

**DESIGN AND CHARACTERIZATION OF *Gymnema*
sylvestre SILVER NANOPARTICLES-INVITRO
ANTIDIABETIC ACTIVITY**

Bachelor of Pharmacy

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Declaration

I do hereby declare that the work presented in the thesis entitled "**DESIGN AND CHARACTERIZATION OF GYMNEMA SYLVESTRE SILVER NANOPARTICLES- IN VITRO ANTI DIABETIC ACTIVITY**", submitted by us to the **Sathyabama Institute of Science & Technology**, Chennai-600119 for the award of the degree of **Bachelor of Pharmacy (2019-2023)** by research is a original record of research work carried out by me under the supervision of **MR.V. VIJAYA KUMAR, M.Pharm, (Ph.D)**, Assistant Professor, School of Pharmacy. The contents of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma, membership, fellowship, associateship etc. In keeping with the general practice in reporting scientific observation, due acknowledgement has been made whenever the work described is based on the findings of other investigators.

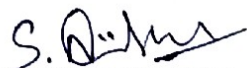
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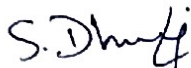
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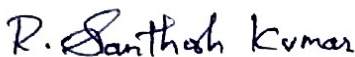
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ABSTRACT

AIM:

Design and Characterization of *Gymnema sylvestre* Silver Nanoparticles In-vitro Antidiabetic Activity.

PLACE AND DURATION OF THE STUDY:

The present work has been carried out between July 2021-April 2022, at School of Pharmacy, Sathyabama Institute of Science and Technology, Chennai -600119.

METHODOLOGY:

The synthesis of silver nanoparticles was carried out using ethanolic and aqueous extract of *Gymnema Sylvestre* leaves. The formulated silver nanoparticles were subjected to UV, Particle Size Analysis, FTIR, SEM analysis. In-vitro antidiabetic activity was evaluated with the formulated *Gymnema sylvestre* silver nanoparticle.

RESULT:

The reduction of silver nitrate to silver nanoparticles was confirmed by UV spectrophotometer, at 400nm. FTIR analysis. The reduction of silver nitrate to silver nanoparticles was confirmed by UV-Visible spectrophotometer at about 400 nm which is specific for Ag-NPs. In FTIR analysis showed that no interaction between *Gymnema sylvestre* and silver nitrate. SEM analysis reveals that silver nanoparticles are spherical in shape low particle size. In-vitro antidiabetic activity of *Gymnema Sylvestre* nanoparticles showed their antidiabetic activity by blocking carbohydrates hydrolyzing enzyme

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LIST OF ABBREVIATIONS:

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2.	CDDS	Conventional Drug Delivery System	7
3.	WHO	World Health Organization	4
4.	DM	Diabetes Mellitus	15
5.	GDM	Gestational Diabetes Mellitus	13
6.	SEM	Scanning Electron Microscope	25
7.	HR-SEM	High resolution-Scanning Electron Microscope	23
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15.	Ka	Dissociation constant	7
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17.	RNA	Ribonucleic Acid	13
18.	H ₂ O ₂	Hydrogen peroxide	13
19.	ROS	Reactive Oxygen Species	13
20.	ETC	Electron Transport Chain	13
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23.	FYM	Farmyard Manure	21

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36.	HNO ₃	Concentrated Nitric Acid	31
37.	Conc.HCl	Concentrated Hydrochloric Acid	38
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LIST OF UNITS:

S.NO	UNITS	EXPANSION	PAGE.NO
1.	mm	millimeter	21
2.	Nm	Nanometer	10
3.	kcps	kilo counts per second	51
4.	kev	electronarcotic	5
5.	Abs	Anti-lock braking system	29
6.	µg/dl	milligrams per deciliter	59
7.	kg/m ²	kilogram per square meter	17
8.	Kg	kilogram	21
9.	mg	milligram	37
10.	ml	milliliter	43
11.	cm	centimeter	21
12.	g/cm ³	gram per cubic centimeter	31
13.	mpa*s	millipascal second	52
14.	ms/cm	milli siemens per centimeter	57
15.	cm ² /vs	square centimeter	53
16.	°C	Celsius	31

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CHAPTER- I

1. INTRODUCTION

1.1. HERBAL MEDICINE

Herbal medicine plays an important role in the development of potent therapeutic agents. Herbal remedies, also known as plants materials or herbals, are made by using whole plants or plant parts to treat cuts or illnesses. Herbal medicines are the oldest form of health care known to mankind. Herbal medicines are currently in high demand, and their acceptance is expanding every day. Ancient literature refers to 500 plants as having therapeutic properties, while indigenous medical systems have used 800 plants. The use of medicinal herbs as drugs is done to treat and prevent illness, as well as to promote health and healing.

1.1.1. Advantages of Herbal Drugs

- Complete accessibility
- Enhanced Tolerance
- More Protection
- Low risk of Side effects

1.1.2. Disadvantages of Herbal Drugs

- Not able to cure rapid sickness and accidents
- Medication interactions
- Lack of dosage instructions and regulations
- Poison risk associated with wild herbs
- Complexity in standardizations

1.1.3. Various systems of Herbal Medicine

Various types of herbal medicines are AYUSH.

- Ayurveda
- Yoga & Naturopathy,
- Unani,
- Siddha
- Homeopathy.

1.1.3.a. Ayurveda

The word Ayurveda derived from *AYU* and *VEDA*. *AYU* means life *VEDA* means science or knowledge. Ayurveda means the science of life. Charaka defines "That science is designated as Ayurveda which deals with advantage and disadvantage as well as happy and unhappy states of life along with what is good and bad for life.

It is divided into three main branches viz.,

- i. NaraAyurveda dealing with human life,
- ii. Satva Ayurveda the science dealing with animal life and its diseases,
- iii. Vriksha Ayurveda the science dealing with plant life, its growth and diseases.

Ayurveda believes that positive health is the basis for attaining four cherished goals of life (*chaturvidh purushartha*) viz., Dharma, Artha, Kama, Moksha. All these four goals cannot be achieved without sound positive health.

Positive health is defined as

- i. Dosha Dhatu samya (*well-balanced metabolism*)
- ii. Prasanna Atma, Indriya, Manah (happy state of the soul, senses and the mind).

The philosophy of ayurveda is based on the theory of panchabhutas (five element theory) of which all the objects and living bodies are composed of ayurveda belongs to India's vedic culture. Ayurveda is a very ancient tradition and is about 2,000 to 4,000 years old. Ayurveda focuses on balancing energy and bodily order in order to prevent diseases and ailments.

1.1.3.b. Yoga and Naturopathy

Yoga is an Art and Science of healthy living. It is a spiritual discipline-based on an extremely subtle science, which focuses on bringing harmony between mind and body. The term Yoga is derived from the Sanskrit root 'YUJ', meaning 'to join' or 'to yoke' or 'to unite'. The aim of Yoga is Self-Realization, to overcome all kinds of sufferings leading to 'the state of liberation'. This is one of the oldest sciences of the world, which originated in India. The holistic approach of Yoga is well established and it brings harmony in all walks of life and thus, known for disease prevention, promotion of health and management of many lifestyle-related disorders

Naturopathy is an art and science of healthy living and a drug less system. It has its

own concept of health and disease and also principle of treatment. In today's age, Naturopathy is Recognized and well accepted as an independent System of medicine. Naturopathy advocates aiding human system to remove the cause of disease i.e., toxins by expelling the unwanted and unused matters from human body for curing diseases. Nature is the greatest healer.

1.1.3.c. Unani:

Unani-tibb or **Unani Medicine** is a form of traditional medicine practiced in Middle-East and South-Asian countries. It is not only the original science of medicine but also a rich store house of principles and philosophies of medicine which can be of immense value to the medicine in particular and science in general. It provides promotive, preventive, curative and rehabilitative healthcare. The fundamentals, diagnosis and treatment modalities of the system are based on scientific principles and holistic concepts of health and healing. Accordingly, it considers individual in relation to his environment and stresses on health of body, mind and soul.

1.1.3.d. Siddha

The Siddha system of medicine is mainly practiced in the Southern part of India. It is one of the earliest traditional medicine systems in the world which treats not only the body but also the mind and the soul. The word Siddha has its origin in the Tamil word Siddhi which means "an object to be attained" or "perfection" or "heavenly bliss". India being the birth place of many traditional philosophies also gave birth to Siddha. The roots of this system are intertwined with the culture of ancient Tamil civilization. "Siddhargal" or Siddhars were the premier scholars of this system in ancient times. Siddhars, mainly hailing from Tamil Nadu laid the foundation for Siddha system of medicine.

1.1.3.e. Homeopathy:

Homeopathy is a distinct medical specialty being practiced across the world. It is a recognized medical system in India through the **Homeopathy Central Council Act, 1973**. Homeopathy can be defined as a "dynamic, holistic and vitalistic system of individualistic drug therapeutics, based on the law of similar, potentially capable to cure diseases that are curable and relieves symptoms of incurable nature". The key principle of the system is Similia Similibus Curentur, while the crucial principles are

doctrine of Drug-proving, doctrine of drug- dynamization, theory of chronic diseases, totality of symptoms, direction of cure and the important supporting principles are vital force / dynamism, Single remedy, and Susceptibility / individualization.

1.1.4. Future Importance of Herbal Medicine:

Herbal medicines are the result of the synthesis of traditional practices from different indigenous medical systems and a diversity of therapeutic experiences from many older decades. It provides practical advice on how to choose, make, and use herbal medicine to heal, control, and handle a variety of ailments. According to WHO estimates that the trade in herbal medications, raw materials, and medicinal plants is growing at a rate of about 15% annually. In the past two decades, research efforts have increased in both developed and developing nations to test and verify herbal treatments using clinical trials and to uncover scientific support for claims made by tribal healers regarding Indian herbs. People will know more about efficient pharmacological therapy and healthier lifestyles once these regional ethno-medical formulations have undergone investigation. In light of the increased future potential for herbal medicines, we thus made an attempt to examine the existing status of employing herbal medicines to treat various diseases as well as any associated pharmacological problems. In Future, the necessity for more research to develop herbal medicines into cutting-edge therapeutic agents is discussed.

1.2. NOVEL DRUG DELIVERY SYSTEM

New developments in understanding of the pharmacodynamic and pharmacokinetic behaviour of drugs are known as novel drug delivery systems (NDDS). Medication delivery systems maintain their therapeutic concentrations of drugs for extended periods of time. NDDS is a formulation or device that distributes a drug to the body at a predetermined rate.

The way a medicine is administered can have a significant effect on its efficacy. Novel delivery systems can effectively deliver drugs with improved bioavailability when properly designed and developed for a particular drug, for example, drugs that partially or completely degrade before reaching the site of action could be delivered with these novel concepts of timed or pulsatile release, or gastro-resistant delivery.

A NDDS can be defined as a new approach that combines innovative development, formulations, new technologies, novel methodologies for delivering pharmaceutical compounds in the body as needed to safely achieve its desired pharmacological effects.



Figure 1.1. Novel Formulation

1.2.1. Need for Novel Drug Delivery System

The traditional drug delivery system fails to provide the following:

- The proper drug absorption does not happen because of various physiochemical barriers.
- The drug gets non-specifically distributed which contributes to its side effects by affecting non-target cells.
- The drug many times becomes vulnerable to metabolizing and excretory organs leading to losses.
- Due to all these reasons, the drug does not match the requires concentration in target.

1.2.2. Advantages of NDDS over Conventional Drug Delivery System

- Long-term maintenance of the optimal therapeutic medication concentration in the blood or tissue is possible.
- It is possible to obtain pre-set release rates over longer periods of time.
- In order to eliminate side effects, the site of action must be targeted.
- The frequency of dose and medication waste can be decreased or eliminated.
- Better patient compliance may be ensured.

1.2.3. Disadvantages of NDDS over Conventional Drug Delivery System

- May be costly, cause dose dumping, if the release design is failed
- Provide less scope for dosage adjustment
- May increase the first pass clearance
- Poor systemic availability in some cases

1.2.4. Differences Between Conventional Drug Delivery System / Novel Drug Delivery System

Table 1.1. Comparison Between CDDS/NDDS

S.NO	CONVENTIONAL DRUG DELIVERY SYSTEM	NOVEL DRUG DELIVERY SYSTEM
1.	After administration, the medication releases completely and quickly.	Medication distribution that is slow or controllable and lasts a long time
2.	Absorption is the rate-limiting step ($K_r \gg K_a$)	Drug release from the dosage form is the rate-limiting step ($K_a \gg K_r$)
3.	Blood level fluctuates (Peak and valley)	Constant blood level is maintained over a prolonged period (Reduced fluctuation)
4.	It perfectly Zero order Kinetics	Do not attain Zero order release kinetic
5.	There is a chance of taking excessive or insufficient medicine at times.	Reliable treatment since the danger is reduced
6.	Frequent Dosing	Reduced frequency of dosing
7.	Patient non-compliance. Therapeutic inefficiency/failure	Improved patient compliance
8.	Inconvenience of patient	Increased patient convenience with the use of daytime and nighttime medications

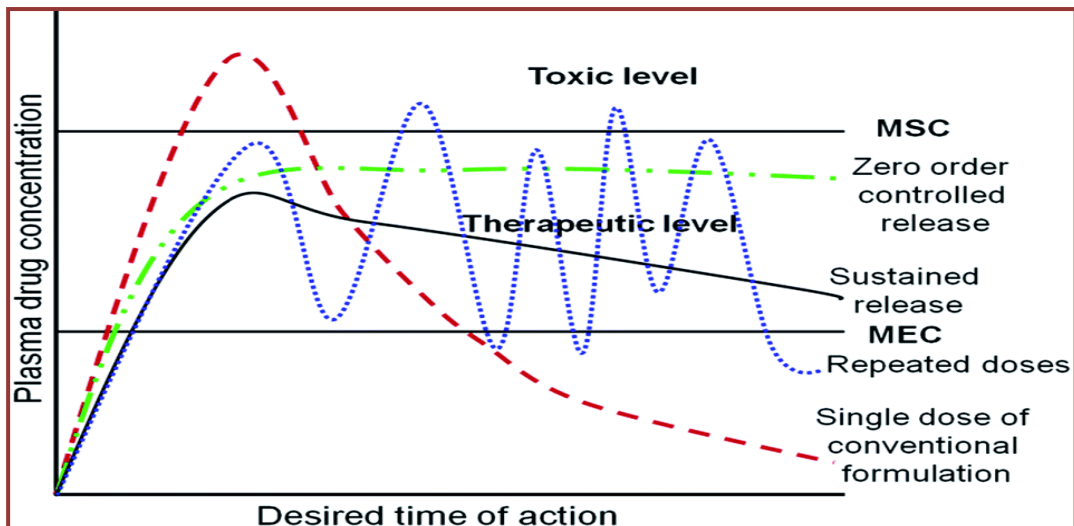


Figure 1.2. Plasma drug concentration

1.2.5. Classification of Novel Drug Delivery System

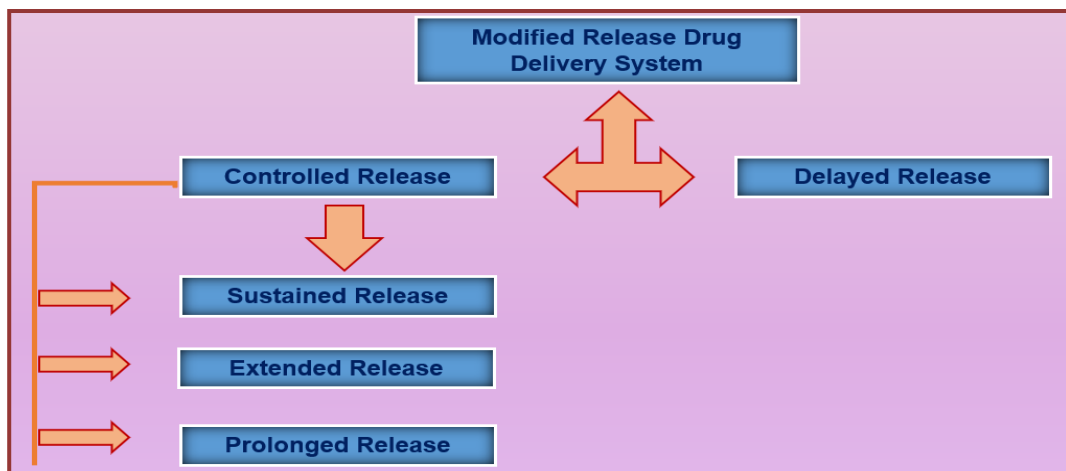


Figure 1.3. Classification of Novel Drug Delivery System

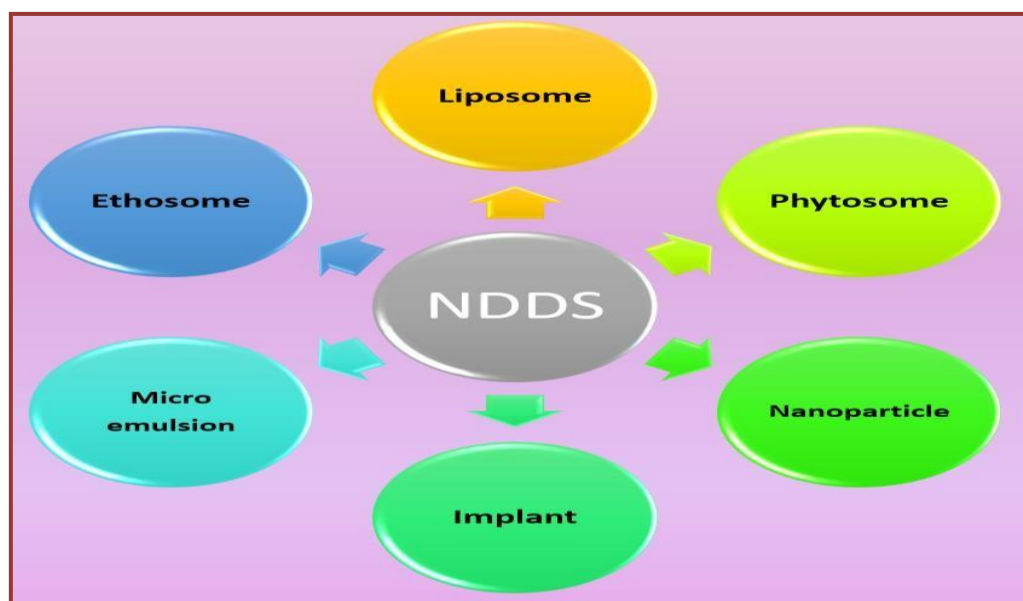


Figure 1.4. Different Technologies to deliver the Novel Drugs

TABLE: 1.2. Classification of Novel Drug Delivery System

S.NO	TYPES	DEFINITIONS
1.	Controlled Release Drug Delivery System	The limitations with conventional drug delivery were addressed by the development of controlled drug delivery systems. Drug delivery system that keeps medication levels constant in the blood and tissues for a long period of time
2.	Delayed Release Drug Delivery System	When a dosage form does not release the medication immediately after administration, such as an immediate release or conventional dosage form, but instead releases the drug in parts over a specified period or at intervals, it is referred to as a delayed release dosage form. Nonetheless, a part of the medication may be released shortly after delivery in rare circumstances
3.	Sustained Release Drug Delivery System	Sustained release is drug delivery system that achieve slow release of drug over an extended period of time after administration of single dose.
4.	Extended-Release Drug Delivery System	When compared to the frequency of administration of an initial release or traditional dosage form, an extended-release dosage form decreases the rate of administration at least twice as much.
5.	Prolonged Release Drug Delivery System	With this dosage form, the drug is delivered at a slower pace but over a longer length of time, allowing the therapeutic activity of the drug to last longer. With this dosage form, one dose of the medicine is delivered immediately after ingestion, followed by the second dose.

