ANTIDIABETIC POTENTIAL OF SILVER NANOPARTICLES OF *GYMNEMA SYLVESTRE*

PREETHI.P

(20PBC017)

M. Sc Biochemistry

In Partial Fulfilment of the Requirement for the Degree of

Master of Science in Biochemistry

DEPARTMENT OF BIOCHEMISTRY,

BIOTECHNOLOGY AND BIOINFORMATICS

Avinashilingam Institute for Home Science and Higher Education for Women,

Coimbatore-641043

May 2022

CERTIFICATE

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Vasundhara
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Signature of the Guide

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Signature of the Head of the Department

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PREETHI. P

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INTRODUCTION

Diabetes is a group of metabolic disorder marked by hyperglycemia which is caused by several problems with insulin secretion, insulin action or both. Diabetes related chronic hyperglycemia is linked to long-term damage, dysfunction and failure of various organs such as eyes, kidneys, nerves, heart and blood vessels. Diabetes is caused by a number of different pathogenic mechanisms. In 2014, 8.5% of adults aged 18 years and older had diabetes. In 2019, diabetes was the direct cause of 1.5 million deaths and 48% of all deaths due to diabetes occurred before the age of 70 years (WHO., 2021).

Diabetes mellitus (DM) is a persistent hyperglycemic condition in patients with diabetes mellitus, chronic hyperglycemic in combination with other metabolic abnormalities, can damage various organ systems, leading to the development of disabling and life-threatening health complications, the most prominent of which are microvascular (retinopathy, nephropathy and neuropathy) and macrovascular complications (coronary heart disease, cardiomyopathy, arrhythmias and peripheral artery disease) which lead to a 2 to 4 fold increased risk of cardiovascular diseases (Goyal *et al*., 2021).

The classification of the diabetes mellitus was categorized into two major groups one is known as Juvenile-onset diabetes mellitus also known as type 1 diabetes mellitus and adult-onset diabetes mellitus or type 2 diabetes mellitus. This has expanded to include over 50 subcategories caused by distinct pathogenic mechanisms or accompanied by other diseases and syndromes (Genuth *et al.,* 2018).

Type 1 diabetes mellitus (T1DM) is an autoimmune disease in which insulin producing pancreatic beta cells are destroyed. Insulin is a vital anabolic hormone that regulates glucose, lipid, protein and mineral metabolism as well as growth. Insulin is important because it allows glucose to enter muscle and adipose cells, stimulates the liver to store glucose as glycogen and synthesize fatty acids, accelerates amino acid uptake, inhibits fat breakdown in adipose tissue and stimulates potassium uptake into cells (Lucier *et al*., 2021).

Type 2 diabetes or non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes is predominant than the type 1 diabetes, accounting for over 90% of all diabetes. At the outset of the condition, the patients are usually elderly and have very minor symptoms. Insulin levels are usually elevated, although they can also be normal or low. Obesity is quite widespread and losing weight helps to reduce hyperglycemia. The condition usually appears after the age of 40 (Galicia-Garcia *et al.*, 2020).

Free radicals are defined as atoms or molecules with at least one unpaired electron. They are very reactive because they contain unpaired electrons. They can give or receive electrons, making them either reductants or oxidants (Bo *et al.*, 2019). They participate in a variety of cellular processes, including redox system disruption, DNA damage and the activation of procarcinogens, all of which lead to cancer (Maddu, 2019). Free radicals are divided into two types reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS are oxygen-derived free radicals, while RNS are nitrogen-derived free radicals. Hydroxyl radicals (•OH), peroxyl radicals (ROO•), superoxide anion ($O^{\bullet-}_2$), alkoxyl radicals (RO•), singlet oxygen ($^1O_2$) and hydrogen peroxide are examples of ROS (Ramos and Muriel, 2019). Nitric oxide (NO•) is the major RNS involved in numerous free radical-related diseases. Excessive generation of these harmful free radicals in the body causes oxidative stress, which can result in a variety of ailments *(*Masuko *et al*., 2021).

Antioxidants are chemicals that protect the body from free radical damage. Antioxidants work by sharing their additional electrons with free radicals, preventing them from causing damage to biological components (Jamshidi *et al*., 2020). Enzymic and non-enzymic antioxidants are the two major kinds of antioxidants based on their nature. Catalase, glutathione peroxidase, superoxide dismutase and glutathione reductase are only a few examples of enzymic antioxidants. Flavonoids, ascorbic acid, carotene and α-tocopherol are the principal non-enzymatic antioxidants. The body maintains a healthy homeostasis by maintaining a balance between free radicals and antioxidants. (Anju *et al.*, 2019). Natural antioxidants can be found in a variety of plant parts, including barks, roots, leaves, nuts, seeds, vegetables and fruits. These plant phytochemicals or secondary metabolites operate as natural antioxidants (Glucin, 2020).

Herbal medications are studied as a component of complementary and alternative medicine (CAM) and is gaining popularity due to their potent antioxidant action, minimal side effects and

cost effectiveness (Ishmeet *et al.*, 2018). Effective ideas derived from natural sources have aided in the development of new medical plant medications. Despite the fact that antioxidant properties of phytochemicals have been the focus for many years. Antioxidant effects on cell signaling and gene expression have also been identified as significant (Lee *et al*., 2017).

Nanotechnology, a developing field of nanoscience which involves the creation, manipulation and application of the nano sized particles in the field of nanomedicine. It works with particles ranging from 1 to 100 nanometers in size. These nanoparticles are referred to as "intelligent" or "smart" materials because of their high utilization in the preparation of nanocarriers, which are essential in the controlled drug delivery systems and have a high biocompatibility (Shi *et al*., 2014).

Nanotechnology is an area of science that is becoming more prevalent in numerous facets of human existence. It is the process of making nanoparticles with structural properties similar to atoms and bulk materials in the nano range. Nanoparticles are 100 to 10,000 times smaller than biological molecules including enzymes, receptors and DNA. Nanoparticles unique properties such as visible absorption, a large surface to volume ratio, surface functionalization potential and specific physicochemical, electrical, optical, magnetic and mechanical properties enable nanoparticles to be used in a variety of fields including electronics, optoelectronics, chemistry, energy, medicine and controlled drug release (Ajitha *et al*., 2015).

Nanoparticles can be produced either by top-down or bottom-up procedures. In top-down procedures, bulk materials are reduced to the nanoscale, whereas in bottom-up methods, beginning materials are grown to larger structures by joining atoms or molecules. Many physical and chemical approaches have been established for the manufacture of mono-dispersed metal nanoparticles (Singh *et al.*, 2019).

Preparation of nanoparticles can be carried out by several methods such as laser ablation, pyrolysis, chemical or physical vapour deposition, sol-gel and lithography electro deposition. However, the majority of them are either prohibitively expensive or require the use of harmful chemicals and solvents. Recently, there has been a lot of effort put into using environmentally friendly nanoparticle manufacturing processes. They allow for easier manipulation, crystal growth control and crystal stabilisation. This has sparked an increase in research into synthetic approaches

that allow for improved form and size control in a variety of nanotechnological applications. The use of environmentally benign materials such as plant extracts, bacteria, fungi and enzymes for the synthesis of silver nanoparticles offers numerous benefits of ecofriendly and compatibility for pharmaceutical and other biomedical applications because toxic chemicals are not used in the synthesis protocol (Ramya *et al.,* 2012). For the manufacture of silver nanoparticles, a variety of methods are available, including reduction in solutions, chemical and photochemical processes in reverse micelles. Recently green chemistry routes include thermal degradation of silver compounds, radiation assisted, electrochemical, sonochemical and microwave assisted processes (Begum, 2009).

In recent times, synthesis employing biological organisms has gained popularity, due to their numerous advantages over traditional methods for that purpose. These approaches are referred to as green synthesis methods. When biological organisms are employed to synthesize nanoparticles, the benefits of being less expensive, clean, quick, nontoxic, easy and environmentally friendly are evitable. Synthetic biology has employed a wide range of biological creatures including bacteria, fungus, actinomycetes, algae and plants. When compared to microbe mediated synthesis, which requires expensive medium and the maintenance of precise culture conditions, plant -based synthesis using a wide variety of plants and plant parts such as leaf, stem, root, flower and fruit extracts is becoming more prominent among the green methods (Arunachalam *et al.,* 2012). Furthermore, by optimising process parameters such as the nature of the plant, concentration of plant extracts, volume of extract and metal salt, pH, temperature, interaction time, varied size and shape, better composition and biocompatibility, less toxicity and long stability for nanoparticles could be achieved for a variety of applications (Ali *et al.,* 2011).

The use of plant leaves extract as a mediator in the generation of nanoparticles has been documented. *Centella asiatica, Murraya koenigii, Alternanthera sessilis* and a variety of other plant leaves were analyzed. *Piper nigrum* leaves have recently been discovered to have an important bioactive component that could be used in the manufacturing of ecofriendly nanoparticles (Jacob *et al.,* 2012).

Nanoparticles are generally characterized by their size, shape, surface area, density and dispersity (Mittal *et al.,* 2013). The most frequent methodologies for evaluating the nanoparticle

properties can be quantitative and qualitative methods. These methods include a range of various sophisticated techniques like dynamic light scattering (DLS), scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), UV-Visible spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR) (Rajasekharreddy *et al.*, 2010; Mittal *et al*., 2013).

GYMNEMA SYLVESTRE

Kingdom: Plantae

Class: Magnoliopsida

Order: Gentianales

Family: Apocynaceae

Genus: Gymnema R.Br.

Species: *G. Sylvestre*

Binomial name: *Gymnema sylvestre*

Gymnema sylvestre (Asclepiadaceae), it is one of the vulnerable species and a slow growing, perennial, medicinal woody climber which is found in central and peninsular India. It is a potent antidiabetic plant and used in folk, ayurvedic and homeopathic systems of medicine. It is also used in the treatment of asthma, eye complaints, inflammations, family planning and snakebite (Komalavalli *et al.,* 2000).

Gymnema sylvestre is one of the plants that has potent anti-diabetic qualities. The active ingredient in plants include a group of acids known as gymnemic acids. Triterpene saponins from the oleanane and dammarene classes are found in the leaves of *Gymnema sylvestre*. Dammarene saponins are gymnemasides, while oleanane saponins are gymnemic acids and gymnemasaponins. Anti-diabetic, anti-sweetener and anti-inflammatory properties are all found in gymnemic acids.

A set of closely related gymnemic acids has been discovered as the antidiabetic array of chemicals (Kanetkar *et al.*,2007).

Besides this, other plant constituents include flavones, anthraquinones, hentri-acontane, pentatriacontane, α and β-chlorophylls, phytin, resins, d-quercitol, tartaric acid, formic acid, butyric acid, lupeol, β-amyrin related glycosides and stigmasterol. The plant extract also possesses alkaloids and the leaves of this species yield acidic glycosides and anthroquinones and their derivative.

Objectives

The objectives of the present study

- To synthesize and characterize silver nanoparticles (AgNPs) from *Gymnema sylvestre* leaf extract.
- To assess the *in vitro* free radical scavenging activity of silver nanoparticles of *Gymnema sylvestre* leaf extract.
- To evaluate the *in vitro* antidiabetic activity of silver nanoparticles of *Gymnema sylvestre* leaf extract.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.Diabetes Mellitus

Diabetes mellitus causes a number of metabolic complications, including diabetes-induced oxidative stress, which plays an important role in the symptoms and course of the disease. Reduced antioxidant defence capacity causes increased production of reactive oxygen species (ROS) in cells and tissues under oxidative stress. Several hypotheses have been proposed to explain the genesis of free radicals in diabetes, including glucose autoxidation, non-enzymatic and progressive glycation of proteins with increased formation of glucose derived Advanced Glycosylated End products (AGEs) and increased glucose flux through the polyol pathway (Maiti *et al.,* 2016).

According to the World Health Organization (WHO), there are more than 220 million diabetics worldwide, which is expected to quadruple by 2040 (Zaid *et al.,* 2016). Currently, diabetes was the direct cause of 1.5 million deaths and 48% of all deaths due to diabetes occurred before the age of 70 years (WHO, 2021). According to the International Diabetes Federation (IDF), there were 382 million people living with diabetes in 2015, with 5.1 million deaths and this number is expected to climb to 592 million by 2035 (Gupta *et al.*, 2016).

2.1. Classification of Diabetes mellitus

Diabetes mellitus is divided into four categories based on the aetiology: Type 1, Type 2 diabetes mellitus and gestational diabetes mellitus. The phrases insulin-dependent and non-insulin-dependent are used to categories diabetes mellitus pathophysiological situations.

2.1.2. Type 1 Diabetes mellitus (IDDM)

Type 1 diabetes, also known as Insulin Dependent Diabetes Mellitus (IDDM), is thought to be caused by the immune system attacking pancreatic beta cells, causing insulin insufficiency. The autoimmune destruction of insulin secreting pancreatic cells starts the pathophysiology of

type 1 diabetes. This observation is based on the existence of a chronic inflammatory infiltration affecting pancreatic islets at the development of type 1 diabetes (Massoud and Massoud, 2012).

It develops in connection with specific hereditary factors, such as Human Leukocyte Antigen (HLA) alleles and proceeds to absolute insulin insufficiency through pancreatic cell death. This illness is common among teenagers and can affect persons of any age group. Autoantibodies against islet antigens have also been observed to grow in the early stages of type 1 diabetes, therefore the illness is also known as 'autoimmune' type 1 diabetes mellitus. Functional deficiencies in the bone marrow and thymus, as well as the immune system and cells, are thought to contribute to the pathophysiology of type 1 diabetes (Oyagbemi *et al.,* 2014).

