3rd and 4th week of the fructosamine assay. Free thiols were significantly protected from oxidation by the plant extracts. The active phytocompounds were isolated by column chromatography by using different fractions of solvents (petroleum ether, chloroform and methanol) on the basis of their polarity and it is stored for further assays. The results of the present study indicated that B.sexangula can provide protection against long term glycation associated complications. However, more in depth studies need to be carried out to decipher its antiglycation potential. Further, the bioactive phytochemicals responsible for this activity may be isolated to access its antiglycation potential in suitable model organism.***

I. INTRODUCTION

Diabetes and its complications are quickly becoming the world's leading cause of morbidity and mortality. Diabetes, which is characterised by weight loss and polyuria, was first documented by the Egyptians.

The Greek physician Aertaeus, on the other hand, coined the term diabetes mellitus. Diabetes is a Greek word that means "to pass through," and mellitus is the Latin word for honey (referring to sweetness). Diabetes is a leading cause of long-term illness and premature death, claiming the lives of more people each year than HIV/AIDS, with nearly one death every 10 seconds. (Jangid et al., 2017). Diabetes is divided into four major types. Type 1 Diabetes (cell destruction, usually leading to absolute insulin deficiency, immune mediated and idiopathic). 2. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance). 3. Pregnancy diabetes 4. Other types (genetic defects in cell function; genetic defects in insulin action; exocrine pancreas diseases; endocrinopathies; drug-induced, chemical-induced; infections; uncommon forms of immune-mediated diabetes; genetic syndromes associated with diabetes).

The negative effects of persistently elevated plasma glucose levels on different body parts vary depending on cell type. Diabetes is expected to double in the next decade as a result of lifestyle changes and obesity in developed countries. These predictions are likely to have long-term implications for the health-care delivery system as the number of patients with type 2 diabetes continues to rise (Sheetz and King 2002). Two landmark studies, the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS), have shown that strict hyperglycemia control reduces the incidence of diabetic microvascular disease. Clearly, identifying the genes and cellular mechanisms responsible for these effects is critical, and this has been the focus of numerous studies.

Several metabolic theories have been proposed to explain this relationship, including increased flux through the aldose reductase pathway, sustained activation of protein kinase C (PKC) by increased levels of diacylglycerol (DAG), and non-enzymatic glycation of macromolecules. The formation of advanced glycation end products(AGEs) is the most compelling of these theories.The development, progression, and complications of diabetes are all thought to be influenced by hyperglycemia(Brownlee 2005). These complications include those that are unique to diabetes, such as retinopathy, nephropathy, and neuropathy, as well as those that are present in non-diabetics but are two to five times more common in diabetics. Blood sugar levels over 125 mg/ dL while fasting and over 180mg/ dL two hours after eating are considered to be hyperglycemia.

A diabetic patient has a fasting blood glucose level of more than 125 mg/ dL. Diabetes treatment is regarded as the most serious global issue, with no successful treatment available. Insulin and oral hypoglycemic agents, two major drugs used as the first line of treatment for diabetes, have some side effects but do not significantly alter the course of diabetic complications.

Untreated hyperglycemia can lead to a variety of serious, sometimes fatal complications, including damage to the kidneys, heart, nerves, eyes, and peripheral vascular system. It is critical to manage hyperglycemia effectively and efficiently in order to prevent illness complications and improve patient outcomes.

Over the past 20 years, there has been a sharp rise in the incidence of hyperglycemia due to rising obesity, declining levels of activity, and an ageing population.

The identification of genes and cellular mechanisms responsible for these effects is obviously important and has thus been the focus of numerous studies. Several metabolic theories have been proposed to explain this relationship, including increased flux through the aldose reductase pathway, sustained activation of protein kinase C (PKC) by increased levels of diacylglycerol (DAG), and non-enzymatic glycation of macromolecules. The most compelling of these theories is the formation of advanced glycation end products (AGEs). Protein glycation is one of the most serious consequences of chronic hyperglycemia, resulting in a partial loss of protein function (Yamagishi and Matsui 2010). Glycation is a non-enzymatic spontaneous event that results in Amadori products by combining free reducing sugars with free amino groups of proteins, DNA, and lipids. Amadori products are subjected to an irreversible chain reaction of dehydration and rearrangement events, which results in the formation of advanced glycation end products (AGEs). In 1912, Louis-Camille Maillard pioneered this method. The glycation reaction is classified into three stages: early, intermediate, and late (Baker et al., 1985). A growing body of research also shows that glycated proteins, AGEs, and their interactions with signal-transducing receptors generate reactive oxygen species, which cause concurrent oxidative stress (OS) and vascular inflammation, and play an important role in the pathogenesis of various diabetic vascular complications. The formation and accumulation of AGEs is aided by oxidative stress, which has been linked to the development of many diseases, including chronic conditions such as diabetes, Alzheimer's disease, and ageing. Advanced glycation is one of the major pathways involved in the development and progression of various diabetic complications such as nephropathy, retinopathy, and neuropathy.

Finding drugs that precisely block each glycation step and the formation of the intermediate products of this critical pathway may thus provide a viable option for developing novel therapeutic techniques to delay and prevent diabetic vascular complications.(Rahbar and Figarola 2003).

However, some anti-diabetic drugs are necessary to keep glycemic control. Oral anti-diabetic medications have had some negative side effects, such as hypoglycemia, fluid retention, osteoporosis, and heart failure, limiting their use in clinical settings. Therefore, it is necessary to find new anti-diabetic solutions with few adverse effects in order to control diabetes. Weight loss and lifestyle modifications can partially decrease hyperglycemia to control T2DM. As a result, we must consider the natural way as an alternative.

Natural products, such as herbal formulas and extracts, have been used for thousands of years to treat human diseases using a unique system of theories and therapies, and are now being increasingly used to treat T2DM. Herbal medicine has grown in popularity in recent years, and these treatments are becoming more popular in both developing and developed countries due to their natural origins and lack of negative side effects. Many well-known traditional remedies are derived from medicinal plants, minerals, and organic substances. Herbal preparations used in Indian traditional health care systems contain a number of medicinal plants known as rasayana, which have been utilized for over 1000 years.

In recent years, there has been a growing interest in the effects and mechanisms of natural products. The goal of this paper was to provide an overview of the main factors/pathways associated with T2DM, as well as a thorough review of current understandings of natural products for T2DM. There is a growing global interest in discovering antioxidant compounds that are pharmacologically potent and have few side effects for use in anti-diabetic therapy. Mangrove species are highly adapted to a wide range of temperatures, strong coastal winds, extreme tidal waves, salinity fluctuations, coastal water turbulence, river run-off, and anaerobic soil and grow at the juncture of the world's coastal and land areas.

They are physiologically and morphologically adapted to extreme environments. Mangroves play distinct biochemical roles in their ecosystem and are thought to be a source of novel natural/biological products. Polyphenols and tannins, for example, are abundant in mangroves. Mangrove leaves also contain phenolic compounds, alkaloids, and flavonoids, which all act as novel bioactive compounds. Despite the growing use of synthetic medications in modern medicine, 50% of all pharmaceutical substances still come from plants.Traditional folk medicine has utilised mangrove plants to treat a number of illnesses, including diabetes (Bandaranayake 1998). However, the anti-glycation potentials of these plants and their relationship with antioxidant potential remain unknown. As a result, the current review emphasizes the link between oxidative stress and diabetes, as well as the potential role of mangrove plants in the management of diabetes and its oxidative stress-related complications. *Avicennia marina, Xylocarpus granatum, and Bruguiera sexangula* have been

Found to be high in flavonoid compounds such as rutin,quercetin, kaempferol, catechin, and epicatechin, which have been shown to have hypoglycemic properties as well as antibacterial, antifungal, antimycobacterial, antimalarial, antiretroviral and antiviral properties(Bandaranayake 1998).

Bruguiera is the largest genus in the *Rhizophoraceae*, and all six described Bruguiera species are members of the "Indo-Malayan" mangrove group, which ranges from East Africa to Australia and the West Pacific. It has viviparous propagules, 16–32 stamens, an explosive pollen discharge, and calyces with 8–16 lanceolate, pointed lobes. It bears the name Jean Guillaume Bruguière after the French adventurer and zoologist (1750-1798).

The upriver orange mangrove, *Bruguiera sexangula*, is a mangrove shrub or tree that can reach a height of 15 to 30 meters. B. sexangula is a tree with multiple stems or a single stem. It has knee-like air-breathing roots known as pneumatophores at the trunk's base. The bark is smooth and grey-brown in color. The smooth, glossy green leaves are elliptic to elliptic-oblong in shape, 9.5-20 cm long, 3-7cm wide, and grow in clusters at the branch tips. B. sexangula trees grow largely in the back mangrove (the landward zone), where tidal flooding is less common, and often in combination with *B. gymnorhiza, Sonneratia ovata, Lumnitzera littorea,* and *Rapanea porteriana.* The calyx colours of B. sexangula, according to our observations in the field, are yellow or yellow-green, and are comparable to those seen in India and Australia.