1.3. NANOPARTICLES

“Nanotechnology” is the newest and one of the most promising and active areas of modern research. The technology deals with the design, synthesis, and manipulation of particles size ranging from 1-100 nm [1]. Within this size range,

- a. chemical,
- b. physical,
- c. biological properties

change in the fundamental way of both individual atoms and their corresponding bulk material [2]. This very small size increases the surface area-to volume ratios of particles. The nanoparticles (NP) synthesized using plant extract have gained huge consideration in recent years due to their remarkable properties and wide range of applications in catalysis, plasmonic, biological sensor, water treatment, pharmaceutical applications, and agriculture and crop protection.

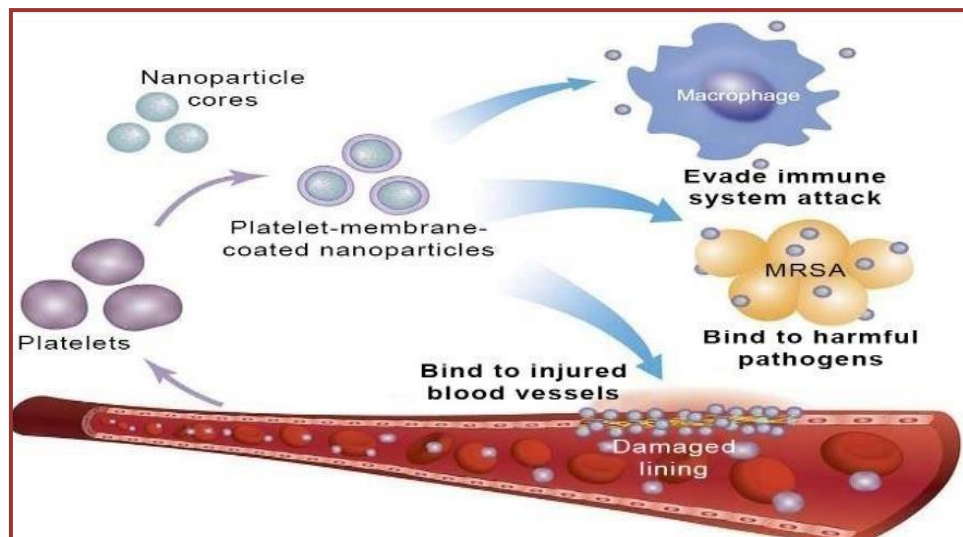


Figure 1.5. Nanoparticle

Among nanoparticles, several noble metal nanoparticles such as

- 1. Silver
- 2. Gold
- 3. copper and
- 4. platinum

1.3.1. Silver Nanoparticle

Silver nanoparticles have an eye-catching role owing to their innumerable physical and chemical properties. Silver nanoparticles are more potent than the silver ions as revealed in avant-garde research. The metallic AgNPs are contemplated as the most promising, as they possess remarkable antimicrobial properties due to their greater

surface area to volume ratio [15]. The biological synthesis methods of NPs reduce hazards to the global efforts. The AgNPs are also reported as medical and pharmaceutical agents that have directly encountered by a human system in such products as shampoos, detergents, soaps, toothpaste, and cosmetics [21]. The biomedical use of AgNPs includes their application as antibacterial, antifungal, anti-inflammatory, antiviral, and anti-diabetic agents. In recent studies, AgNPs were also reported in the diagnosis and treatment of cancer and as drug carriers by either active or passive mechanisms [28].

1.3.2. History and Concept of Nanotechnology

The history of nanoparticles is quite long and, during the last two decades, major developments within nanoscience have taken place. In 1970, Norio Taniguchi coined the term “nanotechnology.” But the concept of nanotechnology was initially defined by Nobel Prize winner Richard Feynman in his renowned address at the California Institute of Technology on 29 December 1959. The topic of nanoparticles was mentioned in one of his articles published in 1960 titled “There is Plenty of Room at the Bottom.” He pointed out that if a single bit of information required only 100 atoms, then all the books ever written could be kept in a cube with sides measuring 0.02-inch in length [31]. Depending upon shape and size, colloidal metal particles play a critical role in different applications. Nanotechnology is a recent invention in scientific research, but its basic concepts have been developed over a long period of time, since people have been coating glass windows with tiny colored metal particles for a long time, particularly silver, to create a glassy yellow color.

1.3.3. Methods of silver nanoparticle Synthesis

In AgNPs synthesizing and stabilization, several physical and chemical methods have been used. In recent years, Nanoparticle synthesis is one of the most fascinating areas of scientific research, and there is an increasing focus on manufacturing nanoparticles in environmentally acceptable ways (green chemistry). Mixed-valence polyoxometalates, polysaccharides, Tollens, irradiation, and biological processes are examples of green synthesis approaches that have advantages over traditional methods which use chemical agents that are detrimental to the environment.

1.3.4. Different methods of Nanoparticles synthesis

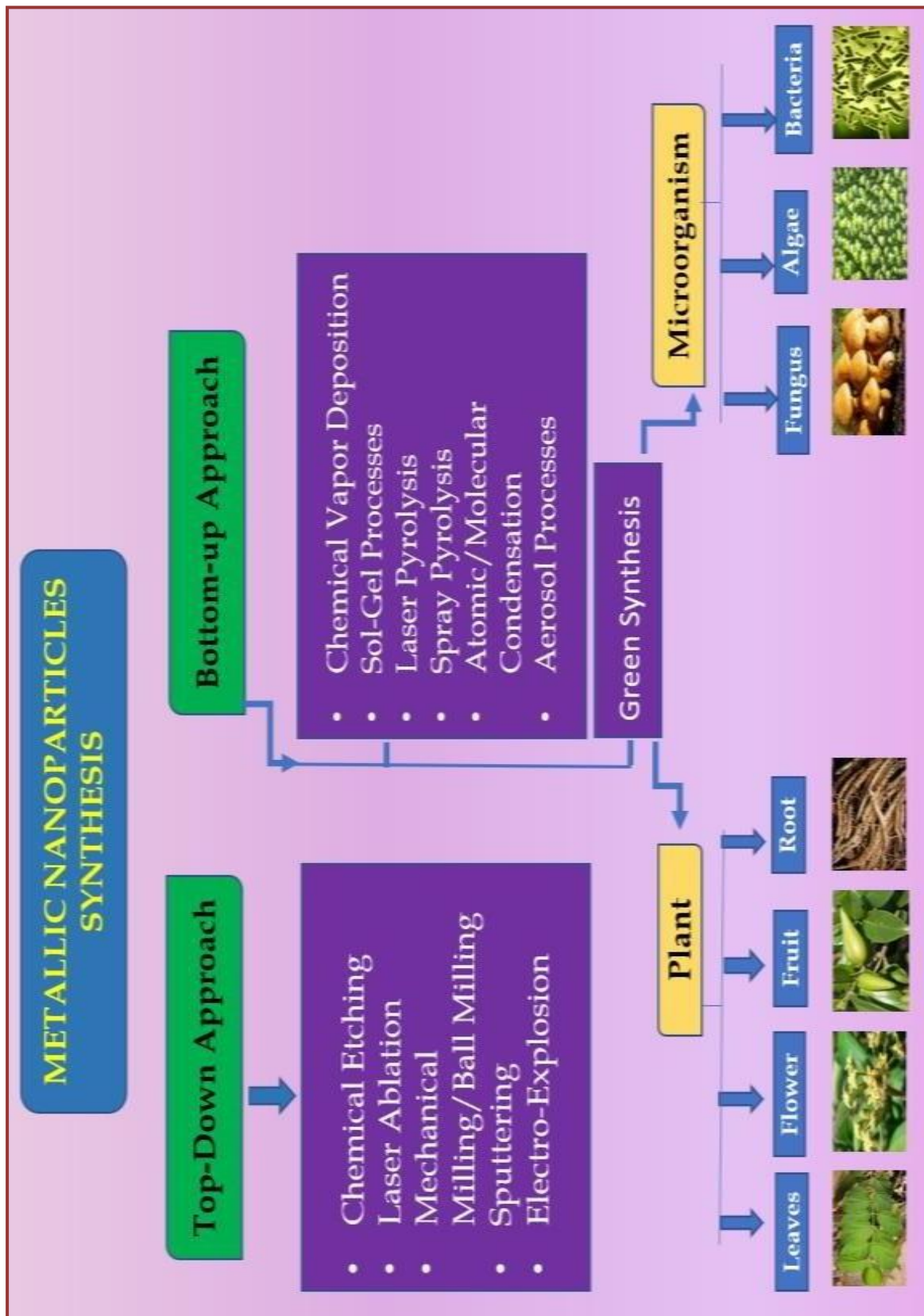


Figure 1.6. Different methods of Nanoparticles synthesis

1.3.5. Mechanism of Nanoparticles

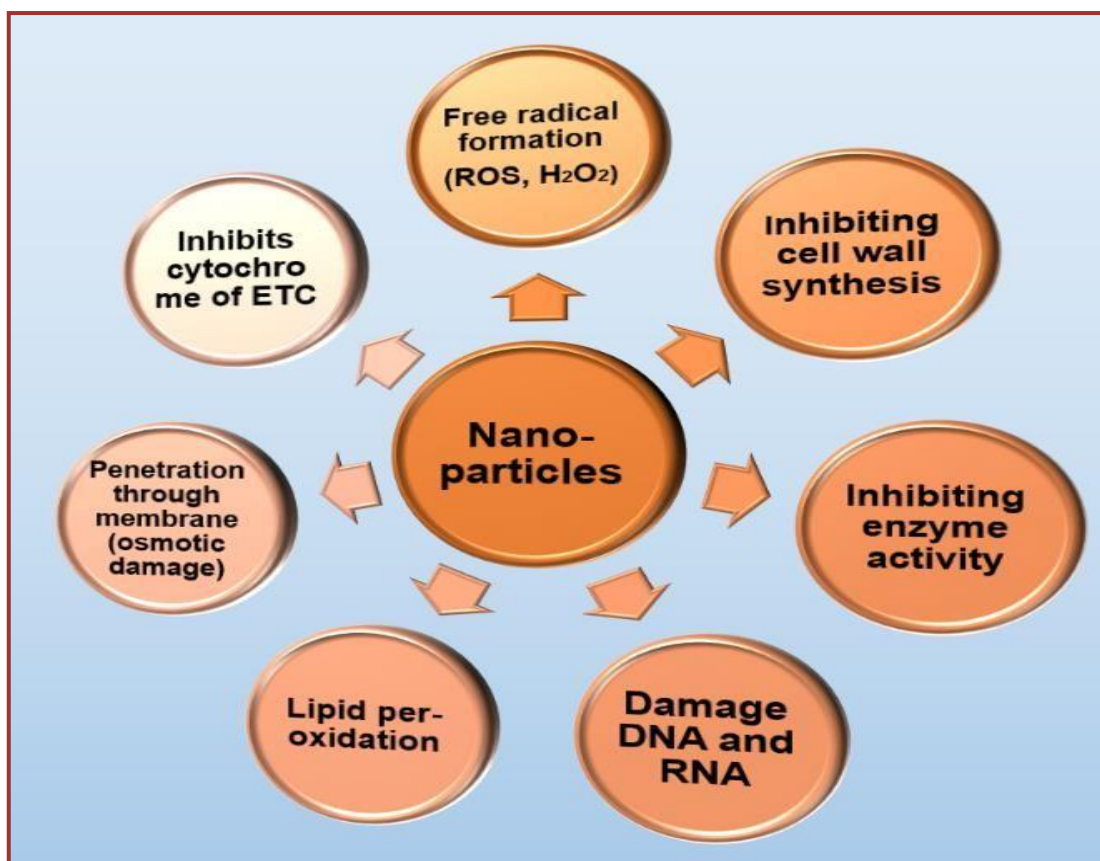


Figure 1.7. Mechanism of Nanoparticles

1.3.6. Different Types of Nanoparticles

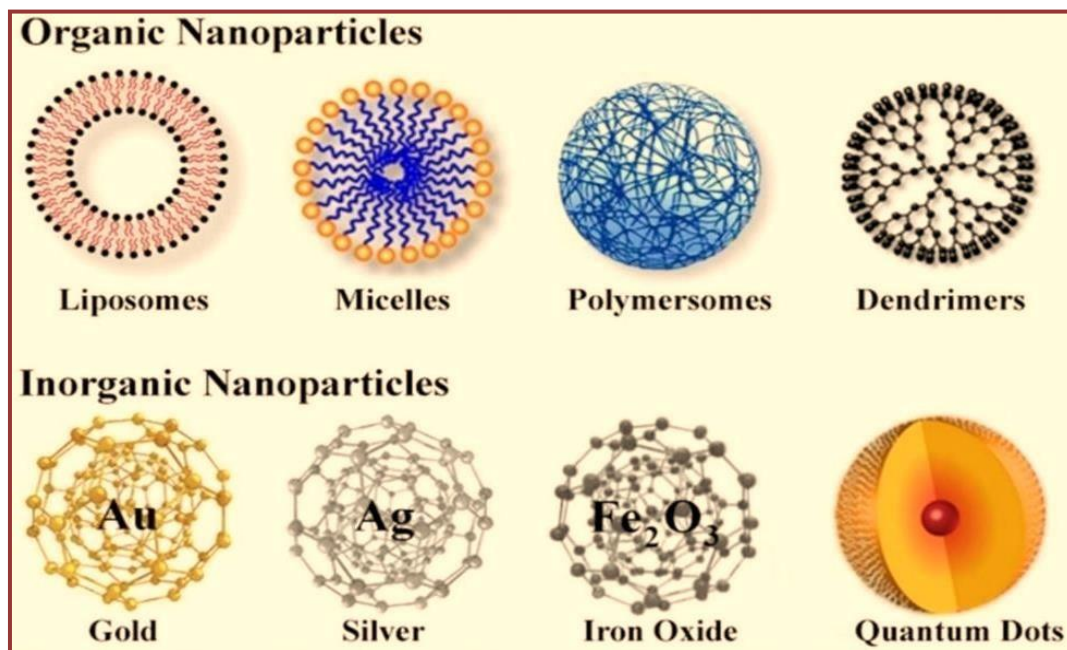


Figure1.8. Different Types of Nanoparticles

1.3.7. Advantages of Nanoparticles

- Targeted drug delivery via specific accumulation
- Extended therapeutic lifetime due to slow elimination
- Controlled drug release and Broad therapeutic index

1.3.8. Disadvantages of Nanoparticles

- High systemic exposure to locally administration drugs
- Nanotoxicity (lung, kidney, liver, brain, germ cell, metabolic, etc.)
- Lack of characterization techniques.

