Characterization of iron nanoparticles synthesized using *Sesbania grandiflora* flower extract and evaluation of its antidiabetic activity

By

M.THARANI

(Roll No. 20PZO018)

Supervisor

Dr. K.S. SANTHY

Thesis Submitted to the
Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore – 641 043

In Partial Fulfilment of the Requirements for the Degree of
Master of Science in Zoology (M.Sc.)

MAY 2022

Characterization of iron nanoparticles synthesized using *Sesbania grandiflora* flower extract and evaluation of its antidiabetic activity

By

THARANI M.

(Roll. No. 20PZO018)

Department of Zoology

Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore- 641043

In Partial Fulfilment of the Requirements for the Degree of Master of Science (M.Sc.)

MAY 2022

K. Ramimegalai 30/5/2022

Signature of the Head of the Department

Sandhya 30.5.2022

Signature of the Supervisor

ACKNOWLEDGEMENT

First and foremost, I thank **Almighty** for his grace, which sustained and helped me to complete my thesis work successfully.

I take this opportunity to pay my respectful gratitudeto (Late) **Dr. P.R. Krishnakumar,** former Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for all the amenities provided for the conduct of my researchwork.

I wish to express my profound sense of gratitude and sincere thanks to **Dr.S. P. Thyagarajan, D.Sc., Ph.D., M.D., Chancellor**, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for all the amenities provided for the conduct of my research work.

I am extremely grateful to **Dr. (Mrs.) V. Bharathi Harishankar, Ph.D., FRSA** Vice Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for supporting me with all facilities needed for the completion of my thesis.

I wish to thank **Dr. (Mrs.) S. Kowsalya, M.Sc., M. Phil., Ph.D.,** Registrar, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing all the facilities needed for the completion of my thesis.

It is my pleasure to acknowledge the wholehearted support and encouragement extended to me by (Late)**Dr. P.R. Padma, M.Sc., Ph.D.,** Former Dean, Faculty of Bioscience and **Dr. (Mrs.) A. Vijayalakshmi M.Sc., MPhil., Ph.D.,** Dean of Bioscience, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

I am happy to express my hearty thanks to **Dr. (Mrs.). K. Manimegalai, M.Sc., Ph.D.,** Professor and Head of the Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, for her unflinching support and constant encouragement given throughout the tenure of my research.

I wish to express my sincere thanks and deep indebtedness to my guide **Dr. K. S. Santhy, M.Sc., M. Phil., Ph.D., M.C.A., PGDBI,** Professor, Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for her amiable suggestions, eminent guidance and encouragement throughout the period of my work.

I express my sincere thanks to **all the members of staff**, Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for their support.

A warm thanks to my friends, **Ms. E. Deepika, Ms. Gunavathi and Ms. E. Amrisa pavithra** for their support, inspiration and good wishes throughout my study period.

My thanks are also due to all those who have not been mentioned by name, but nevertheless, have been of tremendous help in their own ways.

M.Tharani

CONTENT

LIST OF TABLES

LIST OF FIGURES

ABBERIVATION

WHO	-	World Health Organization
DNA	-	Deoxyribonucleic acid
DM	-	Diabetes mellitus
AFM	-	Atomic Force Microscopy
SEM	-	Scanning Electron Microscopy
EBL	-	Electron Beam Lithography
CNS	-	Central Nervous System
TCM	-	Traditional Chinese Medicine
FTIR	-	Fourier Transform Infrared Spectroscopy
MNP	-	Magnetic Nano Particles
NP	-	Nano Particles
MCM	-	Medical Counter Measures
MRSA	-	Methicillin Resistant *Staphylococcus aureus*
EESG	-	Ethanol Extract of *Sesbania grandiflora*
NMR	-	Nuclear Magnetic Resonance
CADD	-	Computer Aided Drug Design
SBDD	-	Structure-Based Drug Design
UV-Vis	-	Ultraviolet Visible Spectroscopy
DPPH	-	2, 2-diphenyl-1-picryl-hydrazyl-hydrate
ADME	-	Absorption, Distribution, Metabolism and Excretion
ISM	-	Industrial, Scientific and Medical
ROS	-	Reactive Oxygen Species
GUI	-	Graphical User Interface

INTRODUCTION

1 INTRODUCTION

Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis. There are nearly 2000 ethnic groups in the world, and almost every group has its own traditional medical knowledge and experiences. Iran is home to several indigenous tribes with a rich heritage of knowledge on the uses of medicinal plants. Man relied on the healing properties of medicinal plants (Roy *et al.,* 2018). Some people value these plants due to the ancient belief which says plants are created to supply man with food, medical treatment, and other effects. (Ullah *et al.*, 2020). It is thought that about 80% of the 5.2 billion people of the world live in the less developed countries and the World Health Organization estimates that about 80% of these people rely almost exclusively on traditional medicine for their primary healthcare needs. All of the medicinal plants were collected from the wild or in the native people's gardens. Some medicinal plants can no longer be found in the region and are only cultivated in the native people's gardens (Ahvazi *et al.*, 2017).

Natural products have evolved over millions of years and acquired a unique chemical diversity, which consequently results in the diversity of their biological activities and drug-like properties (Newman and Cragg, 2016). Therefore, even before the rise of the modern chemical pharmacology, NPs have been used for centuries as components of traditional medicines, in particular as active components of herbal remedies. Nowadays, some of the traditional healing practices, such as Indian Ayurveda, traditional Chinese medicine or African herbal medicines, remain the primary treatment option for many people across the world, due to economic reasons, to personal beliefs or to the difficulty in accessing pharmaceutical products. In modern pharmacology too, NPs have become one of the most important resources for developing new lead compounds and scaffolds (BanoMirza *et al.*, 2015).

The World Health Organization listed between 1999 and 2009 a list of over 21 000 plants used for medicinal purposes all over the world (WHO, 2009). This effort was made for proper identification of safe plants, as it is estimated that plant-based traditional medicines are used by

60% of the world's population (Baessa *et al.,* 2019). In addition to efforts to establish formal, DNA-based identification of such plants for wider use (Palhares *et al.,* 2015), collections of medicinal plant species, and in particular of phytochemicals, NPs produced by plants, associated to their therapeutic activities and physicochemical properties are being established around the world. (Karunamoorthi *et al.,* 2018).This is particularly the case in Asia and Africa, where traditional medicines remain an important part of everyday life for cultural, traditional and economic reasons.

Ayurveda is a medical system primarily practiced in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment .The term comes from the Sanskrit root Au (life) and Veda (Khalifa *et al.,* 2019). As the name implies it is not only the science of treatment of the ill but covers the whole gamut of happy human life involving the physical, metaphysical and the spiritual aspects. Ayurveda is gaining prominence as the natural system of health care all over the world.

Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments(Karunamoorthi *et al.,* 2018). Medicinal plants are considered as a rich resources of ingredients which can be used in drug development either pharmacopoeias, non-pharmacopoeia or synthetic drugs. A part from that, these plants play a critical role in the development of human cultures around the whole world. (Dar *et al.,* 2017). Moreover, some plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values.

Diabetes mellitus (DM) is commonest endocrine disorder that affects more than 100 million people worldwide (6% population). It is caused by deficiency or ineffective production of insulin by pancreas which results in increase or decrease in concentrations of glucose in the blood. It is found to damage many of body systems particularly blood vessels, eyes, kidney, heart and nerves (Cragg, 2016). Diabetes mellitus has been classified into two types i.e. insulin dependent diabetes mellitus (IDDM, Type 1) and non-insulin dependent diabetes mellitus (NIDDM, Type 2) (Mickymaray, 2019). Type 1 diabetes is an autoimmune disease characterized by a local

inflammatory reaction in and around islets that is followed by selective destruction of insulin secreting cells, whereas Type 2 diabetes is characterized by peripheral insulin resistance and impaired insulin secretion (Ragupathi *et al.,* 2017). The presence of diabetes shows increased risk of many complications such as cardiovascular diseases, peripheral vascular diseases, stroke, neuropathy, renal failure, retinopathy, blindness and amputations (Jothivel *et al.,* 2017). Drugs are used primarily to save life and alleviate symptoms.

Insulin replacement therapy is the mainstay for patients with type 1 diabetes while diet and lifestyle modifications are considered the cornerstone for the treatment and management of type 2 diabetes (Ragupathi *et al.,* 2017). Various types of hypoglycemic agents such as biguanides and sulfonylureas are also available for treatment of diabetes. However none of these medications is ideal due to their toxic side effects and diminution of responses is observed sometimes in their prolonged use .The main disadvantage of currently available drugs is that they have to be given throughout the life and produce side effects (Jaji *et al.,* 2020)

According to recent estimates, approximately 438 million people worldwide (7.8%) in the 20–79 year age group will have diabetes in 2030 of the adult population, is expected to have diabetes. India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the "diabetes capital of the world". According to the Diabetes Atlas (2006) published by the International Diabetes Federation, the number of people with diabetes in India, currently around 40.9 million is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken(Jaji *et al.,*2020). The "Asian Indian Phenotype" refers to certain unique clinical and biochemical abnormalities in Indians which include increased insulin resistance, greater abdominal adiposity i.e., higher waist circumference despite lower body mass index, lower adiponectin and higher high sensitive C-reactive protein levels. Higher prevalence of diabetes mellitus often results from in changes in dietary patterns and decreased physical activity in the urban population (Imran and Rani, 2016).

Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease (Joshi and Parikh, 2017) and (Kumar *et al.,* 2018). In 2000, India (31.7 million) topped the world with the highest number of people with diabetes mellitus followed by China (20.8 million) with the United States (17.7 million) in second

and third place respectively. According to Wild et al. the prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India. It is predicted that by 2030 diabetes mellitus may afflict up to 79.4 million individuals in India, while China (42.3 million) and the United States (30.3 million) will also see significant increases in those affected by the disease.

Nanotechnology originates from the Greek word 'Nano' meaning "dwarf". Nanotechnology is defined as the study and use of structures between 1 nanometer and 100 nanometers in size. Nanotechnology promises even more revolutionary advances with potential impacts on nearly every industrial sector, including energy, health care, defense, transportation, medicine, biology, biotechnology, catalysis, electronics and material sciences. Though it is difficult to imagine exactly how this greater understanding of the world of atoms and molecules will affect the everyday objects we see around us, there are a number of areas where nanotechnologies are set to make a difference in the near future.

The main advantage of nanotechnology is the ability to build new compounds and products using much smaller building blocks allowing new flexibility in material design. It was also found that materials re-engineered to Nano scales exhibit very different characteristics and properties than they exhibit on a macro scale. For example copper, an opaque material becomes transparent; normally stable aluminium becomes combustible and solids such as gold can turn into liquids at just room temperature. Much of this is due to the change in the way surface tension and ionic attraction works in nanoscale where there is a dramatic increase in the ratio of surface area to volume. This phenomenon is called the "quantum" size effect where the electronic properties of solids are altered in a nano state. Technologies such as the atomic force microscope (AFM), scanning electron microscopy (SEM) and electron beam lithography (EBL) all have contributed to the ability of man to formulate and process materials in nanoscale.

It is important to distinguish the term nano as a reference to a scale of measurement and process of product formulation and manipulation rather than nano being an actual product itself. The result of utilising nanotechnology is and should be to improve performance over a similar product made using the same materials but with macro sized particles (Mirzaie *et al.,* 2022). Nanoparticles exhibit totally new or improved properties based on specific characteristics such as

size, distribution and morphology. The use of nano particles is gaining impetus in the present century. The size and surface charge of nanoparticles govern its interactions with biological systems, including absorption, distribution, metabolism, and excretion. The species of surfactant used influences the particle size and shape (Mukherjee, 2019). It is important to know whether NPs are in agglomerated (Vander Waals interactions between primary particles) or aggregated state (chemical bonds between primary particles), since their corresponding biological fate and effects will be different (Egbuna *et al.,* 2020*).* The metallic nanoparticles are the most promising as they show good antibacterial properties due to their large surface area to volume ratio, which is coming up as the current interest in the researchers due to growing microbial resistances against metal ions, antibiotics and development of resistant strains.

Nanoparticles have specific optical, fluorescence and magnetic properties and interactions between these properties give NPs great potential environmental screening. NP-based technologies also have applications in improving air, water and soil quality (Ettadili *et al.,* 2022). Nanomaterials often show unique and considerably changed physical, chemical and biological properties compared to their macro scaled counterparts.

In the last few decades eco-friendly, bio-friendly, cost effective and relatively safe, plant-based medicines have moved from the fringe to the main stream with the increased research in the field of traditional medicine. There are several literature reviews by different authors about anti-diabetic herbal agents, but the most informative is the review by (Kabesh *et al.,* 2015) who has documented more than 300 plant species accepted for their hypoglycemic properties. This review has classified the plants according to their botanical name, country of origin; parts used and nature of active agents. One such plant is *Momordica charantia* (Family: Cucurbitaceae). WHO has listed 21,000 plants, which are used for medicinal purposes around the world. Among these, 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called the botanical garden of the world (Tao *et al.,* 2021).

Medicinal plants and their bioactive constituents can be used for treatment of diabetes throughout the world especially in countries where access to the conventional anti-diabetes agents is inadequate (Jaji *et al.,* 2020). Various experimental models are also available to screen ant

diabetic activity of plants (Laladhas *et al.,* 2022). The present review therefore is an attempt to know more precisely about diabetes mellitus, its clinical presentation, epidemiological data, complications and current available treatment of diabetes. Epidemiologically, it is estimated that 366 million people had diabetes in 2011; by 2030 this would have risen to 552 million. The number of people with type 2 diabetes is increasing in every country with 80% of people with diabetes living in low- and middle income countries. Diabetes caused 4.6 million deaths in 2011 (Laladhas *et al.,* 2022). It is estimated that 439 million people would have type 2 diabetes by the year 2030. The incidence of type 2 diabetes varies substantially from one geographical region to the other as a result of environmental and lifestyle risk factors (Dange *et al.,* 2022). It is predicted that the prevalence of diabetes in adults of which type 2 diabetes is becoming prominent will increase in the next two decades and much of the increase will occur in developing countries where the majority of patients are aged between 45 and 64 years.

Sesbania Linn. (Fabaceae) found throughout the plains of India and commonly called as Jayanti. Herbals which form a part of our nutrition and provide us an additional therapeutic effect are in demand and *Sesbania sesban* Linn. is one of such plant. The plant has got good medicinal importance. Flowers contain cyanidin and delphiniding lucosides, pollen and pollen tubes contain alphaketoglutaric, oxaloacetic and pyruvic acids the leaves of *Sesbania sesban* evaluated the topical anti-inflammatory activity, ant diabetic and CNS stimulant effect, in thyroid disorders, dysuria. The leaf of *Sesbania sesban* has traditionally been used as purgative, demulcent, maturant, Anthelmintic and for all pains and inflammation.

It is well adapted to hot, humid environments and does not grow well in the subtropics particularly in areas with a cool season minimum temperature of below 10oC. It has the ability to tolerate water logging and is ideally suited to seasonally water logged or flooded environments. When flooded, they initiate floating adventitious roots and protect their stems, roots and nodules with spongy, arenchyma tissue. *S.grandiflora* is adapted to rain fall conditions of 2000 – 4000 mm but grow well in areas receiving only 800 mm. Another interesting feature is its extraordinary tolerance to saline, alkaline as well as to highly acidic soils. Cutting management has a very important influence on the productivity of perennial *Sesbania* species. *Sesbania grandiflora* cannot survive by repeated cutting. Farmers used to cut only the side branches of trees for fodder leaving the main growing stem untouched.

The trees are grown on rice paddy walls at 1.5 – 2 m intervals and forage is harvested in this manner for 3 – 4 years, yielding up to 2 Kg dry matter per harvest per tree. When the foliage is no longer within easy reach the trees are cut and the long straight poles are used as firewood or for construction purposes (Ettadili *et al.*, 2022).The tree is grown as an ornamental shade tree, and as a fast growing plant used for reforestation also. The tree is extensively used as a pulp source. A gum, resembling Kino, fresh when red, nearly black after exposure, exudes from wounds of the tree. This astringent gum is partially soluble in water and in alcohol, and is applied to fishing cord, to make it more durable. Pepper vines (*Piper nigrum*) are sometimes grown on and in the shade of the Agati. It is a suitable plant for agro forestry, capable of growing in paddy fields, where trees are not normally grown. However, botanist's quote three undesirable features i.e. (i) short lived (ii) shallow rooted and subject to wind throw, and (iii) being prolific seeder, the pods are often considered as a litter.

In India, *Sesbania* has a long history of agricultural use primarily as green manure and as a source of forage. Bark, leaves, gums, and flowers are used for medicinal purpose. Leaves are poulticed onto bruises (Rad *et al.*, 2021). In Yunani the tonic of leaves are used in biliousness, fever and nyctalopia. The juice of leaves is used for headache and nasal catarrh, mixed with stramonium. Malayans apply crushed leaves to sprains. They gargle the leaf juice to cleanse the mouth and throat. In Java leaves are chewed to disinfect the mouth and throat. The tender leaves, green fruit, and flowers are eaten alone as vegetable or mixed into curries or salads. The dried leaves of both *S.grandiflora* and *Sesbania sesban* are used in some countries as tea and are considered to have antibiotic, anti-helminthic, anti-tumor and contraceptive properties.

In small doses, the bark is used for dysentery, in large doses as laxative and in still larger doses used as emetic. Powdered bark is applied to scabies. Philippines use the powdered bark for hemoptysis (Yang *et al.*, 2018). The powdered bark is also recommended for ulcers of the mouth and alimentary canal. In Java, the bark is used for thrush and infantile stomach disorders. The astringent bark is used in treating small pox and other eruptive fevers. Cambodians use the bark for diarrhea, dysentery and paludism. The inner bark can serve as fiber and the white, soft wood not too durable, can be used for making corks. In Asian countries, like bamboo, the wood is used for construction of houses. Dried and powdered bark is used as a cosmetic in Java. An aqueous extract of bark is said to be toxic to cockroaches.

Phytochemicals are bioactive non nutrient components of plants, commonly found in the human diet, that may have beneficial (or harmful) health effects and include flavonoids, glucosinolates, organosulfurcompounds, saponins, monoterpenes, sesquiterpenes, capsaicinoids, and capsinoids (Chaudhary *et al.*, 2018). Currently, there is considerable interest in the potential health effects of dietary phytochemicals such as flavonoids, including isoflavones and other polyphenolic compounds such as resveratrol. Possible health benefits of these dietary components including protection against cardiovascular disease, cancer, osteoporosis, and cognitive-decline are evaluated. Potential mechanisms of action and possible safety concerns are also considered.

Phytochemicals, also referred to as phytonutrients, are found in fruits, vegetables, whole grains, legumes, beans, herbs, spices, nuts, and seeds and are classified according to their chemical structures and functional properties (Brindha, 2016). The terminology used to describe phytochemicals (flavonoids, flavonols, flavanols, proanthocyanidins, procyanidins) can be confusing. Phytochemicals include compounds such as salicylates, phytosterols, saponins, glucosinolates, polyphenols, protease inhibitors, monoterpenes, phytoestrogens, sulphides, terpenes, lectins, and many more.

Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reaction can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions (Rad *et al.*, 2021). They do this by being oxidized themselves. Antioxidants from spices and herbs have the potential for large scale applications. Spices have been used not only for their flavoring properties but also for their food preserving abilities. Over the past few years a number of medicinal plants have been investigated for their quenching activity of specific ROS (Karnan and Subramani, 2015).

The medicinal plants, besides having natural therapeutic values against various diseases, also provide high quality of food and raw materials for livelihood (Umadevi *et al.*, 2018). One of the methods used in drug discovery is the ethno medicinal data, in which the inclusion of a plant is based on the use of the same in traditional medicine. Plants react to surroundings and physiologically adapt by shifting the biochemical profile and producing a spectrum of secondary

metabolites. Secondary metabolites are of individual interest to researchers because of their exclusive pharmacophores and curative properties. Secondary metabolites like polyphenols, trepans and alkaloids have been reported to possess antimutagenic and anticancer properties in many studies (Banik *et al.,* 2019). It is commonly identified that ethno medical data provide substantially increased chance of finding active plants comparative to random method (Mukherjee, 2019). Thus, in the present study *S. grandiflora* of the family Fabaceae, which has been used as a therapy for several ailments, was evaluated for its ant diabetic activities.

Standardization of herbal drugs based on their chemical and biological activity profile is an important prerequisite for phytochemical research. Hundreds of new natural substances are being isolated and identified every year, but data concerning their biological activities are known only to some (Elsharkawy, 2014). In this study, an attempt was made to screen the bioactive constituents of *S. grandiflora* using, FT-IR spectroscopy, XRD and SEM in order to reveal the scope and applications of *S. grandiflora* pharmaceutical field to bring about more drugs and natural products out of it.

In silico prediction systems are cheaper, rapid, and reproducibleand have low compound synthesis requirements. They can undergo constant optimization and have potential to replace the use of animals (Valerio-Jr, 2019). The application of *insilico* methods in herbal sciences is used for the detection of phytochemical compounds bearing known genotoxicity reducing activity. They can help to clarify compounds which are responsible for a proven effect.

Molecular docking is a computational method generally used to explicate the mechanism of action and rationalize structure activity contacts of natural products. *In silico* simulations can be used to offer protein ligand binding features for molecular structures, e.g., known ingredients of a plant tissue. Compounds that show good results in *in silico* predictions can be used as hopeful preliminary materials for experimental work. In the present study, selected diabetic proteins (1V4S) Glucokinase isoinform 2 (1BHS; PDB ID:17 BETA) dehydrogenase, (2nt7) tyrosine protein phosphatase non-receptor type 1 (1GNH) C-Reactive protein) were docked with natural compounds derived from *S. grandiflora* which offer a great hope in the identification of lead compounds for the treatment of diabetes.

Considering all the above information, the present investigation has a broader objective of comprehensive evaluation of the antidiabetic activity of the *S.grandiflora*. Using molecular

docking studies, an attempt has been made to identify the active principle behind *S.grandiflora* flower that may suggest its use as a potential medicine against diabetes.

The present investigation was undertaken with the following objectives

Objectives:

- To synthesize iron nanoparticles using aqueous extracts of *S.grandiflora* flower.
- To determine the phytochemical constituents present in iron nanoparticles of *S.grandiflora* flower.
- To evaluate the antioxidant potential of iron nanoparticles of *S.grandiflora* flower.
- To evaluate the antidiabetic activity of iron nanoparticles of *S.grandiflora* flower.
- To evaluate the anti-bacterial activity of iron nanoparticles of *S.grandiflora* flower.
- To conduct the *in silico* molecular docking studies using the compounds against the diabetes mellitus diseased proteins.