It was evident from earlier studies that So far, B. sexangula has not been researched for its influence on the glycation process. Furthermore, no systematic studies have been conducted to assess the effect of this plant extract on glycation-induced albumin alterations and the cellular consequences of glycated albumin. The current study sought to evaluate the anti-glycation capabilities of the plant extract B. sexangula in depth. The multistage glycation markers fructosamines and AGEs are studied, as well as thiols and β aggregation of albumin using amyloid-specific dyes-Congo Red.

II. LITERATURE REVIEW

“Glycation is a series of irreversible dehydration and rearrangement reactions that produce advanced glycation end products (AGEs). In 1912, Louis-Camille Maillard invented this technique. Protein function loss and decreased elasticity are caused by glycation in tissues such as blood vessels, skin, and tendons. The glycation reaction is greatly accelerated in the presence of hyperglycemia and tissue oxidative stress. This suggests that it plays a role in the development of diabetic complications and the aging process.”

The mechanism by which AGEs form and accumulate, oxidative stress, has been linked to the progression of a number of diseases, including diabetes, Alzheimer's disease, and aging. “As it is associated with changes in the activities of biological compounds and cellular processes that may be linked to a pathological environment, oxidative stress, specifically oxidative protein damage, is increasingly thought to play a central mechanistic role in this context. The generation of excessive reactive oxygen species (ROS) from glucose autoxidation, as well as the nonenzymatic, covalent attachment of glucose molecules to circulating proteins, which results in the formation of AGEs, fuel the formation of oxidative stress.

AGEs were discovered during the cooking process as a result of the Maillard reaction, a non-enzymatic reaction between sugars and proteins in foods. The glycation process begins with a chemical reaction between a reactive carbonyl group of a sugar or aldehyde and a nucleophilic free amino group of a protein, resulting in the rapid formation of an unstable Schiff base. After that, the adduct is rearranged to form a more reversible and stable Amadori product. Over several days to weeks, these intermediate compounds undergo irreversible oxidation, dehydration, polymerization, and cross-linking processes, resulting in the formation of AGEs."

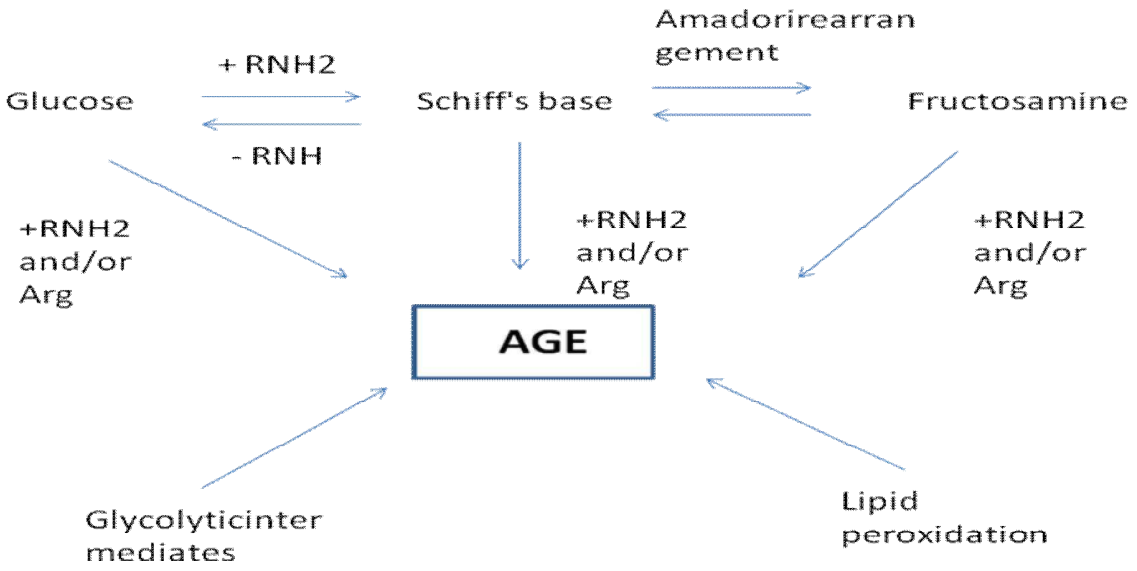

Figure 1. Glycation process leading to the formation of advanced glycation end products(AGEs)

Glycation also lowers insulin production, whereas AGEs have a direct effect on the pancreas and generate the circumstances for oxidation, which leads to the dysfunction and death of insulin secreting beta cells. All of these factors contribute to the chronic hyperglycemia found in type 2 diabetes persons. This hyperglycemia promotes the creation of pathogenic glycated proteins.

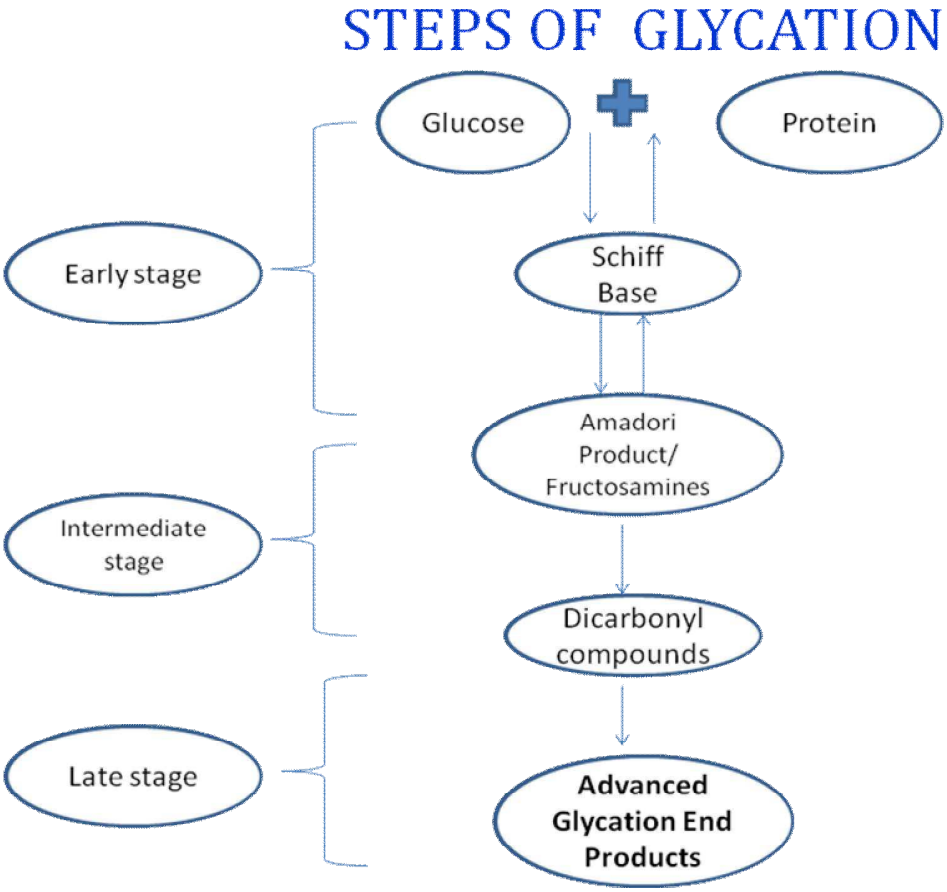

Figure 2. Glycation process showing its different stages

A. Maillard Reaction: Formation Of Advanced Glycation End Product

The Maillard reaction occurs when free amino groups of proteins interact with carbonyl groups of reducing sugars or other carbonyl substances. The three major phases of this reaction are early, moderate, and late. Glucose (or other reducing sugars such as fructose, pentoses, galactose, mannose, or xylose) first reacts with a free amino group of biological amines to form the unstable Schiff base, which then undergoes a rearrangement to form the amadori product. In an intermediate stage, dehydration, oxidation, and other chemical reactions degrade the amadori product to a variety of reactive dicarbonyl compounds such as glyoxal, MGO, and deoxyglucosones.

Through oxidation, dehydration, and cyclization reactions in the late stage of glycation, irreversible compounds known as AGEs are formed. The AGEs are insoluble, fluorescent, yellow-brown adducts that build up on the long-lived proteins and impair their ability to carry out various physiological tasks.

Human cartilage, skin collagen, and pericardial fluid have all been discovered to accumulate AGEs, which are created more frequently as we age. Collagens in particular are long-lived proteins that have a lot of arginine, hydroxylysine,lysine and arginine residuesthat are susceptible to accumulating glycation damage with ageing. AGEs are responsible for the development of a number of conditions, including Alzheimer's disease, diabetes, cataracts, atherosclerosis, diabetic nephropathy, and diabetes. There are three processes that can result in the development of AGEs: The auto-oxidative pathway, which involves the autoxidation of carbohydrates to produce reactive chemicals; the amadori rearrangement; and the Schiff base are all examples of metabolic pathways. Exogenous AGEs are also consumed by humans through diet.