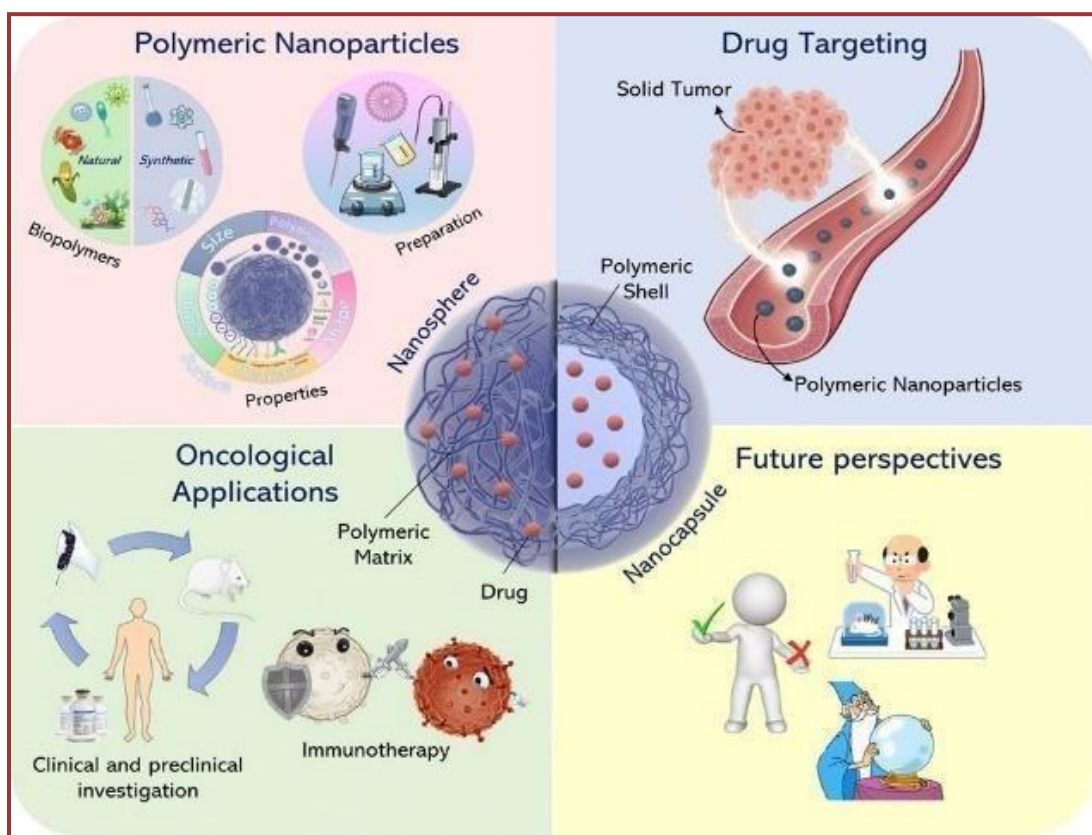


Figure 1.9. Polymeric and Drug Targeting Nanoparticles

1.4. DIABETES MELLITUS:

Diabetes Mellitus (DM) is a metabolic disorder in which there are high blood glucose level over a prolonged period. The Latin phrase diabetes mellitus is a combination of the word's diabetes, which means to syphon or pass through, and mellitus, which means sweet. DM is caused by either inadequate insulin production by the pancreas or insulin-resistant cells. It is characterized by the prevalence of chronic hyperglycemia and varying degrees of impairment in protein, lipid, and carbohydrate metabolism. It is one of the oldest diseases known to man.

Treatment of type 2 diabetes is still a major problem due to therapy failure. Herbal medicines known to be useful in diabetes treatment may be able to lead to compounds with such a combination of ideal therapeutic properties. The herbs that did demonstrate positive clinical effects were *Gymnema sylvestre*. Of particular interest was the herb Gymnema, because of its long history as a treatment for diabetes, and its range of unique and varied effects.

1.4.1. Insulin:

Insulin is the name of the hormone that facilitates the transfer of glucose from the blood into the cells. It helps to maintain appropriate blood sugar levels. High blood glucose levels are caused by the accumulation of glucose in the blood.

1.4.2. Pathogenesis of Diabetes Mellitus:

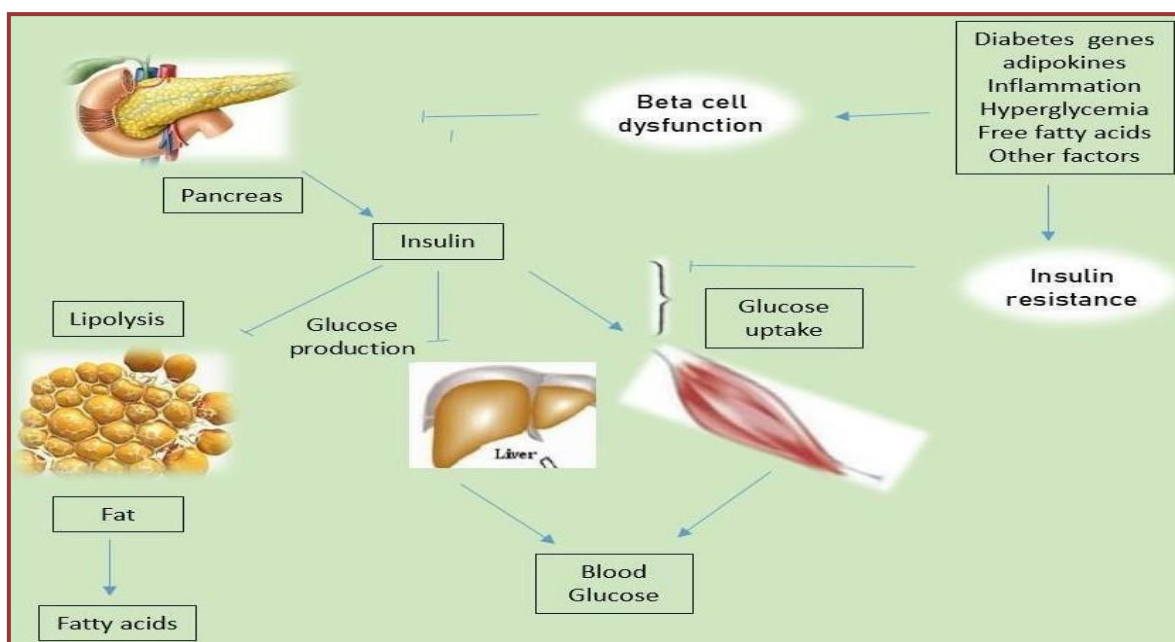


Figure.1.10. a. Pathogenesis of Diabetes Mellitus

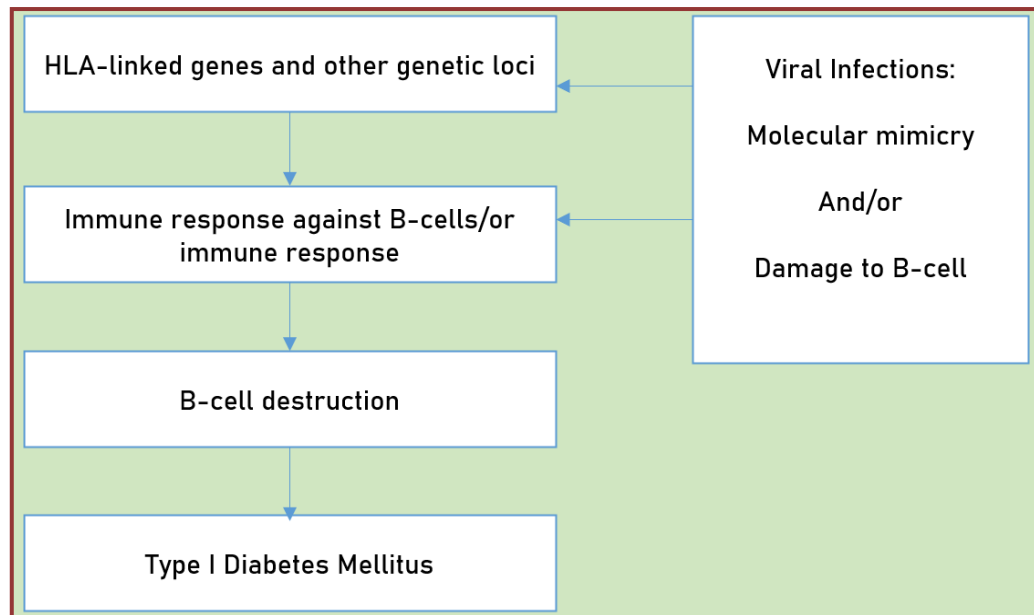


Figure.1.10. b. Pathogenesis of Diabetes Mellitus

1.4.3. Classification of Diabetes Mellitus:

Diabetes is classified into three types namely:

- i. Type 1 Diabetes Mellitus
- ii. Type 2 Diabetes Mellitus
- iii. Gestational Diabetes Mellitus

1.4.4.a. Type 1 Diabetes Mellitus (juvenile diabetes)

An autoimmune process that destroys beta cells in type 1 diabetes mellitus typically results in an absolute lack of insulin. Antibodies against glutamic acid decarboxylase, islet cells, or insulin, which recognize the autoimmune mechanisms that result in beta cell destruction, are typically present in type 1 diabetes. All type 1 diabetic people eventually need insulin therapy to keep normoglycemia.

1.4.4.b. Type 2 Diabetes Mellitus (T2DM)

Most common form of diabetes mellitus and is highly associated with a family history of diabetes, older age, obesity and lack of exercise. 90% of cases are progressive, mostly in adults, with obese body sizes. The respective contributions of deficiencies in insulin secretion or in the hormone's peripheral action to the development of Type 2 Diabetes Mellitus have been and will continue to be relevant. Exhibit intra-abdominal (visceral) obesity, which is closely related to the presence of insulin

resistance. Additionally, these people frequently have dyslipidemia (high triglyceride and low HDL cholesterol levels; postprandial hyperlipidemia) and hypertension.)

1.4.4.c. Gestational Diabetes (GDM):

Diabetes which occurs among women during pregnancy. women who subsequently develop diabetes and are at risk. The future chance of type 2 diabetes in the offspring of pregnant women with the condition is higher.

1.4.4. Risk Factors of DM

- i. Age progression: It mostly occurs in adults, while teenagers are starting to experience it as well.
- ii. Being overweight/obese (BMI is $> 23\text{kg/m}^2$)
- iii. High Blood Pressure
- iv. Addictions like tobacco use, drugs and harmful use of alcohol
- v. Women with polycystic ovarian syndrome

1.4.5. Management of DM:

The aims of treatment are to alleviate and prevent both the symptoms and complications of diabetes

The goals of therapy for type-I or type-II DM are:

- a. Eliminate symptoms related to Hyperglycemia
- b. Reduce or eliminate the long-term micro vascular and macro vascular complications of Diabetes mellitus
- c. Allow the patient to achieve as normal a life style as possible.

1.4.6.a. Methods of Management

- Non-Pharmacological
 - i. Diet and weight control
 - ii. Exercise
- Pharmacological
 - i. Biguanides
 - ii. Incretins
 - iii. Sulfonylureas/Meglitinides

- iv. Alpha glycosidase inhibitors
- v. Thiazolidinediones

1.4.6. Global Impact of Diabetes Mellitus

Today, diabetes mellitus is considered a major public health threat with a growing burden in many parts of the world. Additionally recognized as a significant risk factor for cardiovascular diseases is this disorder. In 1995, the global prevalence of diabetes mellitus was estimated at 135 million. This increased to 171 million (2.8% of the world adult population) in 2000, 193 million (5.1 % of the world adult population) in 2003 (International Diabetes Federation, 2003), 246 million (6% of the world adult population) in 2007 (International Diabetes Federation, 2006), and 284 million (6.4% of the adult world population) in 2010.

Currently, about 366 million individuals (8.5% of the adult world population) suffer from DM, and this number is expected to increase to 552 million (or 8.9% of the world population) by 2030 (International Diabetes Federation, 2011). Not surprisingly, diabetes mellitus - seven years ago not even among the world's top ten principal killers (Mathers et al., 2008) - nowadays occupies ninth place on the list of the leading causes of death worldwide (World Health Organization, 2011c). Diabetes mellitus is a disproportionately expensive disease. Additionally, people with DM used emergency rooms twice as frequently as people without the disease. Indeed, the economic burden of DM is significant and growing. The cost of this disorder globally in 2010 was estimated to be around U\$ 45 billion, or 12% of total health expenditures.

1.5. *Gymnema sylvestre*

Gymnema sylvestre (GS), a plant used in Indian Ayurvedic medicine for the treatment of diabetes mellitus, has also been known since ancient times to have an anti-saccharin flavoring effect. *Gymnema sylvestre* has anti-sweat and hepatoprotective properties, as well as lowering blood sugar and stimulating the heart, uterus, and circulatory system. *G. sylvestre* is an herb native to the tropical forests of India and has been used in herbal medicine as a treatment for diabetes for nearly two millennia, but there is insufficient scientific evidence to draw firm conclusions on its effectiveness.

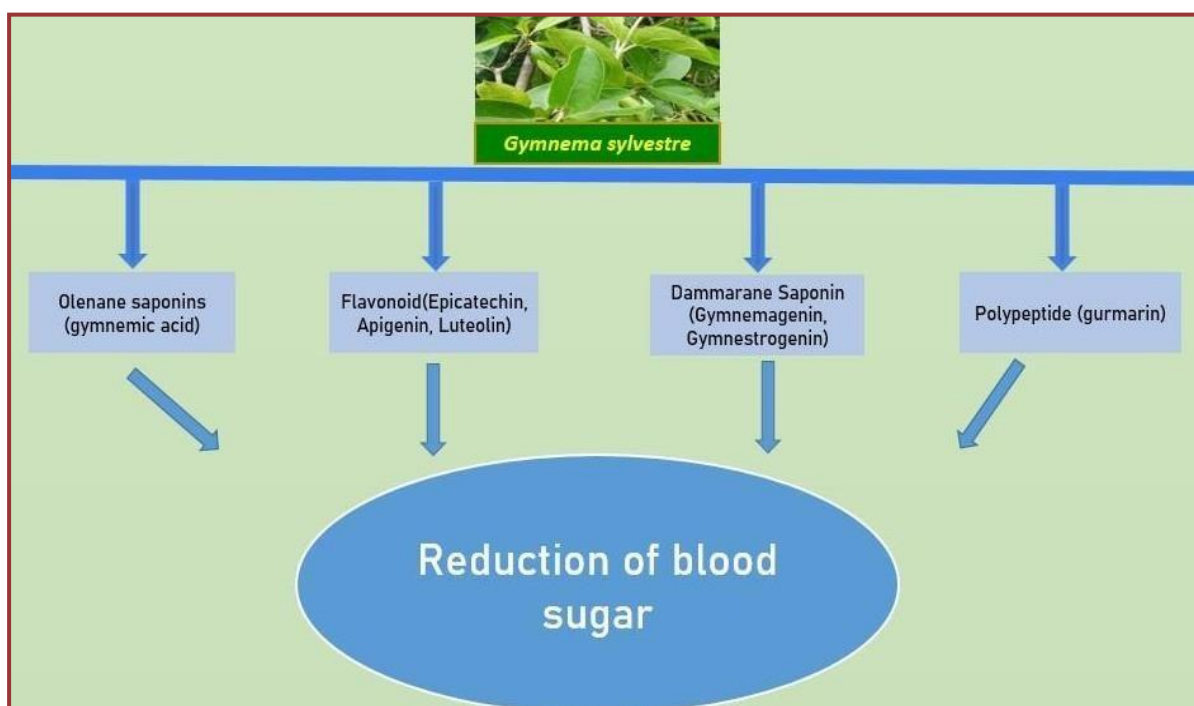


Figure.1.11. Blood Sugar Reduction Potential of Different Bio-Components of *Gymnema sylvestre*



Figure1.12. *Gymnema sylvestre* plant



Figure 1.13. *Gymnema sylvestre* leaves

1.5.1. Dietary Formulation of *Gymnema sylvestre*

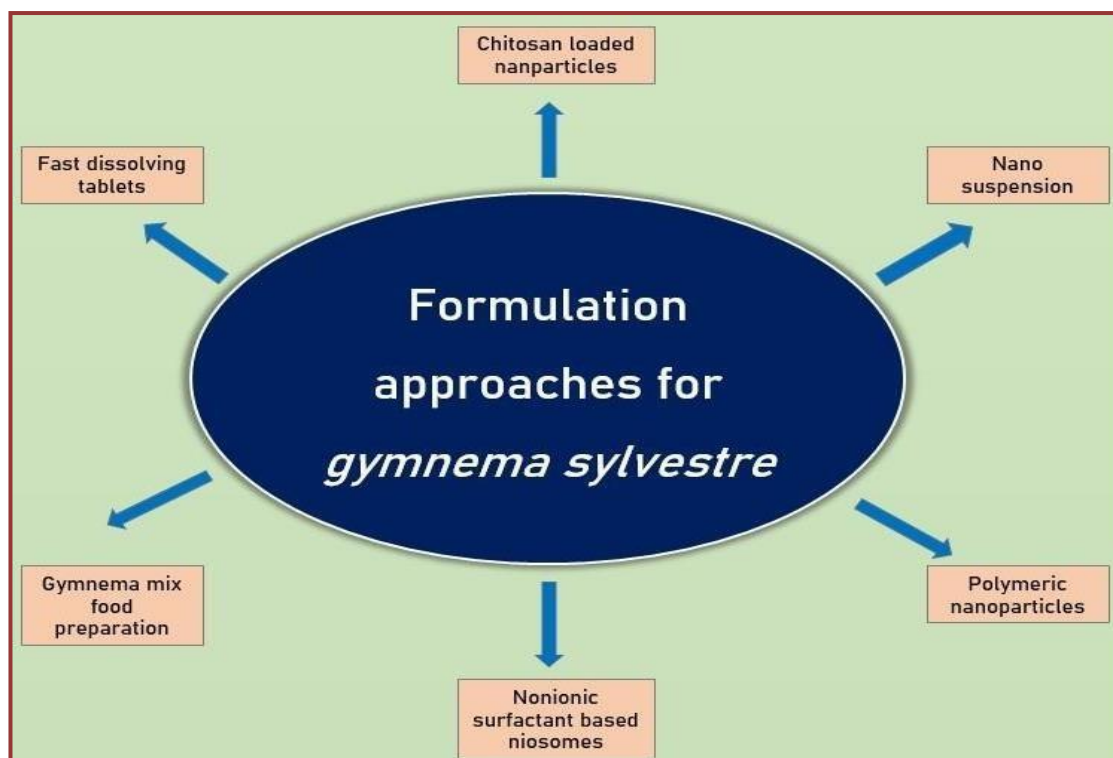


Figure1.14. Formulation Approaches for *Gymnema sylvestre*

1.5.2. Mechanism of *Gymnema sylvestre*

G. sylvestre leaves have been found to cause hypoglycemia in laboratory animals and shown a use in herbal medicine to treat diabetes mellitus in adults. When leaf extract of plant, administered to a diabetic patient, there is stimulation of the pancreas by virtue of which there is an increase in insulin release. These compounds have also been found to increase fecal excretion of cholesterol. There are some possible mechanisms by which the leaves extract of *Gymnema sylvestre* or (Gymnemic acid) possess its hypoglycemic acid effects are:

- 1) It promotes regeneration of islet cells,
- 2) It increases secretion of insulin,
- 3) It causes inhibition of glucose absorption from intestine,
- 4) It increases utilization of glucose as it increases the activities of enzymes responsible for utilization of glucose by insulin-dependent pathways, an increase in phosphorylase activity, decrease in gluconeogenic enzymes and sorbitol dehydrogenase

CHAPTER-II

LITERATURE SURVEY

1. **Ajinkya B. Chavan**, *et al.*, (2022), *Gymnema sylvestre* leaf extract was used to synthesis the silver nanoparticles. By using the technique green synthesis method silver nanoparticles were synthesized. For silver nanoparticles particle size, UV, FTIR and DLS was characterized. It is evaluated for antidiabetic activity. Silver Nanoparticles was confirmed by UV spectrophotometer showing about 400nm. FTIR analysis peak at 3355cm^{-1} in the extract for amine N- H stretching bands. DLS shows good zeta potential and less particle size.
2. **Ashok K. Pandey**, *et al.*, (2012), *Gymnema sylvestre* R.Br. is an important medicinal plant which grows wild in the tropical forests. Different experiments were carried out to standardize seed germination, vegetative propagation and cultivation practices of the species. The species can be cultivated successfully by seeds as well as by rooted cuttings at a spacing of 50 x 50 cm. It was observed that pre-treatment of seeds in cold water for 24 h improves the seed germination. For vegetative propagation, hard wood cuttings of 10-15 mm diameter having three nodes were found most suitable. March and July were found the best time for vegetative propagation. Application of 4000 kg FYM/ha was found promising in terms of growth and yield. Total yield of dry leaves was found to be 1.5 tones/ha. The crop became ready for harvest after two years of plantation and harvesting can be done twice a year in the months of October and June
3. **Aziza A.M. El Shafey**, *et al.*, (2012), The effect of *Gymnema sylvestre* leaves extract on several physiological parameters of diabetic rats. *Gymnema sylvestre* leaves extract (18 mg/kg body weight) was orally administered for 30 days to normal and streptozotocin diabetic rats. It exhibited a significant increase in plasma glucose, liver function enzymes triglycerides, total cholesterol, LDL-cholesterol malondialdehyde, catalase, reduced glutathione and a significant decrease in insulin, HDL-cholesterol and erythrocyte superoxide dismutase levels. Treatment diabetic rats with *Gymnema sylvestre* leaves extract significantly decreased plasma glucose, liver function enzymes,

triglycerides, total cholesterol, LDL-cholesterol, malondialdehyde and significantly increased insulin, HDL-cholesterol and erythrocyte superoxide dismutase levels compared to untreated diabetic rats.