REVIEW OF LITERATURE

2 REVIEW OF THE LITERATURE

2.1. Diabetes mellitus – An overview

Diabetes is a syndrome, initially characterized by loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting in impaired metabolism of glucose and other energy-yielding fuels such as lipids and proteins. Diabetes is the most severe and challenging metabolic pandemic of the 21st century. This is because it affects essential biochemical activities in almost every cell in the body and increases the risk of cardiac and renal disorders. The worldwide survey reported that diabetes is affecting nearly 10% of the population ((Rad *et al.,* 2021). The global diabetes mellitus burden estimate was 246 million, and the International Diabetes Federation (IDF) estimates that this figure is likely to rise to 380 million by the year 2025. In developing countries, those most frequently affected are in the middle, productive years of their lives, aged between 35 and 64 years (WHO, 2002), and 55% deaths occur in women. Currently, many countries face large increases in the number of people suffering from diabetes. The World Health Organization estimated that about 30 million people suffered from diabetes in 1985 and the number increased to more than 171 million in 2000. It is estimated that the number will increase to over 366 million by 2030 and that large increases will occur in developing countries, especially in people aged between 45 and 64 years (Wild *et al.,* 2014).

This pandemic is characterized by excessive sugar in the blood due to deficiency in production of insulin by the pancreas or by the ineffectiveness 27 of the insulin produced to control blood glucose. This disorder affects carbohydrate, protein and fat metabolism (Ponnanikajamideen *et al.,* 2015) and chronic hyperglycemia causes glycation of body proteins that in turn leads to secondary complications that affects eyes, kidneys and nerves (Sudarmani *etal.*,2021). The continued increase in prevalence of diabetes in the developing nations can be largely attributed to urbanization, westernization and economic development. The major contributing risk factors related to these are population ageing, obesity, sedentary lifestyles, over-processed diets, smoking, psychological stress and low birth weight (Ravulapalli *et al.,* 2019).

Diabetes mellitus can be categorized into several types but the two major types are Type 1 and Type-II (WHO, 1985). On the basis of etiology, type 1 is present in patients who have little or no endogenous insulin secretary capacity and who therefore require insulin therapy for survival (Silva *et al.*, 2018). The two main forms of clinical type 1 diabetes are type 1a (about 90% of type 1 cases in Europe) which is thought to be due to immunological destruction of pancreatic ß cells resulting in insulin deficiency; and type 1b (idiopathic, about 10% of type 1 diabetes), in which there is no evidence of autoimmunity. Type 1a is characterized by the presence of islet cell antibody (ICA), anti-glutamic acid decarboxylate (anti-GAD), IA-2 or insulin antibodies that identify the autoimmune process with ß-cell destruction (Buwa-Komoren *et al.*, 2019). Autoimmune diseases such as Grave‟s disease, Hashimoto‟s thyroiditis and Addison‟s disease may be associated with type 1 diabetes mellitus. There is no known etiological basis for type 1b diabetes mellitus. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. This form is more prevalent among individuals of African and Asian Origin (Sudarmani *et al.*, 2021).

Type-II diabetes is the commonest form of diabetes and is characterized by disorders of insulin secretion and insulin resistance. Globally; it affects 5-7% of the world's population. There is a higher incidence of Type-II diabetes in urban than in rural areas (Newman and Cragg, 2016). Traditionally, Type-II diabetes is common in individuals over the age of 40. It is often associated with obesity and decreased physical activity. Recent data from several countries show that Type-II diabetes is increasingly becoming a problem among adolescents and even children. In some countries, childhood diabetes Type-II is more common than type-I. The disease is usually controlled through dietary therapy, exercise and hypoglycemic agents (Saiganesh *et al.*, 2021).

In type-II diabetes mellitus, there is an increased accumulation of visceral fat which contains pro-inflammatory molecules such as α-tumor necrosis factor (TNF-α), which is involved in the regulation of insulin sensitivity in the body (Newman and Cragg, 2016). Other molecules such as adiponectin whose levels are low in obesity improves insulin sensitivity, reduce glucose output and fatty acid oxidation in the liver (Qatanani and Lazar, 2018).Poor dietary choice is a major contributing factor to obesity and associated disorders like Type-II diabetes mellitus. Epidemiological evidence has demonstrated that saturated fatty acid intake is associated with 29 increased risk of insulin resistance, diabetes and impaired glucose tolerance (Rad *et al.*, 2021). The

inclusion of foods rich in trans fatty acids and high ratios of saturated to unsaturated fats results in weight gain and predisposition to diabetes. Foods such as red meats, refined grains, sweets and high fat dairy products have been linked to risks of Type-II diabetes. In contrast, weight loss is characterized by reduction in fat cell mass especially visceral fat which contain inflammatory markers associated with insulin resistance and decreased insulin sensitivity. Reduced visceral fat due to weight loss is accompanied by decreased adipose TNF-α release resulting to improved insulin sensitivity (Mlinar *et al.,* 2016).

Herbal medicines involve the integration of several therapeutic experiences and practices of indigenous systems of medicine that may span many previous generations, which often provide valuable guidelines to the selection, preparation and application of herbal formulation with a view to providing therapeutic benefits. (Newman and Cragg, 2016)There are several literature reviews by different authors about anti-diabetic herbal agents, but the most informative is the review by Atta-ar-Rahman who has documented more than 300 plant species accepted for their hypoglycaemic properties. Currently, medicinal plants continue to play an important role in the management of diabetes, especially in developing countries, where many people do not have access to conventional anti-diabetic therapies (Yuan and Haidan, 2016)

Plants have rich potent sources of phyto-constituents which can be responsible to solve various health problems. Medicinal plants have curative properties due to 30 the presence of various complex chemical substance of different composition, which are found as secondary plant metabolites in one or more parts of plants. Ethno-pharmacological surveys indicate that more than 1,200 plants are used in traditional medicine systems following claims of their hypoglycemic properties (Upasani *et al.,* 2018). The hypoglycemic activity of a large number of plant products have been evaluated and confirmed in animal models. In some cases, the bioactive principles of the medical plants have been isolated and identified. Nevertheless, the mechanisms of action of most of these antidiabetic bioactive principles are not well defined and remain largely speculative. However, reports suggest that the array of anti-diabetic bioactive principles in medicinal plants may act in synergy to exert glycemic control (Yuan and Haidan, 2016) through interference with one or more processes involved in glucose metabolism and homeostasis.

Many therapeutic approaches were used to prevent postprandial hyperglycemia to retard the digestion and absorption of carbohydrates in the gastrointestinal tract through inhibition of enzymes such as α-amylase and α-glycosidase (Upasani *et al.,* 2018). Over the last 2,500 years, there have been very strong traditional systems of medicine such as Chinese, Ayurvedic, and the Unani, born and practiced more in the eastern continent. These traditions are still flourishing, since; approximately 80% of the people in the developing countries rely on these systems of medicine for their primary health care needs. These plants contain substances that can be used for therapeutic 31 purposes, of which are precursors for the synthesis of drugs. A lot of research work has been carried out on some medicinal herbs and they have been found to have definite action on the nervous, circulatory, respiratory, digestive and urinary systems; as well as the sexual organs, the skin, vision, hearing and taste.

2.2. Natural plant product research

Since prehistoric times, humans have used Natural Products (NPs), such as plants, animals, microorganisms, and marine organisms, in medicines to alleviate and treat diseases. According to fossil records, the human use of plants as medicines may be traced back at least 60,000 years (Yuan and Haidan, 2016)The use of natural products as medicines must, of course, have presented a tremendous challenge to early humans. It is highly probable that when seeking food, early humans often consumed poisonous plants, which led to vomiting, diarrhea, coma, or other toxic reactions—perhaps even death. However, in this way, early humans were able to develop knowledge about edible materials and natural medicines (Yuan and Haidan, 2016). Subsequently, humans invented fire, learned how to make alcohol, developed religions, and made technological breakthroughs, and they learned how to develop new drugs.

NPs have evolved over millions of years and acquired a unique chemical diversity, which consequently results in the diversity of their biological activities and drug-like properties. Therefore, even before the rise of the modern chemical pharmacology, NPs have been used for centuries as components of traditional medicines, in particular as active components of herbal remedies. Nowadays, some of the traditional healing practices, such as Indian Ayurveda, traditional Chinese medicine or African herbal medicines, remain the primary treatment option for many people across the world, due to economic reasons, to personal beliefs or to the difficulty in

accessing pharmaceutical products. In modern pharmacology too, NPs have become one of the most important resources for developing new lead compounds and scaffolds (Khalifa *et al.*, 2019).

The World Health Organization listed between 1999 and 2009 a list of over 21 000 plants used for medicinal purposes all over the world (WHO, 2009). This effort was made for proper identification of safe plants, as it is estimated that plant-based traditional medicines are used by 60% of the world's population. In addition to efforts to establish formal, DNA-based identification of such plants for wider use (Palhares *et al.*, 2015), collections of medicinal plant species, and in particular of phytochemicals, NPs produced by plants, associated to their therapeutic activities and physicochemical properties are being established around the world. This is particularly the case in Asia and Africa, where traditional medicines remain an important part of everyday life for cultural, traditional and economic reasons.

Traditional Chinese Medicine (TCM) is naturally part of the Chinese public health system (Yuan *et al.*, 2016). It is therefore coherent that in this country the scientific study of natural compounds from plants used in TCM is very advanced and is receiving strong governmental support, and they have developed a plethora of databases containing NPs, their sources and effects. The biggest database containing NPs used in TCM. It contains over 58,000 entries and is directly feeding I SMART (Chang *et al.*, 2011), an integrated cloud computing web server for online virtual screening, evolution studies and drug design.

2.3. Traditional medicine

Medicinal plants are only source and an important contribution for primary healthcare during ancient times. Knowledge about use of medicinal plants for treating various diseases was highly valued among ancient civilizations. (Arji *et al.*, 2019*)* Until the mid-nineteenth century, plants were the main therapeutic agents used by humans and still have an important role in medicinal preparations. About 80% of people in developing countries depend on traditional medicine for their primary health care needs, because of their low costs, effectiveness, frequently inadequate provision of modern medicine, cultural and religious preferences. 80% of people in India use non-allopathic (Ayurveda, Siddha, Unani and Homeopathy) herbal based medicines for their healthcare which are collected from wild and cultivated sources.About 8,000 herbal remedies have been codified in AYUSH systems in INDIA. Ayurveda, Unani, Siddha and Folk (tribal)

medicines are the major systems of indigenous medicines. Among these systems, Ayurveda and Unani Medicine are most developed and widely practiced in India.

Perhaps the inherent traditional systems of medicine, along with information from conservative folklore, are serving a large section of the populace, particularly in rural and tribal areas, despite the dawn of modern medicine (Upasani *et al.,* 2018). Plants are the basis of both traditional medicines (TMs) and modern drug discoveries. More than 50,000 plant species are used in TM worldwide and majority of them are being used in Asian medicines. Asian medicines comprise oral-based folklore medicines (local healing system) and the scholarly TM systems (Wangchuk *et al.,* 2015). While most of the folklore medicines remain neglected, undocumented, and are becoming rare or extinct due to fast-paced modernization, the scholarly TM systems still thrive in many Asian countries including Bhutan (Wangchuk *et al.,* 2017).

Traditional treatments are still a part of the health care system in many communities despite the fact that well-established alternatives are available (Mirzaie *et al.,* 2019). In reality, current interest in traditional medicine has resulted in quick development and investigation of many remedies applied in various ethnic groups across the world. India has wealthy tradition background on plant-based drugs both for use in precautionary and medicinal medication. In the Indian system of medicine, the Vaidya is known as doctor of herbs, who makes a diagnosis of illness and compounds medicinal preparations, such as asava, aristha, churna (powders), lotions, liniments, pills, syrup, and taila. Furthermore, many old-aged persons (such as a grandmother) are familiar with the application of various herbs.

Traditional Medicinal System (TMS) is one of the centuries-old practices and long-serving companions to the human kind to fight against disease and to lead a healthy life. Every indigenous people have been using their unique approaches of TMS practice where among, the Chinese, Indian and African TMSs are world-wide renowned (Mirzaie *et al.,* 2019). India has a unique Indian System of Medicines (ISM) consisting of Ayurveda, Siddha, Unani, Naturopathy and Homoeopathy. Siddhars are the saints as well as the eminent scholars, who have attained Ashta-mahasiddhiorenlightment. They have postulated, practiced, immensely contributed and have established the concept of the Tamil medicinal system called Siddha System of Medicine (Karunamoorthi *et al.,* 2018).

2.4. Iron nanoparticles

Fe_3SO_4 is a medicine used to treat and prevent iron deficiency anaemia. Iron helps the body to make healthy red blood cells, which carry oxygen around the body. Some things such as blood loss, pregnancy or too little iron in your diet can make your iron supply drop too low, leading to anaemia (Ettadili, 2022). Ferrous sulfate appears as a greenish or yellow-brown crystalline solid. Density 15.0 lb /gal. Melts at 64°C and loses the seven waters of hydration at 90°C. The primary hazard is the threat to the environment.

Fe_3SO_4 nanoparticles were successfully synthesized using pomegranate (Punicagranatum) leaf extract by (Rao *et al.,*2019) The facile green synthesis of magnetite nanoparticles was performed by using plantain peel extract used waste plantain peel extract for reduction of iron salt to form Fe3O4 nanoparticles. Biomolecules present in the plantain peel extract was characterized by FTIR. The well dispersed spherical magnetic NPs (MNPs) sized below 50 nm were seen in a transmission electron microscopic image iron oxide nanoparticles have demonstrated great potential in biomedical applications due to their non-toxic role in biological systems.(Rajendran *et al.,*2017) Also, the magnetic and semiconductor properties of iron oxide nanoparticles can lead to multifunctional applications in medicine. These nanoparticles have been developed as antibacterial, antifungal, and anticancer. For cancer treatment and diagnosis, iron oxide nanoparticles have been functionalized with drugs. The synthesis of iron oxide nanoparticles, the different kinds of coatings used to functionalize them, and the different applications they have had in cancer treatment and diagnosis (Hernández-Hernández *et al.,* 2020)

The purpose is to minimize the negative impacts of synthetic procedures, their accompanying chemicals and derivative compounds. (Melkamu *et al.,* 2021)The exploitation of different biomaterials for the synthesis of nanoparticles is considered a valuable approach in green nanotechnology. Biological resources such as bacteria, algae fungi and plants have been used for the production of low-cost, energy-efficient, and nontoxic environmental friendly metallic nanoparticles (SadiaSaif *et al.,* 2016)

Green synthesis of nanoparticles is becoming one of the robust techniques, which may be suitable alternatives for chemical and physical methods. This study reports a cost effective and environmental friendly green synthesis of titanium dioxide nanoparticles (TiO_2 NPs)

using *S.grandiflora*. The model adapted in this study was suitable to explore the toxicity in Zebrafish (Srinivasan *et al.*, 2019). TiO_2 NPs increased bioavailability and uptake into cells and organisms. In order to assess the TiO_2 NPs toxic level we employed the fish model as aquatic environment.

2.5. Types of nano particles

The synthesize nanosized magnetite (Fe_3O_4) particles using a small amount of cyclohexane as the oil phase, NP5 + NP9 as the surfactant phase, and a Fe(II)/Fe(III) salt solution as the aqueous phase. The Fe_3O_4 powder thus derived from the emulsion containing 88 wt% 0.3 M $FeSO_4$ + Fe $(NO_3)_3$ in the aqueous phase possesses an equiaxial morphology and an average particle size of <10 nm. Studies on the electrical conductivity of the emulsions as a function of Fe^{2+}/Fe^{3+} concentration in the aqueous phase revealed the complexation effect of the NP5 + NP9 surfactant towards Fe^{2+}/Fe^{3+} ions.

It can be used for nanoparticle synthesis and highlight key approaches for the collection of large datasets. (Tao *et al.*, 2021)We examine ML-guided synthesis of semiconductor, metal, carbon-based and polymeric nanoparticles, and conclude with a discussion of current limitations, advantages and perspectives in the development of ML-assisted nanoparticle synthesis.

The nickel oxide nanoparticles (NiO NPs) have been synthesized via photo irradiation as a novel method. It is a simple and cost effective method. The values inhibition zone indicates that nanoparticles effect on different bacteria. The outcome considered a new synthesis of NiO nanoparticles to promise antimicrobial agents against bacteria (Rheima *et al.*, 2021)

The different methods for removal of parts from bulk materials may include chemical, electrochemical, and mechanical methods. The choice of a particular method is based on the material of bulk substrate and desired sizes of NPs. This technique however does not provide a full control on particle size (Din *et al.*, 2016). The metal salts like nitrates, chlorides, oxides, and sulphates have high reduction potential due to attachment of metal with the chloride, oxide, and sulphide parts and their tendency to donate electrons.

Nickel nanoparticles (NiNPs) have been investigated for various potential applications due to their superior ferromagnetic properties such as magneto-crystalline anisotropy, high coercive

forces, and chemical stability. Therefore, there has been a tremendous enhancement in the synthesis techniques, proposed reaction mechanisms, and applications of NiNPs (Jaji *et al.*, 2020).

Silver nanoparticles (AgNPs) were synthesized using water extract of *Clerodendrumviscosum* Vent leaf as reducing agent. These biosynthesize AgNPs exhibited significant antiplasmodial activity against 3D7 sensitive strain of *Plasmodium falciparum* with $IC_{50} = 2.30$ µg/ml. Further, AgNPs were investigated for anticancer property against human cervical cancer (HeLa) cell lines (Sahoo *et al.*, 2020)

The formation of $NiAl_2O_4$ nanoparticles was confirmed by HR-SEM and HR-TEM and their possible formation mechanisms were also proposed. MCM could produce $NiAl_2O_4$ with uniform size and well-defined shape with crystallinity (Ragupathi *et al.*, 2017).

Zinc oxide nanocomposites show also selective toxicity toward normal and cancerous cells, which is explained by reactive oxygen formation (ROS). Yet despite the potentially interesting antitumor activity of ZnO nanoparticles, it has been proven that they can be also cytotoxic and genotoxic for multiple types of human cells (i.e. neuronal or epithelial cells) (Melkamu *et al.*, 2017). The methods of synthesizing zinc oxide nanocomposites as well as their characteristics, antimicrobial activity and cytotoxicity against normal and tumor cells

2.6. *S .grandiflora* – An overview

Kingdom	Plantae
Clade	Rosids
Order	Fabales
Family	Fabaceae
Subfamily	Faboideae
Tribe	Sesbanieae
Genus	*Sesbania*
Species	*S. grandiflora*

The exact origin of *S. grandiflora* (in Tamil Agati) is not known but it is considered as native to many South East Asian countries. *S. grandiflora* is a loosely branched tree grows up to

15 m height. Its leaves are pinnately compound up to 30 cm long with 20 – 50 leaflets in pairs, dimensions 12.44 x 5.15 mm and oblong to elliptical in shape (Jamdagni *et al.*, 2018). Flowers are large, white, yellowish, rose pink or red with a calyx of 15-22 mm long, the standard dimensions up to 10.5 x 6 cm. Pods are long (20 – 60 cm) and thin (6 – 9 mm) with broad sutures containing 15-50 seeds.

It is well adapted to hot, humid environments and does not grow well in the subtropics particularly in areas with a cool season minimum temperature of below 10°C. It has the ability to tolerate water logging and is ideally suited to seasonally water logged or flooded environments (Sekhon-Loodu *et al.*, 2019). When flooded, they initiate floating adventitious roots and protect their stems, roots and nodules with spongy, arenchyma tissue. *S. grandiflora* is adapted to rain fall conditions of 2000 – 4000 mm but grow well in areas receiving only 800 mm. Another interesting feature is its extraordinary tolerance to saline, alkaline as well as to highly acidic soils. Cutting management has a very important influence on the productivity of perennial *Sesbania* species. *Sesbania grandiflora* cannot survive by repeated cutting (Sahu *et al.*, 2021). Farmers used to cut only the side branches of trees for fodder leaving the main growing stem untouched. The trees are grown on rice paddy walls at 1.5 – 2 m intervals and forage is harvested in this manner for 3 – 4 years, yielding up to 2 kg dry matter per harvest per tree. When the foliage is no longer within easy reach the trees are cut and the long straight poles are used as firewood or for construction purposes (Rani *et al.*, 2021).

The tree is grown as an ornamental shade tree, and as a fast growing plant used for reforestation also. The tree is extensively used as a pulp source. A gum, resembling Kino, fresh when red, nearly black after exposure, exudes from wounds of the tree (Nikolova *et al.*, 2020). This astringent gum is partially soluble in water and in alcohol, and is applied to fishing cord, to make it more durable. Pepper vines (*Piper nigrum*) are sometimes grown on and in the shade of the Agati. It is a suitable plant for agro forestry, capable of growing in paddy fields, where trees are not normally grown (Omara *et al.*, 2020). However, botanist's quote three undesirable features i.e. (i) short lived (ii) shallow rooted and subject to wind throw, and (iii) being prolific seeder, the pods are often considered as a litter (Mukherjee *et al* ., 2020). In India, *Sesbania* has a long history of agricultural use primarily as green manure and as a source of forage. Bark, leaves, gums, and flowers are used for medicinal purpose.

Leaves are poulticed onto bruises. In Yunani the tonic of leaves are used in biliousness, fever and nyctalopia. The juice of leaves is used for headache and nasal catarrh, mixed with stramonium (Chaudhary *et al.,* 2018). Malayans apply crushed leaves to sprains. They gargle the leaf juice to cleanse the mouth and throat. In Java leaves are chewed to disinfect the mouth and throat. The tender leaves, green fruit, and flowers are eaten alone as vegetable or mixed into curries or salads (Behravan *et al.,* 2018). The dried leaves of both *S. grandiflora* and *Sesbania sesban* are used in some countries as tea and are considered to have antibiotic, anti-helminthic, antitumor and contraceptive properties.

In small doses, the bark is used for dysentery, in large doses as laxative and in still larger doses used as emetic. Powdered bark is applied to scabies. Philippines use the powdered bark for hemoptysis. The powdered bark is also recommended for ulcers of the mouth and alimentary canal (Pirtarighat *et al.,* 2019). In Java, the bark is used for thrush and infantile stomach disorders. The astringent bark is used in treating small pox and other eruptive fevers. Cambodians use the bark for diarrhoea, dysentery and paludism. The inner bark can serve as fiber and the white, soft wood not too durable, can be used for making corks (Agarwal *et al.,* 2019). In Asian countries, like bamboo, the wood is used for construction of houses. Dried and powdered bark is used as a cosmetic in Java. An aqueous extract of bark is said to be toxic to cockroaches.