B. Source and Classification of AGEs

AGEs are classified as endogenous or exogenous depending on where they originate. The vast majority of endogenous AGEs form spontaneously in the body and accumulate during physiological metabolic conditions and normal aging. Exogenous AGEs are primarily derived from dietary intake, and animal-sourced foods high in fat and protein contain far more AGEs than plant-sourced foods high in water, antioxidants, and vitamins. Heat-treated foods, for example, have 10-100 times the AGE levels of untreated foods. Another important factor in determining the AGE content of food is cooking. Goldberg and colleagues examined the AGE content of 250 different food preparation methods. Fried and barbecued foods had the highest levels of AGE, followed by baked foods, and boiled foods had the lowest levels of AGE. So far, more than 20 different AGEs have been discovered in tissue proteins, including glucosepane, carboxymethyl lysine (CML), and carboxyethyl lysine (CEL). Other AGEs found in tissue proteins include pentosidine, pyrroline, and the imidazolines imidazoline A and imidazoline B. These AGEs are classified into three types based on their chemical properties: crosslinked fluorescent products, crosslinked nonfluorescent products, and noncrosslinked fluorescent products. Fluorescent AGEs account for a significant portion of AGEs and are an excellent target for AGE total measurement.

C. Biological Effects of AGEs

The accumulation of AGEs in our bodies activates a number of signaling pathways through a network of cell membrane receptors. As previously stated, “AGEs are a class of molecules with distinct chemical properties, but their biological differences remain unknown. Different AGEs have very similar biological effects; AGEs with a higher molecular weight have the highest pathogenic potential; crosslinking AGEs have a high affinity for proteins and are resistant to degradation, making them potentially more toxic to the human body. AGEs cause a variety of biological effects by activating cell surface receptors. Scavenger receptors and multi ligand receptors are the two types of AGE receptors. RAGE is currently the most researched AGE receptor. When AGE and RAGE interact, an intracellular cascade is initiated that inhibits insulin-induced GLUT-4 translocation and results in insulin resistance.”

D. Diabetic Complications

Diabetes mellitus is a complex metabolic condition caused by a lack of or malfunctioning of insulin. “Diabetes type I (insulin-dependent) is characterised by a lack of functioning beta cells, which results in insulin deficit. Patients with this condition are thus fully dependent on an exogenous source of insulin, whereas Type II diabetes (insulin dependent) patients are unable to respond to insulin and can be managed with dietary change, exercise and medication. Type II diabetes is the most common type of diabetes, accounting for 90% of the diabetic population. Symptoms of both diabetic conditions include: I high blood sugar levels; (ii) unusual thirst; (iii) frequent urination; (iv) extreme hunger and weight loss; (v) blurred vision; (vi) nausea and vomiting; (vii) extreme weakness and tiredness; and (viii) irritability and mood changes.

Diabetes complications appear to be complex, but the biochemical process of advanced glycation has been proposed to play a critical role in these illnesses, which is accelerated in diabetes due to persistent hyperglycemia and increased oxidative stress.

AGEs accumulate within the various organs damaged by diabetes, with hyperglycemia hastening their accumulation. Because of the intermolecular collagen cross-linking caused by AGEs, there is less arterial and myocardial compliance as well as increased vascular stiffness, which may explain some of the rise in diastolic dysfunction and systolic hypertension seen in diabetics. AGEs build up in the kidney, retina, and atherosclerotic plaques, all of which are associated with diabetes complications." The long-term effects of prior glycemic control on the development of vascular complications have been linked to AGEs.

E. Diabetic Nephropathy

The kidney is a target for AGE-mediated damage and a contributor to the high levels of circulating AGE seen in diabetes because it is the primary site of AGE clearance. Diabetic animals have significantly higher levels of renal AGEs, which have been linked to a variety of diabetic nephropathy structural features such as glomerular basement membrane thickening, mesangial enlargement, glomerulosclerosis, and tubulointerstitial fibrosis. In murine models, AGE albumin administration caused changes similar to diabetic nephropathy, such as glomerular basement membrane thickening, mesangial matrix expansion, and increased collagen IV and TGF expression. Studies focusing on the AGE-RAGE pathway have provided the most convincing evidence for an AGE function in the development of diabetic nephropathy.

F. Accumulation of AGES In Diabetic Nephropathy

It is assumed that AGE accumulation is proportional to time-integrated blood glucose. Using fluorospectrometric analysis, Monnier et al. discovered that the glycation of skin collagen increased with the severity of diabetic complications and ageing, implying that the degree of collagen-linked fluorescence represents long-term glycemic control and that non-enzymatic glycation may be involved in the pathogenesis of diabetic complications (Monnier et al., 1986). Pentosidine, a fluorescent cross-link formed between arginine and lysine residues, is one of the AGE structures. Recent research has discovered pentosidine in diabetic patients' skin collagen, glomerular basement membrane, and plasma proteins (Dyer et al., 1993). We recently discovered that several factors, including the patient's age, renal function, and glucose control, influenced the plasma pentosidine level in diabetic patients [Sugiyama and Miyata, unpublished observation]. Renal function has been identified as the most critical factor among these. This advances our understanding of the pathogenesis and management of diabetic nephropathy. Increased plasma glucose levels in the early stages of diabetic nephropathy may hasten the formation of AGEs and cause AGE accumulation in renal matrix tissue. However, as renal function declines, the accumulation of AGEs may accelerate, resulting in the development of renal lesions at a faster rate. Attempts to control glucose levels may not help prevent AGE accumulation at this stage. As a result, it appears critical to normalize glucose and prevent AGE accumulation in matrix proteins prior to the development of renal failure.

G. Diabetic Retinopathy

AGEs have been found to be localized to retinal blood vessels in patients with type 2 diabetes and to correlate with the degree and clinical progression of retinopathy, according to studies. AGEs were measured in various ocular tissues and found to be higher in diabetic subjects when compared to non-diabetic control subjects. This includes vitreous collagen, where AGE levels have been linked to diabetic retinopathy. AGEs accumulation in diabetic retina has been observed in vascular cells, neurons, and glia, which may have pathogenic implications in individual cells and retinal function. AGEs have been found to be localized to retinal blood vessels in patients with type 2 diabetes and to correlate with the degree and clinical progression of retinopathy, according to studies. AGEs have been found to be localized in type 2 diabetes patients' retinal blood vessels and to correlate with the degree and clinical progression of retinopathy (Bucala and Vlassara 1995). AGEs were found to be higher in diabetic subjects when compared to non-diabetic control subjects in various ocular tissues. This includes vitreous collagen, which has been linked to diabetic retinopathy due to high AGE levels. In diabetic retina vascular cells, neurons, and glia, AGEs accumulation has been observed, which may have pathogenic implications in individual cells and retinal function (Murata et al., 1997). Diabetes causes morphological and functional changes in the retina, such as increased basement membrane thickness, pericyte loss, and permeability (Chappey O et al.,1997). Increased AGE accumulation may contribute to increased permeability of retinal endothelial cells, resulting in vascular leakage (Vlassara et al., 1994). Angiogenesis and neo vascularization are caused by thickening and coagulation of vessel walls, which leads to occlusion and ischemia, as well as the induction of growth factors such as vascular endothelial growth factor (VEGF). During diabetes, AGEs accumulate in retinal pericytes, affecting pericyte survival and function and eventually contributing to pericyte loss. Other characteristics observed in addition to pericyte loss include basement membrane thickening, hyperpermeability, and microaneurysm formation (Frank 1991).

Pericytes are important in the maintenance of microvascular homeostasis, and their loss may predispose the vessels to angiogenesis, thrombogenesis, and endothelial cell (EC) injury, leading to full-blown diabetic retinopathy clinical manifestations (Yamagishi and Imaizumi 2005). According to research, the AGE-RAGE interaction generates ROS in cultured retinal pericytes, resulting in apoptotic cell death. By activating nuclear factor-B (NF-B) and decreasing the Bcl-2/Bax ratio, AGEs increased the activity of caspase-3, an enzyme involved in the execution of pericyte apoptosis (Yamagishi et al., 2002). AGEs raise RAGE mRNA levels in pericytes by generating intracellular ROS (Yamagishi et al., 2002). The AGE signals were transduced by these positive feedback loops, resulting in pericyte dysfunction by increasing the cytotoxic effects of AGEs on retinal pericytes.

H. Diabetic Peripheral Neuropathy

Diabetes patients' peripheral nerves were found to have elevated AGE levels. AGEs have been shown in murine models to worsen diabetic neuropathy by lowering sensorimotor conduction velocity and decreasing blood flow to peripheral nerves. Protein modification and cross-linking by AGEs can cause significant changes in their structural and functional properties. AGEs, unlike Amadori products, are formed irreversibly. As a result, they may accumulate in vivo on long-lived proteins such as nerve myelin. Protein AGE content is influenced by factors such as AGE chemical stability and protein substrate turnover.The minimal modification of proteins found in vivo has three major effects on protein structure and function: (i) decreased functional polymerization of tubulin and actin to form cytoskeleton microtubules and microfilaments, (ii) decreased AGE-modified protein solubility and susceptibility to enzymatic digestion; and (iii) binding to cell surface receptors.It is unclear whether the glycation of tubulin and actin in diabetes results in a significant deficit of the microtubular and microfilament cytoskeleton (Cullum et al., 1991; Williams et al., 2001; Mclean et al., 1992). Sugimoto and colleagues (2001) discovered AGEs in peripheral neurons and endoneurial cells as irregular aggregates. This could be due to AGE-mediated protein cross-linking as well as a change in protein surface charge and hydrophobicity caused by AGE modification (Westwood and Thornalley 1995).