4. **Branislav ruttkay-nedecky, et al.,** (2018), Silver nanoparticle was evaluated for anti-oxidant activity and shows unique properties, such as good electric conductivity, photoelectrochemical activity and antimicrobial activity are widely used. AgNPs were characterized spectrally by determining the zeta potential and the size of nanoparticles (30-80 nm). Furthermore, antioxidant activity was monitored using ABTS (670 nm), DPPH (517 nm), and FRAP (595 nm) methods. Antioxidation assays were evaluated using calibration curve equations in which the standard was gallic acid. It shows results for the ABTS and DPPH methods were also expressed as percentage of inhibition of the radicals and in the FRAP method as percentage of reduction activity. The results were calculated using the ABTS method in the range (25.9 - -84.9%), in the DPPH method (19.2-86.6%) and in the FRAP method (8.5 --93.2%). Most AgNPs prepared by green synthesis showed significant antioxidant activity.
5. **Chakrapani Pullagummi, et al.,** (2014), *Gymnema sylvestre* is an herb native to the tropical forest of southern and central India and Sri Lanka. It belongs to family Apocynaceae. Chewing the leaves suppresses the sensation of sweet. This effect shows the presents of gymnemic acid as active compound. Secondary metabolites like alkaloids, terpenoids, phenolics, steroids and flavonoids plays an important in the plant activities like anti-bacterial.
6. **LIU Yue, et al.,** (2014), To synthesis the chemical constituents of stems of *Gymnema sylvestre* (Retz.) Schult. Chromatographic techniques using silica gel, C18 reversed phase silica gel, and prep-HPLC were used. The structures were elucidated on the basis of MS and spectroscopic analysis (1D and 2D NMR), as well as chemical methods. Seven compounds were isolated and their structures were elucidated. Compound 7 is a new triterpenoid saponin. The inhibition activities of compounds 1, 5–7 on non-enzymatic glycation of protein in vitro were evaluated. It was shown that compounds 1, 5–7 have

weak inhibition activities for non-enzymatic glycation of protein in vitro.

7. **M.S. Subashini, et al.,** (2015), Analysis of potential bioactive constituents of leaves of *Gymnema sylvestre* R.Br. Methanol extracts of the leaves were subjected to thin layer chromatography (TLC), Fourier transform infrared spectroscopy (FTIR) and Gas chromatography- mass spectroscopic (GCMS) analysis. TLC analysis with methanol: Chloroform solvent revealed 6 fractions with R_f values of 0.23, 0.35, 0.45, 0.59, 0.69 and 0.85. FTIR spectroscopy shows characteristic peak values with various functional compounds such as alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes and aromatics. GC-MS analysis gave a spectrum of compounds with 10 major peaks which are bioactive compounds, act as good antimicrobial, antiviral, antioxidant and anti-inflammatory agents.
8. **N. Supraja, et al.,** (2017), Green synthesis silver nanoparticles was synthesized. By using *Gymnema sylvestre* leaf aqueous extract nanoparticles, anti-microbial activity was evaluated. HR-SEM, FTIR, DLS was characterized for silver nanoparticles. HR-SEM images shows that the particles are spherical and uniform shape of AgNP was conformed in the range of 30-50nm. FTIR analysis peak at 3355cm⁻¹ in the extract for amine N- H stretching bands. DLS shows good zeta potential and particle size.
9. **Rofiyat Omolara Raj, et al.,** (2021), *Gymnema sylvestre* is commonly for the management of diabetes and other ailments in traditional medicine. The phytochemical compositions and effect of crude extract, hexane, and ethyl-acetate fractions of *Gymnema sylvestre* on biochemical and hematological parameters of Wistar rats. Albino rats were distributed randomly into a control group and nine treatment groups orally administered crude extract and fractions of *Gymnema sylvestre* (100, 300, and 600 mg/kg BW) for 21 days. Liver and kidney function indices, as well as hematological parameters, were monitored. The result shows crude methanol extract contains flavonoids, alkaloids, total phenol, saponins and tannins. The crude methanol, ethyl acetate and n-hexane fractions of *Gymnema sylvestre* had LD₅₀ greater than 5000 mg/kg BW in acute oral toxicity test. The extract and fractions at 300 and

600 mg/kg BW cause loss of body weight of the rats, significantly altered ($p < 0.05$) the levels of biochemical indices including the aspartate transaminases, alanine transaminase, alkaline phosphates, bilirubin, proteins, urea, creatinine, and white blood cells (WBC) counts when compared with the non-treated control. *Gymnema sylvestre* at sub-acute doses of 300 and 600 mg/kg BW could compromise the functional integrity of the rats' liver and kidney.

10. **Shirish Sadashiv Pingale, et al.,** (2018), *Gymnema sylvestre* stem and root aqueous extract used to biosynthesis the silver nanoparticles. The synthesis was done at room temperature and their usage in the photodegradation of methyl orange dye. UV-Visible, FTIR, XRD was characterized. UV-Visible shows 430nm with spherical reference to the excitation of surface plasmon vibration by AgNP. FTIR analysis reveals the presents of molecular functional groups such as phenolic compounds, amides, carboxylic acid. XRD patterns shows the occurrence of crystalline silver ion. Photocatalytic degradation of methyl orange was measured spectrophotometrically by using AgNP as nano-catalyst under solar light effect. The biosynthesized nanoparticles were found to be notable in degrading methyl orange dye under the influence of sunlight.
11. **Sureka.J, et al.,** (2018), *Gymnema sylvestre* silver nanoparticle was evaluated for anti-bacterial and anti-oxidant properties. It shows extremely potent anti-bacterial activity as well as anti-oxidant activity by blocking DPPH and scavenging nitric oxide. The significant anti-oxidant activity could be related to the preliminary phytochemical screening shows acidic compounds, phenols, flavonoids, saponins, tannins and triterpenoids.

CHAPTER-III

3.1. AIM

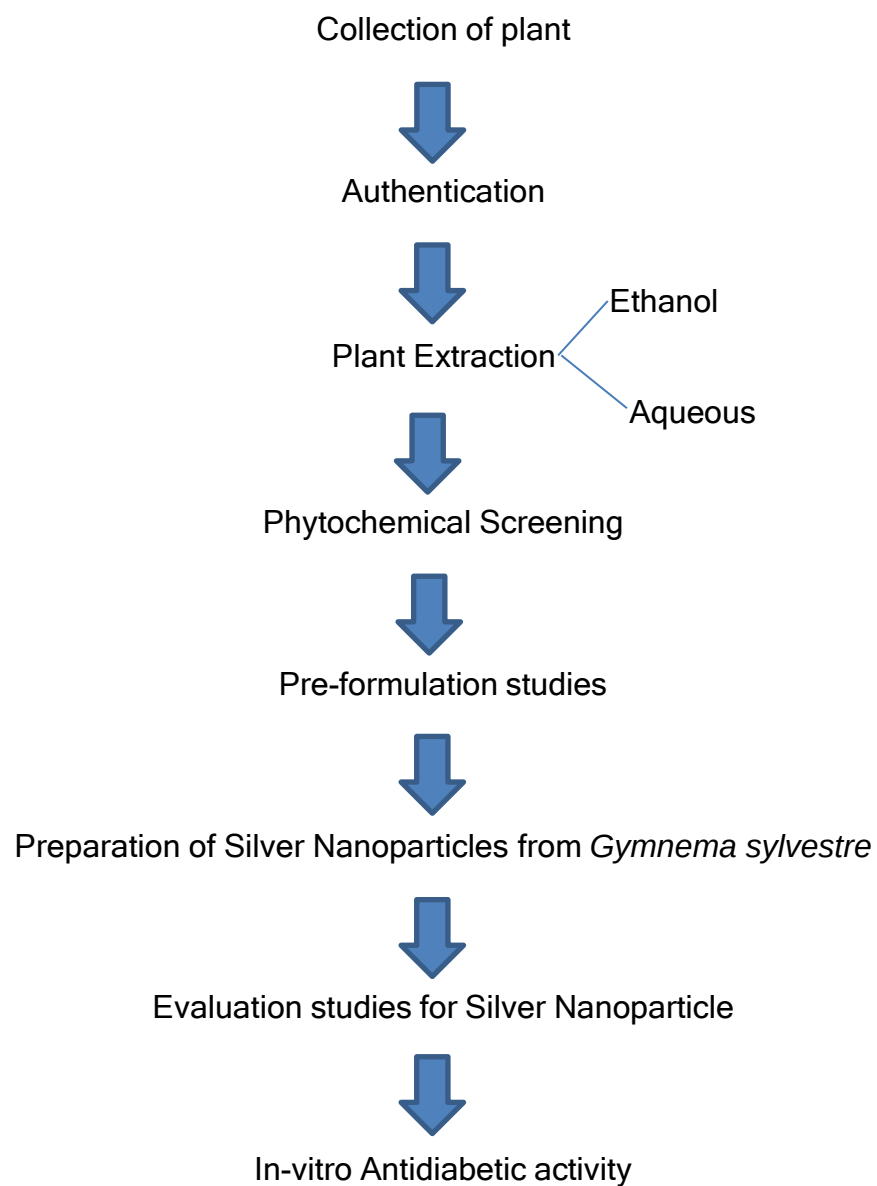
The aim of the current investigation was to design and characterize the *Gymnema sylvestre* silver Nanoparticle and to evaluate the prepared nanoparticles for its *In Vitro* antidiabetic activity (*In-Vitro* study).

3.2. OBJECTIVE OF THE STUDY

The objective of the work are as follows

- Collection and Authentication of the *Gymnema sylvestre*
- To perform the ethanolic and aqueous extraction for the *Gymnema sylvestre*
- To perform the phytochemical screening for *Gymnema sylvestre*
- To perform the pre-formulation studies compatibility studies using FTIR
- To synthesize the silver nanoparticles of *Gymnema sylvestre*
- To characterize the silver nanoparticles using
 - a. UV-Visible Spectroscopy
 - b. Particle size and Zeta potential
 - c. SEM analysis
- To perform the invitro anti diabetic activity of *Gymnema sylvestre*

3.3. PLAN OF WORK



3.4. PROFILE FOR PLANT AND EXCIPIENT

3.4.1. *Gymnema sylvestre*:

Gymnema sylvestre is a staple herb of the Ayurvedic apothecary, traditionally used for supporting balanced blood sugar levels and dulling the palate to the taste of sweets. *Gymnema* is fascinating in that it works a multifaceted path towards maintaining healthy blood sugar. The pharmacological properties of *Gymnema sylvestre* are attributed to a group of triterpene saponins, known as gymnemic acid.



Figure:3.1. *Gymnema sylvestre*

Synonyms

Gymnema, Madhunashini

Biological Source

This consists of the leaves of a perennial woody climber plant known as *Gymnema sylvestre* R.Br. family Asclepiadaceae. It contains not less than 1.0 per cent of Gymnemic acids calculation on gymnemagenin on dried basis

Geographical Distribution

It is a woody climber found in India, common in Deccan-peninsula and also in northern and western parts of India. It is occasionally cultivated for medicinal purposes. It is widely distributed in Malaysia, Sri Lanka, Indonesia, Japan, Vietnam, tropical Africa and South Western region of the people's republic of China.

Scientific Name: *Gymnema sylvestre*

Common Name: *Gymnema*, Australian cow plant.

Other Names:

Bengali - meshashruna

English- Periploca of the woods

Hindi - gurmar

Kannada - madhunashini

Malayalam - chakkarakolli

Marathi - bedakicha pala, gudmar

Sanskrit - madhunaashini, meshasringa

Tamil - sirukurinjan, kokilam

Telugu - Podapatri

Urdu - gurmar.

Macroscopical Characters of leaves

Gymnema sylvestre is slow growing, perennial and medicinal plant.

Color- Green

Odour- Pleasant and aromatic odour

Taste- Tasteless, Slightly bitter and astringency

Size - 2-6 cm long and 1-4 cm wide

Smell- Characteristic odor

Shape- Elliptic or ovate with acute or acuminate apex

Extra Features

The leaves when chewed, have remarkable property of paralyzing the taste glands for few hours against sweet and bitter taste. Lower surface of leaf is more pubescent and baser is rounded or cordate. Non-glandular-trichomes are present on both the surfaces of leaf. It bears small yellow-colored flowers in the form of cymes.

Cultivation and Collection

Climatic Conditions

It grows well in vivid agro- climate conditions in tropical and subtropical regions, under shady situations.

It can grow upto 600 meters from the sea level, favorable temperature is ranging from 25⁰ to 30⁰ C at rainfall of 50-100 cm per annum. It can grow on variety of soils, preferably loamy soil with rich organic matter and well drained property

Method of Cultivation

Gymnema can be propagated from seeds and also vegetatively by using stem cuttings and apical portion of plants. For sowing seeds in nursery beds, December-January is appropriate season: and seedlings are trans-parented in field during February-March. It is a perennial crop Stem cuttings are planted in field, at the onset of monsoon i.e., in months of June-July. The land is ploughed well, weeds are removed, levelled and during last ploughing, soil is mixed with farmyard manure.

Collection

After a year of cultivation, leaves are ready for harvesting. Leaves are usually collected during winter season i.e., October to February. During the same period,

roots are ready for harvesting but they are collected or harvested during summer season i.e., during March-May. The harvested leaves are cleaned of external matter by washing with water and are shade-dried. The roots are also cleaned, washed and cut into equal pieces and are shade-dried

Chemical Constituents

The leaves contain pentriacontane, hentriacontane, phytin, alpha and beta chlorophylls, resin, tartaric acid, formic acid, butyric acid, mucilage inositol, d-quercitol, gymnemic acids (anti-sweet compounds), the mixture of triterpene saponins and anthraquinone derivatives. The plant is reported to contain alkaloids, betaine, choline and trimethylamine in the leaves. Lupeol, beta amyrin, stigmasterol are also present in the leaves

Gymnemic acid is a mixture of at least nine closely related acidic glycosides fractionated by successive extraction with different solvents. Ethyl acetate fraction of such gymnemic acid mixture has a paralyzing effect on taste glands. Gypenosides have also been isolated from leaves

Taxonomical Classification:

Kingdom - Plantae
Sub Kingdom - Traceobionta
Super Division - Spermatophyta
Division - Magnoliophyta
Class - Magnoliopsida
Sub class - Asteridae
Order - Gentianales
Family - Apocynaceae
Genus - *Gymnema*
Species - *sylvestre*

3.4.1.c. Microscopical Character

Leaves are ovate or elliptic, petiolate, glabrous, margin entire, rounded to cordate base, pubescent on both the surfaces.

Petiole:

Transverse section of petiole is horse shoe shaped. The epidermis is barrel shaped single layered, thick walled covered with uniseriate, multicellular, non-glandular

trichomes. The cortex is collenchymatous and vascular bundles are amphicribal and three in number. Well-developed phloem consists of sieve tubes, companion cells and phloem parenchyma. The xylem consists of vessels, tracheids and tracheidal fibres. The starch grains are polygonal, simple or compound in two or many groups.