Rheumatic swellings are poulticed or rubbed with aqueous decoctions of the powdered roots of the red flowered variant. Paste of the root is poulticed onto painful swellings. Indians apply the roots for rheumatism (Rohmah *et al.,* 2020). The juice of flowers is used for headache and nasal catarrh, mixed with stramonium. The juice from the flowers is used to treat headache, head congestion, or stuffy nose. As a snuff, the juice is supposed to clear the nasal sinuses. In Amboina, flower juice is squeezed into the eye to correct dim vision. Cambodians use the flowers as emollient and laxative (Hemmati *et al.,* 2019). Flowers may be dipped in butter and fried in ghee.

2.7. Antioxidant – *S. grandiflora*

The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities (Sahoo *et al.,* 2020). The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity.

The *in vitro* using explants of S. grandiflora together with the estimation of total phenolic content and antioxidative activity of various extracts obtained from the plant (Vinothini *et al.,* 2017). These extracts exhibited anticancer properties in breast cancer cells in a dose-dependent way. The plant was also found to be non-genotoxic and also found to inhibit genotoxicity induced by EMS (Anusmitha *et al.,* 2022). The concentrations (0.04, 0.5, 1, 2, 4, 6, 8 and 10 mg/mL) and results were calculated as percentage relative to a negative control containing ultrapure water or ethanol. The IC_{50} values were calculated for samples with activities higher than 50% at the concentration of 10 mg/mL. Assays were made in 96-well microplates and absorbances were measured using a microplate reader. The extraction yields were 16.6%, 18.4% and 3.15 for infusions, decoctions and tinctures of *S. grandiflora* and 13.8, 13.7% and 4.6% for infusions, decoctions and tinctures of *B. monosperma*. (Baessa *et al.,* 2019).

2.8. Antimicrobial – *S.grandiflora*

Medicinal plants, both as potential antimicrobial crude drugs as well as a source for natural compounds that act as new anti-infection agents. In the case of *Melaleuca alternifolia*, for example, the use of the essential oil (tee tree oil) is a common therapeutic tool to treat acne and other infectious troubles of the skin. Javid *et al.,*2015 described the use of bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) to treat urinary tract infections is reported in different manuals of phytotherapy, while species such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tee tree (*Melaleuca alternifolia*) are described as broad-spectrum antimicrobial agents. Medicinal plants, both as potential antimicrobial crude drugs as well as a source for natural compounds that act as new anti-infection agents.

In the antimicrobial study done by Kubo *et al.,* 2022, aqueous extracts of seed of *Sesbania* and flowers of Calendula officinalis exhibited better antibacterial activity compared to their 26 petroleum ether, methanol and ethanol extracts. Among the organisms tested, *S. aureus* was the most susceptible to the aqueous extracts of Sesbania. The antimicrobial compound obtained from *Parthenum argentatum* showed activity against *C. albicans*, Torulopsis, Hansemula, *K. pneumoniae* and *P. aeruginosa* (Ahmed *et al.,* 2019). Harborne (1998) studied the effects of phytochemicals and observed the antimicrobial activity of anacardic acid on *S. aureus*, *Brevibacterium ammoniagenes*, *Streptococcus mutans* and *Propionibacterium acnes*. Later, the bactericidal activity of anacardic acid and totarol was tested against methicillin resistant strains of

S. aureus (MRSA) and the synergistic effect of these compounds with methicillin was noted. Minimum inhibitory concentrations of *Sesbania* leaf extract against the growth of *B.subtilis* and *E.coli.*

(Kasture *et al.,* 2022) observed that the aqueous extract of *S.grandiflora* leaves inhibited *S.aureus, P.aeruginosa* and *E.coli* at a concentration of 100µg/mL, 200 µg/mL and 250 µg/mL respectively but it did not inhibit the growth of *B.subtilis* at any of the concentration tested. The antimicrobial activity of *S.grandiflora* flower PE against selected pathogens was evaluated using both *in vitro* and *in situ* methods. *S.grandiflora* is a fast-growing tree. The leaves are regular and rounded and the flowers white, red or pink. The fruits look like flat, long, thin green beans.

Antimicrobial activity and antifungal activity of nanoparticles depends on physiochemical properties of nanoparticles and type of microbial pathogen, they can seize the functions of cell of *E. coli* significantly. Bacillus spp contain thicker peptidoglycan cell wall layer so the toxicity of NiO nanoparticles is lesser to them compare to *E.coli*. The antibacterial activity has not been researched much. So the neodymium doped nickel oxide nanoparticles can be used in current Nano medicine and also can be used in alternative to the bleaching process of waste water treatment.

The antibacterial activity of NiO NPs was studied using the zone inhibition method. Gram-positive and Gram-negative bacterial strains, *E. coli* and Bacillus spp., respectively, were selected as test organisms (Venkateswarlu *et al.,* 2017). The bacterial cultures were inoculated in Luria broth (liquid phase) in 100 mL conical flasks, and the flasks were incubated overnight at 37 ◦C. The overnight active Escherichia coli (E. coli) and Bacillus spp. cultures were seeded into Luria agar medium using spread plate techniques. The standard antibiotic streptomycin was used as a positive control. The zone of inhibition was measured using digital Vernier calipers, and the values were reported in centimeters (Saiganesh *et al.,* 2021).

2.9. Anticancer activity

Anticancer activity of (EESG) of both leaves and flowers were having the medicinal value to cure the tumor cells. The medicinal plant *S. grandiflora* without any undesired toxicity potentiates apoptosis, reduces tumor cell viability and interferes in abrogating prolifera-tive signals which are otherwise conducive for tumor growth (Ponnanikajamideen *et al.,* 2015).

2.10. Antiulcer activity

Ulcers are open sores on the skin or mucus membrane characterized by a superficial loss of tissue. Ulcers are mainly occurs due to the disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance to possible ulcerative agents. Many Medicinal plants have the potential for the prevention and treatment of ulcer (Manjunatha *et al.,* 2022).

2.11. Molecular docking studies

Molecular docking techniques aim to predict the best matching binding mode of a ligand to a macromolecular partner (here just proteins are considered). It consists in the generation of a number of possible conformations/orientations, i.e., poses, of the ligand within the protein binding site. For this reason, the availability of the three-dimensional structure of the molecular target is a necessary condition; it can be an experimentally solved structure (such as by X-ray crystallography or NMR) or a structure obtained by computational techniques (Salmaso *et al.,* 2018).

Molecular docking is composed mainly by two stages: an engine for conformations orientations sampling and a scoring function, which associates a score to each predicted pose (Salmaso *et al.,* 2018). The sampling process should effectively search the conformational space described by the free energy landscape, where energy, in docking, is approximated by the scoring function. The scoring function should be able to associate the native bound-conformation to the global minimum of the energy hyper surface.

Computer Aided Drug Design (CADD) techniques are used principally for three reasons: virtual screening hit/lead optimization and design of novel compounds. In virtual screening a huge database of compounds is examined searching for binding capacity for a target and a subset of compounds is picked out and suggested for *in vitro* testing; the purpose is to increase the hit rate of novel drugs by reducing the number of compounds to test experimentally. The second application of CADD is the optimization of a hit/lead compound driven by the rationalization of a structure-activity relationship. After the individuation of key elements for binding, the design of new compounds can be attempted (Salmaso *et al.,* 2018).

CADD methods may be classified as ligand-based (LB) and structure-based (SB), depending on the availability and employment of the target structure (Sliwoski *et al.,* 2014). In the

framework of CADD, structure-based drug design (SBDD) methods take advantage of the abundance of experimentally solved structures in the Protein Data Bank (Salmaso *et al.,* 2018) which can possibly be used also as templates for homology models if the structure of interest is lacking. SBDD is based on the premise that the knowledge of the target structure can help to rationalize and optimize binding since ligand-target interactions are mediated by their complementarity. With the evolution of the binding models, it is clear that speaking of "target structure" is an approximation, given that proteins fluctuate among an ensemble of structures (Salmaso *et al.,* 2018).

Similarly, a study confirmed the inhibitory nature of gallic acid to PBP2a (the penicillin-binding protein 2a) protein through molecular docking technique using the tool Accelrys Discovery studio. Cleistanthus is a plant genus of the family Phyllanthaceae and is well known for its toxicity. Pratheepa (2012) conducted in silico molecular docking analysis using Autodock and found that the compound dioctyl phthalate in the plant exhibited anti-cancerous ability to bind with the p53 receptor.

MATERIALS AND METHODS

3 MATERIALS AND METHODS

The materials used and methods adopted in the present study entitled **"Characterization of iron nanoparticles synthesized using *Sesbania grandiflora* flower extract and evaluation of its antidiabetic activity"** is furnished below

3.1. Collection and authentication of the plant

Healthy plants of *S. grandiflora* were collected from surrounding gardens Annur, Coimbatore, India. The flower was identified and its authenticity was confirmed at the herbarium of Botanical Survey of India, Southern Regional Centre, T.N.A.U.Campus, Coimbatore, Tamil Nadu.

Figure 1: *Sesbania grandiflora*

Figure 2: *S. grandiflora* flower powder

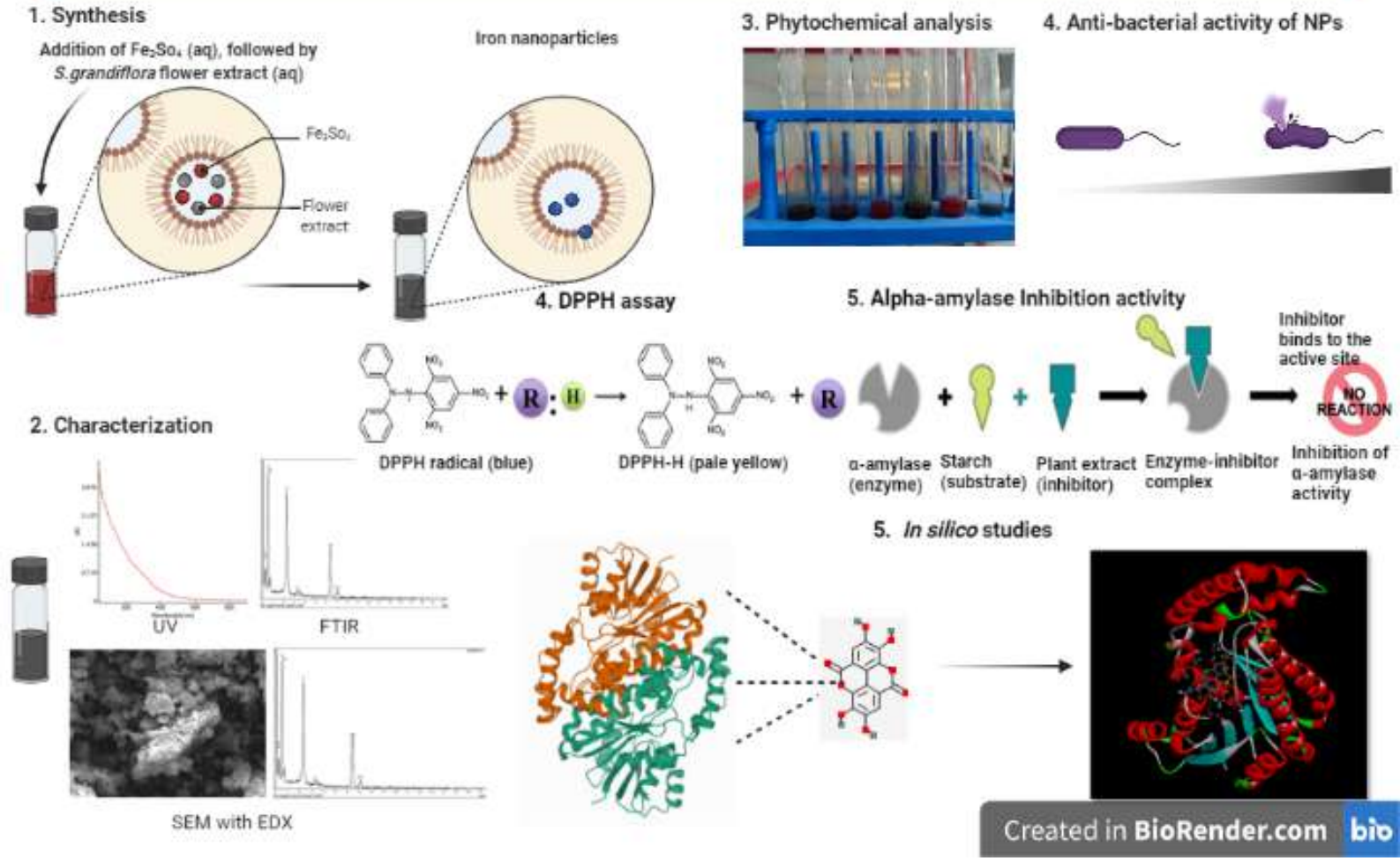
3.2. Preparation of flower powder

Flowers used for extraction were shade dried and powdered using a mechanical grinder. Fine powder was obtained by sieving. The powder was collected in clean air tight containers. Powdered plant material in the container was used for extraction.

3.3. Extraction

Fifty grams of fine sieved powder was weighed using an electrical balance (Denver 210) and subjected to kept in shaker for 10 hours at 37˚C temperature and 200 rpm speed, using water as solvent (500ml). Then the content was boiled for about 30 minutes with intermediate stirring. The suspension was allowed to cool and filtered through Whatman no. 1 filter paper and the filtrate was stored in refrigerator until testing.

Characterization of iron nano particles synthesized using *Sesbania grandiflora* flower extract and evaluation of its antidiabetic activity



3.4. Synthesis of Iron nanoparticles

The ferrous sulphate Fe_3SO_4 was purchased from Hi-Media Laboratories Pvt. Ltd. was used in the present study. The aqueous solution of ferrous sulphate was prepared in different concentrations (0.1mM-1Mm). The prepared flower extract was added to each of the concentrations of ferrous sulphate solution in equal volume. These prepared solutions were kept for incubation at dark room temperature (24-48 hours). The color change was visually observed from blue to black which detected the synthesized iron nanoparticles in the flower extract.

3.5. UV-Vis Spectroscopy

The UV-Vis Spectroscopy was used to confirm the synthesis of FeNPs based on their optical properties. Ultraviolet– visible spectroscope (EBI LAB INC) was used to record the absorbance spectra at 300-700 nm wave length. This was measured by loading the sample of 2ml in quartz cuvette in UV-Vis Spectroscopy.

3.6. Fourier Transform Infrared Spectroscopy (FTIR)

The Fourier Transform Infrared Spectroscope (Shimadzu, japan) is a chemical analytical method meant for the measurement of infrared intensity, wavelength or wave number of lights of the green synthesized FeNPs. Therefore, for FTIR analysis a sample of 0.5 g of FeNPs was used and the spectra was scanned in the range of 3600-600 cm^{-1} for the characterization of chemical functional groups present over the surface of green synthesized FeNPs. The FTIR data measures the interaction between Ag and protein molecules. Therefore, when an infrared light interacts with the sample of FeNPs the chemical bonds show stretching, contracting and bending nature of nanoparticles as indicated by peak in FTIR and the result was matched with the standard library search of Infrared charts.

3.7. X-ray diffraction analysis

The X-Ray Diffraction pattern of the powder samples were recorded on a PANalytical X PERT PRO X-ray diffract meter using Cu K$\propto$ radiation (=1.54060 Å). The particle characterization was done by measuring the crystallite size of the sample from the line broadening analyses using Debey –Schherer formula after accounting for instrumental broadening. The crystallite size in nm is expressed as D XRD = 0.89 / β cos, λ Where is the wavelength of x-ray

radiation used in Å, θ is the diffraction angle and β is the full width at half maximum (FWHM) in radians in the 2 angle.

3.8. Scanning Electron Microscope (SEM) WITH EDX

The size and internal morphology of the synthesized FeNPs were examined by scanning electron microscope (SEM) using TESCAN Mira 3 XMU instrument. SEM has the magnification ranging from 20X to approximately 30,000X with spatial resolution of 50 to 100nm. For imaging a sample of 0.5mg of FeNPs was dusted on one side of the double-sided adhesive carbon conducting tape. The tape was then mounted on 8mm diameter aluminum stub. The sample was observed at different magnification and the images were pictured. In order to carry out EDAX analysis, the bark extracts reduced silver nanoparticles were dried and drop coated on to carbon film and performed on Hitachi S-3400 N SEM instrument equipped with a Thermo EDAX attachments.

3.9. QUALITATIVE PHYTOCHEMICAL ANALYSIS:

3.9.1. Test for alkaloids

- Dragendroff's test: 1ml of each test extract was treated with 1ml of Dragendroff's reagent (sodium iodide, basic bismuth carbonate, glacial acetic acid and ethyl acetate) and observed for the presence of orange brown precipitate.
- Hager's test: 1ml of each test extract was treated with 1ml of Hager's reagent and observed for the presence of reddish-brown precipitate.

3.9.2. Test for flavonoids

- Shinoda test: Few mg of each extract was treated with 1ml of ethanol and heated in boiling water bath followed by addition of 1 drop of Concentrated HCL and few pieces of magnesium fillings. The reaction mixture was incubated at room temperature for 10-15 minutes. Appearance of red color indicates the presence of flavonoids.
- Ammonia test: Filter paper strips were dipped in the test extracts and ammoniated change in the filter paper color to yellow indicate the presence of flavonoids. To the yellow color filter paper 10ml of H_2SO_4 was added. Disappearance of yellow color further confirmed the presence of flavonoids.

3.9.3. Test for sterols and triterpenoids

- Libermann-burchard test: 5ml of each test extracts were boiled with two drops of acetic anhydride and cooled, then concentrated sulphuric acid was added along the side of the test tube. Appearance of brown ring at the junction of two layers is taken as reference. If the upper layer turns green, sterols are present whereas formation of deep red color indicates the presence of triterpenoids.
- Salkowski's test: Each test extracts were treated with few drops of concentrated sulphuric acid and shaken well. The solution was allowed to stand for some time. Appearance of red color in the lower layer indicates the presence of sterols whereas yellow color formation in the lower layer indicates the presence of triterpenoids.

3.9.4. Test for phenols

- Ferric chloride test: 2ml of each test extract was treated with 2ml of 5% ferric chloride solution and formation of deep blue or black color indicate the presence of phenols.
- Libermann's test: 1ml of each extract was heated with a pinch of sodium nitrite. To this solution 0.5ml of dilute H_2SO_4 was added followed by addition of dilute NaOH. Formation of deep red or green or blue color indicates the presence of phenols.

3.9.5. Test for glycosides

- Borntrager's test (Anthraquinone glycosides): 0.5g of each extract was shaken with benzene and organic layer separated. One part of 10% ammonia solution was added to 2 parts of organic layer. A pinkish red or violet coloration in the ammonical phase indicated the presence of anthraquinone glycosides.
- KellerKilliani test (Cardiac glycosides): 0.5gm of each extract was added with 0.4ml of glacial acetic acid containing trace amount of ferric chloride. Contents were transferred to small test tube and 0.5ml of H_2SO_4 acid was added along the sides of the test tube. Appearance of blue color in the acetic acid layer indicates the presence of cardiac glycosides.

3.9.6. Test for tannins

- Lead acetate test: 5ml of each test extract was added with few drops of 10% lead acetate. Appearance of yellow color precipitate indicates the presence of tannins.
- Ferric chloride test: 5ml of each test extract was added 5% of ferric chloride solution. Appearance of intense green or blue color indicates the presence of tannins.

3.9.7. Test for saponins

- Sodium bicarbonate test: Few ml of each test extract was added with three drops of sodium bicarbonate was added and shaken well. Formation of honey comb indicates the presence of saponins.
- Froth test: To each test extract, 20ml of distilled water was added and agitated on a graduated cylinder for 15min. Persistence of characteristic honey comb froth at least 1cm in height for 30min indicates the presence of saponins.

3.9.8. Test for carbohydrates

- Molish's test: To small quantities of solvent free each test extract, few drops of 1%-naphthol in ethanol were added. Concentrated sulphuric acid were then added to the sides of the test tube. Formation of brown purple ring formed at the junction of the two liquids indicates the presence of sugars.
- Benedict's test: 0.5ml of each test extract was added with 2ml of Benedict's solution. Formation of reddish-brown precipitate indicates the presence of carbohydrates.

3.10. Antibacterial activity

3.10.1. Test organisms

Gram positive bacteria (*Staphylococcus aureus, seratia marcescens, and Streptococcus pyogenes*) and Gram negative bacteria (*Escherichia coli, Klebsiella pneumoniae*) were used for antibacterial activity.

Procedure

The antimicrobial activity of the extract of *S. grandiflora* flower was determined using agar well diffusion method. The antibacterial activity of extract were tested against Gram positive

bacteria (*Staphylococcus aureus, seratia marcescens, Streptococcus pyogenes*), Gram negative bacteria *(Escherichia coli, Klebsiella pneumoniae*). Three wells were made in sterile Nutrient agar plates using a sterile cork borer, 50µl of the each bacterial cultures were swabbed on the respective plates. 20µl of each extracts were added in the respective wells separately. The plates were incubated at 37^0C for 24 hours to measure the zone of inhibition of the test extracts against standard antibiotics. Ampicillin was used as positive control.

3.11. Antioxidant studies

3.11.1. Free radical scavenging activity

The radical scavenging activities of the different extracts were measured *in vitro* against a battery of radicals namely DPPH and reducing power assay.

3.11.2. DPPH free radical scavenging activity

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Aldrich Co., St. Louis, USA. The diluted working solutions of the test extracts were prepared in methanol. About 1ml of graded concentration (20, 30, 40, 50, 75, 100 µg/ml) of extracts were taken in different test tubes and assorted with 1ml of DPPH (0.1Mm in methanol) and shaken well. This solution was then incubated in room temperature for 30 minutes. The optical density was recorded at 517 nm using UV spectrophotometer. Corresponding blank sample was prepared. Mixture of 0.5ml methanol and 0.5ml DPPH solution was used as control. The absorbance change was compared with the standard Quercetin (20-100µg/ml) and was determined. The scavenging activity was then calculated using the formula:

Radical scavenging activity (%) = Abs (control)–Abs (sample)/Abs (control) x 100

Where, Abs (control) is the absorbance of DPPH radical with methanol and Abs (sample) is the absorbance of DPPH radical with a sample extract or standard.