I. Diabetic Cardiomyopathy And Peripheral Arterial Disease(Pad)

Diabetes patients are more likely to develop cardiomyopathy and heart failure than nondiabetic subjects. Furthermore, collagen cross-linking has been linked to the development of diabetic cardiomyopathy and PAD. In people with type 1 diabetes, there is a positive relationship between serum AGE levels and the isovolumetric relaxation time, a parameter measured on echocardiography to assess heart function. Increased blood levels of the fluorescent AGE pentosidine have also been linked to increased carotid intima media wall thickness and arterial stiffening. Levels of pentosidine and malondialdehyde (indicators of lipid peroxidation) have also been found to be higher in diabetic patients with PAD.

J. Atherosclerotic Disease

AGEs are likely to contribute to atherosclerosis in a variety of ways, including promoting plaque destabilization by reducing LDL uptake, promoting plaque destabilization via effects on matrix metalloproteinases, inducing neointimal proliferation, and inhibiting vascular repair in response to injury. Serum AGE levels in patients with type 2 diabetes and coronary heart disease have increased.

K. Natural Agents That Reduce/Inhibit AGE Formation

The main mechanisms that prevent AGE formation are “the reduction of active dicarbonyl compounds, inhibition of ROS formation, protein structure protection, and AGE degradation”. Some chemically synthesized drugs are effective. “The first drug with clinical therapeutic potential in this area, aminoguanidine, for example, inhibits the formation of AGEs by trapping the carbonyl group of Amadori products via nucleophilic addition reactions and inhibiting further rearrangement. Aminoguanidine can also bind to active-dicarbonyl intermediates to form triazines, preventing the conversion of Amadori products to AGEsHowever, serious side effects such as pernicious anaemia, gastrointestinal symptoms, lupus, influenza-like syndrome, vasculitis, and oxidative stress limit its clinical applications (Schalkwijk 2012). "ALT-711, Alt-462, Alt-486, and Alt-TRC4186 are synthetic AGE breakers capable of breaking carbon-carbon bonds between carbonyl groups and thus removing cross-linked products." Angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, metformin, and other hypoglycemic drugs can also inhibit AGEs (Luevano and Contreras 2010). All of the compounds mentioned above, however, will inevitably cause adverse reactions and cannot be used indefinitely.” “In terms of safety, the most promising research direction is the search for natural compounds that inhibit AGE formation while exhibiting good activity and safety. Several natural compounds with antioxidant properties currently show good inhibitory activity against AGE formation and appear to have minimal toxic effects. There has been an increase in interest in screening natural substances for novel glycation inhibitors in recent years.”

"Natural compounds" are chemical substances derived from plants or animals that have distinct pharmacological effects. “Natural compounds that may inhibit the formation of AGEs are classified into six classes based on their structural properties:” “polyphenols, polysaccharides, terpenoids, vitamins, alkaloids, and peptides.”

L. Mechanism Of Inhibition Of Ages By Natural Compounds

Because natural compounds have a vast variety of structures and functions, the processes by which they suppress AGE development are diverse as well. “In summary, current research suggests that the mechanisms used to inhibit AGE formation fall into seven categories: “covering protein glycation sites, scavenging oxidative free radicals, regulating AGE receptors, trapping active dicarbonyl compounds, chelating metal ions, inhibiting aldose reductase, and lowering blood glucose levels”(Song etal., 2021). “The primary mechanisms are the reduction of active carbonyl compounds and the scavenging of oxidative free radicals.”

M. Current Management Of Diabetes Mellitus

To treat post-prandial hyperglycemia, agents such as “Voglibose, acrabose, and miglitol are used. These agents reduce glucose absorption by cells and thus manage diabetes at the digestive level. Different therapies used to manage diabetes mellitus have limitations in that they are not cost effective and can cause side effects such as liver toxicity, weight gain, gastrointestinal disturbances, and hypoglycemia. Metformin, a biguanide, is used to boost glucose uptake by peripheral cells. Sulphonylureas, such as glibenclamide, are insulinotropic and act as a secretagogue for pancreatic cells (Joseph and Jini2011).” Though the pathophysiology of diabetes is not completely understood, experimental evidence suggests that free radicals play a role in the pathogenesis of diabetes, and more importantly, in the development of diabetic complications (Oberlay 1988). “Free radicals can cause the degradation of biological components such as DNA, proteins, and lipids, leading to changes in physiological activity. Numerous recent studies indicate that antioxidants capable of neutralizing free radicals are advantageous inavoiding experimentally induced diabetes and reducing the severity of diabetic effects in animal models (Kubish et al., 1997).”

N. Dietary Management Of Diabetes Mellitus

Diabetes dietary management aims to achieve the “best blood lipid concentrations, adequate energy for a healthy weight, normal growth and development during pregnancy and lactation, avoid diabetic complications, and improve health by consuming a well-balanced diet (Asif 2014). Diabetes mellitus is the fourth or fifth leading cause of death in most developed countries, and it can reach epidemic proportions in most developing and newly industrialised countries. Because of their traditional way of life, very few rural people have type 2 diabetes mellitus (Steyn et al., 2004).” Low calorie intake and weight loss are essential for good glycemic control. The appropriate carbohydrate, fat, and protein ratio to consume is uncertain (Ajala et al., 2016).Medical nutrition therapy is important for preventing, managing, or slowing the development of diabetes complications (Bantle 2008). In the case of type 1 diabetes mellitus, the role of diet is doubled; it aids in keeping blood glucose levels nearly constant and reduces long-term complications. “When it comes to diabetes management through diet, the principles are the same for both type 2 and type 1 diabetes mellitus (Bastaki 2005).For the treatment of diabetes, medicinal plants are being researched once more. Many pharmaceuticals have been developed from prototypic molecules found in medicinal plants. Metformin is an example of an effective oral glucose-lowering agent. The usage of Galega officinalis to treat diabetes prompted its development. Galega officinalis has a lot of guanidine, which is a hypoglycemic compound. Because guanidine is too toxic for therapeutic usage, the alkyl biguanides synthalin A and synthalin B were launched in Europe as oral anti-diabetic medicines in the 1920s but were phased out after insulin became widely available. However, metformin was developed as a result of experience with guanidine and biguanides. To far, over 400 traditional plant remedies for diabetes have been recorded, but only a limited number have been scientifically and medically tested to determine their efficacy.

Some herbal extracts have been shown to have a hypoglycemic effect in human and animal models of type 2 diabetes. The World Health Organization's Diabetes Expert Committee has recommended that traditional medicinal herbs be studied further. Herbal medicine cannot be fully incorporated into contemporary medical practises since there is a lack of scientific and clinical evidence supporting its safety and efficacy.

Clinical research on herbal medications is essential, as is the development of simple bioassays for biological standardisation, pharmacological and toxicological evaluation, and the creation of numerous animal models for toxicity and safety assessment. The active component(s) of these plant extracts must also be identified.”

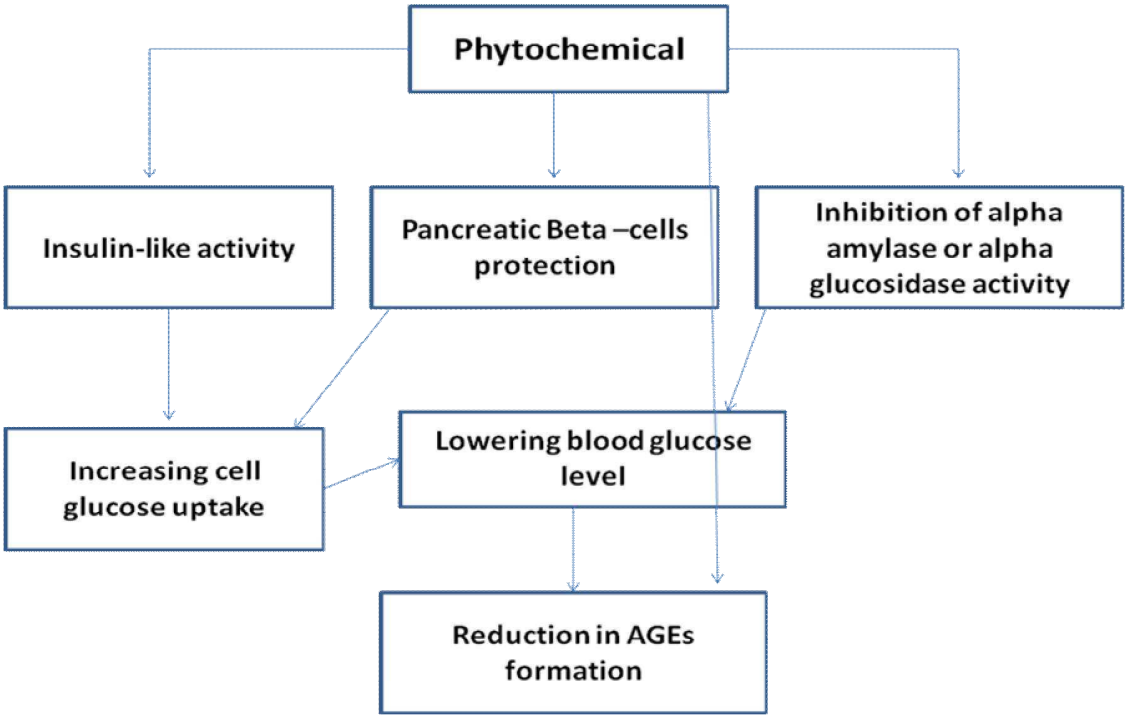

Figure 3. Phytochemicals role in inhibition of AGEs formation by lowering glucose levels