Lamina:

The epidermal cells of lamina are square shaped with outer convex wall and thin cuticle. When viewed transversally, epidermal cell surface is interrupted with trichomes, which are uniseriate, multicellular with 2 to 5 celled, present in abundance on both the surfaces. Single layered closely arranged palisade cells are present just below the adaxial epidermis. Vascular bundles are amphicribal and the mesophyll is 3-5 celled thick

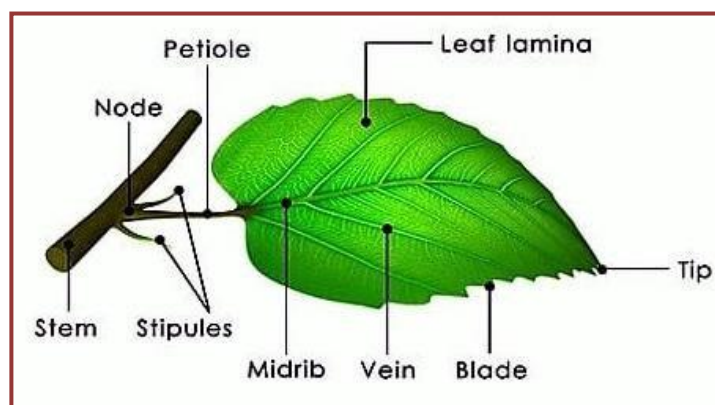


Figure 3.2. Microscopical parts of the leaf

3.4.2. Silver Nitrate

Silver nitrate is the most important silver compound and is used extensively for the manufacture of silver halide photosensitive material. Pure silver nitrate crystal is stable to light and is easily to be reduced to black metallic silver in the presence of organic matter. Wet silver nitrate and silver nitrate solution can be easily subject to decomposition upon light. Silver nitrate is a kind of oxidizing agent and can cause protein coagulation with corrosive effect on the human body. Silver nitrate is an inorganic silver salt and can dissociate silver ions with sterilization, corrosion, erosion, and convergence effect. Its dilute solution has antibacterial and astringent effect while its concentrated solution has a corrosive effect. Topical application of silver ion can bind with bacterial protein to form silver protein precipitate with bactericidal effect. It can be used for treating dentin hypersensitivity, mucosal ulceration, early caries and cavity disinfection.

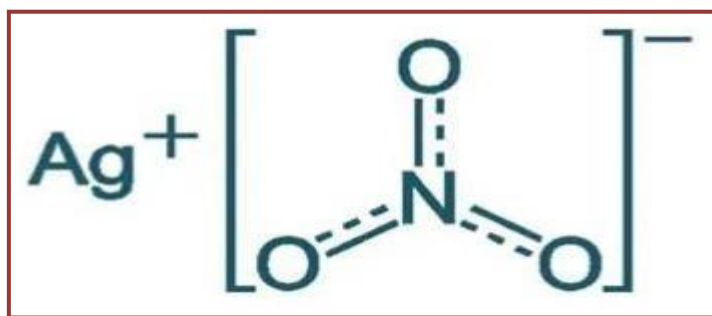


Figure 3.3. Silver Nitrate structure

USES:

It is caustic, and a strong oxidizing agent. Used in photographic film, silver plating, silvering mirrors, and as an antiseptic.

Physical properties

Pure compound is not sensitive to light but trace organics promote photo reduction, turning the salt to grayish black on exposure to light; density 4.35 g/cm³, melts at 212°C; decomposes at 440°C; very soluble in water, soluble in ethanol and acetone.

Toxicity

It is corrosive to the skin and mucous membranes and has convergence effect. Skin, upon contact with silver nitrate, will turn dark upon light and is prone to get inflammation. If the skin is contaminated by silver nitrate, you can use iodine remove graze; if skin get injured upon contact, you can soak in salt water for washing. Upon working, the production staff should wear masks, cotton overalls, and latex gloves and other protective equipment.

Properties of Silver Nitrate:

CHEMICAL NAME: Silver nitrate

MOLECULAR FORMULA: AgNO³

MOLECULAR WEIGHT: 169.87

MELTING POINT: 212° c

BOILING POINT: 444° c

STORAGE TEMPERATURE: 2-8° c

SOLUBILITY: Water, slightly in ether, ethanol, Insoluble in HNO₃

FORM: Solid

COLOUR: White

ODOUR: Odorless

TASTE: bitter, caustic, metallic taste

SHAPE: Orthorhombic Crystal

pH RANGE: 7-9

SENSITIVE: Light Sensitive

3.4.3. MATERIALS TABLE:

Table:3.1. Chemicals Used

S.NO	CHEMICAL NAME	MAKE	MANUFACTURED BY
1.	ETHANOL 99.9%	KAVIN'S SCIENTIFIC LAB	Changshu Hongsheng Fine Chemical Co., Ltd
2.	SILVER NITRATE	KAVIN'S SCIENTIFIC LAB	Ayra synthesis Pvt Ltd



Figure.3.4. Chemicals Used in the Extraction

3.4.4. DEVICE TABLE

Table 3.2. Devices Details

S.NO	DEVICE NAME	MODEL NO	MANUFACTURE BY
1.	UV	Uv-1900i	SHIMADZU DOUBLE BEAM, Japan
2.	FTIR	Cary 630	Agilent
3.	SEM	SEM-Sigma 300	Carel zeiss, German
4.	NANOPARTICLES	Nanopartica SZ-100V2	Horiba, Japan

CHAPTER-IV

4. METHODOLOGY

4.1. COLLECTION OF PLANT

The medicinal plant (leaves) of *Gymnema sylvestre* used in the study were collected from kothamarikuppam, Andhra Pradesh, India.

4.2. AUTHENTICATION OF PLANT

We got authenticated for our *Gymnema sylvestre* plant from siddha central research institute, Arumbakkam, Chennai



சித்த மருத்துவ மைய ஆராய்ச்சி நிலையம்
மத்திய சித்த மருத்துவ ஆராய்ச்சிக் குழுவும், ஆயுஷ் அமைச்சகம், இந்திய அரசு)

சித்த கेंद्रीय अनुसन्धान संस्थान
(सी.सी.आर.एस., चेन्नई, आयुष मंत्रालय, भारत सरकार),
अण्णा सरकारी अस्पताल परिसर, अरुम्बाक्कम, चेन्नई - 600106

SIDDHA CENTRAL RESEARCH INSTITUTE

(Central Council for Research in Siddha, Chennai, Ministry of AYUSH, Government of India)

Anna Govt. Hospital Campus, Arumbakkam, Chennai – 600106,

E-mail: crisiddha@gmail.com/stores.cris@gmail.com/Phone: 044-26214925, 26214809, Web: http://crisiddha.tn.nic.in

Form No. PCOG002-ACF

07.02.2023

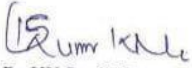
AUTHENTICATION CERTIFICATE FOR 445.121022201

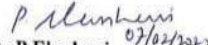
Certified that the sample submitted by Nithish Kumar S, B.Pharm, Sathyabama Institute of Science and Technology, Chennai -119 was identified as:

Code	Botanical Name	Part
G12102201S	<i>Gymnema sylvestre</i> (Retz.) R.Br. ex Sm.	Leaf



G12102201S


Dr KN Sunil Kumar
Research Officer and HOD
Department of Pharmacognosy
செ. கெ.அ. நுனில் குமார்/Dr. KN. Sunil Kumar
கருவிகளின் அறிஞர் (அ.அ.செய்தல்கள்)/RO/Pharmacognosy
சித்த கेंद्रीय अनुसन्धान संस्थान,
(सी.सी.आर.एस., चेन्नई, आयुष मंत्रालय, भारत सरकार)
अण्णा सरकारी अस्पताल परिसर, अरुम्बाक्कम, चेन्नई-600 106
SIDDHA CENTRAL RESEARCH INSTITUTE
(Central Council for Research in Siddha, Chennai-600106, Govt. of India)
Anna Govt. Hospital Campus, Arumbakkam, Chennai-600106


Dr P Elankani
Research officer (Siddha),
Sci-III/In charge

Dr. P. Elankani / தீ. பி. இலங்கனி
அலுவலக அதிகாரி (சித்த)/செய்தல்கள்-III)
Research officer(Siddha) (Sci-III) IC
சித்த கेंद्रीय अनुसन्धान संस्थान
(செந்திர மிக அலுவலகம் சித்த அலுவலகம், அரசு அலுவலகம்),
அரம்பாக்கம், சென்னை-600 106
SIDDHA CENTRAL RESEARCH INSTITUTE,
(Central council for Research in Siddha,
Ministry of Ayush, Govt. of India),
Arumbakkam, Chennai-600 106.

Figure 4.1. Authentication

4.3. EXTRACTION OF PLANT

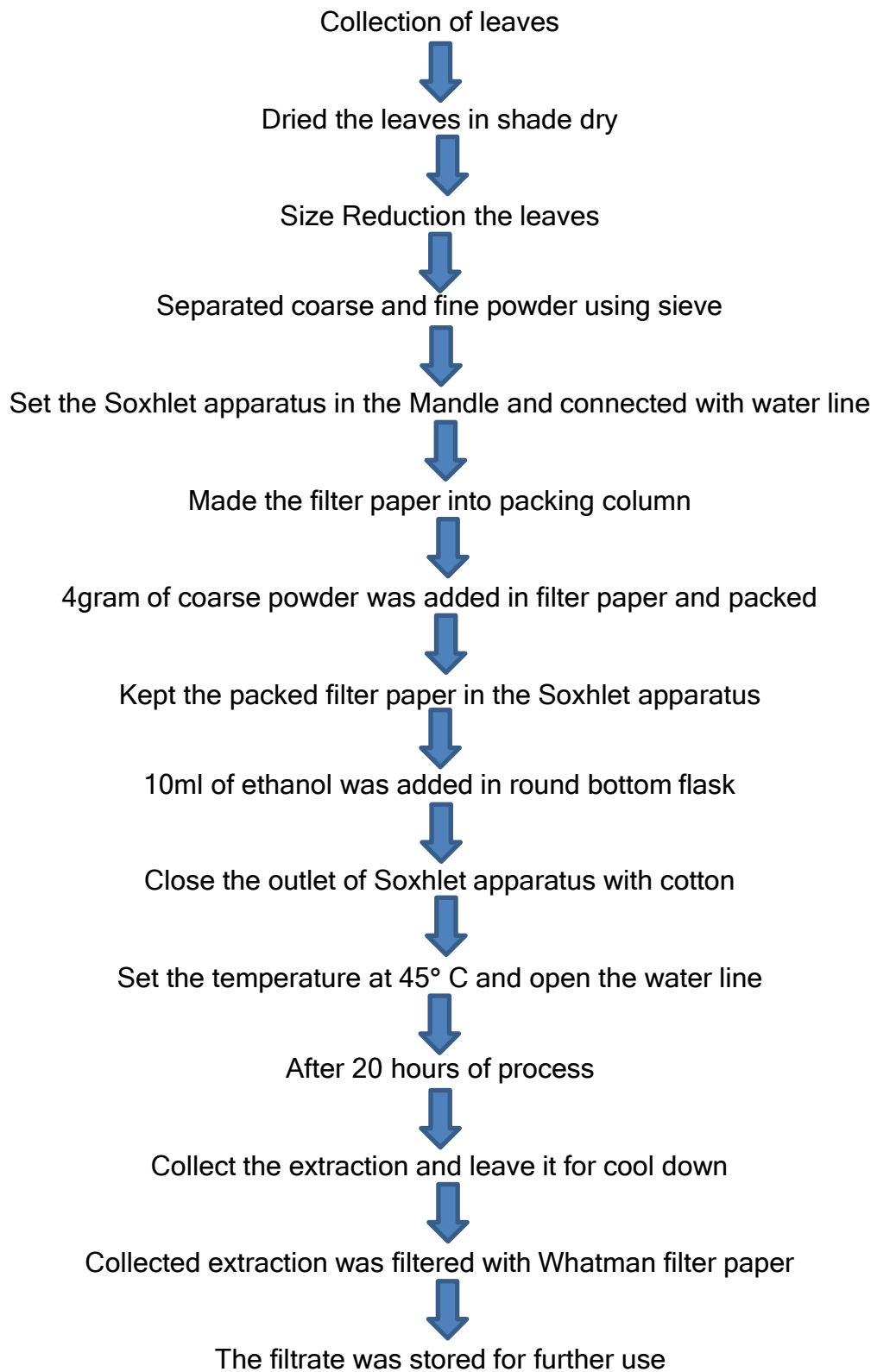


Figure.4.2. Soxhlet Apparatus

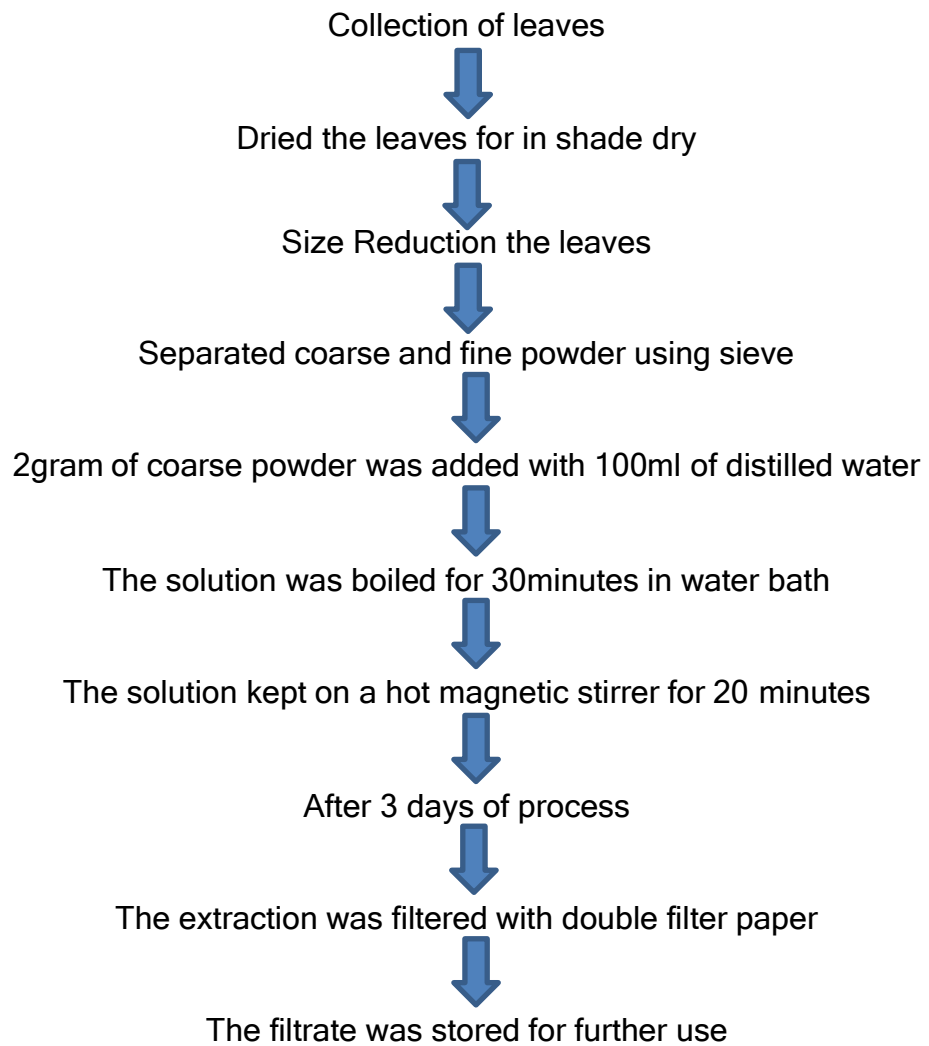


Figure 4.3. Cold maceration

4.3.1. *Ethanollic Extraction*



4.3.2. Aqueous Extraction



4.4. PHYTOCHEMICAL SCREENING

Table 4.1. Phytochemical Screening

S.NO	TEST NAME	PROCEDURE
1.	Test for Saponins: FOAM TEST	Small amount of extract was shaken in a test tube with a little quantity of water
2.	Test for Glycosides: LEGAL'S TEST	The extract of the substance was mixed with a little amount of anthrone on a watch glass. One drop of concentrated sulphuric acid was added made into a paste and warmed gently over water bath.
3.	Test for Alkaloids	Few ml of the extract was dissolved in concentrated HCL and filtered. A few drops of solution are poured into the centre of watch glass. Mayer reagent is added along the sides of the watch glass with the help of a glass rod.
4.	Test for Steroids Libermann's test	The plant extract was mixed with 3ml of acetic anhydride, warmed and cooled. Then few drops of concentrated sulphuric acid were added along the side of test tube.
5.	Test for Phenols	To the ethanolic extract, a few drops of 5 % ferric chloride solution was added.
6.	Test for Flavonoids SHINADO'S TEST	The substance was shaken with 5ml of alcohol and filtered. To the filtrate 1ml of lead acetate solution was added.