3.12. Alpha-amylase inhibition assay (Nikavar, 2009)

The alpha-amylase inhibitory activity was assessed based on the colorimetric assay where Acarbose as the reference compound. Briefly, a starch solution was prepared by dissolving 0.25 g of soluble potato starch in 50 ml of deionized water (0.5% w/v). The enzyme solution made was by mixing 10 mg α-amylase in 100 ml of sodium phosphate buffer (20 mM, pH 6.9) which contains

6.7 mM sodium chloride. The extracts were subject to dissolve in DMSO to give concentrations from 100 to 500 µg/ml.96 mM 3, 5-dinitrosalicylic acid (20 ml), 5.31 M sodium potassium tartrate in 2 M sodium hydroxide (8 ml) and deionized water (12 ml) was mixed to prepare the colour reagent. The reaction was initiated by adding 500 µl of the extract to 500-µl enzyme solution and left at room temperature for 30 min. After, to 500 µl of the reaction mixture 500 µl of potato starch solution was mixed and incubated at 25° C for 3 min. DNS reagent (500 µl) was added to it and subjected to water bath at 85° C. After 15 min it was let to cool and was diluted with 4.5 ml of distilled water. The absorbance value at 540nm in a spectrophotometer. Finally, to prepare the blank starch solution was added after the color reagent and carried out as same above. To a control, where 500 µl DMSO replacing the plant extract was added with 500µl enzyme solution and followed same procedure. Antidiabetic drug Acarbose solution at various concentrations (100 to 500 µg/ml) was a standard here. The inhibition percentage was calculated as follows:

$$I\alpha\text{-amylase}\% = 100\times (\Delta A\ \text{Control} - \Delta A\ \text{Sample}) / \Delta A\ \text{Control}$$

$$\Delta A\ \text{Control} = A\ \text{Test} - A\ \text{Blank}$$

$$\Delta A\ \text{Sample} = A\ \text{Test} - A\ \text{Blank}$$

3.13. *In silico* studies

Computer-based methods are becoming increasingly important and complementary to wet laboratory experiments in studying the structure and function of biomolecules. The integration of computational and experimental strategies has been of great value in the identification and development of novel promising compounds. Docking studies are used at different stages of drug discovery such as to predict a ligand-receptor interaction and also to rank the compounds based on the binding energies or fitness score. Molecular docking plays a significant role in structural based drug designing by predicting the binding orientation of small molecule drug candidates to their known 3D structures of the protein targets. The compounds from the extensive literature survey were selected for *in silico* docking valuation at particular active binding sites of the target proteins.

3.13.1. Evaluation of drug likeliness and toxicity prediction

Lipinski's rule of five was used to scrutinize the ligands for drug-like properties. This rule defines molecular properties significant for a drug's pharmacokinetics in the human body and

provides the evidence concerning the exploitation of the ligands as a drug (Lipinski *et al.*, 1997). The rules are:

1. Molecular weight < 500 daltons,
2. Number of hydrogen bond donors <5,
3. Number of hydrogen bond acceptors < 10 and
4. Calculated water partition coefficient (LogP) < 5.
5. Molar refractivity should be between 40-130

3.13.2. SwissADME

To serve as an effective drug, the ligand molecule should reach the diseased protein target in the body in adequate concentration, and remain there in active state long time for the expected biological events to follow. Drug discovery involves assessment of absorption, distribution, metabolism and excretion (ADME) increasingly earlier in the development process, at a stage when candidate compounds are many but access to the physical samples is limited. In that scenario, computer models constitute valid alternatives to laboratory investigations. In this study, the new SwissADME web tool that gives free access to a pool of fast yet robust predictive models for drug-likeness, physicochemical parameters, pharmacokinetics, and medicinal chemistry friendliness has been used (Hay *et al.*, 2014).

3.13.3. Bio activity score

The bioactivity score of the selected ligands was calculated for a nuclear receptor, GProtein Coupled Receptor, ion channel, kinase, and protease. These were done by obtaining SMILES notations of the chosen compounds from pubchem database and feeding them in the online Molinspiration software version 2011.06 (www.molinspiration.com) (Zhao *et al.*, 2002).

3.13.4. Target proteins

The Protein Data Bank (PDB) is an important resource in divisions of structural biology such as structural genomics. A majority of scientific journals, and a few funding agencies, such as the NIH in the USA, now insister searchers to deposit their structure data to the PDB (Porollo *et al.*, 2007). In the present study three- dimensional structure of diseased protein such as, 11ß-hydroxysteroid dehydrogenase type I (1BHS), Glucokinase (1V4S), Protein-tyrosine phosphatase 1B (2NT7) C-reactive protein (1GNH) were selected from the protein data bank.

3.13.5. Molecular docking

Autodock was used to perform docking of each selected ligand with the target protein. AutoDock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. AutoDock 4 actually consists of two main programs: *autodock* performs the docking of the ligand to a set of grids describing the target protein; *autogrid* pre-calculates these grids. In addition to using them for docking, the atomic affinity grids can be visualised. This can help, for example, to guide organic synthetic chemists design better binders. AutoDock has applications in X-ray crystallography; structure-based drug design; lead optimization; virtual screening (HTS); combinatorial library design; protein-protein docking; chemical mechanism studies. It is very fast, provides high quality predictions of ligand conformations, and good correlations between predicted inhibition constants and experimental ones. AutoDock has also been shown to be useful in blind docking, where the location of the binding site is not known (Morris *et al*., 2009). Molecular docking procedures are given Annexure.I.

3.13.6. Discovery studio visualizer

Discovery Studio is a comprehensive software suite for analyzing and modeling molecular structures, sequences, and other data of relevance to life science researchers. The product includes functionality for viewing and editing data along with tools for performing basic data analysis. This opens a new Sequence Window that allow to visualize the amino acid sequence and the corresponding 3D structure simultaneously.

3.13.7. LIGPLOT

In bioinformatics LIGPLOT is a computer program that generates schematic 2-D representations of protein-ligand complexes from standard Protein Data Bank file input. The LIGPLOT is used to generate images for the PDB sum resource that summarizes molecular structure (Wallace, 1995).


RESULT

4 RESULT

The results pertaining to the study entitled **"characterization of iron nanoparticles synthesized using *Sesbania grandiflora* flower extract and evaluation of its antidiabetic activity"** are presented under the following headings:

4.1. Nanoparticle synthesis

Reduction of iron ions into iron nanoparticles using aqueous extract of *S. grandiflora* flower was evidenced by the visual change of color from pinkish brown to dark black well at the 0.9 Mm concentration (2ml of plant extract in 2 ml of 0.9mM FeNP Solution) compared to other proportion (Figure 5).

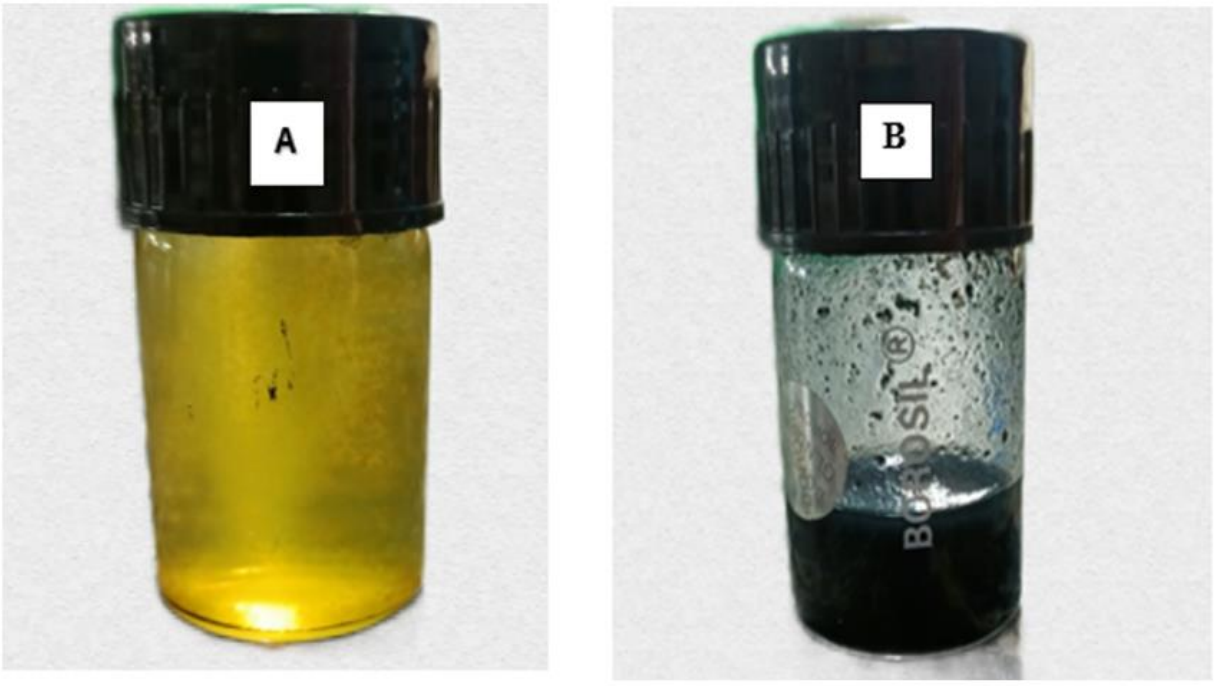


Figure 3: A. Ferrous sulphate solution

B. synthesized Iron nanoparticle of *S. grandiflora*/flower

4.2. UV Visible spectroscopy

The UV-Visible spectra show an absorption band at 300nm, 400nm, 500nm, 600nm, which corresponds to the absorbance of iron nano particles. The excitation of surface Plasmon vibrations in iron nano particles. After 3hours, no significant color change was observed. Increased concentrations of ferrous sulphate resulted in a brown solution of nano iron indicating the completion of reaction.

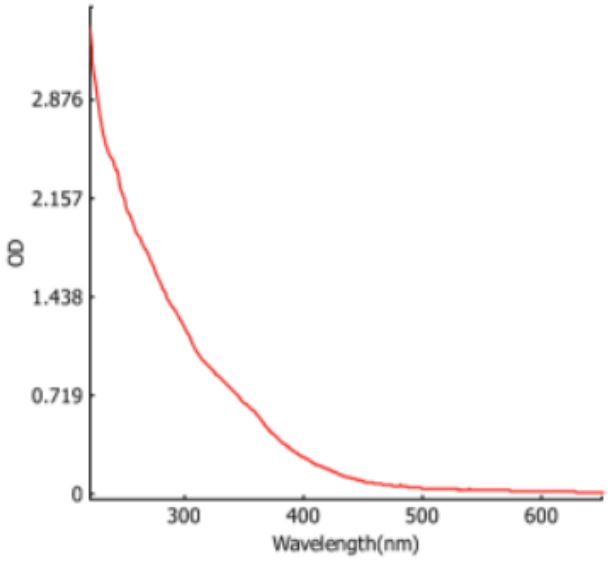


Figure 4: UV Visible spectroscopy *S. grandiflora*

4.3. Fourier-transform infrared spectroscopy (FTIR)

The FTIR analysis was performed to understand the presence of chemical groups and also to elucidate the compounds in the synthesized nanoparticles solution of *S. grandiflora* flower. The results of the present analysis exhibited the typical bands and peak characteristics for the sample. Two styles of vibrations stretching and bending were attained from the FTIR data which ranges from 3600 to 600 cm^{-1}. The compounds were reported in the peaks such as 424.34, 447.49, 493.78, 601.79, 694.37, 1095.57, 1635.64, and 3294.42. The weak bond is noted to be 424.34, 1095.57 which attributes to alkyl halides, amines, aldehydes and alkanes.

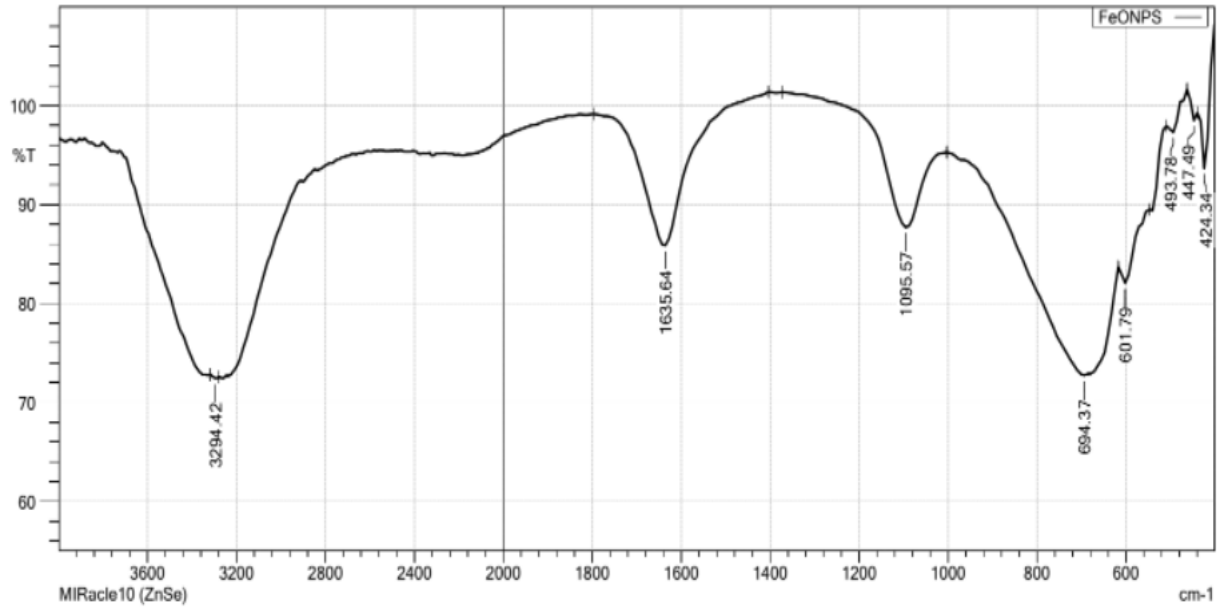


Figure 5: FTIR Spectra of nanoparticles of *S. grandiflora* flower

Table I: Peak values and functional groups present in nanoparticles of *S. grandiflora* flower

Wave number	Functional groups
424.34	Si-O Stretch
447.49	Si-O Stretch
493.78	Si-O Stretch
601.79	C-Br Stretch of alkyl halides group
694.37	C–H bend of alkynes
1095.57	C-N Stretch of aliphatic amines
1635.64	CH Stretch of amide
3294.42	C-H Stretch of aldehyde

4.4. XRD

Synthesized iron nanoparticles from 20% flower extract concentration were characterized using Fe X-ray diffract meter for confirming the presence of nanoparticles and analyzing its structure which was shown respectively. From the result the peaks were identified at $^{31.77}$°, $^{34.44}$°, $^{36.28}$°, $^{47.60}$°, $^{56.52}$°, $^{62.88}$°, and $^{67.96}$°. The X-ray diffraction pattern of iron nanoparticles was illustrated in Figure.8 which indicated that the synthesized iron nanoparticles were crystalline phase.

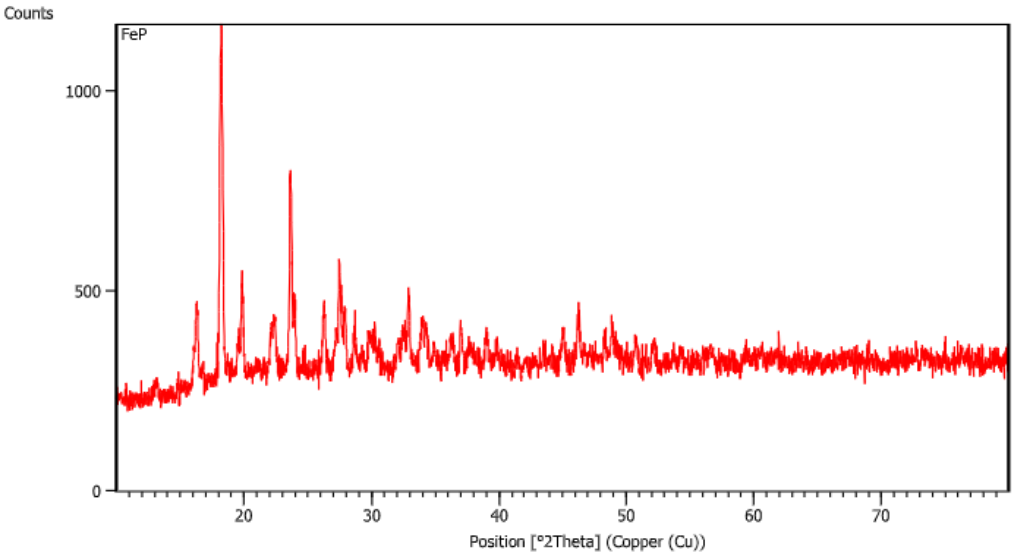


Figure 6: XRD of S. grandiflora flower

4.5. Scanning Electron Microscopy (SEM) with EDX

SEM micrograph shows the high-density morphology of iron nanoparticles. The particles were observed to be granular, irregular and spherical in shape. The nano particles showed without any aggregation and the overall size of nanoparticles was less than 70nm which could be effective for the drug delivery.

The elemental composition through EDX displayed the presence of a strong characteristic peak at 1keV, 6 keV and 7 keV, which is specific to Fe with additional weak signals of respectively.

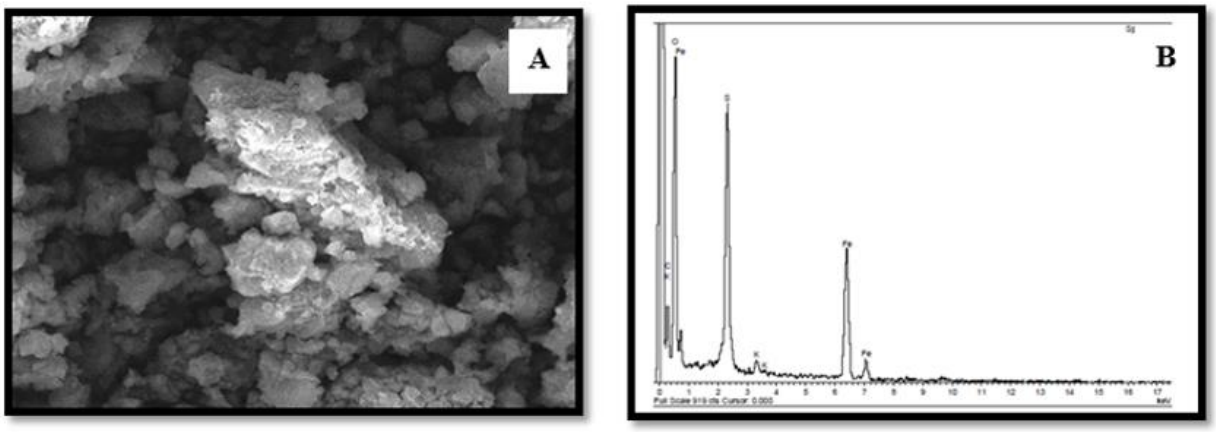


Figure 7: A.SEM Image of FeNP

B.EDAX image of FeNP

4.6. Qualitative phytochemical analysis

To identify the presence of the key phytochemicals in *S. grandiflora*, a qualitative chemical analysis was done. Alkaloids, sugars, flavonoids, tannins, phenols, saponins, glycosides, anthraquinone, sterols, and terpenoids were highest in the synthesized nanoparticles. The presence or absence of different phytochemicals is shown in the table III.

Table II: Qualitative analysis of the primary and secondary metabolites.

Phytochemicals	Nanoparticle
Alkaloids	+ + +
Flavonoids	+ + +
Sterols	+ + +
Tannins	+ + +
Protein	+ + +
Phenol	+ + +
Glycoside	+ + +
Saponin	+ + +
Carbohydrates	- - -

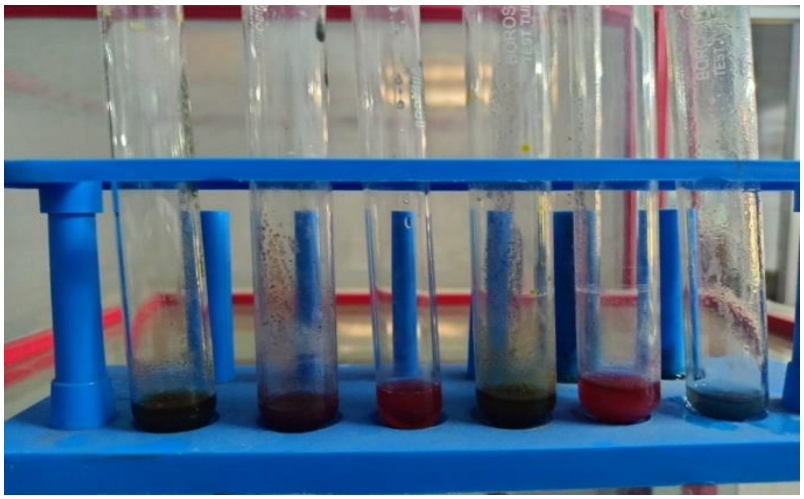


Figure 8: Phytochemical of FeNP

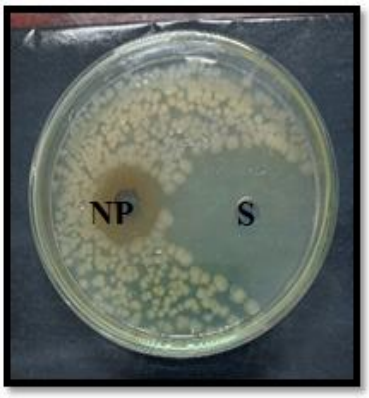
4.7. Antibacterial activity

The FeNP of *S. grandiflora* flower extract were evaluated for its antibacterial activity against five clinical bacterial isolates Gram positive bacteria (*Staphylococcus aureus, Seratia marcescens, Pseudomonas aeruginasa*) and Gram negative bacteria (*Escherichia coli, Klebsiella pneumoniae*). Table VI describes the antibacterial activity of Ampicillin, FeNPs of *S. grandiflora* flower against the selected bacterial isolates. Fig 11 shows specific activity of each extracts in zone formation against each bacterial isolates.

From the Table VI it was observed that the zone of inhibition of The synthhesized FeNPs exhibited the zone of inhibition of *Escherichia coli* (13.3 mm), *Seratia marcescens* (12 mm), *Staphylococcus aureus* (12 mm), *Pseudomonas aeruginasa* (11.6 mm) and *Klebseilla pneumonia* (6 mm).