Table 1. Medicinal plants of India with anti-glycation activity and related beneficial properties

Plant Name	Family	Ayurvedic/common name	Active constituents	Part used	Anti-glycation properties	References
Derris indica	Fabaceae	Pongame Oiltree	Flavonoids, sterols	Bark	Pyranoflavonoid isolated from the plant has inhibitory effects on the AGEs	Pornpat et al. 2014
Bruguiera sexangula	Rhizophoraceae	Upriver orange mangrove	Phenolics, steroids, alkaloids, tannins,Saponins	Bark	good inhibitory potential to glycation reaction at in vitro conditions	Hogg et al. 1984
Azadirachta indica	Meliaceae	Neem Tree	"Nimbocinone, nimolinone, nimocinolides, isonimocinolide, nimbin, salanin, azadirachtin, flavonoids, quercetin"	Leaf	1-The primary mechanism for protein glycation inhibition was AI's capacity to react with carbonyls. 2-Under diabetic circumstances,AI reduced oxidative stress.	Gutierrez 2014

Emblica officinalis	Phyllanthaceae	Amla, Indian gooseberry	Gallic acid, tannins, minerals, flavonoids	Fruit	Inhibited album,in AGEs formation	(Nampoothiri et al. 2011)
Terminalia bellerica	Combretaceae	Belliric myrobalan, bahera	Flavonoid, glycosides, stilbenoids, ellagitannins, proanthocyanidins	Fruit	Inhibited albumin and insulin glycation	(Kasabri et al. 2010)
Syzygiumcumini	Myrtaceae	Jambolan, Indian blackberry, Jamun, jambul	Phenolic compounds, such as Kaempferol 7-O-methylether and sterols.	Seed	Potent antiglycation activity	Perera et al., 2014
Cinnamomum zeylanicum	Lauraceae	Cinnamon	procyandin B2, epicatechin, catechin, proanthocyanidin oligomers and polymers	Bark	Capabilities of reactive carbonyl compounds such as methylglyoxal in trapping (MGO)	Xiaofang et al., 2008

Table 2: Medicinal properties and chemical constituents of true mangroves

Name of Mangrove Plant	Family	Genus	Part used as medicine	Common Name	Chemical Constituents	Medicinal properties	References
Acanthus ilicifolius	Acanthaceae,	Acanthus	Bark, Fruits, Leaves, Roots	Sea holly, holly mangrove	Steroids, Alkaloids, long chain alcohols,sulphur, triterpenes, saponins, Flavonoids	Analgesic, anti-inflammatory, blood purifier, antidiabetic, antiviral, Regeneration of β-cells of pancreas,etc	Jongsuvat et al. (1981)
Aegiceras corniculatum	Myrsinaceae	Aegiceras	Bark, Leaves and Stem	Black mangrove	Flavonoids, tannins, saponins, polyphenols	Utilization of glucose; either by direct stimulation of glucose uptake or via the mediation of enhanced insulin secretion; Antidiabetic, asthma,	Roome et al. (2008)

						antiviral	

Avicennia africana	Acanthaceae	Avicennia L	Bark	janju	Naphthoquinones,	Anticancer, antiulcers	Ito C et al. (2000)
B. cylindrica	Rhizophoraceae	Bruguiera	Fruits, Roots Leaves	blume	flavonoids, phenolic acids, alkaloids, tannins anthocyanins	Stimulation of β-cells to release more insulin	Krishnamoorthy et al. (2011)
Bruguiera sexangula	Rhizophoraceae	Bruguiera	Bark	Upriver orange mangrove	Phenolics, steroids, alkaloids, tannins	Anticancer, antidiabetic	Hogg RW et al. (1984)
Ceriops decandra	Rhizophoraceae	Ceriops	Bark, Fruits and Leaves	Ceriopsdecandra	Polyphenols, tannin, triterpenes	Stimulation β-cells to release more insulin; The increased hexokinase activity may result in increased glycolysis and increased utilization of glucose for energy production.	Nabeel et al. (2010)
Ceriops roxburghiana	Rhizophoraceae	Ceriops	Whole plant		Gibberellins, Procyanidins	Antiucler, antidiabetic	Seshadri TR (1959)
Dalbergia ecastophyllum	Fabaceae	Dalbergia	Bark	Coinvine	Chalcones, steroidisoflavanoids	Antidiabetic	Donnelly DMX (1973)
Excoecaria agallocha	Euphorbiaceae	Excoecaria	Whole plant	Blind your eyes, milky mangrove	Flavonoids, tannins, saponins, polyphenols phorbol	Pancreatic secretion of insulin; ↑ Uptake of glucose.	Thirumurugan G (2009)
Heritiera littoralis	Malvaceae	Heritiera	Stem, Bark, Fruits and Leaves	Dungun, Looking glass tree	Alkaloids, tannins, polyphenols, saponins	Treatment for diarrhea, antifungal	Saxena H (1975)
Heritiera fomes	Sterculiaceae	Heritiera	Whole plant	Sunderi	Tannins, terpenoids,	Enhancement of pancreatic	Ali M (2011)

					flavonoids,saponins, alkaloids, phytosterols	secretion of insulin; ↑ The glucose uptake; Inhibition in glucose absorption in gut	
Intsia bijuga	Fabaceae	Intsia	Bark	Merbau Ipil, Borneo teak	Stilbenes, polyphenols	antiulcer	Rollet B (1981)

Kandelia candel	Rhizophoraceae	Kandelia	Whole plants	Pisang pisang	Alkaloids, tannins, saponins, polyphenols	Antidiabetic	Rollet B (1981)
Oncosperma tigillarium	Arecaceae	Oncosperma	Flowers	Nibung	Sterols	Antispasmodic	Rollet B (1981)
Rhizophora mucronata	Rhizophoraceae	Rhizophora	Bark, Fruits, Flower, Root Leaves	Red mangrove	Alkaloids, tannins, gibberellins, Inositol saponins, lipids	Antiviral (antiHIV), antiulcers	Richer A (1990)
Rhizophora apiculata	Rhizophoraceae	Rhizophora	Bark, Flowers, Fruits and Leaves	Bakau Minyak	Tannin, steroids, triterpenes, phenolic compounds	Improved level of insulin secretion and its action; Insulin mimetic activity β-Cell protection	Lakshmi V (2006)
Rhizophora racemosa	Rhizophoraceae	Rhizophora	Flowers and Leaves	tukaump	Tannins, steroids	Antiviral, antidiabetic	Padmakumar K (1997)
Salicornia brachiata	Amaranthaceae	Salicornia	Stems and Leaves	Pickle grass	Steroids, triterpenes	Antiviral, antidiabetic, toothache	Padmakumar K (1993)
Sonneratia caseolaris	Lythraceae	Sonneratia	Fruits	Crab apple Mangrove	Steroids, glycosides	Intestinal α-glucosidase inhibitory activity; Potentiation pancreatic	Hasan et al. (2013)

						secretion of insulin	
Sonneratia alba	Lythraceae	Sonneratia	Fruits	Mangrove Apple	Tanins, phenolic compunds	Modifying glucose utilization	Morada et al. (2011)
Thespesia populnea	Malvaceae	Thespesia	Bark, Stem	Portia tree	Glycoside, gossypol, mansonones, sterols triterpenes, quinones	Antibacterial and antisteroidgenic	Rollet B (1981)
Xylocarpus granatum	Meliaceae	Xylocarpus	Bark	Cannonball mangrove	Alkaloids, steroids, tannins, triterpenes, Limonoids	Treat fever, malaria, cholera and antidiabetic	Srivastava et al. (2011)
Xylocarpus moluccensis	Meliaceae	Xylocarpus	Bark, Fruits	Nyireh batu	Alkaloids, steroids, tannins, triterpenes, proanthocyanidins	Treat fever, malaria, naphrodisiac and antidiabetic	Srivastava et al. (2014)

There are 84 species of mangrove plant worldwide, divided into "24 genera and 16 families. Seventy of them are true mangroves belonging to sixteen genera and eleven families, while fourteen are semi mangroves belonging to eight genera and five families. According to Wu et al., the family Rhizophoraceae is a true mangrove family with 21 species divided into four genera. The Rhizophoraceae family includes seven Bruguiera species, five Ceriops species, two Kandelia species, and two Rhizophora species (ten species)."

"B. cylindrica, B. exarista, B. gymnorrhiza, B. hainessi, B. parviflora, B. sexangula, and B. sexangula var rhynchopetala are all species derived from B. sexangula. This genus' metabolic pattern has been extensively characterised by a suite of diterpenes and triterpenes. Furthermore, these species generate flavonoids, tropane derivatives, and cyclic polysulphides. So far, 22 B. cylindrica metabolites, 54 B. gymnorrhiza metabolites, nine B. exaristata metabolites, six B. parviflora metabolites, two B. sexangula metabolites, and 40 B. sexangula var rhynchopetala metabolites have been identified. This genus has been linked to 114 different metabolites."