4.5. CHARACTERIZATION OF PLANT

4.5.1. Thin Layer Chromatography

TLC was performed on a pre-coated silica gel TLC plates grade F254 (E-Merck, Darmstadt, Germany) to determine the number of compounds present in the plant crude extract. A total of 5 l (10 mg/ml) of sample was spotted at 1 cm from the bottom of silica gel plates using capillary tubes. Different solvents at

various combinations and concentrations were used for metabolites profiling. Development of the chromatogram was done in closed tanks, in which the atmosphere has been saturated with eluent vapour by wetting a filter paper lining. The chromatogram was visualized under UV light (365 nm and 254 nm), white light and iodine vapour and sulpho-vanillin reagent spray. The R_f values of the compounds were calculated using the following formula.

$$R_f = \frac{\text{distance travelled by the compound}}{\text{distance travelled by the solvent front}}$$

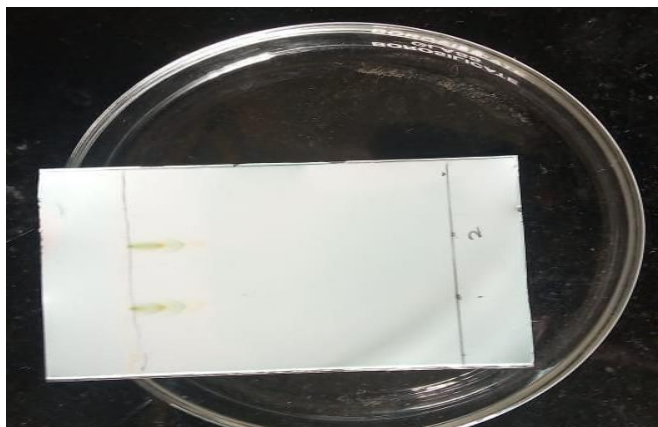


Figure.4.4. Thin Layer Chromatography

4.6. PREFORMULATION STUDIES

4.6.1. Compatibility studies of FTIR

4.6.1.a. *Gymnema sylvestre* Plant Powder

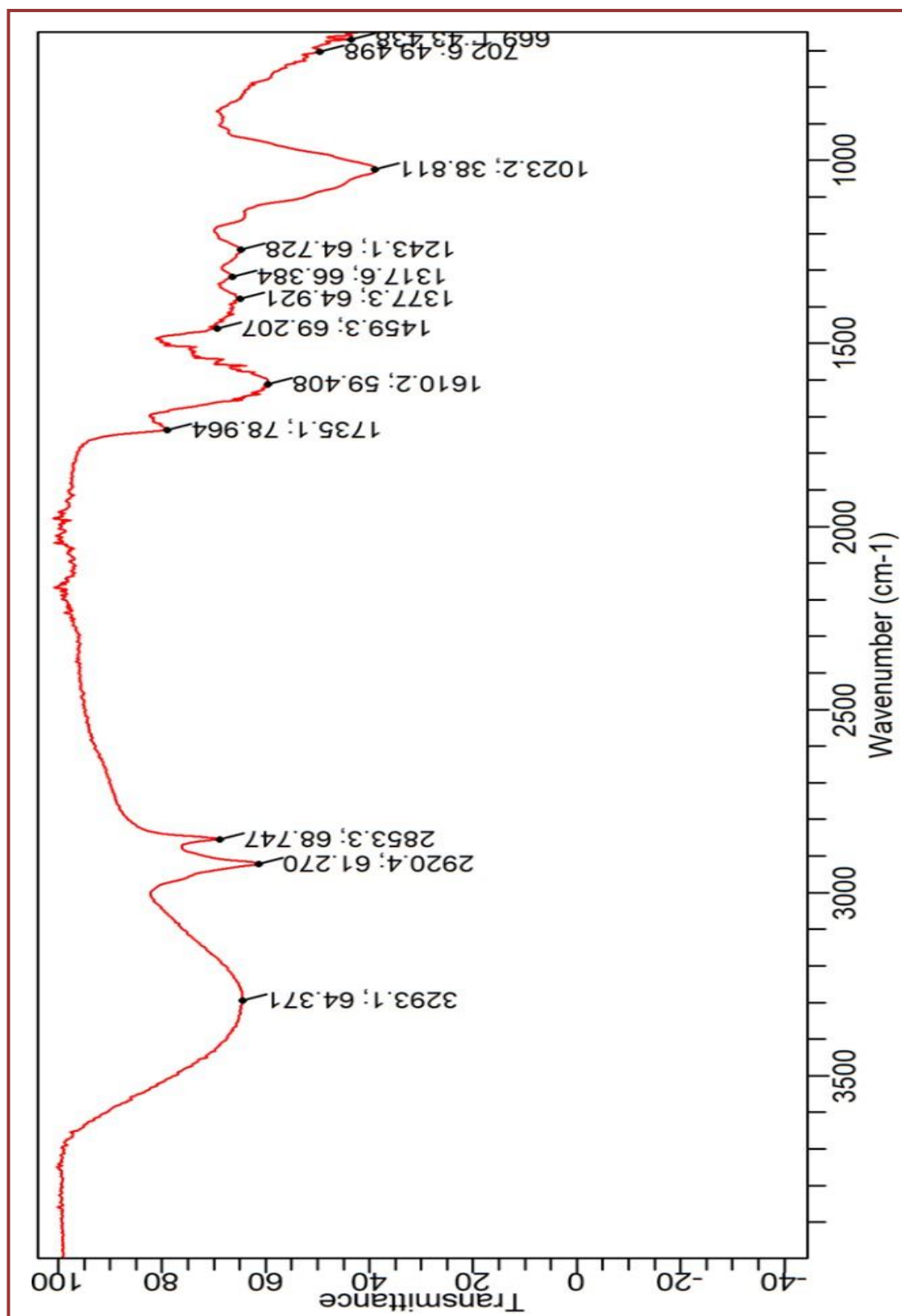


Figure 4.5.a. Pure Plant Powder of *Gymnema sylvestre*

4.6.1.b. Combination of *Gymnema sylvestre* Silver Nitrate

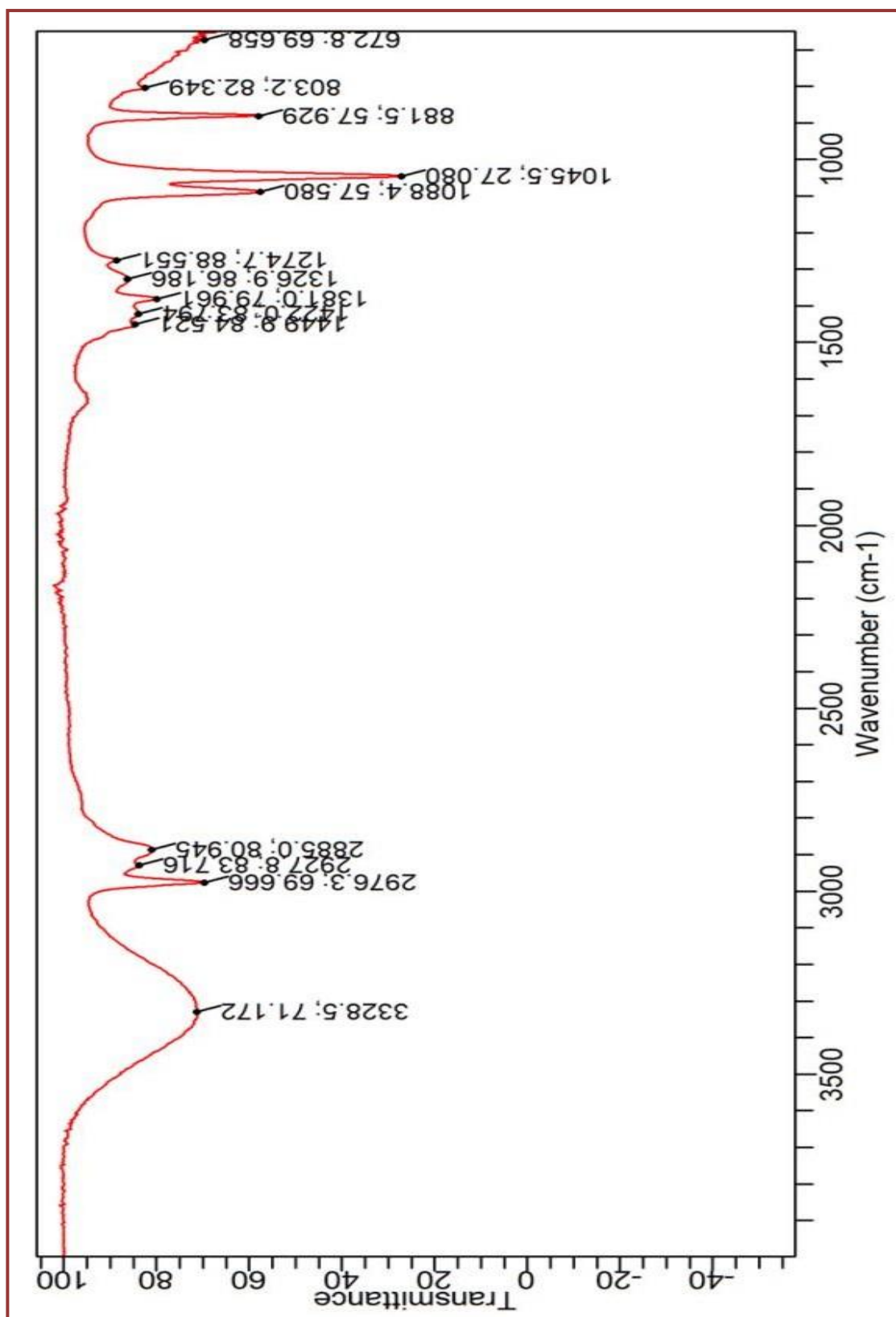


Figure 4.5.b. Combination of *Gymnema sylvestre* and Silver Nitrate

4.7. METHOD OF PREPARATION:

4.7.1. GREEN SYNTHESIS OF SILVER NANOPARTICLE PROCEDURE: Green chemistry is an emerging area that fosters the implementation of a set of principles intended to reduce the utilization and generation of chemical hazardous wastes. AgNPs are considered noble metalNPs (i.e., metals resistant to corrosion and oxidation) and have caught the attention due to their unique physicochemical and biological properties. The use of plant extracts is increasing in usefulness and is conceived as an environmentally and economically friendly alternative for the synthesis of AgNPs by several techniques, such as using AgNO_3 at room temperature within a few minutes to a couple of hours. Extracts can be obtained from multiple parts or products of the plant, such as leaves, bark, stem, shoots, seeds, latex, secondary metabolites, roots, twigs, peels, fruits, seedlings, essential oils, and and tissues. These phytochemicals are extracted and directly employed as reducing and stabilizing agents for the extracellular biosynthesis of metallic NPs, replacing potentially hazardous chemicals like sodium borohydride (NaBH_4).

- 2 ml of *Gymnema sylvestre* leaf extract (aqueous and alcoholic) respectively taken into a round bottom flask containing 60 ml of 1mM AgNO_3 solution.
- Using aluminum foil covered the round bottom flask and kept for incubation for 2-8 hours.
- All the reaction was carried out in a dark place.
- The progress of the reaction was monitored via color change.

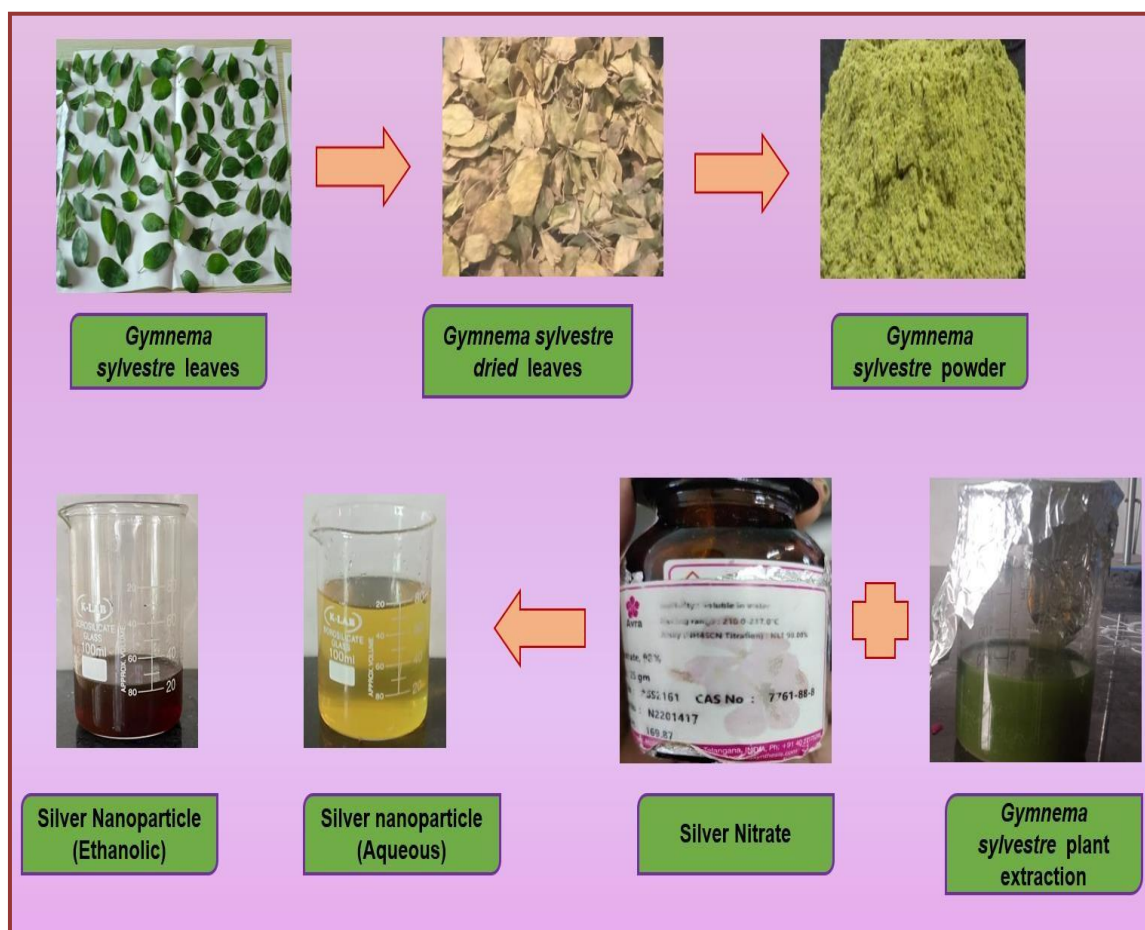


Figure 4.6. A schematic view of green synthesis of *Gymnema sylvestre*

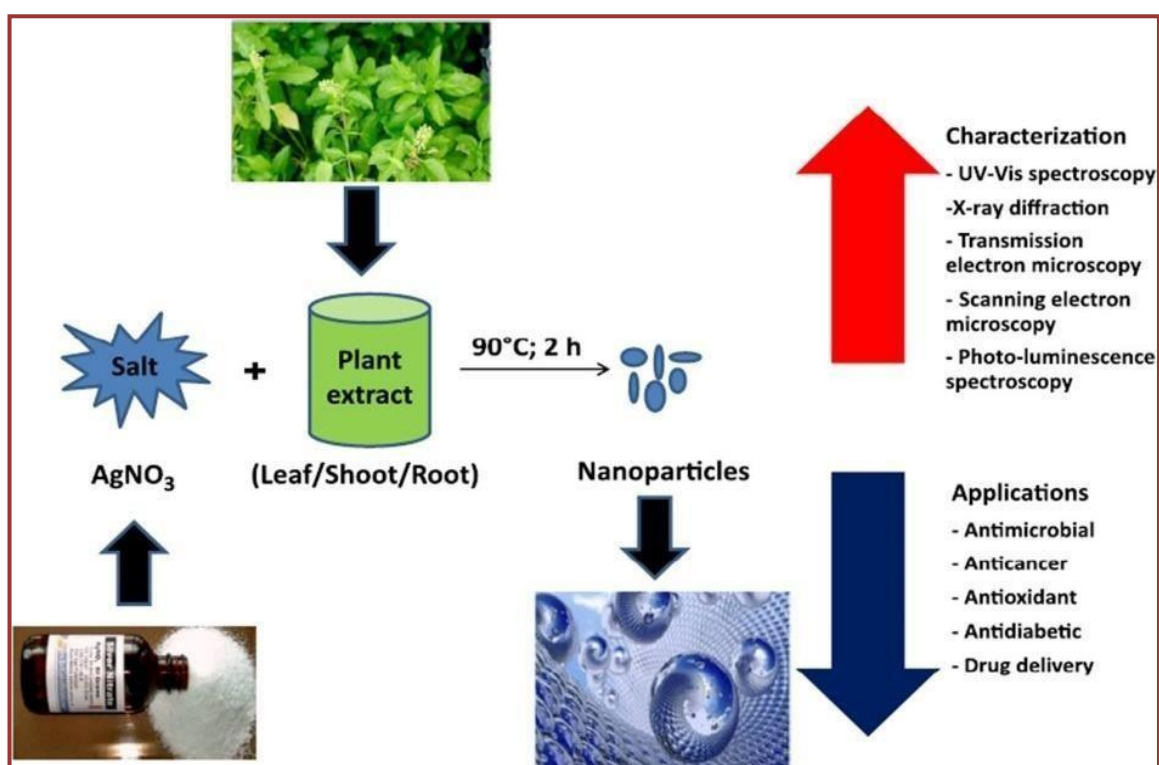


Figure 4.7. Characterization and Application of Nanoparticles

4.7.2. Applications of Plant-Derived NPs

NPs are currently in high demand commercially due to their wide range of applications in industries, electronics, environment, energy, and more particularly in biomedical fields. NPs, such as the most commonly known AgNPs, have been widely explored in this sector and are of tremendous interest for biological applications. In general, plant-derived green NPs are also less likely to cause severeside effects in humans when compared to chemically synthesized NPs, and have a great application potential with applications in a variety of areas, including but not limited to Nanomedicine and human health protection (antimicrobial, antiparasitic, antiproliferative, pro-apoptotic, pro- or anti-oxidative depending on the context, anti-inflammatory activities, etc.) Agriculture (precision farming with controlled release of agrochemicals, target-specific delivery of biomolecules, more efficient nutrients absorption, detection and control of plant diseases, etc.) Food science and technology (processing, storage, and packaging processes), in bioengineering (biocatalysts, photocatalysts, biosensors, etc.) Cosmetics (sunscreen, anti-aging, hair growth, bioactive compounds delivery, nano-emulsion, etc.)

4.8. CHARACTERIZATION OF THE SYNTHESIZED SILVER NANOPARTICLES

The Synthesized Silver Nanoparticles from *Gymnema sylvestre* Leaf Extract were characterized using the following spectroscopic techniques.

4.8.1. Ultra Violet – Visible Spectroscopy Characterization

Mixing of the *Gymnema sylvestre* Leaf Extract in the AgNO₃ solution makes the color change of the solution which is an indication of the formation of silver nanoparticles in the stabilized form. Confirmation of the silver nanoparticle generation is carried out via estimation of the UV spectra which indicated a typical silver surface Plasmon resonance at about 400 nm.

4.8.2. Particle size and zeta potential Analysis

The aqueous suspension of the synthesized nanoparticles was filtered through a 0.22 µm syringe driven filter unit and the size of the distributed nanoparticles were measured by using the principle of Dynamic Light Scattering (DLS).