The autoimmune death of pancreatic cells causes a lack of insulin secretion, leading to the metabolic abnormalities associated with IDDM. In addition, the loss of insulin secretion, pancreatic cell function is aberrant, resulting in increased glucagon release. The metabolic abnormalities caused by insulin insufficiency are exacerbated by improperly increased glucagon levels, which resulted in uncontrolled lipolysis and elevated amounts of free fatty acids in the plasma. This inhibits glucose utilization and lowers the expression of a number of genes required for optimal insulin response in target tissues, such as glucokinase in the liver and the GLUT 4 glucose transporter family in adipose tissue (Ozougwu *et al.*,2013).

2.1.3. Type 2 Diabetes mellitus (NIDDM)

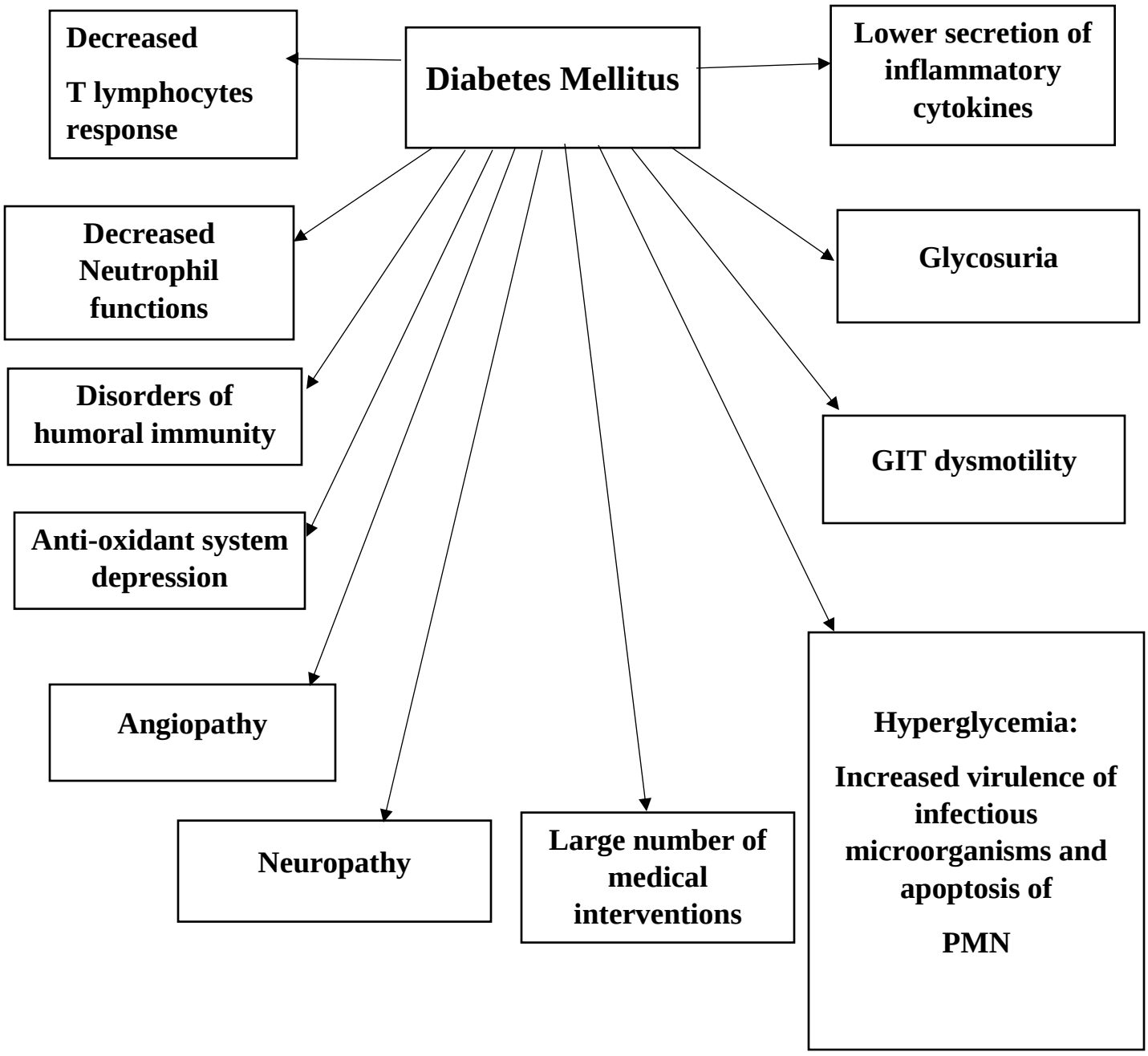
Type 2 diabetes (also known as non-insulin dependent diabetes) is the most common type of diabetes, characterized by hyperglycemia, insulin resistance and insulin deficiency. Genetic, environmental and behavioural risk factors are all strongly linked. Insulin sensitivity is a symptom of type 2 diabetes mellitus, which is characterised by insulin resistance, decreased insulin production leading to the pancreatic beta-cell failure. Insulin resistance and hyperinsulinemia are the symptoms of this chronic metabolic condition, which leads to reduced glucose tolerance (Olokoba *et al.,* 2012). Under normal conditions, despite fluctuations in supply and demand, plasma glucose concentrations are kept within a small range via a finely regulated and dynamic relationship between tissue sensitivity to insulin and insulin secretion. These pathways fail in type 2 diabetes, resulting in reduced insulin production via pancreatic cell dysfunction and impaired insulin action via insulin resistance.

Other specific kinds of diabetes include diabetes mellitus caused by a variety of etiologies. Idiopathic diabetes is also known as primary diabetes. Persons with genetic defects of beta-cell function (formerly known as MODY or maturity-onset diabetes in youth) or defects of insulin action, exocrine pancreas diseases such as pancreatitis or cystic fibrosis and people with pancreatic dysfunction caused by drugs, chemicals or infections are all included in this group**.** The pathophysiology of infections associated with diabetes mellitus is given in the Figure. 1. However, 2% of cases are caused by another well-defined disease or predisposing factor such as pancreatitis or steroid excess and are referred to be 'secondary diabetes. Single gene abnormalities influencing insulin secretion or resistance, injury to the exocrine pancreas, other endocrine disease, drug induced diabetes, unusual presentations of autoimmune diabetes and genetic syndromes associated with diabetes can all be subdivided (Gale and Frits, 2014).

2.1.4. Gestational Diabetes

Type 2 diabetes and metabolic abnormalities are more likely in women with gestational diabetes mellitus (GDM) and their offspring. It happens in roughly 4% of all pregnancies. It is most commonly detected in the later stages of pregnancy in women who have never had diabetes before. The World Health Organization defines gestational diabetes mellitus as "any degree of glucose intolerance with onset or first detection during pregnancy", which includes glucose values that fall within the impaired glucose tolerance range (Balaji *et al.,* 2014).

Figure. 1: Pathophysiology of infections associated with Diabetes Mellitus



2.1.5. Signs and symptoms of Diabetes mellitus

Diabetes typically goes unnoticed since symptoms can be attributed to a variety of other factors, and people either don't notice or ignore warning indications. Excessive thirst (polydipsia), excessive urination (polyuria), dehydration, unexplained weight loss, blurred vision, nearsightedness or other vision problems, frequent infections, such as skin infections, thrush, gingivitis, urinary tract infections and yeast infections, slow healing of sores, itchiness, fatigue, lethargy or drowsiness, shakiness or tremors are all possible (Siddhant *et al.,* 2012).

2.1.6. Oxidative stress and Diabetes mellitus

A free radical is a chemical molecule with an unpaired electron spinning on the nucleus peripheral layer. The group of free radicals produced by the oxygen is known as reactive oxygen species (ROS) and Reactive nitrogen species (RNS) are the free radicals formed from nitrogen that can damage all other molecules by withdrawing an electron from them to achieve stability (Kaneria *et al.,* 2012). Amongst Reactive nitrogen species, with a molecular weight of 30 Dalton, nitric oxide is the most important nitrogen-derived physiological free radical and one of the ten smallest molecules in the nature are involved in the cause or recovery of a variety of disorders. Different pathophysiological conditions are thought to entail both nitric oxide deficit and excess (Montecucco *et al.,* 2010).

Oxidative stress is classified as the excess production or insufficient clearance of highly reactive molecules, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). Superoxide ($\bullet O_2$-), hydroxyl (•OH), peroxyl ($\bullet RO_2$) and hydroperoxyl ($\bullet HRO_2$-) are free radicals, while hydrogen peroxide (H_2O_2) and hydrochloric acid are non-radical species (HOCl). Nitric oxide (•NO) and nitrogen dioxide ($\bullet NO_2$) are free radicals, while peroxynitrite (ONOO-), nitrous oxide (HNO_2) and alkyl peroxynitrates are non-radicals (RONOO). $\bullet O_2$-, •NO and ONOO- are three reactive chemicals that play essential roles in cardiovascular problems. Many disorders, including atherosclerosis, parkinson's disease, heart failure, myocardial infarction, alzheimer's disease, fragile X syndrome and chronic fatigue syndrome are linked to oxidative stress in humans. Reactive oxygen species are useful because they are used by the immune system to target and kill infections, as well as in cell signalling.

Reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI) are constantly created under physiological settings and is a critical event in living organisms. ROI and RNI bind to proteins, carbohydrates and lipids, causing changes in intracellular and intercellular balance, as well as the possibility of cell death and regeneration (Rahman *et al.*, 2012).

Chronic hyperglycemia in diabetes mellitus causes numerous abnormalities in many organs, particularly those where glucose absorption is insulin-independent. Hyperglycemia, oxidative stress and numerous hyperglycemia driven intracellular pathways that might contribute to diabetic complications have all been highlighted. The polyol route, the generation of advanced glycation end products (AGEs), the activation of protein kinase C(PKC) isoforms and enhanced flow through the hexosamine pathway are the key mechanisms. All these mechanisms have shown to have a key role in the development of long-term diabetic problems (Arcaro *et al.*, 2014).

Hyperglycemia, increased free fatty acids (FFA), cytokines and other factors all contribute to an increase in reactive oxygen (and nitrogen) species production and oxidative stress. Multiple stress-sensitive serine/threonine kinase signalling cascades are activated as a result of this. These kinases can phosphorylate various targets, including the insulin receptor and insulin receptor substrate (IRS) proteins IRS-1 and IRS-2. The degree of insulin-stimulated tyrosine phosphorylation is reduced when the insulin receptor or IRS proteins are phosphorylated on discrete serine or threonine sites. As a result, downstream signalling molecules (phosphatidylinositol 3-kinase, PI3K) become less associated and active, resulting in lower insulin action. Antioxidant ability to maintain intracellular redox equilibrium and prevent the activation of stress sensitive kinases may explain their protective effects against oxidative stress induced insulin resistance.

2.1.7. Complications of Diabetes mellitus

Long term diabetes leads to damage and organ failure in a variety of organ systems with the eyes, nerves, kidneys and heart being the most affected. The distinction between the pathogenic causes of microvascular and macrovascular problems of diabetes mellitus, as well as their varied responses to therapeutic approaches, is increasing. Diabetic microangiopathy is characterised by the alterations in the microvasculature, including extracellular matrix protein production and capillary basement membrane thickening, which are pathogenomic characteristics

of the disease (Chawla *et al.,*2016). Macrovascular complications include cardiovascular abnormalities such as atherosclerosis, heart disease and stroke. Dylipidemic condition is one of the major causes defined by higher triglycerides (TG), low levels of high-density lipoprotein cholesterol (HDL-C) and the predominance of low-density lipoprotein cholesterol (LDL-C) particles. Cardiovascular diseases (CVDs) are one of the most common diabetic complications and the major cause of premature death in Type 2 diabetes patients. Controlling hyperglycemia, dyslipidemia and oxidative stress are reported to be more important in lowering the incidence of diabetic complications in patients (Oyenihi *et al.,* 2014).

2.2. Role of antioxidants

Antioxidants are compounds that prevent or slow the oxidation of a target molecule. A single free radical can be neutralized by an antioxidant molecule contributing one of its own electrons, resulting in a carbon-stealing process. Free radicals that have not been activated have the ability to inflict significant damage to biological macromolecules such as proteins, lipids, carbohydrates and nucleic acids. The balance between free radical generation and antioxidant defence aids in disease prevention when the creation of free radicals exceeds the antioxidant defences ability to protect, oxidative stress. As a result, oxidative stress is defined as a loss of balance between the formation of free radicals and reactive oxygen species (Neeraj *et al.,* 2013).

Antioxidants may have a significant impact on the beginning of degenerative illnesses. (Alam *et al.,* 2013). The basic role of healthy skin is to protect the organism from harmful assaults by forming a physical and chemical barrier between the external environment and the internal milieu. Harmful stimuli, such as trauma, viruses or allergens, cause inflammation, which is a complex reaction. Inflammation defends organisms against pathogenic intruders and heals wounds to avoid additional tissue damage (Wagener *et al.,* 2013).

Antioxidants have two main mechanisms of action. The first is a chain breaking process in which primary antioxidants give an electron to the current free radical system. The second process involves quenching chain-initiating catalysts to remove reactive oxygen or nitrogen species initiators (Pankaj *et al.,* 2015).