III. OBJECTIVES

1) To study the process of glycation using *in vitro* techniques.
2) To evaluate the anti-glycation potential of aqueous bark extracts of *Bruguiera sexangula* using *in vitro* methods.
3) To isolate bioactive phytocompounds.

IV. MATERIALS AND METHODS

A. *Collection Of Mangrove Plant*

The bark of the mangrove plant *Brugeira sexangula* is obtained from the coastal parts of the Kendrapara district of Odisha's mangrove forest. The bark was dried in the shade and powderedbefore being extracted with distilled water.

Figure 4: Plant of B. sexangula

B. Extract Preparation

The maceration technique is being carried out for 72 hours in a glass jar in room temperature in aqueous solution by the help of a magnetic stirrer at a concentration of 30gm/500 ml distilled water and then the solvent was filtered through a filter paper. This process was repeated five times. After that it is kept inside the hot air oven until the sample is completely dry so that it can be extracted in its powdered form and stored for further studies in favourable conditions. In terms of dried starting material, the extract yielded 20% (w/v).

Figure 5: Preparation of B. sexangula plant extracts

C. Recovery Of Sample Extract

After filtering through the filter paper, the sample is allowed to pass through a rotary evaporator which functions according to the maintained temperature and pressure. So that the sample will get condensed to a thicker form from which the plant extracts can be recovered easily after drying it in the hot air oven.

Figure 6: A Rotary evaporator

D. Glycation Sample Preparation

"The Glycated samples were prepared by incubating 1ml of BSA(10mg/ml), 1ml of fructose(250mM) in 1 ml of potassium phosphate buffer(200mM) along with aqueous plant extracts(1ml) in one test tube,the standard compound "quercetin" in the second test tube and the positive control in the third test tube and it is incubated at 60°for 4 weeks. And all the incubations were carried out in triplicates and it is ensured that all the samples were free from microbial contamination."

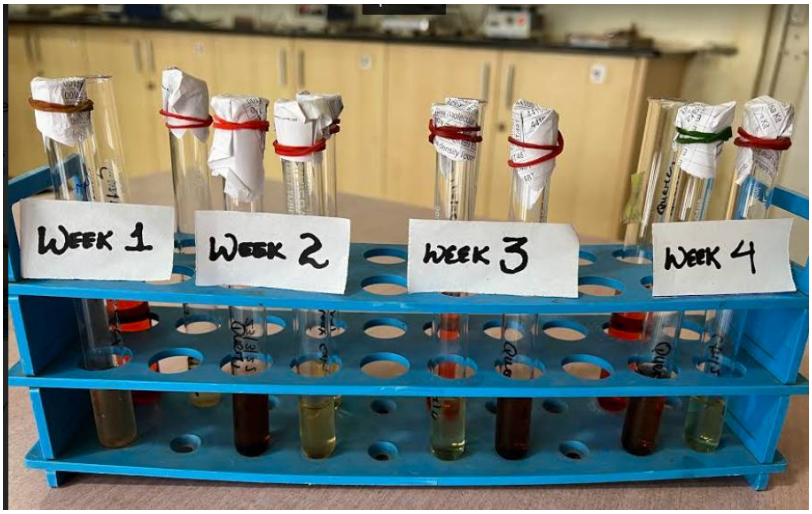

Figure 7:All Samples are in triplicates

E. Anti-Glycation Study

Several assays were carried out to evaluate the anti-glycation potential of the plant extract B. sexangula and the analysis is done on a weekly basis for a time period of 4 weeks for each assay.

F. Congo Red Assay

"500 µL of glycated samples and a positive control were incubated for 20 min at room temperature with 100µL of congo red in 10 ml PBS. In a UV-Vis spectrophotometer, the samples that had been incubated with congo red had their absorbance measured at 530 nm. The results were calculated as a percentage of inhibition using the formula: "Inhibition percent =[(A0−A1)/A0]×100, where Ao is the absorbance at 530 nm of the positive control and A1 is the absorbance at 530 nm" of the samples of glycated albumin that were co-incubated with plant extracts."

G. Estimation of Fructosamines

"The Nitroblue tetrazolium assay was used to determine the level of fructosamines." "40 µL of glycated samples and positive control were added to the 0.8ml of nitroblue tetrazolium and it is incubated at 37 degree Celsius for 30 minutes. After incubation, absorbance at 530nm was measured using a (UV-Vis spectrophotometer)". The following equation was used to determine how much of a percentage plant extract inhibited fructosamine absorption: Inhibition percent is equal to " [(A0−A1)/A0]×100 where Ao is the absorbance at 530 nm of positive control and A1 is the absorbance at 530 nm "of the glycated albumin samples co-incubated with plant extracts.

H. Thiol Estimation

DTNB was used to calculate the thiol groups in glycated albumin samples. In a brief, 210 µL of the reaction mixture were incubated at room temperature for 15 minutes with 390 µL of 5 mili molar DTNB (in 0.1 M PBS). A UV-Visible spectrophotometer was then used to measure absorbance at 412 nm.

I. Bioactivity Guided Isolation By Silica Gel Column Chromatography

5gm of plant sample was taken and it is washed with 3 chemical compounds-Toluene, Dichloromethane and methanol, each one at a time, with the help of a magnetic stirrer based on their polarity gradient and each one of the solutions was filtered through a filter paper. Then the column was set up for the chromatography. Silica gel of 60-120 mesh size was used as the adsorbent or the stationary phase for the chromatography. And then the filtered plant sample was loaded and then the mobile phase was allowed to pass through the column. 3 different chemicals (chloroform, Petroleum benzine and methanol) were passed through the column in varying proportions (100%, 25%-75%, 50%-50%, 75%-25%) (at a time two of the chemicals were allowed to pass) and the chromatography was carried out to collect the eluted molecules. After that the active plant compounds were determined by carrying out the LC-MS technique and further in silico approach can be done to find new drugs to prevent the formation of AGEs.

Figure 8: Column chromatography set up

V. RESULTS

A. Congo Red Assay

The present study showed aqueous bark extract of B. sexangula at 1mg/ml concentration could inhibit amyloid cross beta aggregation from the 0th week itself followed by the first week. The same was also observed for the 3rd and the 4thweek.However, no inhibition was observed in the second week. On the other hand, the standard anti-oxidant compound. Quercetin could inhibit the amyloid beta aggregation in "week 0, week 1, week 2, week 3" and the maximum inhibition was observed in the 4th week for the congo red assay.

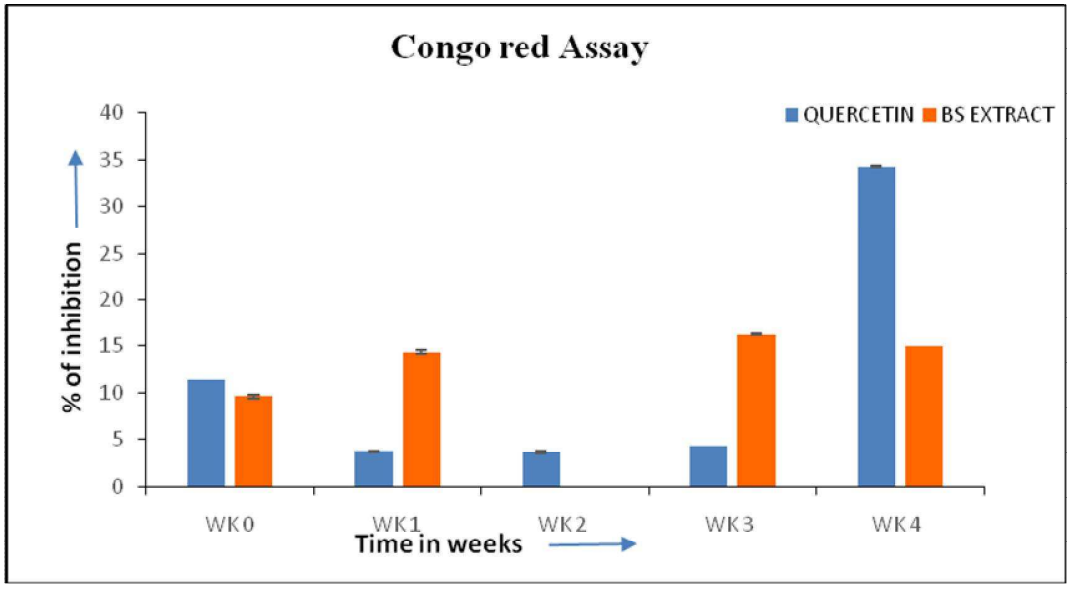

Figure 9:Graphical representation of congo red assay results

Table 3: Results for 4 weeks

Sample	WEEK 0 (%inhibition)	WEEK 1 (% inhibition)	WEEK 2 (% inhibition)	WEEK 3 (% inhibition)	WEEK 4 (% inhibition)
Quercetin	11.33 ± 0.004	3.70 ± 0.110	3.64 ± 0.100	4.31 ± 0.01	34.25 ± 0.037
Extract	9.6 ± 0.198	14.37 ±0.151	0	16.25 ± 0.04	14.93 ± 0.002

B. Fructosamine Inhibition Assay

The present study showed aqueous bark extract of *Bruguiera sexangula* 1mg/ml concentration could inhibit the Amadori product formation in the 0th week. But no inhibition percentage was observed for the 1st, 2nd and the 3rd week. The 4th week recorded the highest inhibition percentage. On the other hand, the standard anti-oxidant compound Quercetin could inhibit the Amadori product formation in week 1 and week 3 slightly and the maximum inhibition was observed in the 4th week for the fructosamine assay. But no such inhibition was found for week 0 and week 2 respectively.