4.8.3. FTIR analysis

Fourier transform infrared (FTIR) spectral measurements are commonly utilized for the estimation of the functional group. The FTIR analysis was utilized in the confirmation of the silver nanoparticles via estimation of the peak values which accounts for the various functional groups which are normally responsible for reducing and capping these silver nanoparticles.

4.8.4. Scanning Electron Microscopic (SEM) Analysis:

Surface morphology and size of biosynthesized AgNPs using both extracts of *Gymnema sylvestre* were studied by SEM images at higher resolution with different magnifications. The SEM images showed the existence of small spherical nanoparticles. Generally, it is known that AgNPs give a typical optical absorption peak in the range 2.1-3.7 KeV due to surface Plasmon resonance.

4.8.5. In-vitro Anti-diabetic Activity (α -Amylase Inhibition Activity)

Anti-diabetic activity of plant extract was studied via the α -Amylase inhibition technique. In vitro α -amylase inhibition was studied by DNS method. In brief, 500 μ L of the test compound (10, 30, 100 μ g/ml) was allowed to react with 500 μ L of 0.1M phosphate buffer pH 6.9 containing α amylase enzyme (Fungal distage (0.5%). After 10-minute incubation at 250°C, 500 μ L of 1% starch solution in 0.1M phosphate buffer pH6.8 was added and incubated at 250°C for 10 min. The same was performed for the controls where 500 μ L of the enzyme was replaced by the buffer. After incubation, 1000 μ L of dinitrosalicylic acid reagent was added to both control and test. They were kept in a boiling water bath for 10 min and cooled. The blank was prepared by replacing the enzyme with buffer for each set of concentrations of a test sample. The control was maintained without the addition of a sample that represented 100% enzyme activity. The absorbance was recorded at 540nm using a spectrophotometer and the percentage inhibition of α -amylase enzyme was calculated using the formula. Acarbose was used as the positive control.

$$\text{Inhibition (\%)} = \frac{\text{Abs 540 (control)} - \text{Abs 540 (extract)}}{\text{Abs 540 (control)}} \times 100$$

CHAPTER-V

RESULTS

5.1. PHYTOCHEMICAL SCREENING

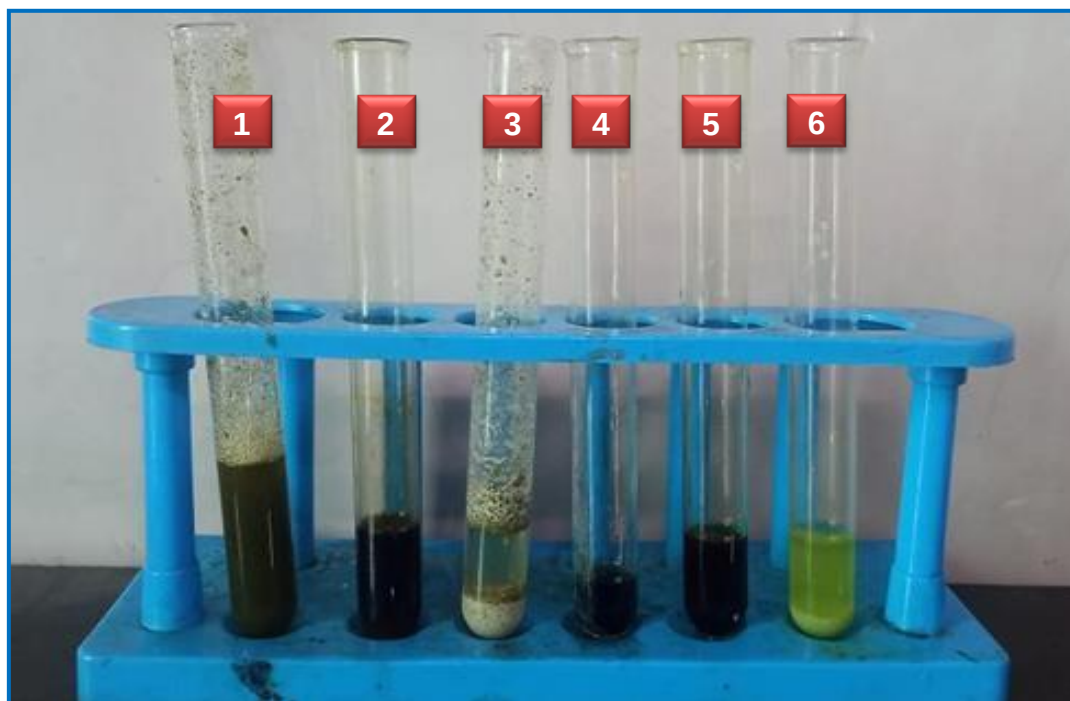


Figure 5.1. Phytochemical Screening

1. TEST FOR SAPONINS
2. TEST FOR GLYCOSIDES
3. TEST FOR ALKALOIDS
4. TEST FOR STEROIDS
5. TEST FOR TANNINS
6. TEST FOR FLAVONOIDS

Table.5.1. Phytochemical Screening

S.NO	TEST NAME	PROCEDURE	INTERFERENCE	RESULTS
1.	Test for Saponins: FOAM TEST	Small amount of extract was shaken in a test tube with a little quantity of water	Persistent foam was formed	Presence of saponins
2.	Test for Glycosides: LEGAL'S TEST	The extract of the substance was mixed with a little amount of anthrone on a watch glass. One drop of concentrated sulphuric acid was added made into a paste and warmed gently over water bath.	Appearance of Dark green color	Presence of glycosides
3.	Test for Alkaloids	Few ml of the extract was dissolved in concentrated HCL and filtered. A few drops of solution are poured into the centre of watch glass. Mayer reagent is added along the sides of the watch glass with the help of a glass rod.	Formation of a gelatinous white precipitate at the junction of two liquid.	Presence of alkaloids
4.	Test for Steroids Libermann's test	The plant extract was mixed with 3ml of acetic anhydride, warmed and cooled. Then few drops of concentrated sulphuric acid were added along the side of test tube.	Appearance of blue colour	Presence of Steroids

5.	Test for Phenols	To the ethanolic extract, a few drops of 5 % ferric chloride solution was added.	Deep black colour was formed	Presence of phenols
6.	Test for Flavonoids SHINADO'S TEST	The substance was shaken with 5ml of alcohol and filtered. To the filtrate 1ml of lead acetate solution was added.	Yellow precipitate was formed.	Presence of flavonoids

5.2. GREEN SYNTHESIS OF SILVER NANOPARTICLE

5.2.1. ETHANOLIC EXTRACTION

The green synthesis of silver nanoparticles was done by using Soxhlet apparatus method for alcoholic extract of *Gymnema sylvestre* leaf as a reducing agent. The confirmation of the formation of silver nanoparticles was done with the formation of the dark color after the reaction. The confirmation of the silver nanoparticles was carried out by using UV-visible characterization.



Figur.5. 2. a. Ethanolic Extraction

5.2.2. AQUEOUS EXTRACTION

The synthesis of silver nanoparticles was ascertained by the color change from faint yellow color to the dark brown color solution. After completion of the incubation period, the color of the mixture of silver nitrate solution and *Gymnema sylvestre* leaf extract was changed to dark brown color which ascertained the formation of the silver nanoparticles. The confirmation of the nanoparticles was carried out by using UV-

visible characterization. the silver surface was known for typical plasmon resonance at 400nm, the UV visible spectra of synthesized silver nanoparticles showed Plasmon resonance peak at 400nm.

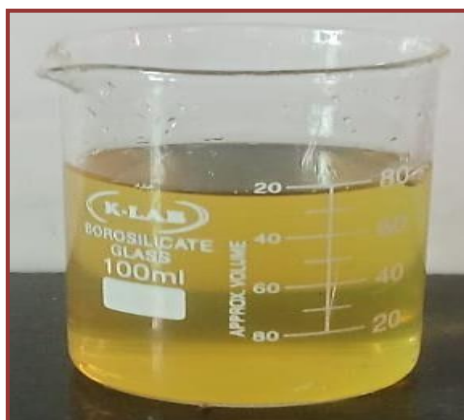


Figure.5.2. b. Aqueous Extraction

5.3. CHARACTERIZATION OF SILVER NANOPARTICLE

5.3.1. UV-VISIBLE SPECTRAL ANALYSIS

It is well known that silver nanoparticles exhibit a brown color, which results from the excitation of Plasmon vibrations from the surface of the silver nanoparticles. After the addition of a 1 mM silver nitrate solution to the aqueous extract, the color of the composition had changed to a dark brown color. The maximum absorbance peak is observed at 390nm for the *Gymnema sylvestre*. General observations suggest that the bio-reduction of Ag^+ (silver ions) to $\text{Ag}^{(0)}$ has been confirmed.

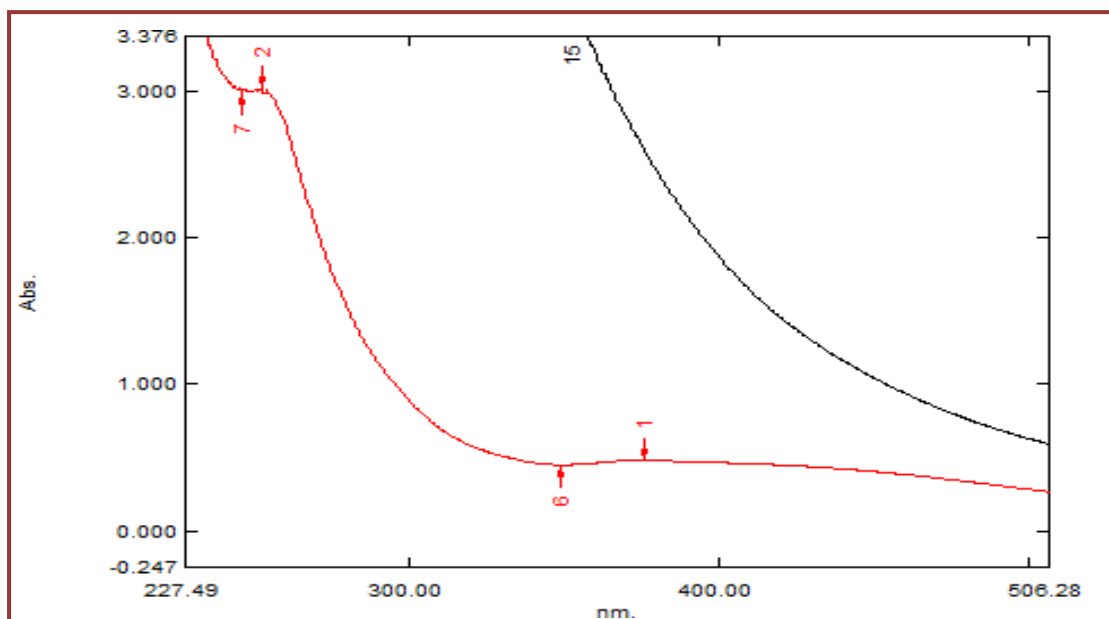


Figure 5.3. UV spectra of Extract

5.3.2. PARTICLE SIZE AND ZETA POTENTIAL ANALYSIS

a. Particle Size

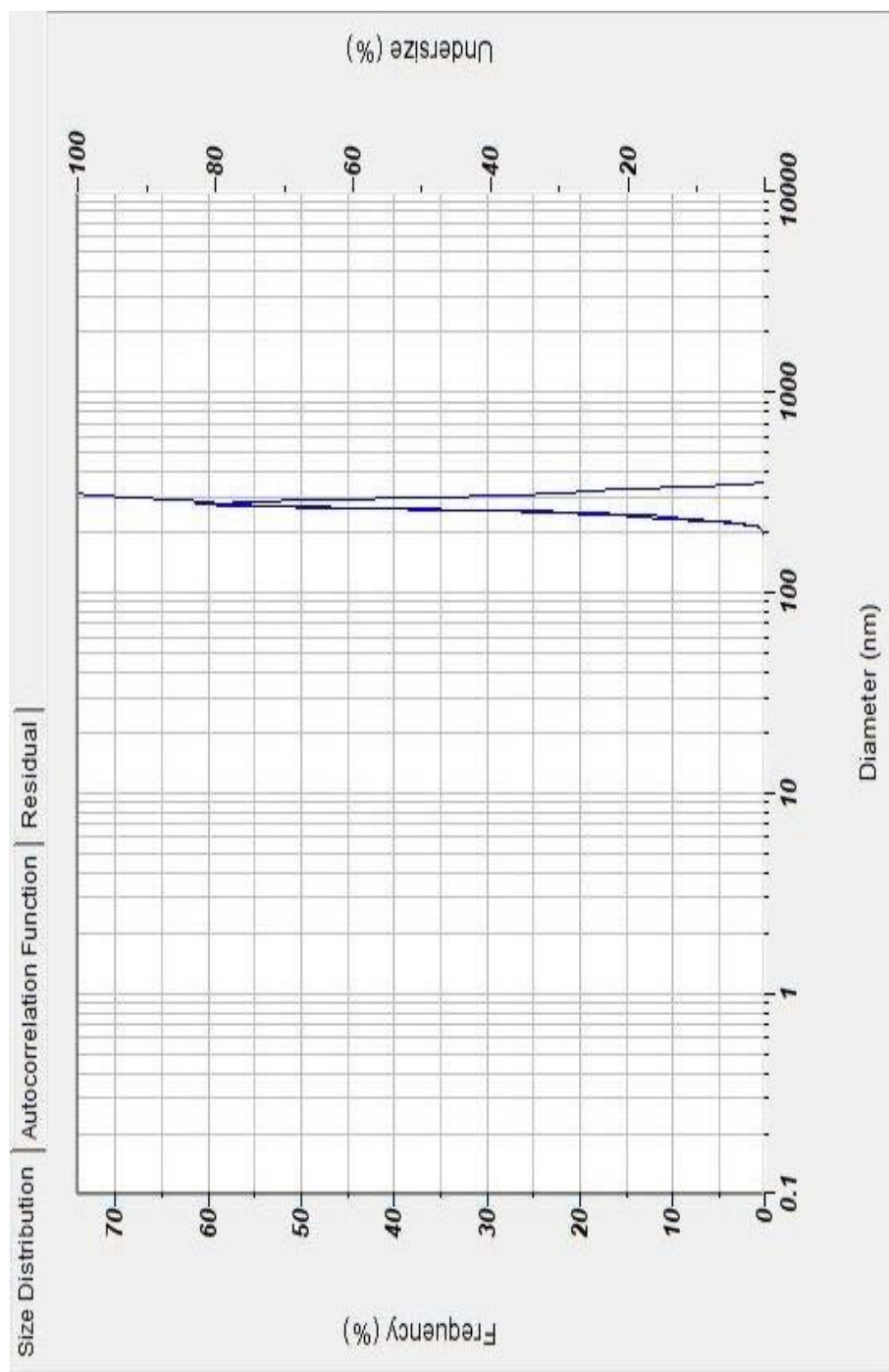


Figure 5.4.a. Particle size

Table:

Date	27 February 2023 15:36:29
Measurement Type	Particle size
Sample Name	AGNPS PLANT
Scattering Angle	173
Temperature of the holder	25.1°C
Dispersion Medium viscosity	0.893 mPa*s
Transmission intensity before Measurement	5728
Distribution form	Standard
Distribution form (Dispersity)	Monodisperse
Representation of result	Scattering light intensity
Count rate	1075kcps

CALCULATION RESULTS

Table: 5.2.a. Particle Size Peak Value

Peak no	S. p. Area Ratio	Mean	S. d	Mode
1	1.00	265.1nm	20.5nm	264.4nm
Total	1.00	265.1nm	20.5nm	264.4nm

CUMULANT OPERATIONS

Z-average: 9454.8 nm

PI: 2.537

b. Zeta Potential

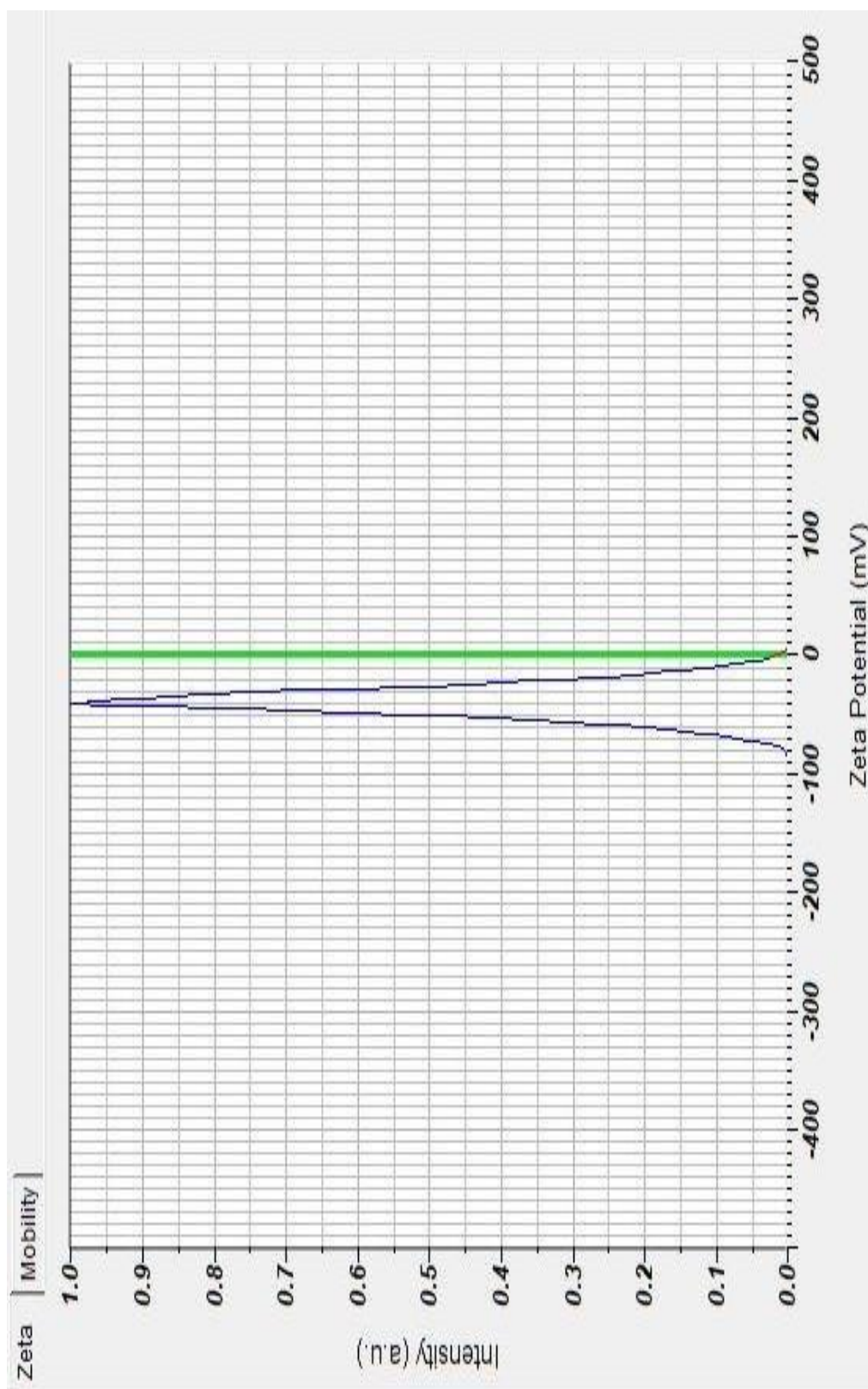


Figure 5.4.b. Zeta Potential

Date	28 February 2023 14:16:33
Measurement Type	Zeta potential
Sample name	Silver Nanoparticles
Temperature of the Holder	25.1 °C
Dispersion Medium Viscosity	0.893 mPa*s
Conductivity	0.333 mS/cm
Electrode Voltage	3.3 V