TABLE III: Antibacterial activity of *S. grandiflora* flower against the selected bacterial isolates

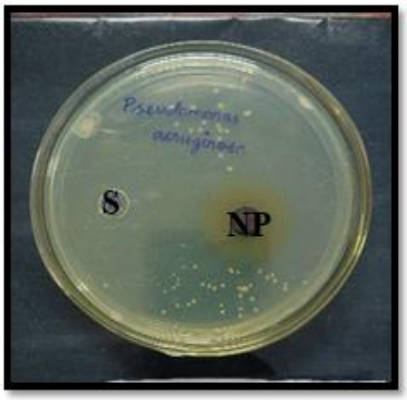
Bacteria isolation	**Zone of inhibition**	
	Control	**Nanoparticle**
Gram positive		
Staphylococcus aureus	9.3	12
Seratia marcescens	9	12
Pseudomonas aeruginasa	6.3	11.6
Gram negative		
Escherichia coli	8	13.3
Klebseilla pneumonia	7.3	6



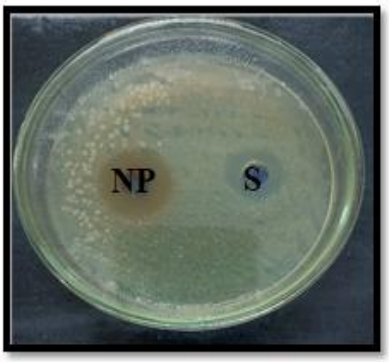
Klebsiella pneumoniae

Staphylococcus aureus

Serratia



Pseudomonas aeruginasa



E.coli

Figure 9: *Antibacterial activity of* S. grandiflora flower

4.8. Antioxidant activity

4.8.1. Radical scavenging activity of *S. grandiflora* flower

The radical scavenging activities of these extracts were determined *in vitro* against a variety of radical's namely Ascorbic acid. The highest scavenging efficacy of FeNP were 95.08%.

4.8.2. DPPH Assay

In the DPPH assay, The DPPH reducing activity of the FeNP was measured based on color change which was shown due to the reduction reaction. The extent of DPPH scavenging by nanoparticle solution was significant, where the stable radical was effectively reduced to the yellow-colored compound di phenylpicryl hydrazine. The assay is based on the scavenging capacity of antioxidants towards a stable free radical α, α-diphenyl-β-picrylhydrazyl (DPPH). The high percentage of FeNP of S.grandiflora flower was recorded at the concentrations 10µl and 50µl with the inhibition percentage of 95.08% and 85.25%.

Table IV: DPPH Radical Scavenging Assay

Samples	Concentration(µl)	% inhibition
Standard (Ascorbic acid)	30	68.85
	60	77.87
	90	86.89
	120	93.44
	150	97.54
Sample (FeNP)	10	95.08
	50	85.25
	150	71.31
	250	56.56
	500	24.59

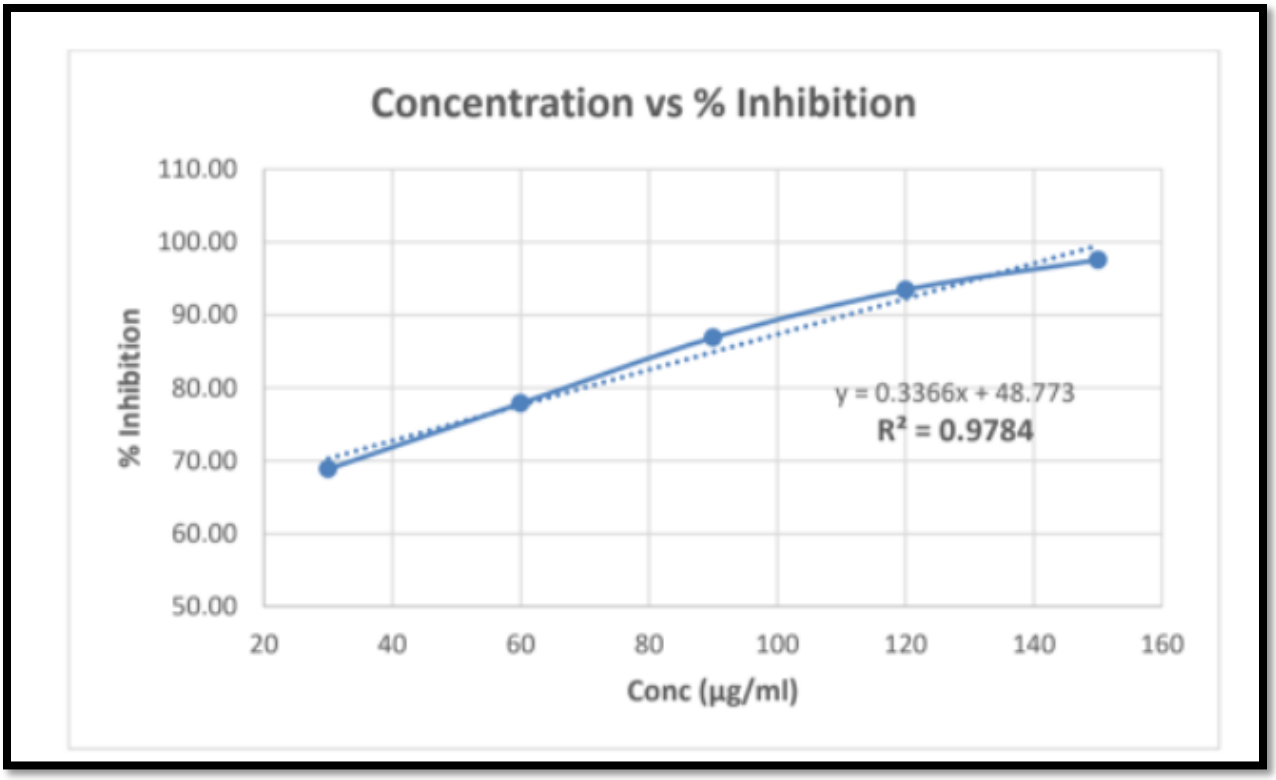


Figure 10: DPPH Assay of *S. grandiflora*

4.9. α-Amylase Inhibition Assay:

S. grandiflora flower extract was screened with synthesized iron nano particles for *invitro* amylase inhibition efficacy. Synthesized iron nano particles showed a significant α-amylase inhibition activity as shown in Table V. Bio synthesized iron nano particles and Acarbose indicated a highest inhibition percent at 500 µg/ml and lowest at 100 µg/ml revealing a dose dependent effect. The biosynthesized nanoparticle solution showed the highest absorbance of 43.21 ± 0.63 at 500 µg/ml which correlates almost to the standard acarbose which showed the highest absorbance of 46.12 ± 0.43at 500 µg/ml.

Table V. Inhibition % of α-amylase enzyme assay

Concentration (µg/ml)	Inhibition (%)	
	Acarbose	Biosynthesized FeNPs
100	30.44 ± 0.63	26.00 ± 0.16
200	33.75 ± 0.46	30.73 ± 0.39
300	36.32 ± 0.22	35.69 ± 0.13
400	41.68 ± 0.31	38.60 ± 0.41
500	46.12 ± 0.43	43.21 ± 0.63

4.10. *Insilico* studies of *S. grandiflora* flower

4.10.1. Ligand retrieval

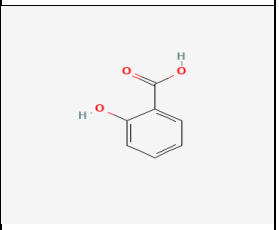
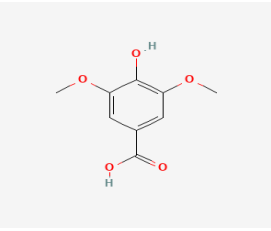
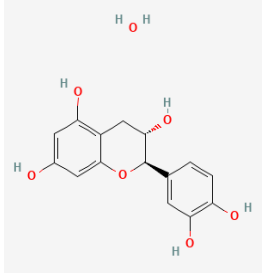
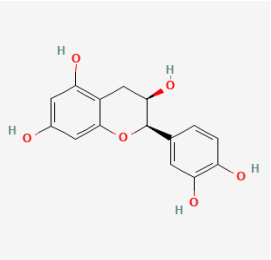
PubChem is a huge database for small molecule deposition from NMR and XRD derived structures. The structure of eight molecules which had CAS numbers was collected from the PubChem database. The detail of the selected compounds was given in the Table VII.

4.10.2. Drug likeliness prediction of selected molecule

Out of 8 molecules, only 4 molecules were found to satisfy drug-like properties based on Lipinski's rule of five. Lipinski's rule of five defines a molecule as drug like only if the molar weight (MW) is less than 500 Daltons (Da); the logarithm of the octanol/water partition coefficient (QPlogPo/w) was not more than 5, the number of hydrogen bond acceptors (HBA) not more than 10 and the number of hydrogen bond donors (HBD) not more than 5. The distributions of the compound MW, log P, HBA and HBD were calculated and used to assess the drug like nature of the compounds present in *S. grandiflora* flower.

All the selected ligands were found to have drug like property, obeying Lipinski's rule of five except nicotiflorin, luteolin-7-o-glucoside (Table VIII).

Table VI: Ligands chosen for docking

Compound name	Molecular formula	CAS number	Smile	Molecular structure
Salicylic acid	$C_7H_6O_3$	69-72-7	C1=CC=C(C(=C1)C(=O)O)O	
Syringic acid	$C_9H_{10}O_5$	530-57-4	COC1=CC(=CC(=C1O)OC)C(=O)O	
Catechin hydrate	$C_{15}H_{16}O_7$	88191-48-4	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C=C3)O)O)O.O	
Epicatechin	$C_{15}H_{14}O_6$	17334-50-8	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C=C3)O)O)O	

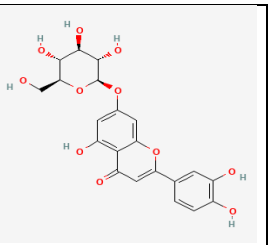
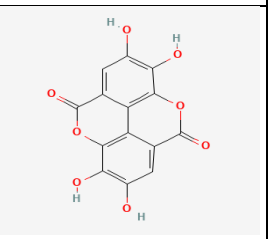
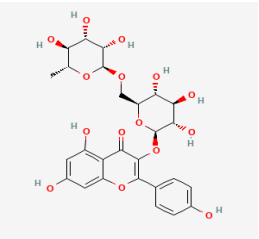
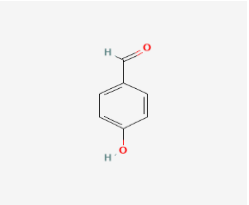
Luteolin-7-o-glucoside	$C_{21}H_{20}O_{11}$	5373-11-5	C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)OC4C(C(C(C(O4)CO)O)O)O)O)O)O	
Ellagic acid	$C_{14}H_6O_8$	476-66-4	C1=C2C3=C(C(=C1O)O)OC(=O)C4=CC(=C(C(=C43)OC2=O)O)O	
Nicotiflorin	$C_{27}H_{30}O_{15}$	17650-84-9	C[C@H]1[C@@H]([C@H]([C@H]([C@@H](O1)OC[C@@H]2[C@H]([C@@H]([C@H]([C@@H](O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC=C(C=C5)O)O)O)O)O)O)O	
4-hydroxybenzaldehye	$C_7H_6O_2$	123-08-0	C1=CC(=CC=C1C=O)O	

TABLE VII: Compliance of compounds to computational parameters of drug likeness

S.no	Compound name	Mw(g\mol)	Hba	Hbd	Qplog p3	Rule of violence
1	Salicylic acid	138.12	3	2	2.3	0
2	Syringic acid	198.17	5	2	1	0
3	Catechin hydrate	308.28	7	6	1.37	0
4	Epicatechin	290.27	6	5	0.4	0
5	Ellagic acid	302.19	8	4	1.1	0
6	4-hydrobenzaldehyde	150.17	2	1	0.8	0
7	Nicotiflorin	594.5	15	9	-0.9	2
8	Luteolin-7-o-glucoside	448.4	11	7	0.5	2

4.10.3. Bioactivity score

The bioactivity score of ligands which was chosen from *S.grandiflora* flower was calculated for GPCR (G-Protein Coupled Receptor) ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitors.

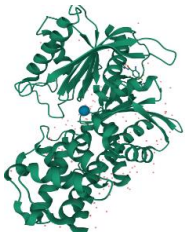
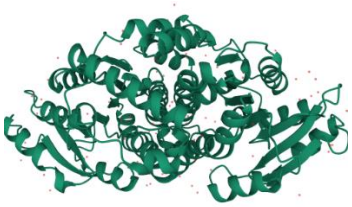
4.10.4. Diseased Protein for diabetes

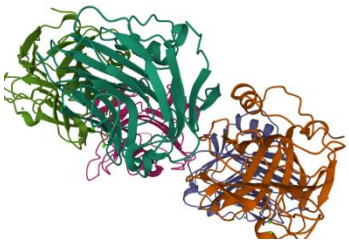
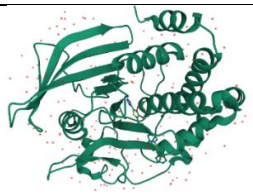
The selection of target protein for diabetes was made through literature survey. There are four protein retrieved from PDB. Accordingly, the protein structures were retrieved on basis of a number of criteria such as single monomer, less than 2 resolution, Homo sapiens and single domain in the protein complex. All ligands chosen from *S. grandiflora* flower were docked against the four chosen diabetes proteins such as 11ß-hydroxysteroid dehydrogenase type I (1BHS), Glucokinase (1V4S), Protein-tyrosine phosphatase 1B (2NT7) C-reactive protein (1GNH). The structure of the diabetes proteins derived from PDB is shown table X.

TABLE VIII: Bio activity score of chosen ligands

Compound name	Gpcr Receptor	Ion channel receptor	Protein kinase receptor	Nuclear receptor	Protease receptor	Enzyme inhibitor
Salicylic acid	-0.98	-0.43	-1.22	-0.79	-1.14	-0.41
Syringic acid	-0.65	-0.28	-0.69	-0.44	-0.82	-0.15
Catechin hydrate	0.41	0.14	0.09	0.60	0.26	0.47
Epicatechin	0.41	0.14	0.09	0.60	0.26	0.47
Luteolin-7-o-glucoside	0.09	-0.02	0.15	0.27	-0.01	0.42
Ellagic acid	-0.29	-0.27	-0.01	0.11	-0.18	0.17
Nicotiflorin	-0.01	-0.43	-0.09	-0.17	-0.04	0.18
4-hydroxybenzaldehye	-2.38	-1.51	-2.37	-1.93	-2.80	-1.75

TABLE IX: Selected protein for *insilico* analysis

Protein name	PDB ID	Role of the protein	Protein 3D Structure
Glucokinase (GK)	1V4S	Phosphate is transferred from ATP to glucose through this enzyme, resulting in glucose 6-phosphate.	
11ß hydroxysteroid dehydrogenase 1 (11β-HS1),	1BHS	Builds insulin resistance by translating cortisone to cortisol	

C-reactive protein (CRP)	1GNH	Causes chronic inflammation in adipose tissue and leads to insulin resistance	
Protein tyrosine phosphatases (PTP)	2NT7	Eradicates phosphate groups that present in phosphorylated tyrosine residues in liver and fat	

4.10.5. Docking of target proteins with ligands

To investigate the detailed intermolecular interactions between the ligand and the target protein, autodock 4.2.8 was used. It performs grid-based ligand docking with energetics and searches for favorable interactions between one or more typically small ligand molecules and a typically larger receptor molecule, usually a protein. Three-dimensional structural information on the target proteins was taken from the Protein Data Bank (PDB). Protein preparation was done to delete water molecules not associated with active sites and to regenerate the native status and also for the addition of hydrogen atoms. The compounds from *S.grandiflora* were retrieved from extensive literature search and docked with the selected proteins. A correlation was calculated by docking score.

The diseased proteins Glucokinase (GK) (1V4S), 11ß hydroxysteroid dehydrogenase 1 (11β-HS1) (1BHS), C-reactive protein (CRP) (1GNH), and Protein tyrosine phosphatases (PTP) (2NT7) were retrieved from protein data bank. Protein was interacted with screened ligands from *S. grandiflora* flower powder. The entire docked complex was visualized using the software discovery studio visualizer.

Table X: Summary of docking score of the top ranked poses in each protein

Protein	Screened ligand	Binding score
1V4S	Salicylic acid	3.17
	Syringic acid	2.64
	Epicatechin	5.23
	Catechin hydrate	4.97
	Ellagic acid	4.5
	Nicotiflorin	1.06
	Leutotin-7-o-glucoside	2.9
	4-hydroxybenzaldehyde	3.84
1BHS	Salicylic acid	3.84
	Syringic acid	3.85
	Epicatechin	4.71
	Catechin hydrate	5.04
	Ellagic acid	5.43
	Nicotiflorin	2.34
	Leutotin-7-o-glucoside	3.56
	4-hydroxybenzaldehyde	3.79
1GNH	Salicylic acid	2.97
	Syringic acid	4.14
	Epicatechin	4.0
	Catechin hydrate	3.79
	Ellagic acid	4.08
	Nicotiflorin	1.56
	Leutotin-7-o-glucoside	2.93
	4-hydroxybenzaldehyde	3.55
2NT7	Salicylic acid	3.76
	Syringic acid	3.6
	Epicatechin	4.31
	Catechin hydrate	5.01
	Ellagic acid	3.43
	Nicotiflorin	1.09
	Leutotin-7-o-glucoside	3.85
	4-hydroxybenzaldehyde	4.2

4.10.5.1. Docking interaction between Glucokinase (1V4S) and epicatechin

The docking profile of Glucokinase (1V4S) and epicatechin complex showed non-bonded interaction with the docking score of -5.23 kcal/mol which shows the affinity of epicatechin towards the protein 1V4S.

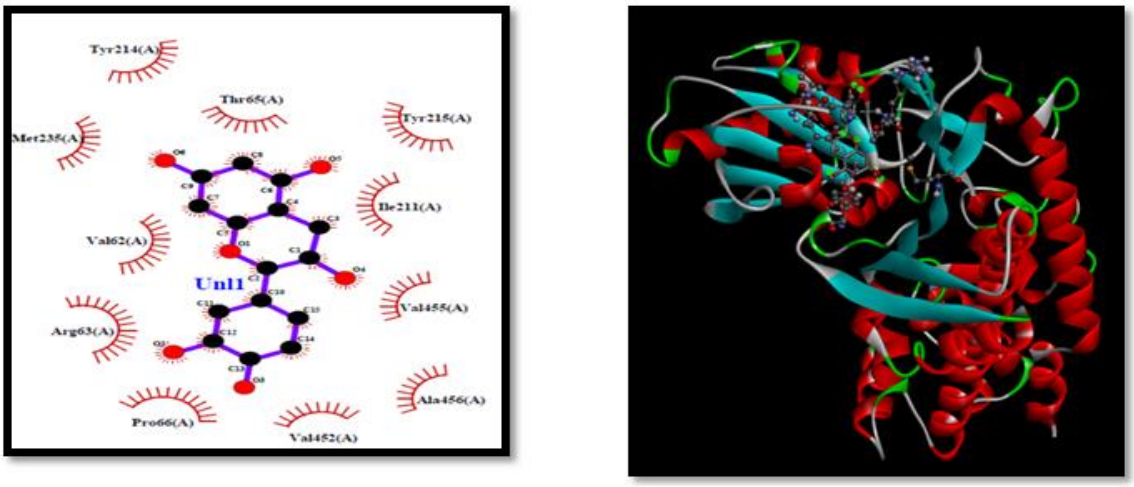


Figure 11: 2D, 3D plot 1V4S complexed with epicatechin

4.10.5.2. Docking interaction between 11ß hydroxysteroid dehydrogenase 1 (1BHS) and Ellagic acid

The docking profile of 11ß hydroxysteroid dehydrogenase 1 (11β-HS1)1BHS and Ellagic acid complex showed six hydrogen bond interaction with aminoacid residue at the position of Gly 186, Lys 159, Gly 92, Asn 90, Ser 12, Thr 190 of 1BHS. The oxygen atom of the amino acid residue Gly 186 (atom red in color) bonded with oxygen atom (atom red in color) of the ligand with the bond length of 3.07 Å. Nitrogen atom of the aminoacid residue Lys 159 bonded (atom blue in color) with oxygen atom (atom red in color) of the ligand with the bond length of 2.77 Å. Nitrogen atom of the amino acid residue Gly92 bonded (atom blue in color) with oxygen atom (atom red in color) of the ligand with the bond length of 3.17 Å. Oxygen atom of the aminoacid residue Asn 90 bonded (atom red in color) bonded with oxygen atom (atom red in color), of the ligand with the bond length of 3.01 Å. Oxygen atom of the aminoacid residue Ser 12 bonded

(atom red in color) bonded with oxygen atom (atom red in color), of the ligand with the bond length of 3.14 Å. And Oxygen atom of the aminoacid residue Thr 190 bonded (atom red in color) bonded with oxygen atom (atom red in color), of the ligand with the bond length of 3.06 Å, which is within the limit. The docking score was -5.43 kcal/mol and the number of hydrogen bonds was found to be 6. A docking score of -5.43 kcal/mol and hydrogen bond provides the efficacy of Ellagic acid in docking with the protein 1BHS (table XI).

TABLE XI: Docking interaction between (1BHS) and Ellagic acid.

Protein complex	**Amino acid**	**Protein atom**	**Ligand atom**	**Bond length**	**Docking score**	**No. Of hydrogen bond**
1BHS	Gly 186	O	O	3.07	-5.43	6
	Lys159	N	O	2.77		
	Gly 92	N	O	3.17		
	Asn 90	O	O	3.01		
	Ser 12	O	O	3.14		
	Thr190	O	O	3.06		

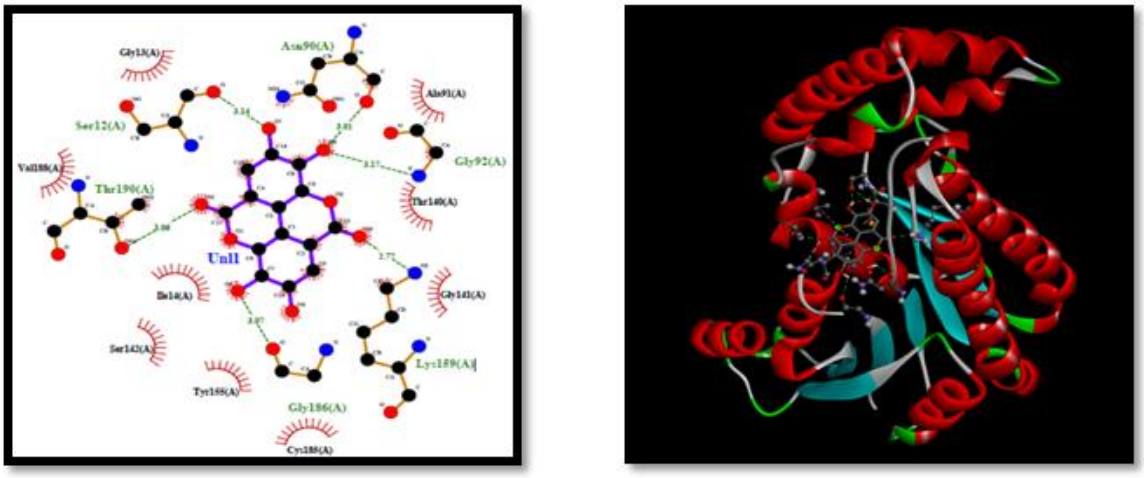


Figure 12: 2D, 3D plot 1BHS complexed with ellagic acid

4.10.5.3. Docking interaction between C-reactive protein (1GNH) and Syringic acid

The docking profile of C-reactive protein (CRP) 1GNH and Syringic acid complex showed two hydrogen bond interaction with aminoacid residue at the position of Tyr 19, Asn 145 of 1GNH. The oxygen atom of the amino acid residue Tyr 19 bonded (atom red in color) with oxygen atom (atom red in color) of the ligand with the bond length of 3.09 Å. And the nitrogen atom of the amino acid residue Asn 145 bonded (atom blue in color) with oxygen atom (atom red in color) of the ligand with the bond length of 2.82Å, which is within the limit. The docking score was -4.14 and the number of hydrogen bonds was found to be 2. A docking score of -4.14 kcal/mol and 2 hydrogen bond proves the efficacy of Syringic acid in docking 1GNH (table XII)

TABLE XII: Docking interaction between 1GNH and Syringic acid.