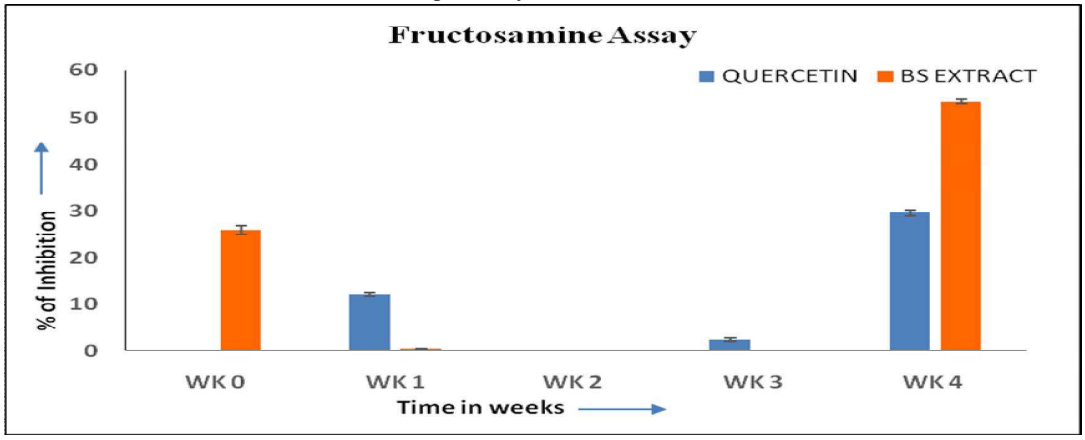

Figure 10: Graphical representation of fructosamine assay results

Table 4: Results for 4 weeks

Sample	WEEK 0 (% inhibition)	WEEK 1 (% inhibition)	WEEK2 (% inhibition)	WEEK 3 (% inhibition)	WEEK 4 (% inhibition)
Quercetin	0	12.15±0.296	0	2.48±0.355	29.63±0.698
Extract	25.92±0.969	0.47±0.052	0	0	53.44±0.464

C. Protein Thiol Assay

In the current study, the effect of B. sexangula plant extracts on thiol group modification was investigated. The free thiols in the positive control samples were significantly reduced, and the plant extracts showed significant protection against thiol oxidation in all four weeks. Results indicated that thiol group protection with the plant extract was the highest in the 3rd week. However, all other weeks also showed significant inhibitory results with extracts. Similarly, the compound quercetin could also protect the thiols from denaturation in all the above-mentioned weeks.

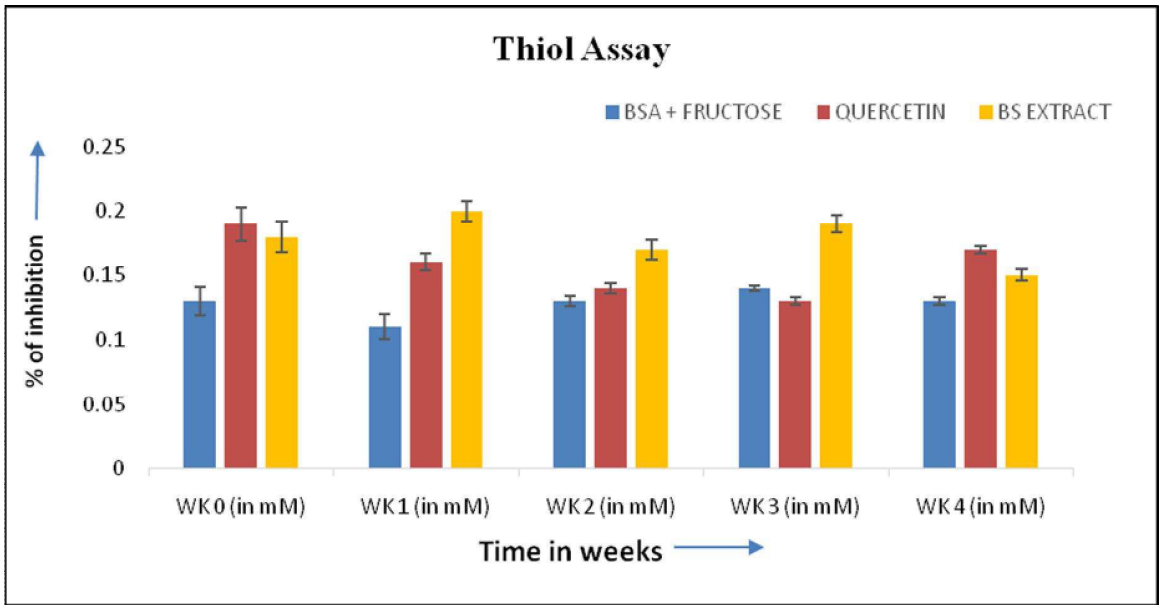

Figure 11: Graphical representation of Protein Thiol Assay

Table 5: Results for 4 weeks

Sample	WEEK 0 (nM of Thiol)	WEEK 1 (nM of Thiol)	WEEK2 (nM of Thiol)	WEEK 3 (nM of Thiol)	WEEK 4 (nM of Thiol)
BSA + Fructose	0.13 ± 0.011	0.11±0.010	0.13±0.004	0.14± 0.002	0.13±0.003
Quercetin	0.19 ± 0.013	0.16±0.007	0.14±0.004	0.13±0.003	0.17±0.003
BS Extract	0.18 ± 0.012	0.2±0.008	0.17±0.008	0.19±0.007	0.15±0.004

1) Isolation Of Phyto-Compounds

After the column chromatography is carried out, the phytocompounds are isolated in different proportions and stored in the fridge for further assays. After that the active plant compounds present in the sample were determined by carrying out the LC-MS technique and further in silico approach can be done to develop new drugs that inhibit AGE formation

VI. DISCUSSION

Protein glycation and the formation of advanced glycation end products (AGEs) are important in the pathogenesis of diabetic complications such as "retinopathy, nephropathy, neuropathy, and cardiomyopathy, as well as other diseases such as rheumatoid arthritis, osteoporosis, and aging. Protein glycation disrupts normal functions by changing molecular conformation, altering enzymatic activity, and interfering with receptor function. AGEs form intra- and extracellular crosslinks not only with proteins, but also with other endogenous key molecules such as lipids and nucleic acids, which contributes to the development of diabetic complications.

Furthermore, serum-advanced glycation end product levels reflect the severity of these problems, whereas treatment therapies aiming at lowering AGE can block or postpone their progression (Rahbar and Figarola, 2003)."

"As a result of their formation from glucose-derived dicarbonyl precursors, AGEs frequently accumulate intracellularly (Brownlee 2001). These intracellular AGEs play critical roles as stimuli for activating intracellular signaling pathways and modifying intracellular protein function (Brownlee 1995). Denaturation and functional decline of the target protein and lipid, organopathy due to AGE accumulation in tissue, activation of receptor mediated signal pathway in cells, generation of oxidative stress and carbonyl stress are some of the mechanisms by which glycation alters cell functions. According to recent research, AGEs interact with plasma membrane-localized AGE receptors (RAGE) to alter intracellular signaling, gene expression, and the release of pro-inflammatory molecules and free radicals."

"Inhibition of protein glycation is a complex process and glycation inhibitors may act by various mechanisms at different steps that can delay or prevent the glycation process, such as: i) at an early stage scavenging hydroxyl radicals and superoxide radicals and reducing the generation of reactive carbonyl or dicarbonyl groups, 2) during the glycation process, blocking the carbonyl or dicarbonyl groups in reducing sugars, Schiff bases or Amadori products, 3) inhibiting the formation of late stage Amadori products, 4) breaking the cross-linking structures in the formed AGEs and 5) AGEs receptor (RAGE) blocking (Wu et al., 2011)."

"Many plants have been used for centuries as a rich source of potent anti-diabetic drugs, and these herbal preparations are thought to be free of side effects. Patients are increasingly interested in using natural products with anti-diabetic activity. Plant remedies have recently gained popularity. More than 400 plants and their secondary metabolites, such as glycosides, alkaloids, terpenoids, flavonoids, carotenoids, tannins, and polyphenolic derivatives, are thought to be used in the treatment of diabetes mellitus around the world. (Bailey and Day 1989).

In recent years, mangroves and associated plants have provided a variety of therapeutic applications, the majority of which have yet to be explored. Various parts of mangrove plants are said to be high in flavonoids, which have anti-inflammatory and antioxidant properties. The pharmaceutical properties of mangrove trees provide a wide domain for medical use , requiring further studies for possible drug development."