Antioxidant status is maintained with the help of antioxidants, which safeguard the cell's physiological activities from the reactive oxygen system. In nature, antioxidants are both endogenous and exogenous. Superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, heme oxygenase (HO), paraoxonase (PON) and the thioredoxin (TXN) system are examples of endogenous antioxidant enzymes. Besides antioxidant enzymes, biological systems contain a variety of redox active substances with antioxidant characteristics. Exogenous antioxidants such as ascorbate (Vitamin C), α-tocopherol (Vitamin E) and reduced glutathione (GSH) prevent LDL oxidation by scavenging reactive oxygen species (ROS) (Lee *et al.,* 2012).

Medicinal herbs are a wonderful gift to human mankind. Medicinal plants are commonly used in medicine delivery systems, either as a single agent or in combination. Traditional medicinal herbs are the source of today's modern medications. Traditional herbal medicine was briefly repressed by the advancement of modern medicine. However, it is presently experiencing a worldwide "herbal revival." Traditional medicinal methods are used by 80% of people in poor nations (Bonifacio *et al.,* 2014). Bioactive components found in medicinal plants are abundant, characteristics that are antioxidative and antitumorigenic (Elansary *et al.,* 2018). Plant-derived bioactive components are used to create green nanoparticles with increased antioxidative and antitumorigenic properties (Mohanta *et al*., 2017).

2.3. Nanotechnology

Nanotechnology, a relatively advanced branch of research that involves manipulating materials at the atomic, molecular and macromolecular scales, where properties differ from those at higher scales. The assertion of nanotechnology is that when an element or compound exists in its nano form, its properties can be easily altered. The nanometer scale ranges from 1 to 100 nanometers. One nanometer is one billionth of a metre (10-9m). At this scale, components respond differently than their larger counterparts, revealing various physical, chemical and biological properties. To avoid single atoms or very small groupings of atoms being put out as nano objects, the size spans from 1nm to 10nm. Nanoscience and nanotechnology are concerned with at least 1nm-sized groupings of atoms (Magykandil, 2016). Nanosized particles acquires different visual, mechanical and electrical properties (Ibrahimkhan *et al.*, 2017).

Nanomaterials are predicted to be employed in a wide range of applications, from biomedical drug delivery to electronics, pollution cleanup and less toxic manufacturing methods, despite the fact that much of nanotechnology has been in the research and developmental stage (Neildesai *et al.,* 2012).

2.3.1. Nanoparticles

Nanoparticles offer a wide range of functional platforms for imaging and therapeutic applications. These platforms can be made out of a variety of inorganic compounds, but inorganic platforms are particularly useful for simultaneous therapy and diagnosis due to their ease of modification, high drug loading capacity and stability (Haruna *et al.,* 2016). Metal oxide ceramics, silicates, magnetic materials, liposomes, dendrimers, emulsions and other materials are utilized to make these nanoparticles (Gareth *et al.,* 2016).

2.3.2. Types of nanoparticles

Nanoparticles can be organized into four material-based categories, they are:

- **Carbon-based nanomaterials:** These nanomaterials are mostly carbon-based and come in shapes including hollow tubes, ellipsoids and spheres. Carbon nanotubes (CNTs), carbon nanofibers, carbon black, graphene (Gr) and carbon onions are all examples of fullerenes (C60) onions (Kumar and Kumbhat, 2016).
- **Inorganic based nanomaterials**: Metal and metal oxide nanoparticles are among these nanomaterials. Metal nanoparticles like Au or Ag, metal oxides like $TiO_2$and ZnO nanoparticles and semiconductors like silicon and ceramics can all be used to make them.
- **Organic based nanomaterials**: Nanomaterials comprised primarily of biological stuff are among them. The use of noncovalent (weak) interaction for molecular self-assembly and design aids in the transformation of organic nanomaterials into desired forms including dendrimers, micelles, liposomes and polymer nanoparticles.
- **Composite based nanomaterials**: Composite nanomaterials are multiphase nanoparticles and nanostructured materials with one phase on the nanoscale dimension, which can combine nanoparticles with other nanoparticles or nanoparticles with larger or bulk-type materials (e.g., hybrid nanofibers) or more complex structures, such as metal-organic

frameworks. Any combination of carbon-based, metal-based or organic-based nanomaterials with metal, ceramic or polymer bulk materials can be used in the composites (Jeevanandam *et al.,* 2018).

2.3.3. Silver nanoparticles

Certain materials have been used in medicine since time immoral because of their exceptional therapeutic capabilities. Silver (Ag) has been used in the treatment and management of numerous ailments since ancient times due to its wide spectrum of antibacterial and therapeutic qualities. Silver nanoparticles are essential in nanomedicine because of their appealing physicochemical features and biological activity, such as their high antibacterial efficacy and non-toxic, broad spectrum of bactericidal activities (Durán *et al.,* 2016), Their anticancer qualities and many other therapeutic abilities, as well as their unique capacity to create a variety of nanostructures and their inexpensive manufacturing costs, make them more attractive candidates (Sohn *et al.,* 2015). Silver nanoparticles are nanostructures with diameters ranging from 1 to 100 nm that are largely used for novel and enhanced biomedical applications such as medication delivery, wound dressings, tissue scaffolding and protective coatings (Almatroudi, 2020).

2.3.4. Methods of silver nanoparticles synthesis

Many approaches and methods have been developed such as physical, chemical (chemical reduction method, electrochemical method, pyrolysis and irradiation-assisted chemical method) and biological procedures for the successful manufacture of silver nanoparticles. While physical and chemical approaches are more cost-effective on a commercial level, biological ones are more environmentally friendly (Hulkoti and Taranath, 2017). The synthetic technique determines the size, shape, structure, physical, chemical and biological properties of nanoparticles. Researchers have described several synthetic approaches, three most important approaches have primarily been discussed. The schematic diagram for the various methods of synthesis of silver nanoparticles in the Figure. 2. The chemical reduction approach, which includes reducing Ag+ species to Ag° using reducing agents such as$NaBH_4$, $LiAlH_4$ and others, is the most popular and widely used method. During synthesis, physical processes (laser, arc discharge, ball milling and vapour condensation)

often consume a lot of energy. AgNPs have been produced biologically utilising fungi, plants and bacteria, with no hazardous reducing agents used (Arif and Uddin, 2021).

The physical method for nanoparticles synthesis includes radiolysis, microwave, ultrasonication, laser ablation and electrochemical processes (Sarsar *et al.*, 2014). The evaporation condensation technique was carried out in a tube furnace at atmospheric pressure, which can be used to make metal nanoparticles. The use of a tube furnace to make silver nanoparticles has a number of disadvantages. Tube furnaces occupies room space and use a lot of energy to raise the temperature of the surroundings around the source material. To achieve a consistent operating temperature, a typical tube furnace requires several kilowatts of power and several tens of minutes of preheating time (Makarov *et al.*, 2014).

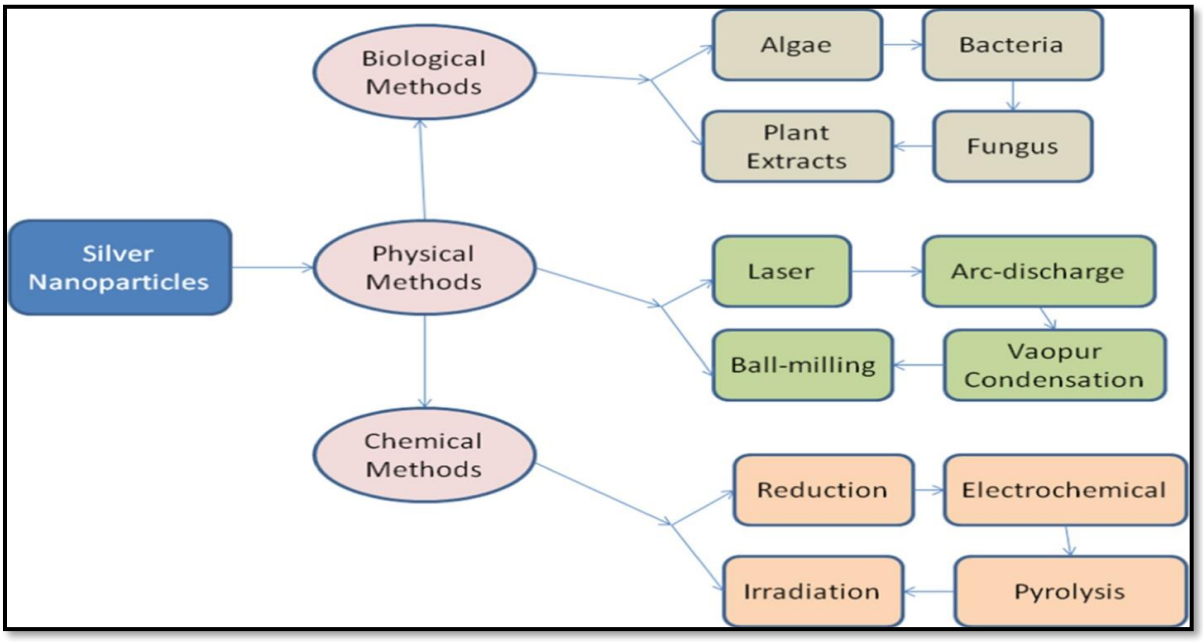


Figure. 2

The schematic diagram for the various methods of synthesis of silver nanoparticles

Biological approaches can overcome the drawbacks of physical and chemical techniques. Green plants and microbes have an amazing ability to synthesize nanoparticles. Biological synthesis of nanoparticles was proven to be ecofriendly and economical (Khan *et al.*, 2018). In the synthesis of nanoparticles, extracts from bio-organisms acts as reducing and capping agents.

Several microorganism extracts have been used to generate intracellular or extracellular silver nanoparticles in the creation of nanoparticles, phytoconstituents found in plant tissues interact (Lokina *et al.*, 2015).

2.3.5. Green synthesized nanoparticles from medicinal plants

The green synthesis of nanoparticles employing renewable natural sources such as plant extract, microorganisms and biodegradable materials as a reducing and capping agent has attracted a lot of attention. Plant sources are commonly used for the rapid synthesis of nanoparticles because they are suitable for large scale biosynthesis. Metallic nanoparticles are typically made from plant parts such as seed, leaf, stem, root and latex (Kharissova *et al.*, 2013). The schematic diagram for the synthesis of silver nanoparticles through green synthesis method is given in the Figure,3. Employing green technology for the synthesis of nanoparticles was found to be the best method, as it does not involve any harmful chemicals (Singh *et al.*, 2018; Gopinath *et al.*, 2013). The composition of the plant extract, pH, temperature, metal salt concentration and contact time all influenced nanoparticle production (Rossi *et al.*, 2014).

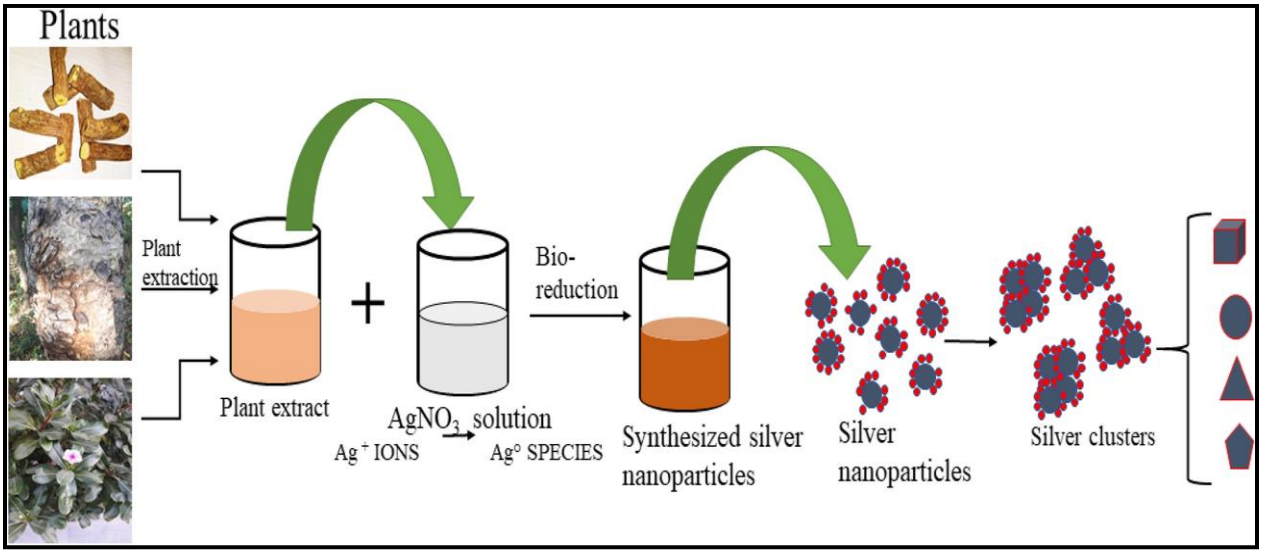


Figure.3

Synthesis of AgNPs through Green synthesis method (Jain *et al.*, 2021)

2.4. Characterization of nanoparticles

The application of analysis methods for the examination and characterisation of nanoparticles has become necessary as nanotechnology has expanded in numerous scientific fields. Microscopic, separation and spectroscopic techniques are among the characterisation techniques accessible. The size distribution, shape, surface characteristics, stability and interactions of nanoparticles are generally described in the literature (Lopez and Mizaikoff, 2016).