Protein complex	Amino acid	Protein atom	Ligand atom	Bond length	Docking score	No. Of hydrogen bond
1GNH	Tyr 19	O	O	3.09	-4.14	2
	Asn 145	N	O	2.82		

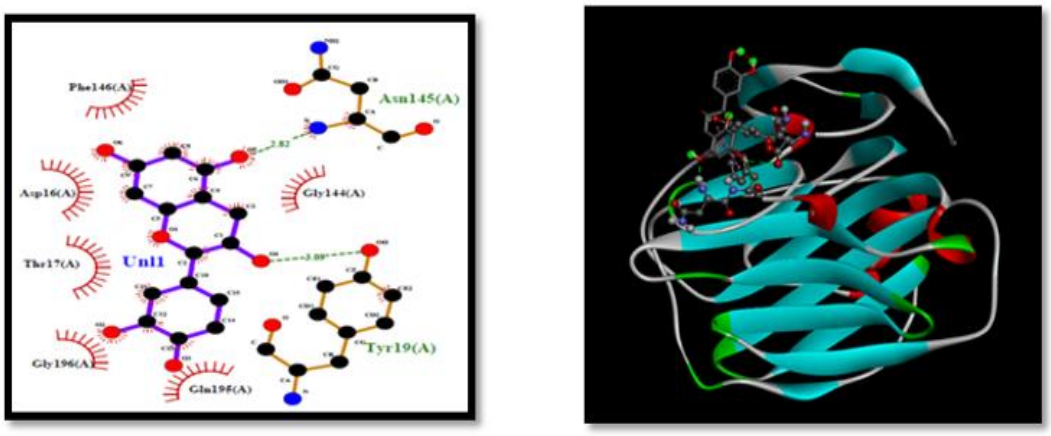


Figure 13: 2D, 3D plot 1GNH complexed with syringic acid

4.10.5.4. Docking interaction between Protein tyrosine phosphatases (2NT7) and catechin hydrate

The docking profile of Protein tyrosine phosphatases (PTP) 2NT7 and catechin hydrate complex showed four hydrogen bond interaction with amino acid residues such as Arg 221, Arg 221, Phe 182 and Cys 215 of 2NT7.

TABLE XIII: Docking interaction between 2NT7 and catechin hydrate.

Protein complex	Amino acid	Proten atom	Ligand atom	Bond length	Docking score	No. Of hydrogen bond
2NT7	Arg 221	N	O	3.06	-5.01	4
	Arg 221	N	O	3.34		
	Phe 182	N	O	3.18		
	Cys 215	S	O	2.91		

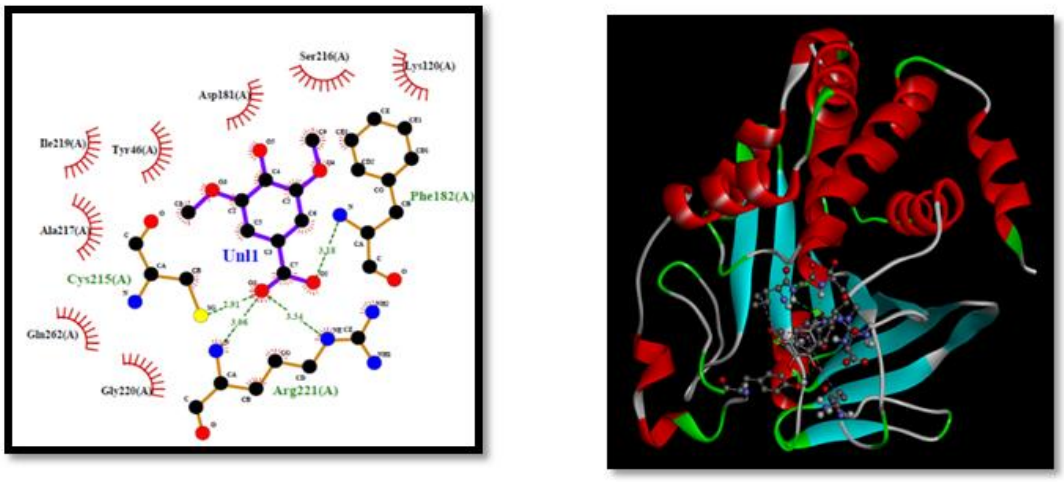


Figure 14: 2D, 3D plot 2NT7 complexed with catechin hydrate

The nitrogen atom of the amino acid residue Arg 221 bonded (atom blue in color) with oxygen atom (atom red in color) of the ligand with the bond length of 3.06Å. The nitrogen atom of the amino acid residue Arg 221 bonded (atom blue in color) with oxygen atom (atom red in color) of the ligand with the bond length of 3.34Å. The nitrogen atom of the amino acid residue Phe 182 bonded (atom blue in color) with oxygen atom (atom red in color) of the ligand with the bond length of 3.18Å. And sulphur atom of the amino acid residue Cys 215 bonded (atom yellow in color) with oxygen atom (atom red in color) of the ligand with the bond length of 2.91Å, which is within the limit. The docking score was -5.01 and the number of hydrogen bonds was found to be 4. A docking score of -5.01 kcal/mol and 4 hydrogen bond proves the efficacy of catechin hydrate in docking 2NT7 (Table XII).

DISCUSSION

5 DISCUSSION

Plants have been used throughout the world for its medicinal powers since ancient time (Chandran *et al.,* 2020). India is a reservoir of numerous high-valued medicinal plants and is one of the major medicinal plants producing Asian country (Anand *et al.,* 2019). The pharmacological properties of plants are based on their phytochemical components especially the secondary metabolites which are outstanding sources of value added bioactive compounds. The most important things for consumers about medications are purity, safety, potency, and efficacy. Although, plant-induced cutaneous reactions can occur and hence this should provide the basis for the creation of a phytovigilance programme and re-evaluation of how traditional medicine is utilised in the general population.

S. grandiflora is a small erect quick-growing short-lived soft-wooded tree sparsely branched. All parts of *S. grandiflora* are utilized for medicine in Southeastern Asia and India including preparations derived from the roots, bark, gum, leaves, flowers, and fruit. Different parts of this plant are used in Siddha system of Indian traditional medicine for the treatment of a wide spectrum of ailments including anemia, bronchitis, fever, headache, ophthalmia, nasal catarrh, inflammation, leprosy, gout and rheumatism (Ahvazi *et al.,* 2018). *S. grandiflora* plant in natural systems of medicine, Ayurveda, Siddha, Unani, Tibbi, and Amchi have tempted us to take up and study about this plant.

Bio fabrication of Metal nanoparticles using plant extract has been utilized in the present work, implementing the use of non-hazardous chemicals and facile methods of synthesis which insist on green nanotechnology. The advantages of the biosynthesized iron nanoparticles as antioxidant, anti-diabetic has been explored (Jamshidi-Kia *et al.,* 2018). Comparing literature studies, in the present work robust formation of FeNPs even with less quantities of the reactants is noted obviously revealing the increased experimental efficiency. The selected plant extracts contain Sterols, soponin and proteins which may reduce the ferric ions. The stabilization of the biosynthesized FeNPs can be attributed to the proteins and prevent agglomeration of FeNPs.

Different ratios of iron into iron nanoparticles using aqueous extracts of *S. grandiflora* flower in separate glass vials which produces nano iron of same dark black color at room temperature at the ratio 0.9 mM concentration (2ml of plant extract in 2 ml of 0.9 mM FeNP Solution) compared to other proportions which was made confirmed by observing the visible color changes. This makes that the effect of variation of concentration influences the formation of nanoparticles to be better.

Within a decade, there were a number of dramatic advances in analytical techniques including UV, FTIR, SEM with EDX and XRD that were powerful tools for separation, identification and structure determination of phytochemicals. UV-visible spectroscopy can be performed for qualitative analysis and for identification of certain classes of compounds in both pure and biological mixtures (Bindhu *et al.,* 2020). UV-visible spectroscopy can be used for quantitative analysis because aromatic molecules are powerful chromophores in the UV range. Natural compounds can be determined by using UV-visible spectroscopy. UV-Vis spectroscopy is well known to investigate shape and size controlled of nanoparticles. The colour change which indicated after the mixture of iron solution with the extract was the formation of Fe_3So_4 and correspondingly due to the excited surface plasmon vibrations with the reduction of iron ions. The zinc oxide and iron oxide nanoparticles had an intense absorbance at ∼250 nm which indicates that the synthesized particles were photosensitive in UV region. The color change of the leaf extract was observed when the extract was incubated with the iron solution. It can be seen that the surface Plasmon resonance (SPR) of FeNPs is 440-458nm (Rajendran *et al.,* 2017).

The interaction of Fe_3So_4 with the bioactive components which are the ones responsible for stabilization of iron ions were analyzed by Fourier transform infrared spectroscopy (FTIR) measurement. The peaks at 1700 cm^{-1} and 1850 cm^{-1} exhibited the C=O stretching vibrations and the strong peak at 1388 indicated the aromatic amine (C-N) stretching and the medium peak at 915 cm^{-1} assigned to carboxylic acid (O-H) bending vibrations. The peak was found at a frequency of 669 cm^{-1} due to Fe-O bond vibration (Bindhu *et al.,* 2020*).* The peaks at 1649 cm^{-1} and 1039 cm^{-1} were corresponding to the >C=O asymmetric stretching and COO− symmetric stretching (Rajendran *et al.,* 2017). The region of 1000-1500 cm^{-1} are assigned to the O-C = O symmetric and asymmetric stretching vibrations and the C-O stretching vibration, but the intensity of the band has weakened, which indicated that the ultrafine powers tend to strong physical absorption to H_2O and CO_2 (Saiganesh *et al.,* 2021).

X-Ray Diffraction, frequently abbreviated as XRD, is a non-destructive test method used to analyze the structure of crystalline materials. XRD analysis, by way of the study of the crystal structure, is used to identify the crystalline phases present in a material and thereby reveal chemical composition information. Peaks were found in a diffractogram at an angle of 18.44°, 19.60°, 23.84°, 33.20°, 35.64°, 40.96°, 49.44°, 54°, and 62.60° which indicated that the synthesized iron oxide nanoparticles were crystalline phase. Similar kinds of results have been found in green synthesis of manetite nanoparticle (Rajendran *et al.*, 2017). The XRD spectrum of the $Nd3^{+}$ ion doped NiO nanoparticles exhibit the different crystalline phases, such as (111), (200), (220) and (311), in which the particle size is in range of 20-50nm.The parameters show the lattice vibrations of the NiO nanoparticles into the structural arrangements of the sample. It is a good analysis of the NiO materials (Saiganesh *et al.*, 2021).

SEM, is probably the most widespread analytical instrument available in analytical laboratories destined to characterize physical properties such as morphology, shape, size and distribution of materials at the micro and nano-scale (Belayneh and Birru, 2018). The performance of a modern, high-resolution SEM, in particular its spatial resolution, can reach to enable the identification and even morphology characterization of nanoparticles down and below 10 nm Hydrogen bond and electrostatic interactions between the bioorganic capping molecules could be reason for the SEM image of the nanoparticles. The iron oxide nanoparticles were nonspherical shapes with rough surface (Rajendran *et al.,* 2017). In the past, researchers evaluated that the spherical nanoparticles can be obtained by green synthesis method. From EDAX spectrum, it is clear that Boswellia ovalifoliolata has recorded weight percent (39.88 %) of nanoparticle followed

Phytochemical evaluation is an efficient way to predict the plant drugs and discover various components present in different polar solvents. An extraction technique with superlative effectiveness with respect to time/yield ratio is fundamental to exactly quantify the phyto constituents (Farooq *et al.,* 2018). Preliminary phytochemical screening is helpful in the prediction of nature of medicines and also useful for the discovery of various constituents existing in solvents of diverse polarity.

The qualitative phytochemical study indicated the presence of compounds such as alkaloids, flavonoids, sterols and triterpenoids, polyphenols, glycosides, tannins, saponins, carbohydrates with maximum in the ethanol extract as compared to all other extracts. This was in agreement with the earlier studies. Flavonoids which are polyphenolic composites include

flavones, flavonols, flavanone and flavanonols. These complexes describe the majority of plant secondary metabolites and stated to possess notable health promotor effects such as anti-inflammatory, antioxidant, antimicrobial and anticancer properties (Pourmorad *et al.,* 2016). The present outcomes are almost similar to the results of Jain *et al.,* 2014 who reported the presence of tannins, phlobatannins, terpenoids and glycosides in the aqueous and ethanolic extracts of *S. grandiflora.*

Based on the results (i.e., the antimicrobial activity) the FeNPs of *S. grandiflora* flower exhibited varying degree of inhibitory activity against the growth both gram positive and gram negative bacteria tested. The mean inhibition zone for the tested bacteria ranged from (13.3 mm- 6 mm) indicating a remarkable antibacterial effect when compared with ampicillin the positive control, which ranged from 9.3mm – 6.3mm.

The infections caused by *E.coli,* Enterococcus faecalis and *Streptococcus pyogenes , can* be treated with ethanol extract as it exhibited the more inhibitory activity against the pathogen. Hence, it can be stated that *E.coli,* Enterococcus faecalis and *Streptococcus pyogenes* were susceptible to *S.grandiflora* flower whereas the remaining bacterial isolates were resistant and moderately susceptible.

Generally gram positive bacteria (*Staphylococcus aureus, Streptococcus*) were more sensitive to plant extracts because of the presence of a mesh-like peptidoglycan layer which is more accessible to permeation by the extracts (Tajkarimi *et al*., 2018). The resistance of the gram negative bacteria (*Klebseilla pneumonia, Escherichia coli*) could be attributed to its cell wall structure. Gram negative bacteria have a powerful permeability barrier, composed of a thin lipopolysaccharide exterior membrane, which could restrict the penetration of the extruding plant extract. It has been discussed earlier that gram negative bacteria are usually more resistant to the plant originated antimicrobials and even show no effect, compared to gram positive bacteria (Tajkarimi *et al*., 2018).

Natural antioxidants present in the nut extracts helped humans in the maintenance of health for thousands of years. “Antioxidant compounds like flavonoids, phenolic compounds and polyphenols scavenge free radicals. The antioxidants contain peroxide, hydro peroxide or lipid peroxyl and hence prevent the oxidative processes that lead to progressive ailments (Bouyahya *et al.,* 2020). The mechanism by which antioxidants play their protective role can be classified into

two types. First mechanism is referred to as hydrogen atom transfer, the free radical removes a hydrogen atom from the antioxidant, that itself becomes a radical. The second mechanism is based on electron transfer, the antioxidant can give an electron to the free radical and itself become a radical cation (Nayik, and Gull, 2020).

The present study showed a dose dependent scavenging power with maximum in the FeNP of *S.grandiflora*. The evaluation of DPPH scavenging activity is the most consistent assay for evaluating the antioxidant activity of the plant extracts. DPPH (di phenylpicryl hydrazine ($C_{18}H_{13}N_5O_6$, M=395.3) is a commercially available organic nitrogen radical. DPPH can only be dissolved in organic media; this condition becomes an important limitation when it comes to interpreting the role of hydrophilic antioxidants (Desmiaty *et al.,* 2018). The potential of the scavenging ability is 95.08percentage. This study, the DPPH radical scavenging assay reveals the proton contribution to the lone pair electron of the radicals. DPPH displays a strong absorption maximum at 517 nm. The color change takes places to yellow from purple that indicate the formation of DPPH by the absorption of hydrogen from the antioxidant present in the sample. This reaction is stoichiometric in relation to the number of hydrogen atoms occupied. Hence, the antioxidant effect can be easily assessed by following the reduction of UV absorption at 517 nm (Abu-Darwish *et al.,* 2018).The present study showed a dose dependent scavenging power with maximum in the ethanol extract. The potential of the scavenging ability has been mentioned in percentage. The efficacy increased with increase in concentration. In this study, the DPPH radical scavenging assay reveals the efficiency of the nut extracts in contributing a proton to the lone pair electron of the radicals.

The antioxidant potential of various extracts (ethanol, chloroform, acetone and aqueous) from nuts of *S. grandiflora* were determined by using DPPH assay. The result revealed that they were highly efficient in aqueous extract 70.38%. All the tested samples showed high level of scavenging activity. Among the extracts of the investigated parts of *S. grandiflora*, the ethanol extract showed remarkable scavenging activity, followed by chloroform, petroleum ether and water (Altemimi *et al.,* 2017). The chloroform extracts exhibited distinguishing activity than petroleum ether that displayed normal antioxidant activity. The water extracts showed minimum level of scavenging activity compared to other extracts. The antioxidant activity of compounds is mainly due to their redox properties, that play important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides, which may be related

to the high amount of flavonoid and phenolic compounds in this plant extract (Djiazet *et al.*, 2021). Based on the results of our study and other previous studies, the findings validated traditional medicinal values and suggest that *S. grandiflora* is potentially a good source of natural anti-oxidants (Li *et al.*, 2021).

Diabetes mellitus is a group of diseases that affect how your body uses blood sugar (glucose). Glucose is vital to your health because it's an important source of energy for the cells that make up your muscles and tissues (Delshad *et al.*, 2018). It's also your brain's main source of fuel. Diabetes mellitus is a metabolic disorder characterized by altered glucose and lipid metabolism leading to persistent hyperglycemia. High fat diets and oxidative damage may contribute to the development of diabetes mellitus which is associated with hyperglycemia, insulin resistance, dyslipidaemia, abdominal obesity, and fatty liver and is characterized by chronic polyuria, polydipsia, polyphagia, and weakness due to disturbance in carbohydrate, fat, and protein metabolism (Duan *et al.*, 2018).

Our earlier study revealed the total phenolic and flavonoid content and *in vitro* antioxidant potential of aqueous extract of *S.grandiflora* flower which may be responsible for potent hypoglycemic and hypolipidemic properties. Inhibition of carbohydrate hydrolyzing enzymes such as α- amylase and α-glucosides presents a way to lower postprandial glucose levels. Since these enzymes are responsible for breaking down oligosaccharides into the readily ingested monosaccharide's their inhibition decreases glucose absorption. Inhibitors of carbohydrate – hydrolyzing enzymes also lengthen the total carbohydrate digestion period (ueno *et al.*, 2015)

Inhibition of alpha amylase by iron nanoparticles of *S. grandiflora* flower were studied and showed a highest inhibition percent at 500 µg/ml and lowest at 100 µg/ml revealing a dose dependent effect. Bhusnure *et al.*, 2017 experimented about the neuroprotective effect of *S. grandiflora* (Linn.) on Streptozotocin induced diabetic neuropathy in rats. This study explained both the cold-water tail immersion test and hot-water tail immersion test in which plant extract improved the tail withdrawal latency. Hypoglycemic studies with *S. grandiflora* roots, leaves, flowers, and stems showed the lowering of blood glucose level notably by the leaves at 6 h, $P < 0.05$ Insulin-sensitive glucose transport gene (GLUT-2 and GLUT-4) are the major ones which facilitate the transport of glucose from blood to the cells through insulin. In the *S. grandiflora* treated animals, the level of this gene increases. The matters of glucose transport gene mRNA were reinstated to near normal values after treating with *S. grandiflora*. Consequently, the uptake of

glucose in liver was developed and thus helped to fight against the hyperglycemic condition (Al-Shaqha *et al.,* 2015). This could be defensible that the presence of some constituents (Alkaloids, Phenols, Saponins, and Terpenoids) in the extract were accountable for being effective inhibitors of α-amylase Hydro-alcoholic extract and four fractions of *A. caudatum* and *C. argentea* were evaluated for their inhibitory effect on a-amylase and a-glucosidase enzymes by in-vitro method. Among all, fraction 2 of *A.caudatum* and fraction 4 of *C. argentea* has shown best enzyme inhibitory activity with an IC_{50} value of 0.241 and 0.211 (a-amylase and a-glucosidase) and 0.294 and 0.249 mg/ml (a-amylase and a-glucosidase) which were comparable with that of acarbose.

Our results support its use as folklore medicine for the treatment of diabetes. Plants may act on blood glucose through different mechanisms, some of them may have insulin like substances and some may inhibit insulinase activity. The mechanism of alloxan induced diabetes has been the subject of many investigations and it is now generally accepted that free radicals are selectively involved in the initiation of the damage that ultimately leads to β cells death (Jais and Brüning, 2022). Therefore, the pancreas is especially susceptible to the action of alloxan induced free radical damage. Many substances have been shown to ameliorate the diabetogenicity of alloxan in animals, which Recently, it was reported that the *S. grandiflora* extract, exhibited significant radical scavenging activity and thus antioxidant activity and the present finding indicates that administration of *S. grandiflora* offers protection of vital tissues including the pancreas, thereby reducing the causation of diabetes in these animals (Luo *et al., 2022*). Therefore, protective effect of *S. grandiflora* flower extract on pancreas of alloxan induced diabetic rats could be attributed directly to scavenging activity and for more extent to the regenerative properties of the extract. The results of the present study indicated that 70% alcoholic extract of *S. grandiflora* flower exhibited dose-dependent antidiabetic activity against alloxan induced diabetes in rats. Thus justifies the traditional use of this plant in the treatment of diabetes mellitus. However, clinical studies should be performed to confirm the similar antidiabetic before put into therapy.