However, very little study has been conducted on the anti-glycation potential of mangrove plants and their impact on glycation-related problems. According to reports from around the world, there are six species in this genus. In India, four species of the genus Bruguiera have been identified: "B. gymnorrhiza, B. cylindrica, B. parviflora, and B. sexangula. Bark part of B. sexangula was extracted with aqueous solvent which displayed anti -glycative effect in conducting several assays. Treatment with aqueous bark extracts(1mg/ml) for 4 weeks reported significant reduction in protein glycation level. The potent anti-diabetic and anti-glycation effect of the plant extract suggests the presence of various potent anti-diabetic and anti-glycative active compounds, which produced an anti-hyperglycemic effect."

Based on this background, a series of experiments were carried out in the current study to fully elucidate the effect of B. sexangula plant extracts at physiologically relevant concentrations on albumin glycation, structural modifications, and cellular effects. Glycation is a key mechanism for inducing protein conformational changes by increasing the level of amyloid cross β-structure,which is essential for protein aggregation. The Congo red binding assay can be used to estimate the degree of protein secondary structure modification. Congo red binds to protein -sheet structures with strong affinity and exhibits unique absorbance at 530 nm following binding. The dye is attracted to hydrophobic clefts between anti-parallel -strands. Using the amyloid marker Congo Red, the ability of plant extracts to retard aggregation of glycated albumin, which leads to amyloidosis, was investigated. The presence of B. sexangula resulted in significant inhibition of the amyloid marker. Our findings show that plant extracts can reduce the level of amyloid cross-structure in albumin. This beneficial effect of B. sexangula should be investigated further in order to determine the mode of inhibition.

The NBT method is based on the ability of fructosamines in alkaline solution to reduce. Amadori rearrangement products, such as fructosamines, have reducing activities with NBT under alkaline conditions and form compounds that emit absorbance at 530nm (Armbruster 1987).

B.sexangula presence was found to inhibit the fructosamine or Amadori product formation in the initial and the final weeks of the assay.

The thiol assay is used to determine the amount or concentration of free thiol groups in a sample. It is carried out with the help of Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid) or DTNB. Thiols react with DTNB to give 2-nitro-5-thiobenzoate(TNB), a yellow coloured compound which exhibits specific absorbance at 412nm. So it was observed that with respect to the compound quercetin, B.sexangula extract was capable of protecting the thiol structure from getting oxidized from the initial weeks to the final weeks of our study.

In recent decades, there has been an increase in conservation research on mangrove plants, particularly from India. Mangrove species from around the world have been studied for their medicinal properties and bioactive potential in the treatment of diseases such as cancer, rheumatism, free radical scavenging, anti-inflammatory, antinociceptive, painful arthritis, inflammation, asthma, antioxidant, diabetes, and as hepatoprotective agents (Arora et al., 2014). However, Indian scientific communities' research on developing drug derivatives from mangroves is very limited. Based on our data compilation on anti-glycative research, we believe that mangrove research is critical because there are many potentially medically significant compounds that have been reported from various regions, but very little work has been done from the Indian coast, specifically on phytochemical speciation.

In order to find more effective and less toxic anti-diabetic drugs, new anti-glycation moieties derived from bioactive compounds must be developed. As a result, additional in vitro and in vivo animal testing, followed by toxicity and clinical tests, should be conducted on the potential of mangrove plants with isolated active compounds that have shown anti-glycation activity. This could provide a promising compound to be optimized and used in the development of new anti-diabetic and anti-glycation compounds.

VII. CONCLUSION

In a nutshell, mangrove plants have a high potential to address diabetes due to their distinct chemical structures. In recent years, the majority of pharmaceutical industries have focused primarily on the development of new drugs for diabetes on a large scale. However, there is promising potential from alternative sources such as herbal medicines/traditional knowledge-based drugs that have multiple targets and have the potential to evolve as new drugs/complementary that requires serious consideration. Natural compounds and their molecular frameworks are currently important starting points for drug discovery. Numerous studies have found that many natural compounds, such as polyphenols, polysaccharides, terpenoids, vitamins, and alkaloids, are promising candidates for the development of new drugs to inhibit AGE formation. By scavenging free radicals, chelating metal ions, capturing active carbonyl compounds, covering protein glycation sites, and lowering blood glucose levels, these compounds prevent AGE formation. As a result, further research into natural compounds and their specific mechanisms of action is required and will be beneficial in inspiring drug discovery. However, the importance of discovering new drugs among natural compounds cannot be overstated, and safety and efficacy issues must be carefully considered.

The extract of the mangrove plant B.sexangula showed promising anti-glycation activity and could be used in clinical studies and drug development.

The aqueous extract of B.sexangula bark showed significant inhibitory activity on the formation of AGEs in the current study.

The present study showed aqueous bark extract of B.sexangula could inhibit amyloid cross beta aggregation in all the 4 weeks except the second week. The extract could inhibit the Amadori product formation only in the 0th week and the 4th week. The free thiols in the positive control samples were significantly reduced, and the plant extracts showed significant protection against thiol oxidation in all four weeks. Results of the present study demonstrated that B.sexangula plant extracts can efficiently inhibit the glycation process.

Furthermore, more phytochemicals with low content or significant biological activities should be discovered, and the synergistic effect of various compounds should be determined. When combined, B.sexangula extracts or isolates could be used as natural antioxidants and anti-diabetic agents. These findings could provide a solid foundation for the traditional uses of B.sexangula in human benefits.

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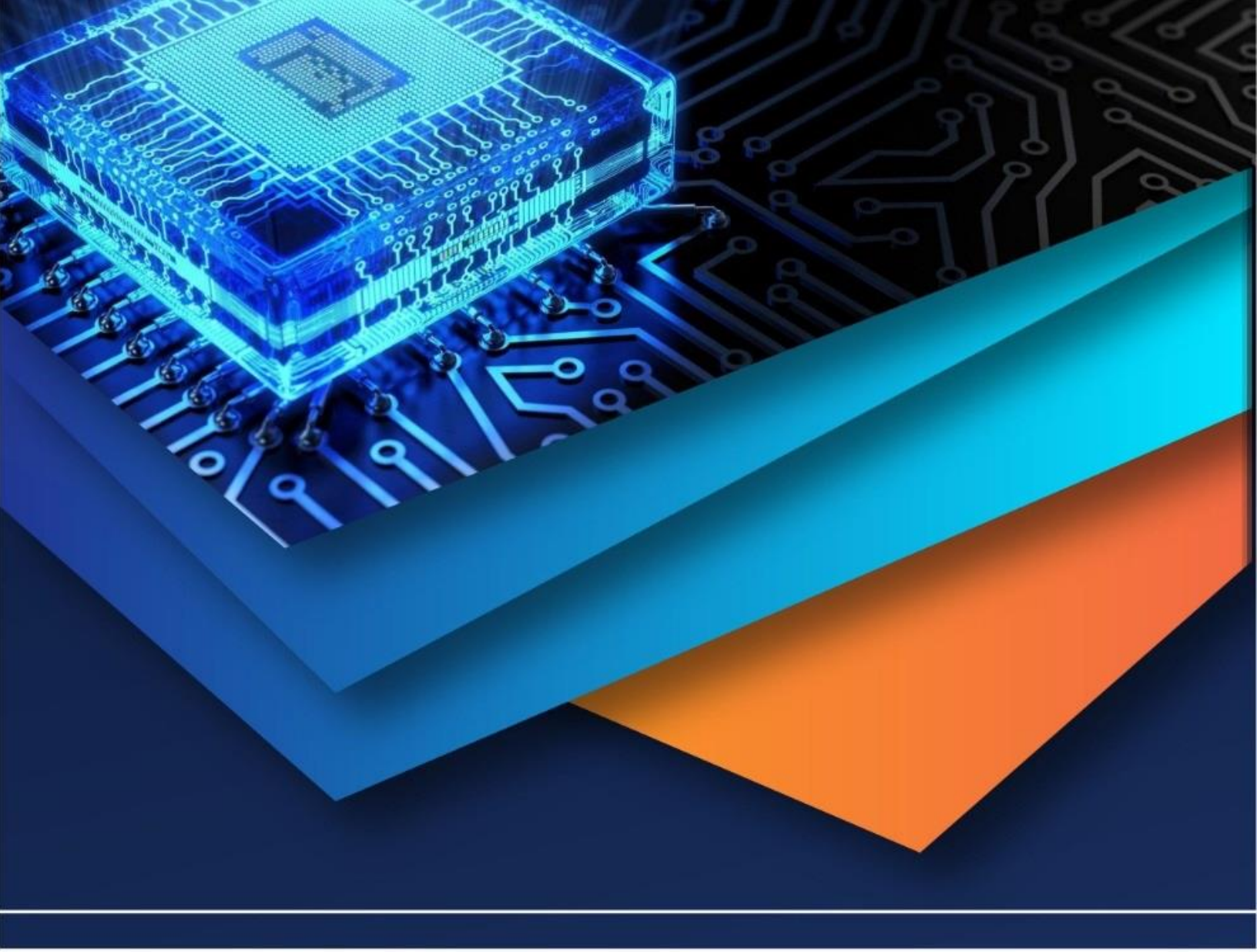

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