2.4.1. UV-Visible spectroscopy

UV-vis spectroscopy is a very helpful and dependable approach for primary characterization of produced nanoparticles, and it's also utilised to monitor silver nanoparticles (AgNPs) synthesis and stability. AgNPs have unique optical features that cause them to interact strongly with specific light wavelengths. Furthermore, UV-vis spectroscopy is quick, simple, sensitive, selective for different types of nanoparticles (NPs), it requires just a brief measurement time and does not require calibration for particle characterization of colloidal suspensions. The conduction band and valence band in AgNPs are relatively near to one other, allowing electrons to freely travel. Due to the collective oscillation of electrons in silver nano particles, these free electrons produce a surface plasmon resonance (SPR) absorption band (Zhang *et al.,* 2016).

2.4.2. Fourier transform infrared spectroscopy (FTIR)

FTIR has the ability to give accuracy, repeatability and a good signal-to-noise ratio. Small absorbance changes on the order of 103 can be detected using FTIR spectroscopy, allowing difference spectroscopy to be performed, allowing the small absorption bands of functionally active residues to be distinguished from the massive background absorption of the entire protein. FTIR spectroscopy is extensively employed in academic and industrial research to determine whether biomolecules are involved in the creation of nanoparticles. FTIR has been used to investigate nanoscaled materials such as the confirmation of functional molecules covalently grafted onto silver, carbon nanotubes, graphene and gold nanoparticles or interactions between the enzyme and substrate throughout the catalytic processes (Shan *et al.,* 2016).

2.4.3. X ray diffraction (XRD)

XRD can be utilised to estimate crystallinity by comparing the integrated intensity of the background pattern to that of the sharp peaks. Powder XRD values are often equivalent, but not identical, to values obtained by other methods like as DSC (Differential scanning calorimetry). The size and shape of the crystalline phases unit cell dictate the position of a diffraction peak, which is independent of atomic positions within the cell. A Miller index can be used to identify each peak, which represents a certain lattice plane. If the symmetry is great, such as cubic or hexagonal, determining the index of each peak, even for an unknown phase, is usually not difficult (Fraundorf *et al.,* 2015).

2.4.4. Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) is a surface imaging approach suitable for determining unique molecule forms, surface morphology, sizes and size dispersions of the controlled nanoparticles at the miniature and nano scale levels, among a few electron microscopy strategies (Buhr *et al.,* 2009). SEM examines the outside of the AgNPs test with a high-energy electron bar and then back dispersed electrons perception provides example signature highlights. Energy Dispersive Xray Spectroscopy (EDX) is a compound research technique that is used in conjunction with SEM to determine the vital AgNPs test arrangement. The EDS method detects x-rays communicated by electron pillar from the example during siege and EDS x-ray locator calculates the general riches of released x-rays vs their energy (Anandalakshmi *et al.,* 2016).

2.5. Therapeutic applications of silver nanoparticles

Despite the fact that AgNPs are used in a variety of applications such as thin films, surface coatings, batteries, cosmetics, textiles, food, energy harvesting and conductors, medical applications have attracted the attention due to the rise in life-threatening diseases around the world and the challenges of multidrug resistance (Shanmuganathan *et al.,* 2019). Silver particles exhibit unique physicochemical properties and biological functions when they reach the nanoscale. The unique nature of silver nanoparticles enables them to be used in antibacterial, antifungal, antiviral, anti inflammatory, anti angiogenic and anti cancer therapies. In diluted concentrations, AgNPs destroy viruses, bacteria and other eukaryotic microorganisms without causing any harm to

humans, according to recent studies (Sukriye and Cigdem, 2019). AgNPs are important in the development of innovative antibacterial agents, biomaterial, medical device coating, drug delivery compositions, prediction and diagnosis platforms, tissue repair and regeneration materials and performance-enhanced therapy options. In addition, AgNPs can be used as an additive in membranes, bone cement, denture bases, dental implants, broken bone, catheters and hydrogel to prevent or limit the production of biofilm or any other medical pathogens, as well as to enhance the bone growth, wound healing and gum healing (Yang *et al.*, 2021).

2.6. Plant description

Gymnema sylvestre is a large woody climber, rooting at nodes, leaves elliptical to acuminate, base acute to acuminate, glabrous above sparsely or densely tomentose beneath. Flowers small, in axillary and lateral umbel like cymes, pedicels long; Calyx-lobes long, ovate, obtuse, pubescent. Corolla pale yellow campanulate, valvate, corona single, with 5 fleshy scales. Scales adnate to throat of corolla tube between lobes. Anther connective produced into a membranous tip, 2 erect pollinia, 2 unilocular carpels, many ovuled locules, with 1 fusiform long follicle.

Gymnema sylvestre leaves have been found to cure hypoglycemia in laboratory animals and have found a use in herbal medicine to help treat adult-onset diabetes mellitus (NIDDM). When *Gymnema* leaf extract is administered to a diabetic patient, there is stimulation of the pancreas by virtue of which there is an increase in insulin release (Kanetkat *et al.*, 2007).

Gymnema sylvestre leaf extract, notably the peptide ‘Gurmarin’, has been found to interfere with the ability of the taste buds on the tongue to taste sweet and bitter. Gymnemic acid has a similar effect. It is believed that by inhibiting the sweet taste sensation, people taking it will limit their intake of sweet foods and this activity may be partially responsible for its hypoglycemic effect (Dahanukar *et al*., 2020).

Experimental Procedures

3. Experimental Procedure

Diabetes got its name from a Greek doctor, Aretaeus of Cappadocia, after the word diabainein which signifies "to siphon" that is to pass through. This is because that in diabetes overabundance of sugar is found in the blood and urine and it was once referred to as "pissing evil" (Sattley, 2008). Type 1 diabetes (T1D) is an immune system problem leading to the destruction of pancreatic beta-cells. Type 2 diabetes (T2D), is fundamentally an issue of progressively impaired glucose regulation due to a combination of dysfunctional pancreatic beta cells and insulin resistance. Type 2 diabetes mellitus (T2DM) is described by dysregulation of carbohydrate, lipid and protein metabolism and results from impaired insulin secretion, insulin resistance or a combination of both (Gardner and Tai, 2012).

3.1. Collection of plant sample

The present study focused mainly on determining the antioxidant and antidiabetic effects of *Gymnema sylvestre* leaf extract. The study was conducted by synthesizing the silver nanoparticles in *Gymnema sylvestre* leaf extract. The leaves of the plant were collected from the Vellingiri hills in Coimbatore. The leaves were washed, dried thoroughly and pulverized. The powder was weighed, packed in air tight container and stored at 4ºC until use.

Plate 1

The anti-diabetic potential of *Gymnema sylvetre* extract was determined using alpha amylase inhibitory assay, alpha glucosidase inhibitory assay, non-enzymatic glycosylation of haemoglobin inhibitory activity, glucose uptake by yeast cells and antioxidant potential was determined by radical scavenging assays.

The experimental conditions and the procedures of the various parameters analyzed in each phase of the present study are explained in detail in this chapter.

Chemicals

All chemicals used were of analytical grade and were used as received without any further purification.

3. 2. Preparation of *Gymnema sylvestre* Leaf Extract

The plant was collected and washed thoroughly with water. The washed leaves were dried at room temperature for 10 days. The dried sample was powdered and stored in air tight container for further use. To 10g of the powdered sample 100ml of ethanol was added. Then the mixture was kept in the mild shaker for about seven days. It was then filtered by Whatmann no.1 filter paper and the filtrate obtained was used for further studies.

3.3. Synthesis of Silver Nanoparticles of Leaves of *Gymnema sylvestre*

Synthesis of silver nanoparticles is carried out by the method explained by Harbone. 1mM silver nitrate solution is prepared using the deionized water. 10 ml of ethanolic extract was added to the 90 ml of 1mM silver nitrate solution and shake well. The mixture of ethanolic extract of leaves of *Gymnema sylvestre* with silver nitrate is exposed to sunlight for the duration till the green solution turns into brown colour.

The synthesized silver nanoparticles of the ethanolic leaf extract of *Gymnema sylvestre* (AgNPsEGs) sample is kept for centrifugation in a refrigerated centrifuge at 5000rpm for 45 minutes and washed 3 times with deionized water. The residue of silver nanoparticles is obtained by freeze drying.

3.4. Characterization of Silver Nanoparticles of Leaves of *Gymnema sylvestre*

The synthesized silver nanoparticles were characterized as per the methods explained below.

3.4.1 UV -Visible spectrum of *Gymnema sylvestre*

The formation of AgNPs is primarily observed by monitoring the change in colour of the extract after treating with $AgNO_3$ (1mM). The bioreduction of Ag ions in aqueous extract is monitored with the UV- visible spectra of the solutions after diluting a small aliquot (0.1 ml) of the sample to 10 times with ddH_2O. UV-visible spectra were recorded with spectrophotometer from 300 to 700 nm wavelength at room temperature. Double distilled water is used as reaction blank (Premasudha *et al*., 2015).

3.4.2 Scanning electron microscopy (SEM) of *Gymnema sylvestre*

Silver nanoparticles synthesized were characterized using high resolution scanning electron microscopy (SEM). The samples were prepared by simple drop coating of the suspension of silver nanoparticles separately on a carbon- coated copper grid, by dropping a very small amount of the sample on the grid and the excess solution is removed by blotting. The film on the scanning electron microscopy grid was the allowed to dry under a mercury lamp for 5 minutes. It is then subjected to SEM analysis (Lee and Cadogan, 2013).

3.4.3 EDAX spectrum measurements of *Gymnema sylvestre*

Synthesized silver nanoparticles were dried, drop coated on to carbon film and tested using EDAX analyses (Nagy *et al.*, 2015).

3.4.4 Fourier transform infrared (FTIR) spectroscopy of synthesized silver nanoparticles of leaves of *Gymnema sylvestre*

FT-IR analysis is done to obtain the infrared spectra of absorption, emission and to ensure the formation of silver nanoparticles. It helps to identify the possible interactions between silver with bioactive molecules, which may be responsible for the formation and stabilization

(capping material) of silver nanoparticles. The advantage of using an FT-IR is that it simultaneously collects spectral data in a wide spectral range to analyze the functional groups which is present on synthesized AgNPs (Djomgoue and Njopwouo, 2013).

3.4.5 X-ray diffraction spectroscopy (XRD) of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

The bioreduced silver nanoparticles solution was drop coated onto glass substrate and powder X-ray diffraction measurements. The pattern is recorded by Cu Kα 1 radiation with λ of 1.5406 A° and nickel monochromator filtering the wave at tube voltage of 40 kV and tube current of 39 mA. The scanning was done in the region of 2θ from 30º to 80º at 0.02- min and the time constant was 2s (Priyarangini *et al*., 2013). The average particle size of the silver nanoparticles formed in the bio reduction process was determined using Scherr's formula:

$$D = K\lambda/\cos\theta$$

Where, λ is the X-ray wavelength, β is the full-width half maximum (FWHM) of a diffraction peak, θ is the diffraction angle, K is constant (0.89).

Strain can be calculated from the formula

$$\varepsilon = \beta/4\tan\theta$$

Dislocation density is calculated using the Williamson-Hall relation,

$$\delta = 1/D^2$$

3.5 Free radical scavenging activity of synthesized silver nanoparticles of *Gymnema sylvestre*

3.5.1 Analysis of 2,2'-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity

In vitro 2,2'-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity was carried out by the method proposed by (Mensor *et al.,* 2001 and given in Appendix I.

3.5.2 Determination of 2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid (ABTS) radical scavenging activity

In vitro 2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid (ABTS) radical scavenging activity was done on the method proposed by (Shirwaikar *et al.*, 2006) and given in Appendix II.

3.6 *In vitro* antidiabetic activity of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

3.6.1 *In vitro* alpha amylase inhibitory activity of synthesized silver nanoparticles of *Gymnema sylvestre* leaf extract

In vitro alpha amylase inhibitory activity of silver nanoparticles of *Gymnema sylvestre* was done by the method explained by Subramanian *et al.* (2008) and it is given in Appendix III

3.6.2 Non enzymatic glycosylation of haemoglobin inhibitory activity of synthesized silver nanoparticles of *Gymnema sylvestre* leaf extract

Non enzymatic glycosylation of haemoglobin inhibitory activity of silver nanoparticles of *Gymnema sylvestre* are measured by the method of Daksha *et al.* (2012) and detailed procedure is given in Appendix IV.

3.6.3 Inhibition of glucose diffusion of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

Glucose diffusion inhibitory activity of silver nanoparticles of *Gymnema sylvestre* is studied by the method of Gallager *et al.* (2003) and detailed procedure is given in Appendix V.

3.6.4 Glucose uptake by yeast cells of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

Glucose uptake capacity of silver nanoparticles of *Gymnema sylvestre* is studied by the method of Vijayalakshimi *et al.* (2015) and detailed procedure is given in Appendix VI.

RESULTS AND DISCUSSIONS

4.RESULTS AND DISCUSSION

Type 1 diabetes (T1D) is an immune system problem leading to the destruction of pancreatic beta-cells. Type 2 diabetes (T2D), is fundamentally an issue of progressively impaired glucose regulation due to a combination of dysfunctional pancreatic beta cells and insulin resistance. Type 2 diabetes mellitus (T2DM) is described by dysregulation of carbohydrate, lipid and protein metabolism and results from impaired insulin secretion, insulin resistance or a combination of both (Gardner and Tai, 2012). Of the three significant kinds of diabetes, T2DM is more common (representing over 90% of all cases) than either type 1 diabetes mellitus (T1DM) or gestational diabetes (Ferrannini and Mari, 2014).