Subsequent studies in the late 1990s that specified that poor pharmacokinetics and toxicity were significant causes of high late-stage failures in drug improvement. It has become broadly valued that these areas should be considered as first as possible in the drug discovery process (Rad *et al.,* 2021). Lipinski's rule of five recognises numerous critical properties that should be considered for compounds for oral delivery. In this study, for *insilico* analysis, ligand optimization

was carried out. Total of 8 bioactive molecules obtained from literature analysis and were retrieved without any ambiguities from the Pubchem deposition (Harrad *et al.,* 2018). Among the selected 8 compounds 6 compounds such as epicatechin, catechin hydrate, salicylic acid, syringic acid, ellagic acid, and 4-hydroxybenzaldehyde obeyed the Lipinski rule of five.

In the present study, bioactivity score was noted for enzyme inhibitor, kinase inhibitor, GPCR ligand, ion channel modulator, nuclear receptor ligand, and protease inhibitor. The good bioactivity score is an indication of the excellent pharmacological activity *invivo* (Nath *et al.,* 2014). The bioactive scores for the compounds can be assumed as active (bioactivity score > 0), inactive (bioactivity score < -5.0) and moderately active (bioactivity score: -5.0-0.0). The present study identified that the epicatechin and catechin hydrate were highly active and moderately active compounds were salicylic acid, syringic acid, luteolin-7-o-glucoside, ellagic acid, nicotiflorin and 4-hydroxybenzaldehye. Our observation corroborates with the findings of Sytar *et al.,* 2018 who reported that boswellic acid and its derivatives exhibit good bioactivity score for drug targets including nuclear receptor ligand, protease inhibitor and enzyme inhibition and thus expected to have excellent pharmacological activity *invivo*. In another study, the antioxidant compounds present in *Aloevera* were screened in search of a new lead compound and found that dihydro coumarin ethyl ester showed good drug likeness score and bioactivity score, on comparison with other compounds and chosen as the best (Ahvazi *et al.*, 2018). In another study, (Valli and Geetha, 2015) made an *in silico* prediction of bioactivity of flavonoids present in *Erythrina varigata* and showed that methoxy phaseolludin exhibited the highest score towards GPCR (G-protein-coupled receptors) ligand, nuclear receptor ligand and enzyme inhibitor activities among the sixteen flavonoids analysed. Similarly molecular properties and bioactivity score of alkaloids present in *Erythrina varigata* leaves were calculated using molinspiration software. As per the comparative scores of fifteen alkaloids studied, alkaloid I exhibited the highest score towards GPCR ligand, exhibited ion channel modulator, kinase inhibitor and enzyme inhibitor activities (Valli and Jayalakshmi, 2015). Hence, the present study also concludes that the compounds such as catechin hydrate and epicatechin will have outstanding pharmacological activity as they had good bioactivity score for nuclear receptor ligand, protease inhibitor, GPCR ligand, ionchannel receptor and enzyme inhibition. Also ellagic acid will also expected to have active pharmacological properties as they have good bioactivity score for nuclear receptor ligand.

The bioactive compounds identified in the *S. grandiflora* flower extract have previously reported to possess many biological activities such as antioxidant, anticancer, anti-inflammatory, antimicrobial and anti-diabetic activity. Ellagic acid is the compound found abundantly in the flower *S. grandiflora*. It is the most extensively used oil in therapy, both internally and externally, being recommended for the treatment of acute and chronic gastritis and enteritis, in disorders of the respiratory tract, and for inflammation of the oral mucosa (Balakrishnan, 2015).

Molecular docking is a method used to predict the binding orientation of small molecular drug candidates to their protein targets in order to predict the affinity and activity of the small molecule (Maresch *et al.,* 2018). Recently docking ligands to receptors utilizing rational drug design is on the increase owing to few problems in the conventional methods of drug designing. Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking.

In the present investigation, eight bioactive components have been selected from extensive literature search and docked against the four different diabetic protein. The selected bioactive components were docked with each of the four diabetic protein Glucokinase (GK) 1V4S, 11ß hydroxysteroid dehydrogenase 1 (11β-HS1) 1BHS, C-reactive protein (CRP) 1GNH, and Protein tyrosine phosphatases (PTP) 2NT7. Molecular docking was performed to examine the complete intermolecular connections among the ligand and the target proteins. Out of the 32 complexes docked using autodock 4.2.8, 4 complexes such as1V4S-epicatechin complex, 1BHS-ellagic acid complex, 1BHS-catechin hydrate complex and 2NT7-catechin hydrate complex showed highest docking score of -5.23, -5.43, -5.08, and -5.01 kcal/mol correspondingly. Among the selected bioactive compounds ellagic acid was the potent drug candidate in comparison to all other compound where the docking score of -5.43 kcal/mol stands top with the diabetic protein 1BHS. The best scoring against the target proteins was mean to be with better agonistic effect leading to Anti-diabetic potency. Low docking energy indicates high binding ability. Based on this observation, it can concluded that ellagic acid can be considered as good drug candidate for anti-diabetic treatments.

The present study is in line with the study of Sreejaya and Santhy (2015) who tried to find out drug candidates for breast cancer from the methanolic extract of *Acorus calamus*. Analysis by

GC-MS of the extract revealed 14 compounds. These compounds were taken for docking studies using highly influential breast cancer proteins. Based on the results, ERBb1 protein was found to be a good target for breast cancer. Their study identified [(2R)-2-[(1S)- 1-hexadecanoyloxy-2-hydroxyethyl]-4-hydroxy-5-oxo-2H-furan-3-yl] as a ligand that act as a good inhibitor for breast cancer. Docking of 26 withaferin and 14 withanolides from *Withania somnifera* in to the PknG of *Mycobacterium tuberculosis* revealed that withanolide E, F and D and withaferin-diacetate 2 phenoxy ethyl carbonate were identified as potential inhibitors of PknG (Santhi and Aishwarya, 2011).

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Medicinal plants are only source and an important contribution for primary healthcare during ancient times. Knowledge about use of medicinal plants for treating various diseases was highly valued among ancient civilizations. Until the mid-nineteenth century, plants were the main therapeutic agents used by humans and still have an important role in medicinal preparations. About 80% of people in developing countries depend on traditional medicine for their primary health care needs, because of their low costs, effectiveness, frequently inadequate provision of modern medicine, cultural and religious preferences. 80% of people in India use non-allopathic (Ayurveda, Siddha, Unani and Homeopathy) herbal based medicines for their healthcare which are collected from wild and cultivated sources.

Diabetes is a syndrome, initially characterized by loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting in impaired metabolism of glucose and other energy-yielding fuels such as lipids and proteins. Diabetes is the most severe and challenging metabolic pandemic of the 21st century. This is because it affects essential biochemical activities in almost every cell in the body and increases the risk of cardiac and renal disorders. The worldwide survey reported that diabetes is affecting nearly 10% of the population. The global diabetes mellitus burden estimate was 246 million, and the International Diabetes Federation (IDF) estimates that this figure is likely to rise to 380 million by the year 2025.

In the present study, the UV-Visible spectra shows an absorption band at 300nm, 400nm, 500nm, 600nm, which corresponds to the absorbance of iron nano particles. Increased concentrations of ferrous sulphate resulted in a brown solution of nano iron indicating the completion of reaction.

The FT-IR spectrum profile showed the presence of amines, alkyl halides, alkanes, and aldehydes compounds which can be isolated and further screened for different kind of biological activities depending upon their therapeutic uses.

In XRD, the peaks were identified at 31.77°, 34.44°, 36.28°, 47.60°, 56.52°, 62.88°, and 67.96°. The X-ray diffraction pattern of iron nanoparticles which indicated that the synthesized iron nanoparticles were in crystalline phase.

SEM micrograph shows the high-density morphology of iron nanoparticles. The particles were observed to be granular, irregular and spherical in shape. The nano particles were seen without any aggregation and the overall size of nanoparticles was less than 70nm which could be effective for the drug delivery.

The elemental composition through EDX displayed the presence of a strong characteristic peak at 1keV, 6 keV and 7 keV, which is specific to Fe with additional weak signals.

Preliminary screening and qualitative phytochemical analysis of aqueous extract revealed the presence of alkaloids, sugars, flavonoids, tannins, phenols, saponins, glycosides, anthraquinone, sterols, and terpenoids were high in the synthesized nanoparticles.

The free radical scavenging action is known to be one of the important mechanisms for measuring antioxidant activity. The highest scavenging efficacy of FeNPs was 95.08%. The DPPH reducing activity of the FeNPs was measured based on color change which was shown due to the reduction reaction. The high percentage of FeNPs of S. grandiflora flower was recorded at the concentrations of 10µl and 50µl with the inhibition percentage of 95.08% and 85.25%.

The assessment of antibacterial activity revealed that the synthesized FeNPs exhibited the zone of inhibition in *Escherichia coli* (13.3 mm), *Seratia marcescens* (12 mm), *Staphylococcus aureus* (12 mm), *Streptococcus pyogenes* (11.6 mm) and *Klebseilla pneumonia* (6 mm).when compared to the standard ampicillin *Escherichia coli* (8 mm), *Seratia marcescens* (9 mm), *Staphylococcus aureus* (9.3 mm), *Streptococcus pyogenes* (6.3 mm) and *Klebseilla pneumonia* (7.3 mm).

The alpha amylase inhibitor activity showed that the bio synthesized iron nano particles and Acarbose indicated a highest inhibition percent at 500 µg/ml and lowest at 100 µg/ml revealing a dose dependent effect. The biosynthesized nanoparticle solution showed the highest absorbance of 43.21 ± 0.63 at 500 µg/ml which correlates almost to the standard acarbose which showed the highest absorbance of 46.12 ± 0.43 at 500 µg/ml.

The present study aimed to find the drug targets for diabetes mellitus from *S. grandiflora* flower. Ligands identified from the literature survey were subsequently taken in for docking studies with highly influential diabetes mellitus protein such as Glucokinase (GK) 1V4S, 11ß hydroxysteroid dehydrogenase 1 (11β-HS1) 1BHS, C-reactive protein (CRP) 1GNH, and Protein tyrosine phosphatases (PTP) 2NT7. Out of various complexes docked using Autodock 4.2.8, 1BHS-ellagic acid complex showed high binding energy with six H-bond interaction.

Altogether this study throws light on the possibility to recommend iron nano particles of *S. grandiflora* flower as a natural remedy which have anti-diabetic, anti-oxidant and anti-bacterial activity. Ellagic acid is the compound found abundantly in the iron nano particle of *S. grandiflora* flower and 1BHS-ellagic acid complex showed high binding energy. Further *invitro* and *invivo* investigations will be helpful to develop natural remedy from iron nano particles of S. *grandiflora* flower for the treatment of diabetes.

BIBLIOGRAPHY

BIBLIOGRAPHY

Agarwal, H., Nakara, A., Menon, S., &Shanmugam, V. (2019). Eco-friendly synthesis of zinc oxide nanoparticles using CinnamomumTamala leaf extract and its promising effect towards the antibacterial activity. *Journal of Drug Delivery Science and Technology*, *53*, 101212.

Ahvazi, M., Khalighi-Sigaroodi, F., Charkhchiyan, M. M., Mojab, F., Mozaffarian, V. A., &Zakeri, H. (2018). Introduction of medicinal plants species with the most traditional usage in Alamut region. *Iranian journal of pharmaceutical research: IJPR*, *11*(1), 185.

Ajitha, B., Reddy, Y. A. K., Rajesh, K. M., & Reddy, P. S. (2016). Sesbaniagrandiflora leaf extract assisted green synthesis of silver nanoparticles: Antimicrobial activity. *Materials Today: Proceedings*, *3*(6), 1977-1984.

Anusmitha, K. M., Aruna, M., Job, J. T., Narayanankutty, A., Benil, P. B., Rajagopal, R., ...&Barcelo, D. (2022). Phytochemical analysis, antioxidant, anti-inflammatory, anti-genotoxic, and anticancer activities of different Ocimum plant extracts prepared by ultrasound-assisted method. *Physiological and Molecular Plant Pathology*, *117*, 101746.

Arji, G., Safdari, R., Rezaeizadeh, H., Abbassian, A., Mokhtaran, M., &Ayati, M. H. (2019). A systematic literature review and classification of knowledge discovery in traditional medicine. *Computer methods and programs in biomedicine*, *168*, 39-57.

Baessa, M., Rodrigues, M. J., Pereira, C., Santos, T., da Rosa Neng, N., Nogueira, J. M. F., ...&Custódio, L. (2019). A comparative study of the in vitro enzyme inhibitory and antioxidant activities of Buteamonosperma (Lam.) Taub. andSesbaniagrandiflora (L.) Poiret from Pakistan: New sources of natural products for public health problems. *South African Journal of Botany*, *120*, 146-156.

Bag, S., Mondal, A., Majumder, A. and Banik, A., 2022. Tea and its phytochemicals: Hidden health benefits & modulation of signaling cascade by phytochemicals. *Food Chemistry*, *371*, p.131098.

Banik, K., Ranaware, A. M., Deshpande, V., Nalawade, S. P., Padmavathi, G., Bordoloi, D., ...&Kunnumakkara, A. B. (2019). Honokiol for cancer therapeutics: A traditional medicine that can modulate multiple oncogenic targets. *Pharmacological research*, *144*, 192-20.

Behravan, M., Panahi, A. H., Naghizadeh, A., Ziaee, M., Mahdavi, R., &Mirzapour, A. (2019). Facile green synthesis of silver nanoparticles using Berberis vulgaris leaf and root aqueous extract and its antibacterial activity. *International journal of biological macromolecules*, *124*, 148-154.

Belayneh, Y. M., &Birru, E. M. (2018). Antidiabetic activities of hydromethanolic leaf extract of calpurniaaurea (ait.) benth. Subspecies aurea (fabaceae) in mice. *Evidence-based complementary and alternative medicine*, *2018*.

Bindhu, M. R., Umadevi, M., Esmail, G. A., Al-Dhabi, N. A., &Arasu, M. V. (2020). Green synthesis and characterization of silver nanoparticles from Moringaoleifera flower and assessment of antimicrobial and sensing properties. *Journal of Photochemistry and Photobiology B: Biology*, *205*, 111836.

Bocquet, L., Rivière, C., Dermont, C., Samaillie, J., Hilbert, J. L., Halama, P., ...&Sahpaz, S. (2018). Antifungal activity of hop extracts and compounds against the wheat pathogen Zymoseptoriatritici. *Industrial Crops and Products*, *122*, 290-297.

Bouyahya, A., Lagrouh, F., El Omari, N., Bourais, I., El Jemli, M., Marmouzi, I., ...&Bakri, Y. (2020). Essential oils of Menthaviridis rich phenolic compounds show important antioxidant, antidiabetic, dermatoprotective, antidermatophyte and antibacterial properties. *Biocatalysis and agricultural biotechnology*, *23*, 101471.

Chaudhary, P., Sharma, A., Singh, B., &Nagpal, A. K. (2018). Bioactivities of phytochemicals present in tomato. *Journal of food science and technology*, *55*(8), 2833-2849.

Dange, S., Jadhav, R., &Vikhe, S. (2022). Phytochemical and Pharmacological Review of Sesbaniagrandiflora. *Asian Journal of Pharmacy and Technology*, *12*(1), 20-24.

Dar, R. A., Shahnawaz, M., &Qazi, P. H. (2017). General overview of medicinal plants: A review. *The Journal of Phytopharmacology*, *6*(6), 349-351Dar, R. A., Shahnawaz, M., &Qazi, P. H. (2017). General overview of medicinal plants: A review. *The Journal of Phytopharmacology*, *6*(6), 349-351.

Dehghan, H., Sarrafi, Y., &Salehi, P. (2016). Antioxidant and antidiabetic activities of 11 herbal plants from Hyrcania region, Iran. *Journal of food and drug analysis*, *24*(1), 179-188.

Delshad, E., Yousefi, M., Sasannezhad, P., Rakhshandeh, H., &Ayati, Z. (2018). Medical uses of Carthamustinctorius L.(safflower): a comprehensive review from traditional medicine to modern medicine. *Electronic physician*, *10*(4), 6672.

Duan, Y., Liu, T., Zhou, Y., Dou, T., & Yang, Q. (2018). Glycoside hydrolase family 18 and 20 enzymes are novel targets of the traditional medicine berberine. *Journal of Biological Chemistry*, *293*(40), 15429-15438.

Egbuna, C., Mishra, A. P., &Goyal, M. R. (Eds.). (2020). *Preparation of phytopharmaceuticals for the management of disorders: The development of nutraceuticals and traditional medicine*. Academic Press.

El Harrad, L., Bourais, I., Mohammadi, H., & Amine, A. (2018). Recent advances in electrochemical biosensors based on enzyme inhibition for clinical and pharmaceutical applications. *Sensors*, *18*(1), 16.

Ettadili, F. E., Aghris, S., Laghrib, F., Farahi, A., Saqrane, S., Bakasse, M., ...& El Mhammedi, M. A. (2022). Recent advances in the nanoparticles synthesis using plant extract: Applications and future recommendations. *Journal of Molecular Structure*, *1248*, 131538.

Farag, R. S., Abdel-Latif, M. S., Abd El Baky, H. H., &Tawfeek, L. S. (2020). Phytochemical screening and antioxidant activity of some medicinal plants' crude juices. *Biotechnology Reports*, *28*, e00536.

Farooq, M., Ilyas, N., Khan, I., Saboor, A., Khan, S., &Bakhtiar, M. (2018). Antifungal activity of plant extracts and silver nano particles against citrus brown spot pathogen (Alternariacitri). *IJOEAR*, *4*, 118-125.

Guan, R., Van Le, Q., Yang, H., Zhang, D., Gu, H., Yang, Y., ...&Peng, W. (2021). A review of dietary phytochemicals and their relation to oxidative stress and human diseases. *Chemosphere*, *271*, 129499.

Gupta, A., Mahajan, S., & Sharma, R. (2015). Evaluation of antimicrobial activity of Curcuma longa rhizome extract against Staphylococcus aureus. *Biotechnology reports*, *6*, 51-55.

Hemmati, S., Rashtiani, A., Zangeneh, M. M., Mohammadi, P., Zangeneh, A., &Veisi, H. (2019). Green synthesis and characterization of silver nanoparticles using Fritillaria flower extract and their antibacterial activity against some human pathogens. *Polyhedron*, *158*, 8-14.

Hernández-Hernández, A. A., Aguirre-Álvarez, G., Cariño-Cortés, R., Mendoza-Huizar, L. H., & Jiménez-Alvarado, R. (2020). Iron oxide nanoparticles: synthesis, functionalization, and applications in diagnosis and treatment of cancer. *Chemical Papers*, *74*(11), 3809-3824.

https://www.3ds.com/products-services/BIOVIA/products/molecular-modeling-simulation/BIOVIA-discovery-studio/visualization.

Imran Din, M., & Rani, A. (2016). Recent advances in the synthesis and stabilization of nickel and nickel oxide nanoparticles: a green adeptness. *International journal of analytical chemistry*, *2016*.

Jais, A. and Brüning, J.C., 2022. Arcuate Nucleus-Dependent Regulation of Metabolism—Pathways to Obesity and Diabetes Mellitus. *Endocrine reviews*, *43*(2), pp.314-328.

Jaji, N. D., Lee, H. L., Hussin, M. H., Akil, H. M., Zakaria, M. R., & Othman, M. B. H. (2020). Advanced nickel nanoparticles technology: From synthesis to applications. *Nanotechnology Reviews*, *9*(1), 1456-1480.

Jamdagni, P., Khatri, P., &Rana, J. S. (2018). Green synthesis of zinc oxide nanoparticles using flower extract of Nyctanthes arbor-tristis and their antifungal activity. *Journal of King Saud University-Science*, *30*(2), 168-175.

Jamshidi-Kia, F., Lorigooini, Z., &Amini-Khoei, H. (2018). Medicinal plants: Past history and future perspective. *Journal of herbmed pharmacology*, *7*(1).

Javid, T., Adnan, M., Tariq, A., Akhtar, B., Ullah, R., &Abd El Salam, N. M. (2015). Antimicrobial activity of three medicinal plants (Artemisia indica, Medicago falcate and Tecomastans). *African Journal of Traditional, Complementary and Alternative Medicines*, *12*(3), 91-96.

Kabesh, K., Senthilkumar, P., Ragunathan, R., & Kumar, R. R. (2015). Phytochemical analysis of Catharanthusroseus plant extract and its antimicrobial activity. *Int. J. Pure App. Biosci*, *3*(2), 162-172.

Kale, I., Khan, M. A., Irfan, Y., &Veerana, G. A. (2012). Hepatoprotective potential of ethanolic and aqueous extract of flowers of Sesbaniagrandiflora (Linn) induced by CCl4. *Asian Pacific Journal of Tropical Biomedicine*, *2*(2), S670-S679.

KamarulZaman, M. A., Azzeme, A. M., Ramle, I. K., Normanshah, N., Ramli, S. N., Shaharuddin, N. A., ...& Abdullah, S. N. A. (2020). Induction, multiplication, and evaluation of antioxidant activity of Polyalthiabullata callus, a woody medicinal plant. *Plants*, *9*(12), 1772.

Karunamoorthi, K., Jegajeevanram, K., Xavier, J., Vijayalakshmi, J., &Melita, L. (2018). Tamil traditional medicinal system-siddha: an indigenous health practice in the international perspectives. *CELLMED*, *2*(2), 12-1.

Khare, C. P. (2018). *Indian medicinal plants: an illustrated dictionary*. Springer Science & Business Media.

Kobayashi, M., Akaki, J., Yamaguchi, Y., Yamasaki, H., Ninomiya, K., Pongpiriyadacha, Y., ...&Morikawa, T. (2019). Salaciachinensis stem extract and its thiosugarsulfonium constituent, neokotalanol, improves HbA1c levels in ob/ob mice. *Journal of natural medicines*, *73*(3), 584-588.

Kothari, S., Thangavelu, L., & Roy, A. (2017). Anti-diabetic activity of Sesbaniagrandiflora-alpha amylase inhibitory effect. *Journal of Advanced Pharmacy Education & Research*| *Oct-Dec*, *7*(4).

Król, A., Pomastowski, P., Rafińska, K., Railean-Plugaru, V., &Buszewski, B. (2017). Zinc oxide nanoparticles: Synthesis, antiseptic activity and toxicity mechanism. *Advances in colloid and interface science*, *249*, 37-52.

Kumar, N. S., &Dhanyaraj, F. S. (2016). Phytochemical analysis and antimicrobial activities of Sesbaniagrandiflora (L) leaf extracts. *Int. J. Pharm. Sci. Rev. Res*, *36*, 144-148.