Nanotechnology has a significant impact on natural medicine as well as the economy and society. It is involved in the creation and development of a variety of innovative formulations for the prevention, treatment and diagnostics of a variety of serious diseases (Kumar, 2017). Nanoparticles includes carbon nanotubes, nanorods, nanocapsules, nanoemulsions, fullerenes, metallic nanoparticles, clay nanoparticles and polymer nanoparticles. These are being used for a wide range of applications including clinical treatment, energy storage employing various aspects of the industrial production, such as solar batteries and oxide fuel batteries and widespread inclusion into everyday items like cosmetics and clothing (Dubchak *et al.,* 2010). Silver nanoparticles have antidiabetic, anticancer and antimicrobial properties. Antidiabetic drugs are delivered to the target site by silver nanoparticles. With the help of silver nanoparticles, targeted drug delivery for diabetes is now possible (Gomes *et al.,* 2021).

Plant extracts rich in biologically active compounds can slow down the growth of cancer cells and induce apoptosis in them at the same time which leads to tumor eradication by hindering angiogenesis and metastasis. Many natural phytochemicals have been proved to possess significant therapeutic effects such as antioxidant, antidiabetic, anti inflammatory and anticancer. Each plant contains a several significant elements that can be employed in the medical industry and used to generate various types of medications (Mohammed, 2019). Hence it has become inevitable to identify such therapeutically effective natural phytoconstituents from different type of plants (Stagos, 2019).

Gymnema sylvestre (Asclepiadaceae) is one of the vulnerable species and a slow-growing perennial medicinal woody climber found in central and peninsular India. It is a powerful antidiabetic plant that is utilised in folk, ayurveda, and homoeopathic medicine. Asthma, eye ailments, inflammations, family planning and snakebite are all treated with *Gymnema sylvestre*. It also has antibacterial, hepatoprotective, antihypercholesterolemic and sweet suppressing properties too (Parijat kanetkar *et al.*,2007).

Hence the present study was formulated to synthesize the silver nanoparticles of ethanolic leaf extract of *Gymnema sylvestre* and to find out their efficacy on the *in vitro* antidiabetic properties. The results obtained were furnished and discussed below.

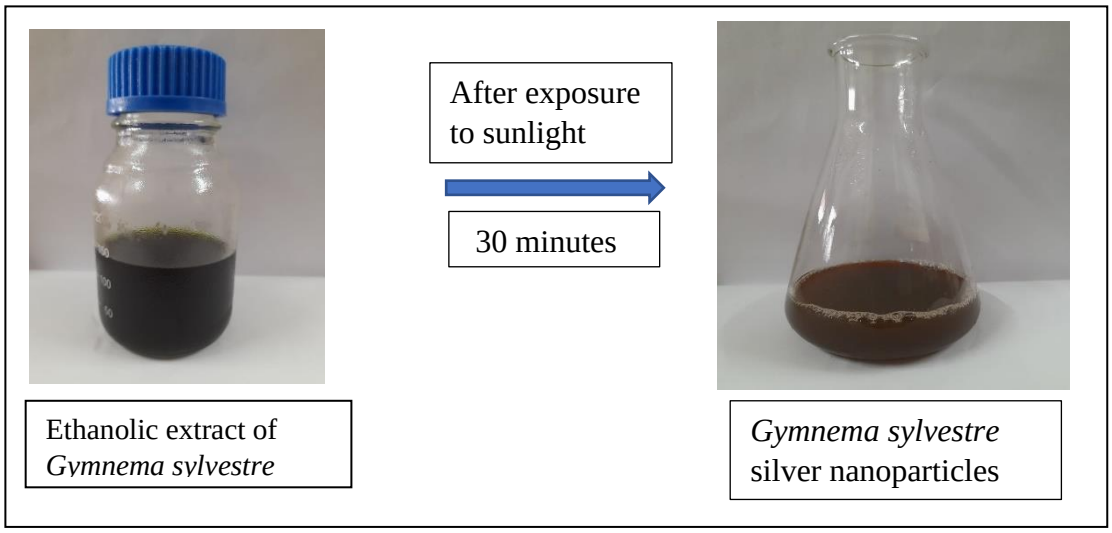
4.1 Synthesis of silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

Silver nanoparticles have received considerable attention due to their good conductivity, chemical stability, catalytic property, photonics and optoelectronics, unique antibacterial, antifungal and UV filtering properties (Chauhan *et al.*, 2015). Plate 2 represents the synthesis of silver nanoparticles of ethanolic extract of leaves of *Gymnema sylvestre.*

The silver nanoparticles of ethanolic extract of leaves of *Gymnema sylvestre* were synthesized on exposure to sunlight for 30 minutes. *Gymnema sylvestre* was found to act as the stabilizing agent for the reduction of Ag+ to Ag°. As the silver nanoparticles are formed, the color of the solution changed from green to dark brown which is an indication of the presence of silver nanoparticles. The variation of the color was due to the change in surface plasmon resonance of silver nanoparticles during the formation.

`PLATE 2

Synthesis of silver nanoparticles of ethanolic extract of leaves of *Gymnema sylvestre*



Mounil *et al.* (2020) reported similar results for the synthesis of silver nanoparticles using *Azadirachta indica* leaf extract. In line with our findings, Fahkun *et al.* (2020) synthesised AgNP from Durian rind extract and observed a colour shift from pale yellow to dark brown and reported that the colour change is caused by electron oscillations on the surface of the nanoparticles, a phenomenon known as surface plasmon resonance. The results of Vivek *et al.* (2021) also proves that synthesised silver nanoparticles from *Onium tenuiflorum* aqueous extract and showed a colour change from light yellow to brownish yellow due to silver ion reduction to AgNPs. It is evident that various phytochemicals found in plant extracts play a vital role in the reduction process.

4.2 Characterization of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

The characterization of silver nanoparticles synthesized from *Gymnema sylvestre* was done by UV-Visible spectroscopy, Fourier-Transform infrared spectroscopy (FT-IR), X ray diffraction spectroscopy (XRD), Scanning Electron Microscopy (SEM) and Energy dispersive analysis of X-rays (EDX).

4.2.1 UV-Visible spectroscopy of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

UV-Visible spectroscopy is a technique for monitoring nanoparticle synthesis during the early phases of synthesis. A significant and well-defined peak arises in the visible spectrum when nanoparticles are formed from their respective salts. In accordance with the previous research, the UV-Vis absorption band between 300 and 800 nm is found optimal for the nanoparticle characterization. Silver nanoparticles conduction and valence bands are usually close, facilitating an electron transport and resulting in a surface plasmon resonance peak. Particle size, dielectric medium and surrounding chemical substances all influences the silver nanoparticle absorption (Almatroudi and Ahmed, 2020).

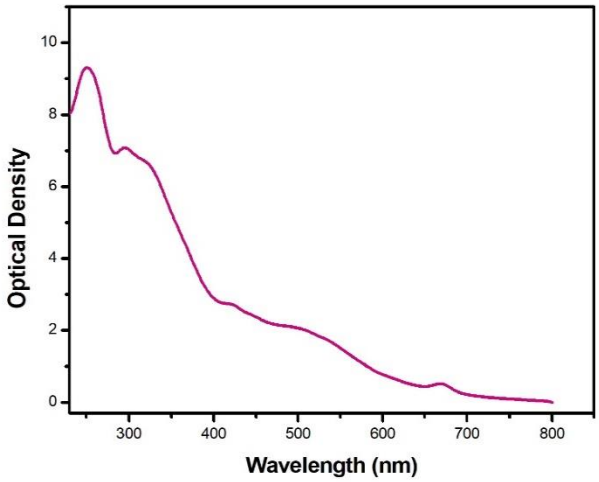


Figure 4 UV-Visible spectroscopy of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

From the figure 4, it is clear that the silver nanoparticles synthesized from the extract of *Gymnema sylvestre* a formed in the reaction media has absorption maxima at 250-300nm and the broadening of peak indicated that the particles are polydispered. The absorption peak of the *Gymnema sylvestre* was found at 250nm. This clearly indicates that there is an interaction between silver nanoparticles and biomolecules present in *Gymnema sylvestre.* Single absorption spectra, according to Mie's theory, indicate that the silver nanoparticles formed will be spherical in shape,

in contrast, anisotropic particles exhibit two or more absorption peaks based on their shape (Francois *et al.,* 2016).

Johnson *et al*. (2020) reported that *O. chinensis* extract showed that the absorption peak (SPR) was observed in the visible range at 250 nm, which clearly indicates the synthesis of silver nanoparticles. Govindappa *et al.* (2018) reported that the plant extract of *C. tomentosum* showed the reduction of silver nitrate using the plant leaf extract which was evidenced by the colour change in the reaction solutions. The UV-Vis spectra recorded for the reaction solution of reduced silver nitrate by leaf extract showed highest absorbance peak at 260 nm indicating the formation of AgNPs

In agreement with the previous reports, the current study confirms the formation of spherical shaped silver nanoparticles of *Gymnema sylvestre* leaf extract, as evidenced by the single peak at 250 nm in UV-Vis spectroscopy.

4.2.2 Fourier transform infrared (FTIR) spectroscopy of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

FTIR is a common technique used by researchers and industrialists to analyse the composition and structure of molecules. It is a simple and reliable method for determining the functional group of a substance. Researchers frequently employ this technique to characterise materials (Fahelelbom *et al.,* 2022).

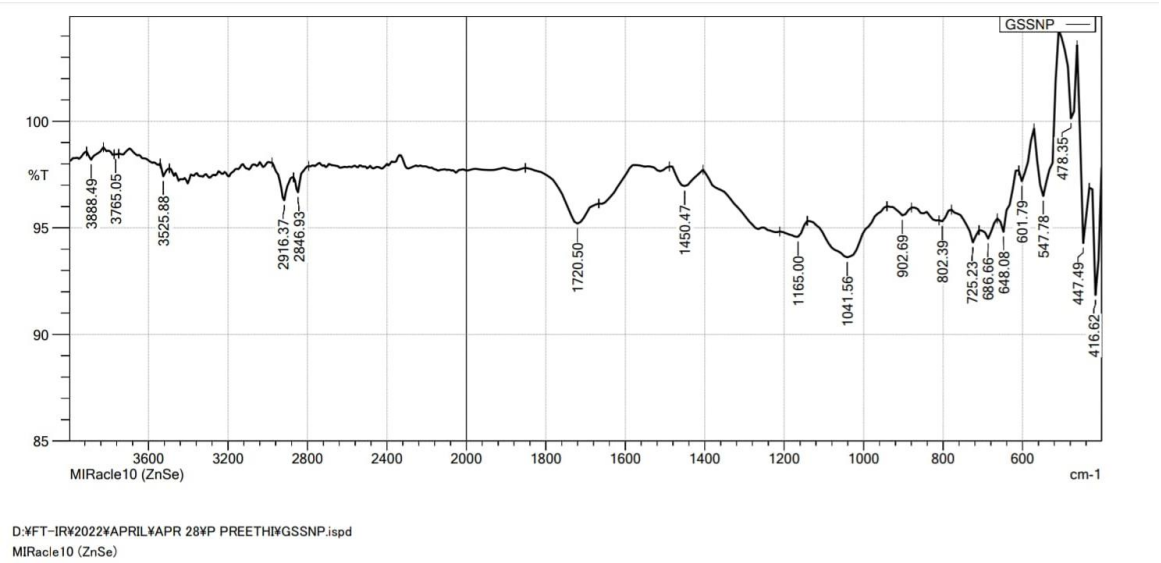


Figure. 5

FT-IR spectroscopy of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

The FT-IR spectrum of synthesized silver nanoparticles is presented in figure. 5. The absorption peak at 3888.49, 3765.05 and 3525.88 cm^{-1} show the presence of O-H stretching which may indicate the alcoholic group in the given sample. The presence of C-H stretching is confirmed by the medium absorption peaks at 2846.93 and 2916.37 cm^{-1}, which may be due to the presence of an aldehyde or alkane in the sample tested. The peak at 1720.50 cm^{-1} shows the presence of C-H bending which indicates the presence of aromatic compound. The presence of carboxylic acid is due to the O-H bending which is confirmed by the peak at 1450.47 cm^{-1}. The absorption peak at 1165.00 cm^{-1} indicates the presence of anhydrous part in the sample. The anhydride part CO-O-CO is confirmed in the sample due to the presence of absorption peak at 1041.56 cm^{-1}. The absorption peaks at 902.69 and 802.39 cm^{-1} indicates that the sample tested may contain C-H bending and might be a 1,3 disubstituted or 1,4 disubstituted compound.

In line with our findings, Chandrasekharan *et al.* (2022) synthesised silver nanoparticles from *Gmelina arborea* extract and discovered that various functional groups such as alcoholic groups, hydroxyl flavonoids, carbonyl or carboxylic groups, phenolic and amino groups are used in the reduction process. These functional groups serve as a capping agent during the formation of silver nanoparticles and are also involved in the stabilisation of the nanoparticles that are

synthesised. The finding of Vinodhini *et al*. (2022) who synthesised silver nanoparticles from *Tabernaemontana divaricate, Basella alba, and Allium fistulosum* extracts and analysed their functional groups using FTIR analysis and claiming that various functional groups are found in plant extracts that act as capping agents and are involved in the production of silver nanoparticles

4.2.3. X-Ray diffraction spectroscopy (XRD) of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

X-ray diffraction is a technique used in the characterization of silver nanoparticles. It is used to determine the crystalline structure, size and phase nature of synthesised AgNPs (Mourdikoudis *et al.,* 2021). It has been carried out for the verification of phase formation of the biosynthesized silver nanoparticles and the recorded Xray diffraction was shown in the Figure 6. The diffraction peaks observed at the 2θ positions of 27.32º, 32.96º, 36.87º, 46.34º, 55.23º, 57.67º, 64.58º, 67.84º and 78.94º confirms the bragg reflections corresponds to (111), (111), (111), (200), (220), (220), (224), (226), (300) and (311) planes and reveals the face centered cubical phase formation of the prepared silver nanoparticles.

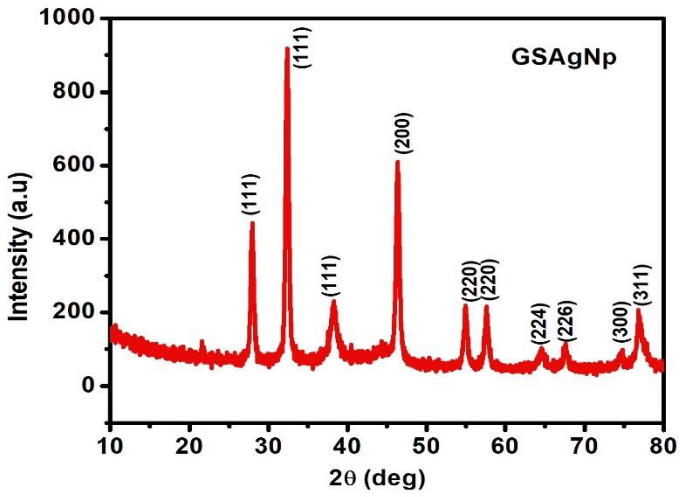


Figure. 6

X-Ray diffraction spectroscopy (XRD) of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

The X-ray diffraction pattern of synthesised silver nanoparticles shows major diffraction peaks at 32.96°, corresponding to the (111) plane respectively. These diffraction patterns indicate that the synthesised nanoparticles have a Face Centered Cubic Crystalline (FCCC) structure. The high intensity of these diffraction peaks indicates that the AgNPs are crystalline in nature. The values are given in the Table. 1.

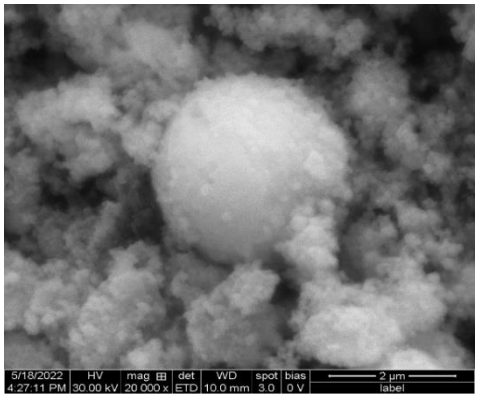
Table. 1

X-ray diffraction analysis of ethanolic extract of Gymnema sylvestre

S. No	2θ (degree)	β(FWHM) (degree)	Crystalline size (nm)	Strain	Dislocation density $(\times 10^{15})/cm^3$
1.	32.96	0.15	17.8	0.0228	0.003156

4.2.4 Field emission scanning electron microscopy (FESEM) of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

FESEM was used to gain a better understanding of the silver nanoparticles shape and size. An image is created when an electron is reflected from the sample's surface. Important features like size, shape, topography, composition and electrical conductivity can be examined using the data generated from high-resolution images of nanoparticle surfaces. An image is created when an electron is reflected from the samples surface. Important features like size, shape, topography, composition and electrical conductivity can be examined from high-resolution pictures of nanoparticle surfaces (Jain *et al.,* 2021). The results of the study showed that the diameter of the silver nanoparticle of *Gymnema sylvestre* was about 5-100nm and they were spherical in shape as shown in Figures. 7 and 8.



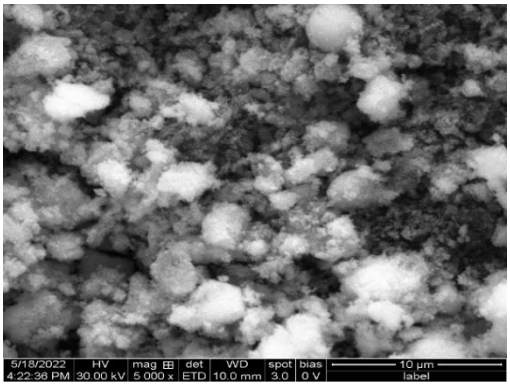


Figure. 7 **Figure. 8**

Figure. 7 and 8

Field Emission scanning electron microscopy (FESEM) of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

Bayrami *et al*. (2018) reported that the plant extract of *Vaccinium arctostaphylus* shows the surface morphology of AgNPs which was analysed through SEM images. The majority of particles were almost spherical in shape, but with different sizes and aggregation. A study carried out by Yadav and Mendhulkar, (2018) demonstrated the presence of uniform and spherical shape of AgNPs synthesized by leaf extract of *Camellia sinensis* in the range of 13- 30 nm in size.

4.2.5. Energy dispersive spectroscopy of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

The elemental composition, relative abundance and pollutants of nanoparticles are all assessed using EDX, which uses X-ray interaction with the sample. The presence of emission peaks at 3 keV verified the existence of silver crystallites, while the absence of any other peaks confirmed the impurity-free character of AgNPs. Other peaks, such as carbon and oxygen, indicate metabolite interaction with AgNPs on the surface (Radhakrishnan *et al.*, 2018).

Figure. 9 shows the EDX image of AgNPs synthesized from ethanolic extract of leaves of *Gymnema sylvestre*, the results clearly indicates that silver nanoparticles of *Gymnema sylvestre* displayed an intense signal at 3 KeV, which is considered to be a characteristic feature for metallic silver nanoparticles due to surface plasmon resonance. The EDAX analysis confirmed the weight

percentage of silver as 44.04 obtained by using *Gymnema sylvestre*, the other signals with characteristic absorption for copper and carbon may be owing to the existence of organic compounds present in the ethanolic plant extract, which represents the capping of phytosynthesized AgNPs by the biomolecules present in the plant.

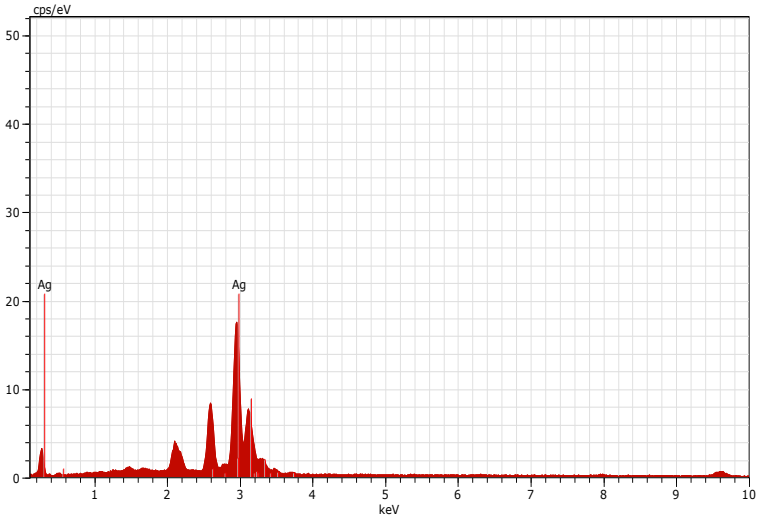


Figure. 9

Energy dispersive spectroscopy of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

Oves *et al.* (2018) reported that the plant extract of *Salvia spinosa* shows the morphology of AgNPs analyzed through SEM images. The majority of particles were spherical in shape and there were a few oval as well. Biosynthesized AgNPs had been spread thoroughly in the solution. The size of some selected biosynthesized nanoparticles was 19–125 nm according to SEM images. The elemental composition of the silver particles synthesized using aqueous leaf extract of pomegranate extract was studied by Sarkar and Kotteeswaran, 2018 and they revealed strong signals in the silver region around 3.2 keV with atomic weight percentage as 0.55.

Bayrami *et al.* (2018) reported that the plant extract of *Vaccinium arctostaphylus* shows the elemental mapping of silver nanoparticles in the range of 55 weight per cent Ag and 19.2 weight per cent of oxygen.

Free Radical Scavenging Activity

4.3.1. Determination of DPPH radical scavenging activity of synthesized silver nanoparticles of ethanolic leaf extract *Gymnema sylvestre*

The stable free radical 2,2-diphenyl-1-picrylhydrazyl is used to assess the antioxidant capacity of plant samples. The antioxidants in the samples convert the purple DPPH to a yellow solution that can be measured at 517nm. The DPPH assay is widely used because it is dependable, quick, time consuming, simple and inexpensive (Lalhminghlui and Ganesh, 2018).

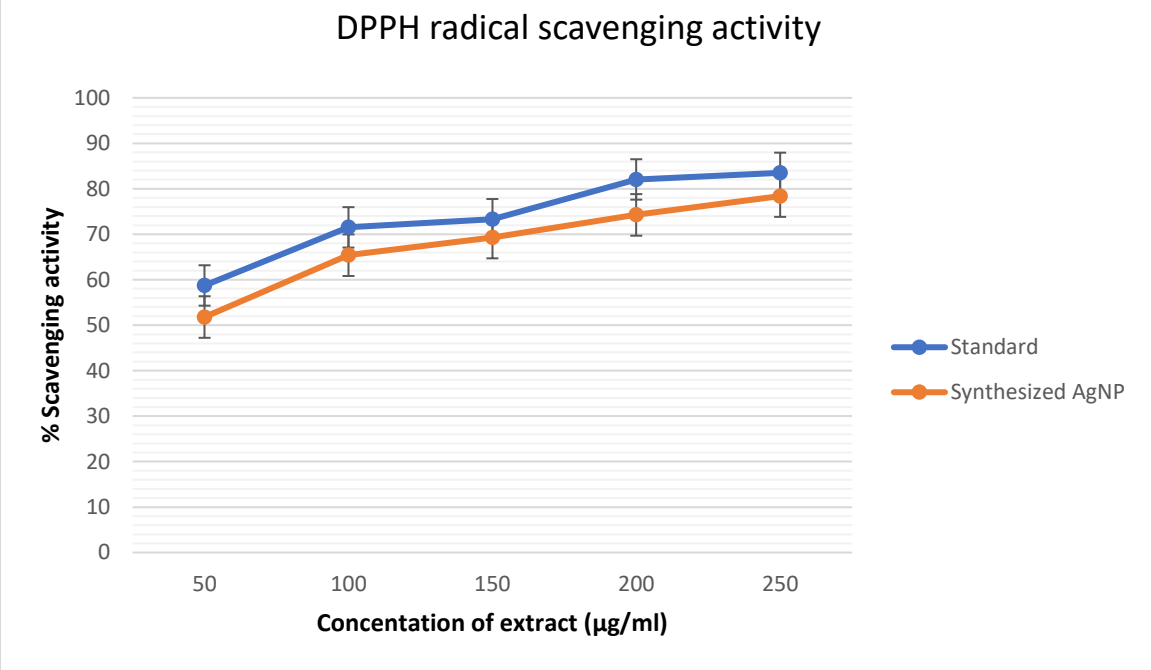


Figure. 10 DPPH radical scavenging activity of synthesized silver nanoparticles of ethanolic leaf extract *Gymnema sylvestre*

The result obtained for the DPPH radical Scavenging assay of *C. pulcherrima* plant extract is shown in the figure 10. The maximum activity for silver nanoparticle leaf extract was recorded for 250µg/ml. The inhibition of the silver nanoparticles leaf extract was 78.40% respectively and that of ascorbic acid was 83.51%. The IC_{50} of ascorbic acid was 175 µg/ml while that of the leaf extract was 135 µg/ml respectively. Leaf extract has a stronger antioxidant activity marked by a stronger inhibition of DPPH radical. When comparing this antioxidant activity with those of the reference molecules, it appears that ascorbic acid shows stronger activity than that of leaf extract.

4.3.2. Determination of ABTS radical scavenging activity of synthesized silver nanoparticles of ethanolic leaf extract of *Gymnema sylvestre*

The antioxidant activity of the leaf extract was analyzed using another type of stable free radical, namely ABTS, and the results obtained are shown in Figure 11. The maximum activity was found to increase with increase in concentration from 0- 250µg/ml. The inhibition of the leaf extract was 79.26% respectively and that of ascorbic acid was 83.51%. The IC_{50} of ascorbic acid was 175 µg/ml while that of the leaf extract was 143 µg/ml respectively. The leaf extract showed good radical scavenging activity. Comparing this antioxidant activity with the reference molecules it appears that ascorbic acid was more active than the extract.