Kumar, R., Janadri, S., Kumar, S., &Swamy, S. (2015). Evaluation of antidiabetic activity of alcoholic extract of flower Sesbaniagrandiflora in alloxan induced diabetic rats. *Asian Journal of Pharmacy and Pharmacology*, *1*(1), 21-26.

Kuruppu, A. I., Paranagama, P., &Goonasekara, C. L. (2019). Medicinal plants commonly used against cancer in traditional medicine formulae in Sri Lanka. *Saudi Pharmaceutical Journal*, *27*(4), 565-573.

Laladhas, K. P., Cheriyan, V. T., Puliappadamba, V. T., Bava, S. V., Unnithan, R. G., Vijayammal, P. L., &Anto, R. J. (2017). A novel protein fraction from Sesbaniagrandiflora shows potential anticancer and chemopreventive efficacy, in vitro and in vivo. *Journal of cellular and molecular medicine*, *14*(3), 636-646.

Li, Y., Kong, D., Fu, Y., Sussman, M. R., & Wu, H. (2020). The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant Physiology and Biochemistry*, *148*, 80-89.

Luo, A., Xie, Z., Wang, Y., Wang, X., Li, S., Yan, J., Zhan, G., Zhou, Z., Zhao, Y. and Li, S., 2022. Type 2 Diabetes Mellitus-associated Cognitive Dysfunction: Advances in Potential Mechanisms and Therapies. *Neuroscience & Biobehavioral Reviews*, p.104642.

Mahamat, O. B., Younes, S., Otchom, B. B., Franzel, S., Mahamat, A. D. O., &Fadoua, A. (2021). Ecology, Morphology, Distribution, and Use of Sesbaniatchadica (SesbaniaSesban) from the Republic of Chad: A Review.

Maresch, C. C., Stute, D. C., Alves, M. G., Oliveira, P. F., de Kretser, D. M., & Linn, T. (2018). Diabetes-induced hyperglycemia impairs male reproductive function: a systematic review. *Human Reproduction Update*, *24*(1), 86-105.

Mathina, M., Shinyjoy, E., Kavitha, L., &Gopi, D. (2021). A preliminary study on the synthesis of biogenic derived hydroxyapatite/medicinal plant extracts composite for potential bone tissue engineering applications. *Materials Today: Proceedings*.

Mazzei, L., Cianci, M., Vara, A. G., &Ciurli, S. (2018). The structure of urease inactivated by Ag (I): a new paradigm for enzyme inhibition by heavy metals. *Dalton transactions*, *47*(25), 8240-8247.

Melkamu, W. W., &Bitew, L. T. (2021). Green synthesis of silver nanoparticles using Hageniaabyssinica (Bruce) JF Gmel plant leaf extract and their antibacterial and anti-oxidant activities. *Heliyon*, *7*(11), e08459.

Mirzaie, A., Halaji, M., Dehkordi, F. S., Ranjbar, R., &Noorbazargan, H. (2020). A narrative literature review on traditional medicine options for treatment of corona virus disease 2019 (COVID-19). *Complementary therapies in clinical practice*, *40*, 101214.

Mishra, A. (2019). Traditional methods of food habits and dietary preparations in Ayurveda—the Indian system of medicine. *Journal of Ethnic foods*, *6*(1), 1-9.

Mitharwal, S., Kumar, A., Chauhan, K. and Taneja, N.K., 2022. Nutritional, phytochemical composition and potential health benefits of taro (Colocasia esculenta L.) leaves: A review. *Food Chemistry*, *383*, p.132406.

Mitra, S. K., Prakash, N. S., &Sundaram, R. (2012). Shatavarins (containing Shatavarin IV) with anticancer activity from the roots of Asparagus racemosus. *Indian journal of pharmacology*, *44*(6), 732.

Morikawa, T., Akaki, J., Ninomiya, K., Kinouchi, E., Tanabe, G., Pongpiriyadacha, Y. & Muraoka, O. (2015). Salacinol and related analogs: new leads for type 2 diabetes therapeutic candidates from the Thai traditional natural medicine Salaciachinensis. *Nutrients*, *7*(3), 1480-1493.

Mukherjee, A., & Bhattacharyya, S. (2020). Nanotechnology in medicine. In *Biotechnology Business-Concept to Delivery* (pp. 57-64). Springer, Cham.

Mukherjee, P. K. (2019). *Quality control and evaluation of herbal drugs: Evaluating natural products and traditional medicine*. Elsevier.

Mwamatope, B., Tembo, D., Chikowe, I., Kampira, E., &Nyirenda, C. (2020). Total phenolic contents and antioxidant activity of Sennasingueana, Meliaazedarach, Moringaoleifera and Lannea discolor herbal plants. *Scientific African*, *9*, e00481.

Nahed, A., Abd El-Hack, M.E., Albaqami, N.M., Khafaga, A.F., Taha, A.E., Swelum, A.A., El-Saadony, M.T., Salem, H.M., El-Tahan, A.M., AbuQamar, S.F. and El-Tarabily, K.A., 2022. Phytochemical control of poultry coccidiosis: a review. *Poultry Science*, *101*(1), p.101542.

Nikolova, M., Slavchov, R., &Nikolova, G. (2020). Nanotechnology in medicine. *Drug discovery and evaluation: methods in clinical pharmacology*, 533-546.

Omara, T., Kiprop, A. K., Ramkat, R. C., Cherutoi, J., Kagoya, S., MoraaNyangena, D., ...&ChepkemoiKoske, M. (2020). Medicinal plants used in traditional management of cancer in Uganda: a review of ethnobotanical surveys, phytochemistry, and anticancer studies. *Evidence-Based Complementary and Alternative Medicine*, *2020*.

Packer, J., Naz, T., Harrington, D., Jamie, J. F., &Vemulpad, S. R. (2015). Antimicrobial activity of customary medicinal plants of the Yaegl Aboriginal community of northern New South Wales, Australia: a preliminary study. *BMC research notes*, *8*(1), 1-7.

Pirtarighat, S., Ghannadnia, M., &Baghshahi, S. (2019). Green synthesis of silver nanoparticles using the plant extract of Salvia spinosa grown in vitro and their antibacterial activity assessment. *Journal of Nanostructure in Chemistry*, *9*(1), 1-9.

Rad, M. K., Ghani, A., &Ghani, E. (2021). In vitro effects of Capparisspinosa L. extract on human sperm function, DNA fragmentation, and oxidative stress. *Journal of Ethnopharmacology*, *269*, 113702.

Ragupathi, C., Vijaya, J. J., & Kennedy, L. J. (2017). Preparation, characterization and catalytic properties of nickel aluminate nanoparticles: A comparison between conventional and microwave method. *Journal of Saudi Chemical Society*, *21*, S231-S239.

Rajendiran, D. E. E. P. A., Packirisamy, S. U. B. B. U. L. A. K. S. H. M. I., &Gunasekaran, K. R. I. S. H. N. A. M. O. O. R. T. H. Y. (2018). A review on role of antioxidants in diabetes. *Asian journal of pharmaceutical and clinical research*, *11*(2), 48-53.

Rajendran, S. P., &Sengodan, K. (2017). Synthesis and characterization of zinc oxide and iron oxide nanoparticles using Sesbaniagrandiflora leaf extract as reducing agent. *Journal of Nanoscience*, *2017*.

Rani, M., Bhattacharjee, A., Singh, P., Basu, S., Das, K., Goswami, K., ...& Kumar, A. (2022). Antimicrobial activities of different nanoparticles concerning to wastewater treatment. In *Development in Wastewater Treatment Research and Processes* (pp. 501-514). Elsevier.

Rheima, A., Anber, A. A., Shakir, A., Salah Hammed, A., & Hameed, S. (2020). Novel method to synthesis nickel oxide nanoparticles for antibacterial activity. *Iranian Journal of Physics Research*, *20*(3), 51-55.

Rioux, J., &Howerter, A. (2019). Outcomes from a whole-systems ayurvedic medicine and yoga therapy treatment for obesity pilot study. *The Journal of Alternative and Complementary Medicine*, *25*(S1), S124-S137.

Roy, A., &Bharadvaja, N. (2017). Medicinal plants in the management of cancer: a review. *Int J Complement Alt Med*, *9*(2), 00291.

Roy, A., Bhoumik, D., Sahu, R.K. and Dwivedi, J., 2014. Phytochemical screening and antioxidant activity of *Sesbania grandiflora* leaves extracts. *Asian Journal of Research in Pharmaceutical Science*, *4*(1), pp.16-21.

Roy, A., Jauhari, N., &Bharadvaja, N. (2018). 6 Medicinal plants as. *Anticancer Plants*, *2*, 109.

Sahoo, R. K., Tamuli, K. J., Narzary, B., Bordoloi, M., Sharma, H. K., Gogoi, K., & Bhattacharyya, D. R. (2020). Clerodendrumviscosum Vent leaf extract supported nanosilver particles: Characterization, antiplasmodial and anticancer activity. *Chemical Physics Letters*, *738*, 136893.

Sahu, M., Kumar, V., & Joshi, V. (2021). Indian medicinal plants with antidiabetic potential: An overview. *Research Journal of Pharmacy and Technology*, *14*(4), 2328-2335.

Saiganesh, S., Krishnan, T., Narasimha, G., Almoallim, H. S., Alhari, S. A., Reddy, L. V., ... &Prabhakar, V. S. V. (2021). Phytosynthetic Fabrication of Lanthanum Ion-Doped Nickel Oxide Nanoparticles Using Sesbaniagrandiflora Leaf Extract and Their Anti-Microbial Properties. Crystals 2021, 11, 124.

Saiganesh, S., Muni Sudhakar, B., Thyagarajan, K., &Narasimha, G. (2021). Structural, Optical and Antibacterial Properties of neodymium (Nd3+) doped nickel oxide (NiO) Nanoparticles using Sesbaniagrandiflora Leaf Extract. *Indian Journal of Science and Technology*, *14*(18), 1442-1451.

Santin, J. R., Lemos, M., Júnior, L. C. K., Niero, R., & de Andrade, S. F. (2010). Antiulcer effects of Achyroclinesatureoides (Lam.) DC (Asteraceae)(Marcela), a folk medicine plant, in different experimental models. *Journal of Ethnopharmacology*, *130*(2), 334-339.

Saso, L., &Firuzi, O. (2014). Pharmacological applications of antioxidants: lights and shadows. *Current Drug Targets*, *15*(13), 1177-1199.

Segun, P. A., Ogbole, O. O., &Ajaiyeoba, E. O. (2018). Medicinal plants used in the management of cancer among the Ijebus of Southwestern Nigeria. *Journal of herbal medicine*, *14*, 68-75.

Sekhon-Loodu, S., &Rupasinghe, H. P. (2019). Evaluation of antioxidant, antidiabetic and antiobesity potential of selected traditional medicinal plants. *Frontiers in nutrition*, *6*, 53.

Shao, H., Laxy, M., Benoit, S. R., Cheng, Y. J., Gregg, E. W., & Zhang, P. (2021). Trends in total and out-of-pocket payments for noninsulin glucose-lowering drugs among US adults with large-employer private health insurance from 2005 to 2018. *Diabetes Care*, *44*(4), 925-934.

Sreelatha, S., Padma, P. R., &Umasankari, E. (2011). Evaluation of anticancer activity of ethanol extract of Sesbaniagrandiflora (AgatiSesban) against Ehrlich ascites carcinoma in Swiss albino mice. *Journal of ethnopharmacology*, *134*(3), 984-987.

Srinivasan, M., Venkatesan, M., Arumugam, V., Natesan, G., Saravanan, N., Murugesan, S., ...&Pugazhendhi, A. (2019). Green synthesis and characterization of titanium dioxide nanoparticles (TiO2 NPs) using Sesbaniagrandiflora and evaluation of toxicity in zebrafish embryos. *Process Biochemistry*, *80*, 197-202.

Stith, S. S., Diviant, J. P., Brockelman, F., Keeling, K., Hall, B., Lucern, S., & Vigil, J. M. (2020). Alleviative effects of Cannabis flower on migraine and headache. *Journal of Integrative Medicine*, *18*(5), 416-424.

Sytar, O., Hemmerich, I., Zivcak, M., Rauh, C., &Brestic, M. (2018). Comparative analysis of bioactive phenolic compounds composition from 26 medicinal plants. *Saudi journal of biological sciences*, *25*(4), 631-641.

Tao, H., Wu, T., Aldeghi, M., Wu, T. C., Aspuru-Guzik, A., &Kumacheva, E. (2021). Nanoparticle synthesis assisted by machine learning. *Nature Reviews Materials*, *6*(8), 701-716.

Tjahjandarie, T. S., Tanjung, M., Saputri, R. D., Rahayu, D. O., Gunawan, A. N. I., &Aldin, M. F. (2021). Two new 2-arylbenzofurans from Sesbaniagrandiflora L. and their cytotoxicity towards cancer cell. *Natural Product Research*, *35*(24), 5637-5642.

Tungmunnithum, D., Thongboonyou, A., Pholboon, A., &Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines*, *5*(3), 93.

Ullah, R., Alqahtani, A. S., Noman, O. M., Alqahtani, A. M., Ibenmoussa, S., &Bourhia, M. (2020). A review on ethno-medicinal plants used in traditional medicine in the Kingdom of Saudi Arabia. *Saudi Journal of Biological Sciences*, *27*(10), 2706-2718.

Vinay, N. S., Babitha, S., Nandeesh, R., Paramesh, S., Manjunath, E., Geetha, J. P., ...&Prasanth, H. V. (2021). Evaluation of acute oral toxicity of methanolic fractions of Sesbaniagrandiflora Linn. roots in albino mice. *Thai Journal of Pharmaceutical Sciences*, *45*(2).

Vinothini, K., Devi, M. S., Shalini, V., Sekar, S., Semwal, R. B., Arjun, P., &Semwal, D. K. (2017). In vitro micropropagation, total phenolic content and comparative antioxidant activity of different extracts of Sesbaniagrandiflora (L.) Pers. *Current Science*, 1142-1147.

Yang, F., Ding, F., Chen, H., He, M., Zhu, S., Ma, X., ...& Li, H. (2018). DNA barcoding for the identification and authentication of animal species in traditional medicine. *Evidence-Based Complementary and Alternative Medicine*, *2018*.

APPENDIX

Annexure – I

Molecular docking

Autodock was used to perform docking of each selected ligand with the target protein. The integration of position and orientation of a ligand comparative to the receptor, as well as its conformation in flexible docking, is denoted as a ligand pose. Receptor ligand docking jobs required a set of previously calculated grid box. Preparation of the ligands before docking is strongly recommended.

Preparing PDBQT format for Target and ligand (Target.pdbqt, Ligand.pdbqt), Grid and Docking Parameter file (a.gpf and a.dpf) using AutoDock 4.2

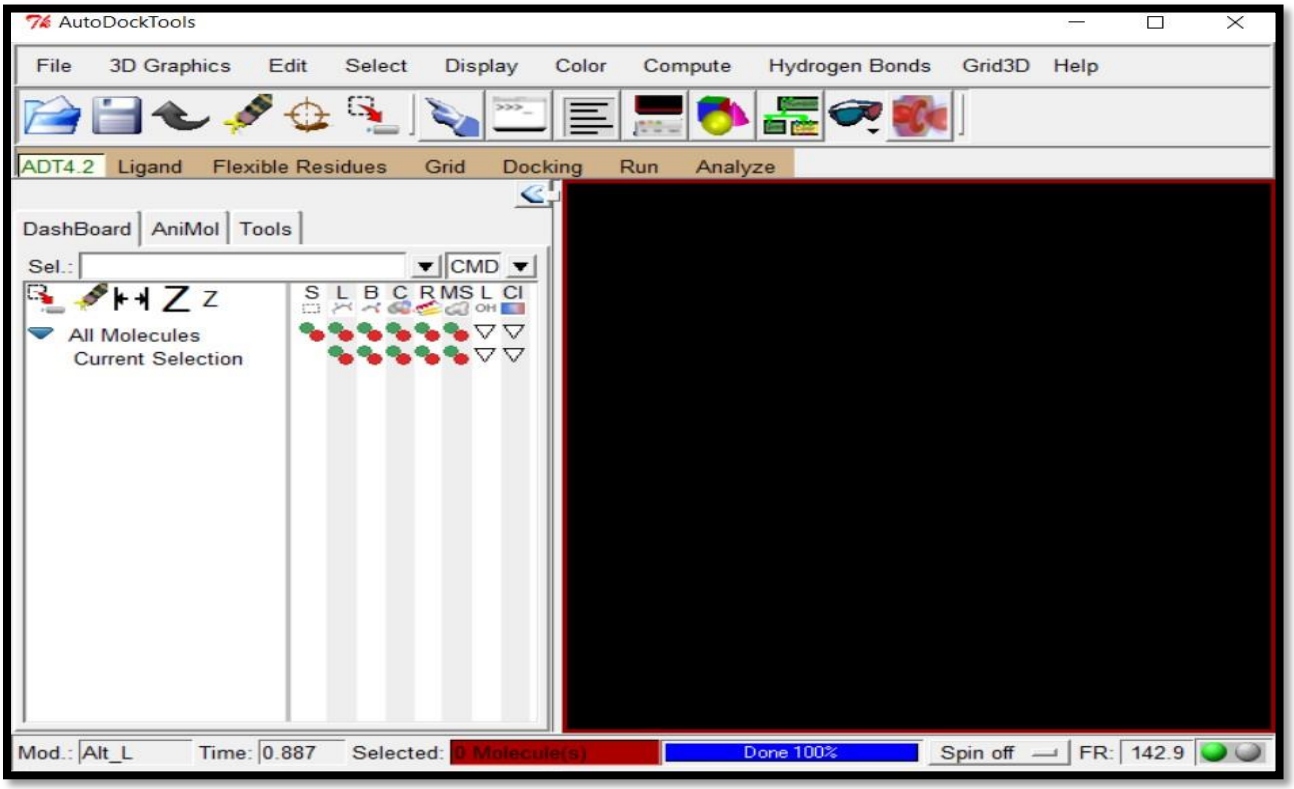


Fig.18. Autodock Graphical User Interface

- Open AutoDock present on desktop

(*Created after successful installation of MGL Tools)

- Select AutoDock 4.2
- Dismiss

The preparation of a protein involves a number of steps, which are outlined below.

Preparation of Target.pdbqt file

- Open File
- Read Molecule
- Select and Open Target.pdb
- Target molecule will appear on screen
- Click on Edit
- Click on Hydrogens
- Click on Add
- Click Polar Only
- Click OK
- Again Edit Click Charges
- Add Kollman Charges
- Click OK
- Open Grid
- Click on Macromolecules
- Click on Choose
- Click Target
- Click Select Molecule
- Click OK

(*Then save the target.pdbqt in the respective workspace)

The preparation of a ligand involves a number of steps, which are outlined below.

Preparation of Ligand.pdbqt file

- Open Ligand
- Click Input
- Click Open
- Change format from .pdbqt to .pdb in appeared dialogue box
- Select Ligand Click Open
- Click OK
- Again Open Ligand
- Click Torsion Tree
- Click Detect Root
- Again Open Ligand
- Click Output
- Click Save as PDBQT
- Save Ligand filei n the same folder and in same way as Target.pdbqt file

Preparation of Grid Parameter File (a.gpf)

- Open Grid
- Click Set Map Types
- Click Choose Ligand
- Click Ligand
- Click Select Ligand
- Again Open Grid Click Grid Box

(*We have used X,Y,Z dimension as 60x60x60. Further X,Y,Zcenter (Center Grid Box) can be changed according to the requirements.

- Click File
- Click Close saving current
- Again Open Grid
- Click Output
- Click Save GPF
- Name the File name as a.gpf
- Save a.gpf file (.gpf format) in the same file where Target and Ligand .pdbqt files were saved)

- Now click Run
- Click Run Autogrid
- Select the Programme pathname from the workspace that is autogrid application file.
- And select the Parameter filename that is .gpf file from the workspace
- Click launch and wait until .glg file is generated.

Preparation of Docking Parameter File (a.dpf)

- Open Docking
- Click Macromolecules
- Click Set Rigid Filename from the respective workspace
- Select Target.pdbqt
- Click Open
- Again Docking Click Ligand
- Click Choose
- Click Ligand
- Click Select Ligand
- Click Accept
- Again Docking
- Click Search Parameters
- Click Genetic Algorithm
- Click Accept (*Using Default but we can change no. of GA runs)
- Again Docking
- Click Docking parameters
- Click Accept (*Using Default)
- Again docking
- Click Output
- Click LamarkianGA(4.2)
- Name the File name as a.dpf
- Save a.dpf file (.dpf format) in the same file where Target and Ligand .pdbqtand a.gpf files were saved
- Now click Run

- Click Run Autodock
- Select the Programme pathname from the workspace that autodock application file.
- And select the Parameter filename that is .dpf file from the workspace
- Click launch and wait until .dlg file is generated.

Analyzing Results

- Open AutoDock
- Click Analyze
- Click Docking
- Click Open
- Select a.dlg
- Click Open
- Click OK
- Again Analyze
- Click Conformations
- Click Play
- Click &
- Click show information
- Click write and built complex and save in .pdbqt format.

APPENDIX

भारतसरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA

दक्षिणीक्षेत्रीयकेन्द्र / Southern Regional Centre
टी.एन.ए.यूकैम्पस/ T.N.A.U. Campus
लाउलीरोड/ Lawley Road
कोयंबत्तूर/ Coimbatore - 641 003

टेलीफोन / Phone: 0422-2432788, 2432123
टेलीफक्स/ Telefax: 0422- 2432835
ई-मैल/E-mail id: sc@bsi.gov.in
bsisc@rediffmail.com

सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2022/Tech. /755

दिनांक/Date: 12th May 2022

पौधे प्रमाणीकरण प्रमाणपत्र / PLANT AUTHENTICATION CERTIFICATE

The plant specimen brought by you for authentication is identified as *Sesbania grandiflora* (L.) Poir. - FABACEAE. The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

12/5/2022

डॉ. एम. यु. शरीफ़/DR. M. U. SHARIEF
वैज्ञानिक 'ई' एवं कार्यालयाध्यक्ष/
SCIENTIST 'E' & HEAD OF OFFICE
वैज्ञानिक 'ई' एवं कार्यालय अध्यक्ष
SCIENTIST 'E' & HEAD OF OFFICE
भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA
दक्षिणी क्षेत्रीय केन्द्र
SOUTHERN REGIONAL CENTRE
कोयम्बत्तूर / COIMBATORE - 641 003

सेवा में / To

Ms. THARANI M
II M.Sc. Student
Department of Zoology
Avinashilingam Institute for Home Science &
Higher Education for Women
COIMBATORE – 641 043