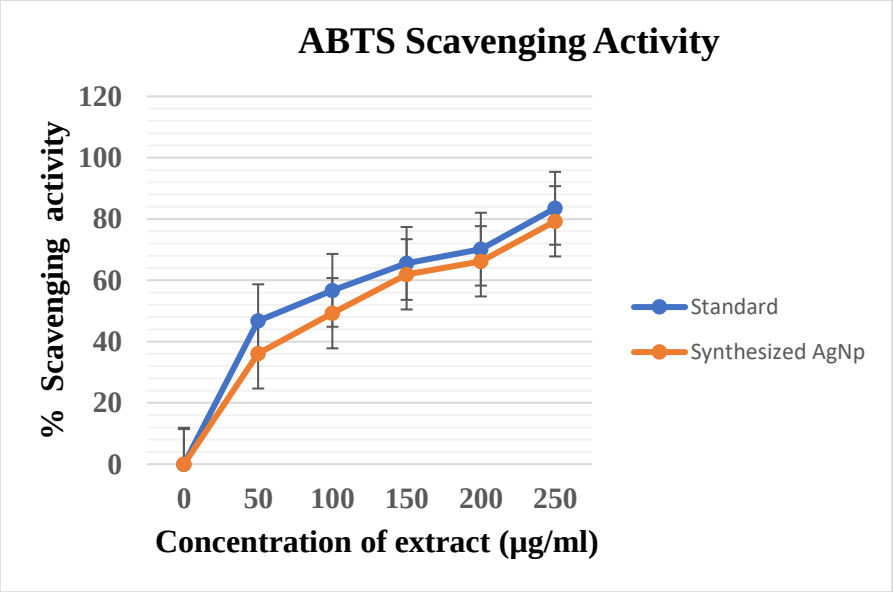


Figure 11

ABTS radical scavenging activity of synthesized silver nanoparticles of ethanolic leaf extract of *Gymnema sylvestre*

Adebiyi *et al.*, 2017 reported that *G. carpinifolia* extract was found to be effective in scavenging the ABTS radical. The percentage inhibition of this radical was dependent on concentration. The inhibition of the leaf and stem extract was 58.15% and 70.25% respectively and that of ascorbic acid was 80.02%. The IC_{50} of ascorbic acid was 0.31 mg/ml while that of the leaf and stem extract was 0.32 and 1.98 mg/ml respectively.

Lalhminghlu *et a.l.*,2018 reported that the extracts of *S. wallichii* showed a concentration dependent rise in the scavenging of the ABTS free radicals. The maximum activity for chloroform extract was recorded for 350µg/ml, whereas ethanol extracts showed maximum ABTS inhibitory action respectively.

4.4. *In Vitro* antidiabetic activity of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

In the present study, the inhibitory activity of different concentrations (250 – 1000 µg/ml) of silver nanoparticles synthesized from ethanolic extract of *Gymnema sylvestre* was investigated for its alpha amylase activity, Nonenzymatic glycosylation, Glucose uptake capacity, Glucose diffusion inhibitory activity and the results are given below.

4.4.1 Inhibition of alpha amylase activity of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

Inhibition of alpha-amylase, which is involved in carbohydrate digestion, can considerably lower post-prandial blood glucose levels, making it a useful technique in the control of blood glucose levels in type 2 diabetic and borderline individuals (Tundis *et al.*, 2010).

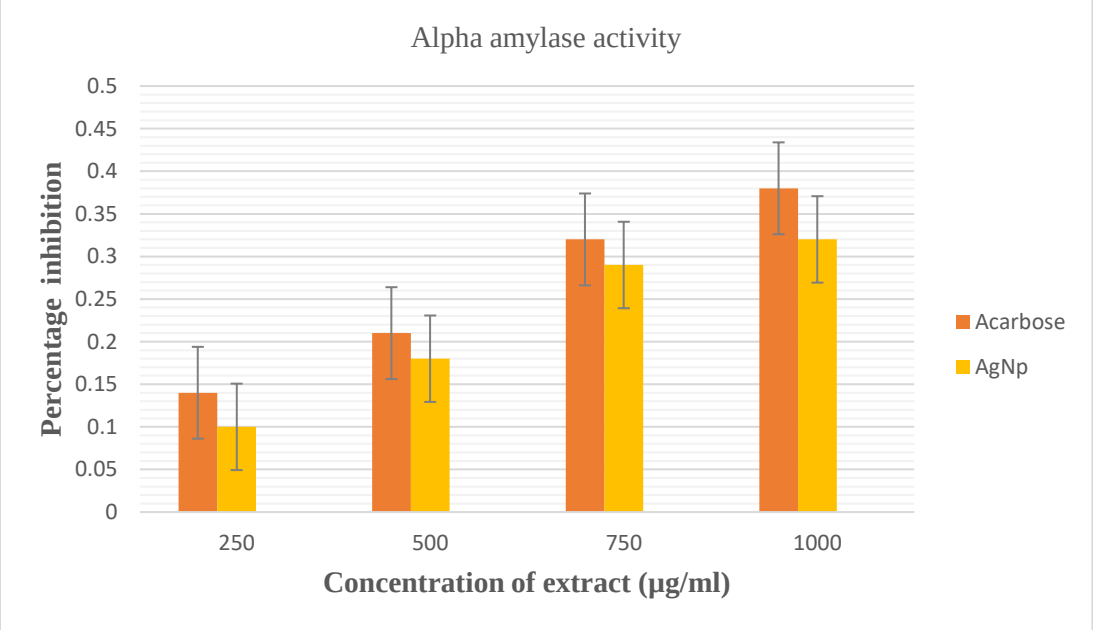


Figure. 12 Inhibition of alpha amylase activity of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

From the above figure, it is clear that the alpha amylase activity of silver nanoparticles of ethanolic leaf extract of *Gymnema sylvestre* was found to be increased with increase in concentration from 250-1000µg/ml. At a concentration of 1000 µg/ml, the silver nanoparticles from the extract of *Gymnema sylvestre* showed 0.32±0.03 percentage inhibition. The positive control, acarbose has exerted the highest potent inhibitory action against alpha amylase (0.36±0.01 per cent).

According to Sangeetha and Vedasree (2012), ethyl acetate and methanol extracts were more efficient than petroleum ether and chloroform extracts in studies of alpha-amylase inhibitory activity of *Thespesia populnea* leaves. Swathi *et al.* (2015) has shown that water, ethanol, ethyl acetate, and chloroform extracts of *Areva lanata* whole plant extracts had dose-dependent increases in % inhibitory activity against alpha-amylase enzyme.

4.4.2. Inhibition of non-enzymatic glycosylation of haemoglobin of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

Nonenzymatic glycation is a post-translational process in which D-glucose interacts slowly with intracellular and extracellular proteins, resulting in the covalent binding of glucose to the protein. The first example of an *in vivo* glycated protein is the glycated human haemoglobin (HbA1C) (Ajitha *et al.*, 2014). Figure 12 represents non-enzymatic glycosylation of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre.*

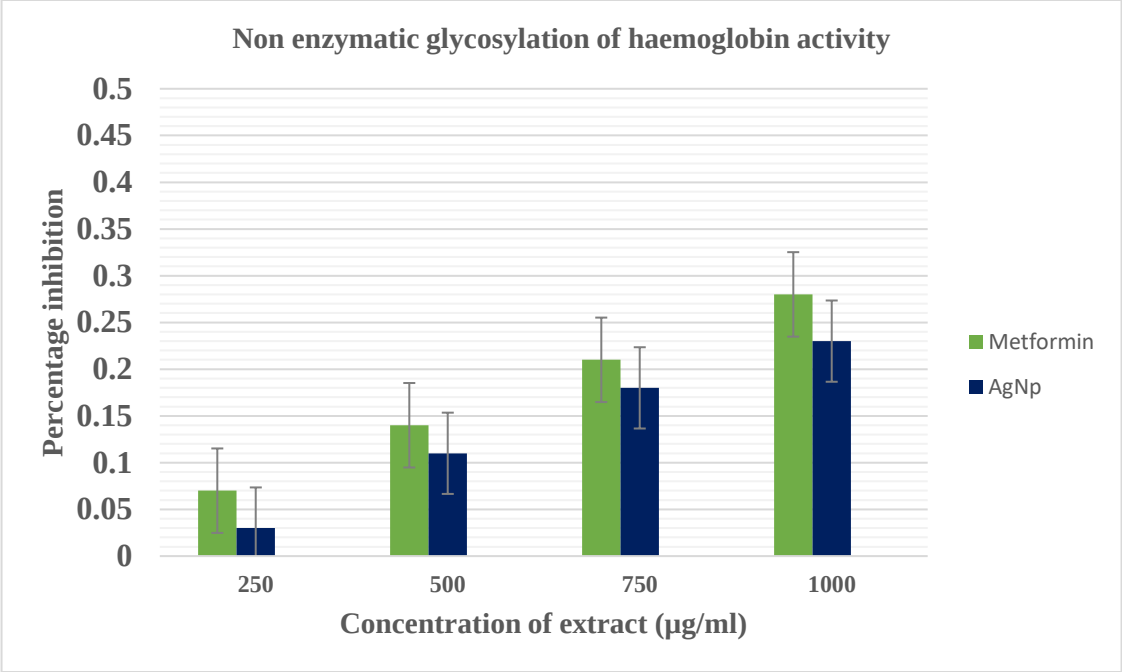


Figure 13

Non-enzymatic glycosylation of haemoglobin of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

From the above figure, it is clear that the non-enzymic glycosylation activity of silver nanoparticles from *Gymnema sylvestre* was found to be increased with increase in concentration from 250 - 1000 µg/ml. At a concentration 1000 µg/ml, the silver nanoparticles of leaves of *Gymnema sylvestre* showed 0.23±0.05 percentage inhibition. The positive control, metformin has exerted the highest potent inhibitory action against non-enzymic glycosylation (0.28±0.03 per cent).

Ning Zhang *et al.,* (2012) showed that when haemoglobin was incubated with increasing glucose concentrations for 72 hours, the plant extract of *Rosa indica* increased glycosylation. *Rosa indica* inhibited glycosylation more effectively than conventional medicines at high concentrations of extract (µg/ml) metformin AgNPs. The plant extracts, on the other hand, considerably reduced haemoglobin glycosylation, as seen by increased haemoglobin concentration.

4.4.3. Glucose uptake assay by yeast cells of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

The transport of glucose across the membrane of yeast cells was seen in an *in vitro* setup in which yeast cells were suspended at various concentrations. In the presence of extracts, the rate of glucose uptake into yeast cells is linear in the (5mM, 10mM) glucose solution. The amount of glucose present in the solution was used to determine the yeast cells glucose absorption after incubation.

The silver nanoparticles of ethanolic extract of *Gymnema sylvestre* promoted the uptake of glucose across the plasma membrane of yeast cells. The effect of standard metronidazole on glucose uptake by the yeast cells at 5Mm and 10Mm glucose concentration was higher as compared to that of silver nanoparticles of ethanolic extract of *Gymnema sylvestre.*

Table. 2

Glucose uptake assay by Yeast cells of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

Glucose uptake at 5mM glucose concentration		
Concentration (µg/ml)	Standard (%)	Silver nanoparticles (%)
250	0.18±0.09	0.15±0.02
500	0.25±0.4	0.21±0.04
750	0.32±0.3	0.27±0.4
1000	0.38±0.3	0.32±0.01
Glucose uptake at 10mM glucose concentration		
250	0.20±0.2	0.18±0.09
500	0.28±0.4	0.25±0.3
750	0.35±0.04	0.31±0.4
1000	0.40±0.02	0.36±0.3

From the above Table 2, it is clear that the glucose uptake by yeast cell activity of silver nanoparticles of *Gymnema sylvestre* in 5mM glucose solution *was* found to be increased in concentration from 250-1000 µg/ml. At a concentration 1000 µg/ml the silver nanoparticles of

Gymnema sylvestre showed (0.32 ±0.04) percentage inhibition. In 10 mM glucose solution, at a concentration 1000 µg/ml the silver nanoparticles of *Gymnema sylvestre* showed (0.36 ±0.04) percentage inhibition. The positive control, has exerted the highest potent of (0.37±0.07 per cent). The silver nanoparticles of ethanolic extract of *Gymnema sylvestre* promoted the uptake of glucose across the plasma membrane of yeast cells. The effect of standard metronidazole on glucose uptake by the yeast cells at 5Mm and 10Mm glucose concentration was higher as compared to that of silver nanoparticles of ethanolic extract of *Gymnema sylvestre.*

Singh *et al.* (2017) has revealed that the oral starch tolerance test in mice was used to test the most active extract *in vivo*. All of the extracts inhibited amylase in a dose-dependent manner and increased glucose transport across yeast cells. *Calocybe indica* extract was the most effective amylase inhibitor (18.07 0.75 mg/mL half-maximal inhibitory concentration) and had the highest glucose absorption by yeast cells (77.53 0.97 percent at 35 mg/mL).

4.4.4. Inhibition of glucose diffusion of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

The inability of the cell membrane to retain glucose molecules causes the blood sugar level in hyperglycemic patients to rise dramatically. Glucose diffusion across the membrane has been demonstrated to be slowed down by some viscous components found in plant extracts. Plant extracts showed a lot of promise in terms of preventing glucose diffusion across the dialysis membrane. As a result, they may operate as a potential barrier to lowering blood glucose levels by preventing glucose molecules from crossing the plasma membrane and reaching the blood vessel (Abas *et al.*, 2016).

Table. 3

Inhibition of glucose diffusion of synthesized silver nanoparticles of ethanolic extract of *Gymnema sylvestre*

Glucose diffusion at 1st hour		
Concentration (µg/ml)	**Standard (%)**	**Silver nanoparticle (%)**
250	0.18±0.09	0.14±0.2
500	0.23±0.3	0.21±0.04
750	0.28±0.2	0.24±0.03
1000	0.32±0.5	0.29±0.02
Glucose diffusion at 2nd hour		
Concentration (µg/ml)	**Standard (%)**	**Silver nanoparticle (%)**
250	0.23±0.3	0.19±0.03
500	0.28±0.5	0.23±0.3
750	0.33±0.2	0.30±0.03
1000	0.37±0.4	0.34±0.2
Glucose diffusion at 3rd hour		
Concentration (µg/ml)	**Standard (%)**	**Silver nanoparticle (%)**
250	0.25±0.3	0.23±0.3
500	0.29±0.2	0.25±0.3
750	0.37±0.4	0.32±0.5
1000	0.40±0.3	0.37±0.4

In the given table, the silver nanoparticles of ethanolic extract of *Gymnema sylvestre* inhibit glucose diffusion across the dialysis membrane. The glucose diffusion inhibition was measured at different time interval like first, second and third hour. At first hour of glucose diffusion, the activity increases with increased concentration upto 1000µg/ml with 0.29±0.04 percentage inhibition. At second and third hour, the silver nanoparticles of *Gymnema sylvestre* showed (0.35 ±0.04) and (0.35 ±0.04) percentage inhibition at 1000µg/ml. The effect of standard acarbose on glucose diffusion measurement at different time interval was a bit higher as compared to that of silver nanoparticles of ethanolic extract of *Gymnema sylvestre.*

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

Diabetes mellitus, a metabolic endocrine illness characterised by chronic hyperglycemia, resulting in biochemical alterations and tissue death. The relevance of insulin as an anabolic hormone causes metabolic irregularities in the metabolism of carbohydrates, lipids and proteins. The type and duration of diabetes determines the severity of symptoms. Some diabetic patients, particularly those with type 2 diabetes in the early stages of the disease, are asymptomatic; others with severe hyperglycemia and especially children with absolute insulin shortage, may experience polyuria, polydipsia, polyphagia, weight loss and blurred vision.

Nanotechnology-based therapeutic and diagnostic techniques have shown promising strategies in the improvement of cancer treatment in recent years. It provides a range of techniques for treating diabetes by overcoming biological barriers and directly delivering therapeutic chemicals. Metal nanoparticles, particularly silver nanoparticles, have physicochemical qualities such as high surface-to-volume ratio, broad optical properties, simplicity of manufacturing and surface functionalization, which open up new possibilities. In addition, the use of nano particulate drug carriers might improve solubility of the drug, extend half-life of the drug in the bloodstream, and reduce adverse side effects at non-target organs. Thus, targeted therapies with nano sized formulations might be a valuable approach to render cytotoxicity towards the proliferating cancerous cells.

Plants are an intriguing source of new therapeutic entities and they contribute to drugs with potential application as antidiabetic agent. A few plant species have been significantly evaluated for their possible medical application. Phytoconstituents are promising alternatives for producing green silver nanoparticles, which have a lot of potentials in treating chronic disorders. Several plants, their parts and products have been successfully used for efficient and rapid green synthesis of AgNPs in non-hazardous ways. Hence the present study aims to synthesize the silver nanoparticles from the ethanolic extracts of *Gymnema sylvestre* and to evaluate its antidiabetic property.

The silver nanoparticles are synthesized from ethanolic extract of *Gymnema sylvestre*. The synthesis of nanoparticles was noticed by change in colour and absorbance. The color of the solution changes from green to dark brown which is an indication of the presence of silver

nanoparticles. The variation of the colour was due to the change in surface plasmon resonance of silver nanoparticles.

UV-Visible spectroscopy is used to determine the particle formation and properties. The absorption spectrum of silver nanoparticles of ethanolic extract of *Gymnema sylvestre* shows intense peak at 250 nm. This clearly indicates that there is an interaction between nanoparticles and biomolecules present in *Gymnema sylvestre.*

Functional groups of the plant secondary metabolites involved in the reduction and capping of nanoparticles were identified by FT-IR technique. The FT-IR spectrum of synthesized silver nanoparticles of *Gymnema sylvestre* showed the peaks assigned for O-H stretching of alcohols, C-H stretching mode in alkenes, O=C=O stretching vibrations of carbon dioxide, C-H bending of aromatic compounds, O-H bending of phenol, CO-O-CO bending of anhydrides and C-H bending that might be a 1.3 disubstituted or 1,4 disubstituted compound.

The size and morphology of the synthesized silver nanoparticles of *Gymnema sylvestre* was determined by the Scanning Electron Microscopy (SEM) and the elemental composition of it was confirmed by Energy Dispersive X-ray (EDX) analysis. The SEM images revealed that the synthesized silver nanoparticle was in the range of 5 -100nm. A spherical shape was observed as well as a variation of the nanoparticle size with variation of the silver nitrate volume in the colloidal solution. EDX quantitative analysis confirms the nanostructure of the silver nanoparticles of extracts of *Gymnema sylvestre*. The silver nanoparticles synthesized from ethanolic extract of *Gymnema sylvestre* contains about 44.04 weight per cent Ag of oxygen.

The crystallinity and purity of green synthesized AgNPs were evaluated by powder X-ray diffraction. The peaks observed in the diffractogram are corresponding with face-centered cubic phase of metallic Ag at the 2θ positions of 27.32º, 32.96º, 36.87º, 46.34º, 55.23º, 57.67º, 64.58º, 67.84º and 78.94º confirms the bragg reflections corresponding to (111), (111), (111), (200), (220), (220), (224), (226), (300) and (311) planes and reveals the face centered cubical phase formation of the prepared silver nanoparticles. The average crystalline size was calculated with respect to the major intensity peak corresponding to the (111) plane and it was estimated to be around 17.8 nm which falls in the acceptable feature of nanoparticles.

The radical scavenging studies using DPPH and ABTS assays, revealed the strong antioxidant potential of *Gymnema sylvestre* leaves which is equivalent to that standard antioxidant such as ascorbic acid.

The inhibitory activity of different concentrations (250 – 1000 µg/ml) of silver nanoparticles synthesized from ethanolic extract of *Gymnema sylvestre* was investigated for its alpha amylase activity, Nonenzymatic glycosylation, Glucose uptake capacity and Glucose diffusion activities. The silver nanoparticles synthesized showed potent inhibitory activity against alpha amylase. The positive control, acarbose has exerted the highest potent inhibitory action against alpha amylase (0.36±0.01 percent). The positive control, metformin has exerted the highest potent inhibitory action against non-enzymic glycosylation (0.25±0.03 per cent).

The silver nanoparticles of ethanolic extract of *Gymnema sylvestre* advanced the uptake of glucose across the plasma membrane of yeast cells. The glucose uptake at an initial concentration of 5 mM and 10 mM was comparable to that of silver nanoparticle of *Gymnema sylvestre*. The effect of silver standard metronidazole on glucose uptake by the yeast cells at 5mM and 10mM glucose concentration was higher as compared to that of silver nanoparticles of ethanolic extract of *Gymnema sylvestre*. The silver nanoparticles of ethanolic extract of *Gymnema sylvestre* reversed the glucose diffusion across the dialysis membrane. However, the effect of silver nanoparticles of ethanolic extract of *Gymnema sylvestre* on glucose diffusion measurement at different time interval was measured. The effect of standard acarbose on glucose diffusion measurement at different time intervals (1^{st} hour, 2^{nd} hour and 3^{rd} hour) corresponding to the values of $0.29 \pm 0.2, 0.34 \pm 0.3,$ and 0.37 ± 0.4, which was higher as compared to that of silver nanoparticles of ethanolic extract of *Gymnema sylvestre.*

The results of the present study demonstrated that the silver nanoparticle synthesized from the ethanolic extract of leaves of *Gymnema sylvestre* possesses significant antidiabetic activity, which was evidenced by the spectral analysis. Hence, these nanoparticles can be further exploited for its use in treating diabetes mellitus and associated disorders. However, future studies have to be carried out to find out the efficacy of *in vivo* antidiabetic activity using animal models.

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Appendices

APPENDICES

Free radical scavenging activity of synthesized silver nanoparticles of *Gymnema sylvestre*

Appendix I

Analysis of 2,2'-Diphenyl-1-Picryl-Hydrazyl-Hydrate (DPPH) Radical Scavenging Activity of *Gymnema sylvestre*

Spectrophotometric quantification of the radical scavenging ability of the extract towards DPPH free radical was carried out by the method of (Mensor *et al.*, 2001).

Principle

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant activity of plant extracts. DPPH produces violet/purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants. The assay measures the reducing ability of antioxidants toward the DPPH radical.

Reagents

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

2. Methanol

Procedure

In a reaction mixture of 1ml, 20µl corresponding to 1 mg of plant extracts were mixed 0.5ml of DPPH in methanol and 0.48 ml of ethanol. After incubating at room temperature for 30 minutes. The decolorization of the purple color was measured at 518 nm. Methanol alone was used as the Reagents 29 blank and DPPH in methanol, without the extracts, was used as positive control. The radical scavenging activity was calculated as follows

$$\text{percent scavenging activity } = 100 - \frac{\text{A518(Sample)} - \text{A518(Blank)}}{\text{A518(Control)}} \times 100$$

Appendix II

Determination of 2,2'-Azino-Bis-3-Ethyl Benzthiazoline-6-Sulphonic Acid (ABTS) Radical Scavenging Activity of *Gymnema sylvestre*

The Procedure for ABTS (2,2′-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolorization assay was based on the method proposed by (Shirwaikar *et al.,* 2006).

Principle

ABTS (2,2′-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolourization assay was employed to assess the radical scavenging effect of the whole plant extract of the plants. ABTS is a chromogen, which damages into a coloured monocation radical form (ABTS+) in the presence of oxidative agent and the ABTS+ has an absorption peak at 750nm. Antioxidants will reduce ABTS+ into its colourless form and the extent of decolourisation corresponds to the present reduction of ABTS+.

Reagents

1. Ethanol
2. ABTS solution (7Mm ABTS with 2.45 mM ammonium persulfate).

The solution was incubated at room temperature for 12- 16 hours before use.

Procedure

The Plant extracts (100µl each) were added to ABTS solution (300µl) and the final volume of each was made up to 1ml with ethanol. The absorbance was read at 745 nm and the percentage inhibition was calculated using the formula,

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

In vitro Antidiabetic Activity of synthesized Silver Nanoparticles of *Gymnema sylvestre* Leaf Extract

Appendix III

In vitro Alpha Amylase Inhibitory Activity of Synthesized Silver Nanoparticles of *Gymnema sylvestre* Leaf Extract (Subramanian *et al.*, 2008)

Alpha amylase inhibition method, 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 were 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 (5min). After cooling, the absorbance was measured colorimetrically at 565nm. The inhibition percentage was calculated using the given formula,

$$\text{Percent Inhibition} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100$$

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.

Appendix IV

Non- Enzymatic Glycosylation of Haemoglobin of Synthesized Silver Nanoparticles of Ethanolic Extract of *Gymnema sylvestre* (Daksha *et al.*, 2012)

Glucose (2 percent), haemoglobin (0.06 percent) and gentamycin (0.02 percent) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. 1 ml each of the above solutions was mixed and 1 ml of the ethanol extract of varying concentrations was added to it, respectively. The reaction mixture was incubated in dark at room temperature for 72hrs and then the degree of glycosylation of haemoglobin was measured colorimetrically at 520nm. Metformin was used as a standard drug for the assay and percentage inhibition was calculated using the formula,

$$\text{Percent Inhibition} = \frac{\text{Absorbance Sample} - \text{Absorbance control}}{\text{Absorbance Sample}} \times 100$$

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

Appendix V

Inhibition of Glucose Diffusion of Synthesized Silver Nanoparticles of Ethanolic Extract of *Gymnema sylvestre* (Gallager *et al.*, 2003)

2 ml of 0.15 M NaCl containing 0.22mM D-glucose was loaded into a dialysis tube containing plant extract (50g/L) and the dialysis tube was sealed. The sealed tube was then placed in a centrifuge tube containing 45 ml of 0.15 M NaCl and kept in an orbital shaker at a room temperature. The diffusion of glucose into the external solution was monitored by measuring the glucose concentration in the external solution every 60 minutes.

Appendix VI

Glucose Uptake Assay by Yeast Cells of Synthesized Silver Nanoparticles of Ethanolic Extract of *Gymnema Sylvestre* (Vijayalakshimi e*t al*., 2014)

Yeast suspension was prepared by repeated washing (by centrifugation 3,000× 5min) in distilled water until the supernatant fluids were clear. A 10% (v/v) suspension was prepared with the supernatant fluid. 1ml of the glucose solution (5, 10 and 25 mM) was added to various concentrations of the ethanol extract (250, 500, 750 and 1000µg) and incubated for 10 minutes at 37ºC. Reaction was started by adding 100µl of yeast suspension, vortexed and further incubated at 37ºC for 60 min, the reaction mixture was centrifuged (2,500g, 5min) and the glucose content was estimated in the supernatant. Metronidazole was taken as a standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula,

$$\text{Percent inhibition} = \frac{\text{Absorbance Sample} - \text{Absorbance Control}}{\text{Absorbance Sample}} \times 100$$

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.