CALCULATION RESULTS

Table: 5.2.b. Zeta Potential peak Value

Peak no	Zeta Potential	Electrophoretic Mobility
1	-38.4 mV	-0.000298 cm ² /Vs
2	mV	 cm ² /Vs
3	mV	 cm ² /Vs

ZETA POTENTIAL (MEAN): -38.4Mv

ELECTROPHORETIC MOBILITY MEAN: -0.000298 cm²/Vs

5.3.3. FTIR ANALYSIS

5.3.3.a. *Gymnema sylvestre* (Powder)

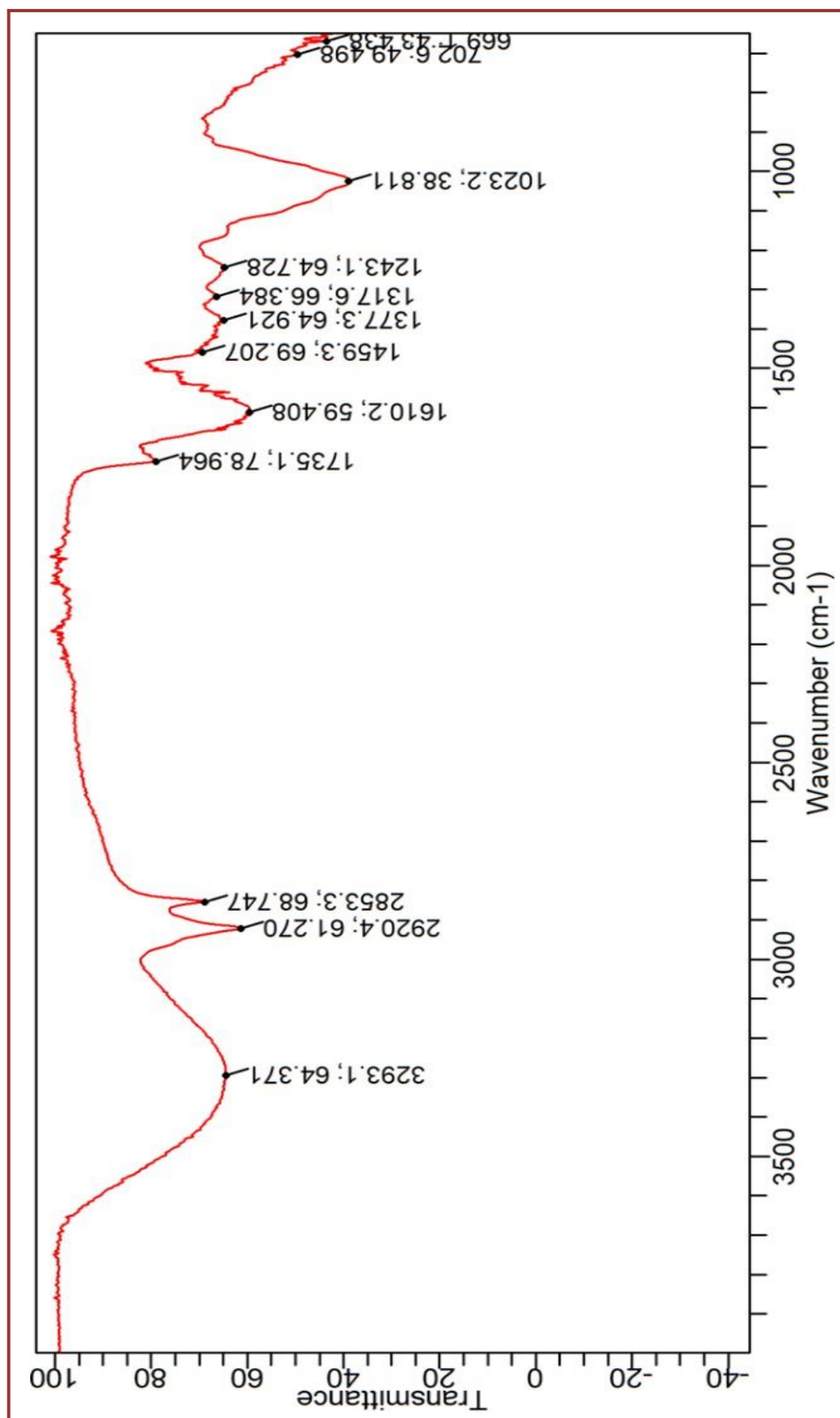


Figure 5.5.a. FTIR plant Powder

5.3.3.b. Green synthesized silver nanoparticle from *Gymnema sylvestre* plant extract (Liquid)

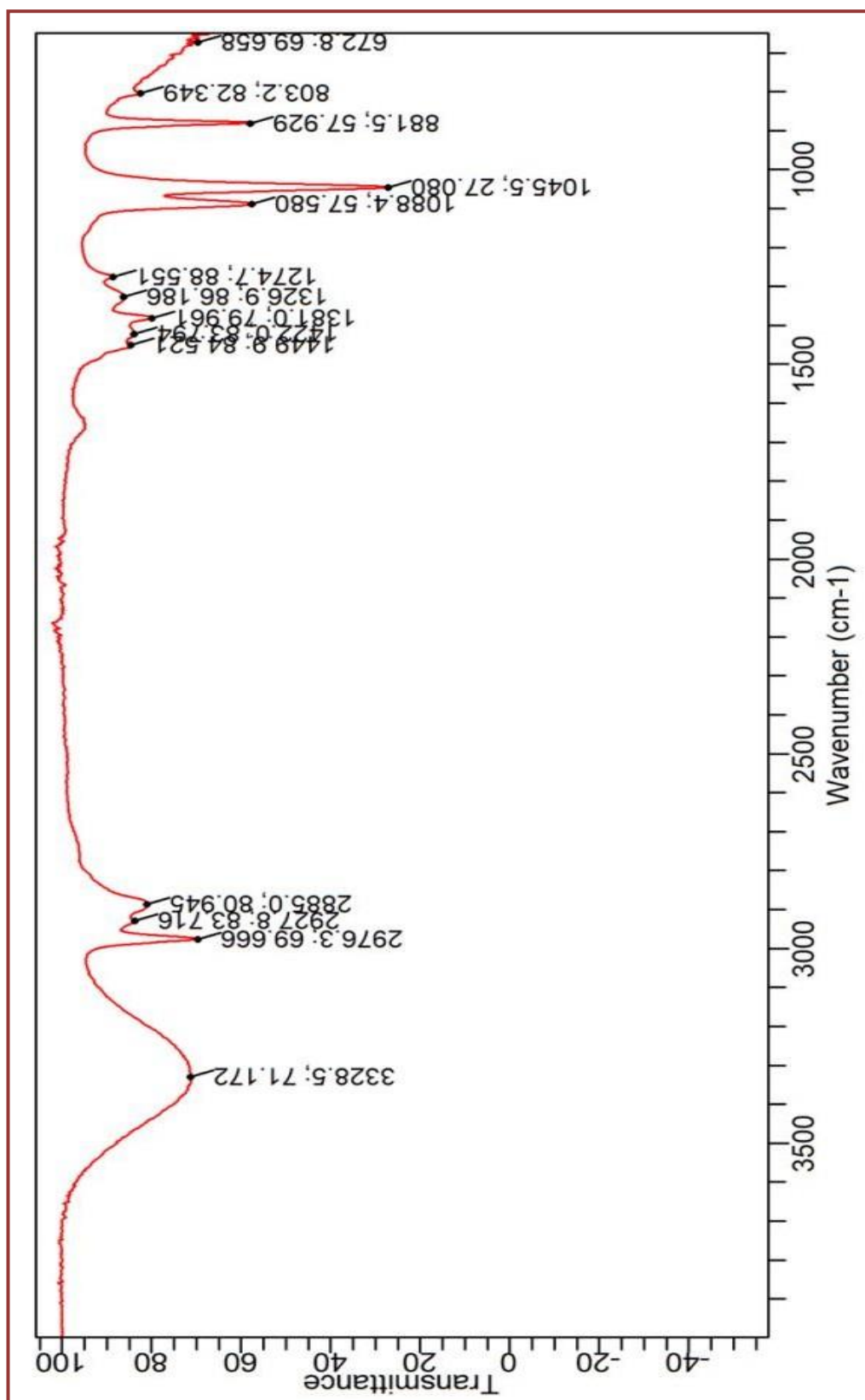


Figure 5.5.b. FTIR Silver Nanoparticles

shows absorption peaks at 3328.5, 2976.3, 2885, 1422, and 1045.5 cm^{-1} .

Table:5.3. Wave number (cm^{-1}) of dominate peak obtained from absorption spectra

S.NO	FUNCTIONAL GROUPS	COMPOUNDS	<i>Gymnema sylvestre</i> VALUES
1.	O-H stretching	primary and secondary amino proteins	3328.5 cm^{-1}
2.	C-H stretching	Alkenes	2976.3 cm^{-1}
3.	CH stretching	Alkanes	2885 cm^{-1}
4.	DC stretching	Aromatics	1422 cm^{-1}
5.	C-C stretching	Aliphatic Amines	1045.5 cm^{-1}

- i. The peak at 3328 cm^{-1} reveals the presence of OH stretching vibration, indicating the group of **primary and secondary amino proteins**,
- ii. 2976 cm^{-1} reveals the presence of CH stretching vibration, indicating the presence of **alkenes**,
- iii. 2885 cm^{-1} reveals the presence of stretching CH vibration, which indicates the presence of **alkanes**,
- iv. 1442 cm^{-1} reveals the presence of DC stretching vibration, which indicates the presence of **aromatics**,
- v. 1045.5 cm^{-1} reveals the presence of C-C vibration of stretching, indicates the presence of **aliphatic amines**,

The position of these bands was comparable to that of primary and secondary amino proteins, alkenes, alkanes, aromatics and aliphatic amines. Therefore, we can confirm that the nano tamponing of *Gymnema sylvestre* extract is responsible for the reduction and subsequent stabilization of Ag-NPs. *Gymnema sylvestre* extract contains the phytochemicals and secondary metabolites such as primary and secondary amino proteins, alkenes, alkanes, aromatics, aliphatic amines and Gymnemic acid derivative of gymnemagenin which contains the functional groups

of amines, aldehydes, carboxylic acids, and alcohols.

5.3.4. SCANNING ELECTRON MICROSCOPIC (SEM) ANALYSIS

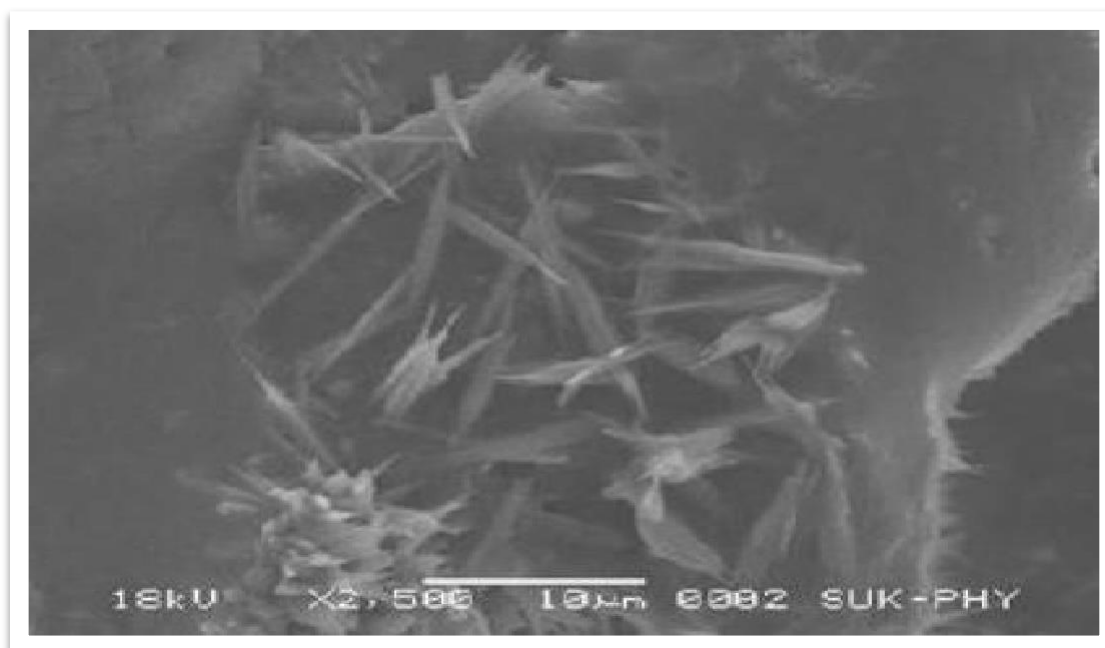


Figure 5.6.a. SEM image of Ethanolic Extract

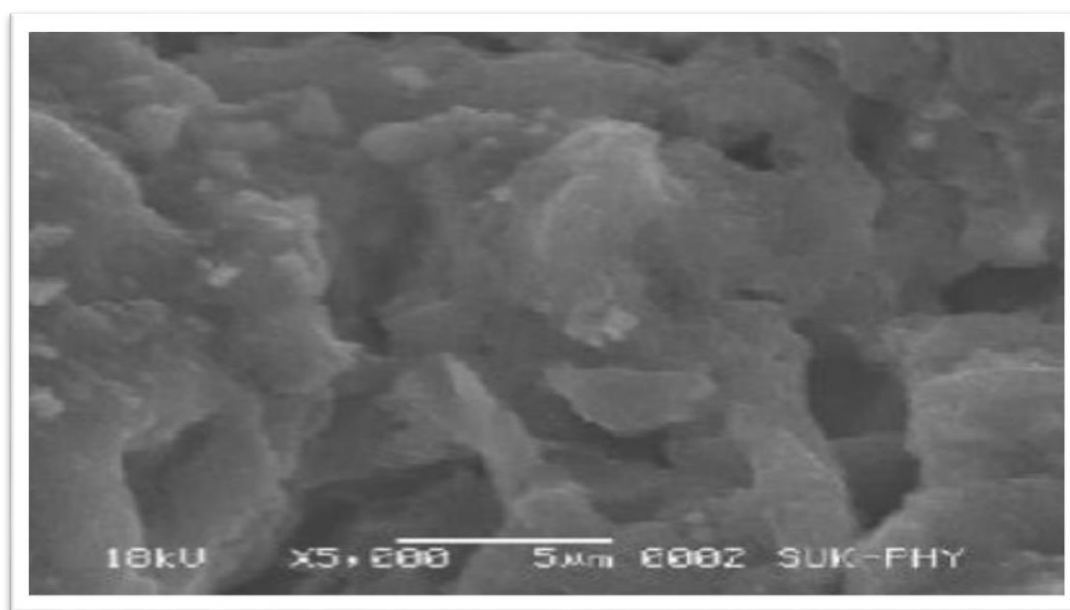


Figure 5.6.b. SEM image of Aqueous Extract

The SEM analysis indicated the morphology and size of the nanoparticles were dependent on the biomolecules which are present in the extract. The microscopic images indicated silver nanoparticles synthesized are having spherical shapes and the size of the particles is in the range of 70-100 nm. A number of the results reported the possible effects of the extracting solvent on the size and shape of the

silver nanoparticles which can be attributed to the concentration and type of the biomolecules which are present in the extract. The type and concentration of the biomolecules in the extract led to the formation of the hydrogen bond and electrostatic interaction between biomolecules and Ag as these molecules are capping the silver molecules.

5.3.5. *IN VITRO* ANTIDIABETIC POTENTIAL OF *GYMNEMA SYLVESTRE*

5.3.5.a. Alpha Amylase Inhibitory Activity

Alpha amylase is a most important digestive enzyme, present in pancreas which catalyzes the hydrolysis of the alpha-1, 4 glycosidic linkages of starch. This is responsible for most of starch digestion in humans. Inhibitors of α -amylase (hydrolyzing enzyme) have been useful as oral hypoglycemic drugs for the control of hyperglycemia especially in patients with Type 2 Diabetes Mellitus. This activity can be measured *in vitro* by hydrolysis of starch in presence of α -amylase enzyme. Plant extracts possess the inhibitory effect of these enzymes that may lead to reduction in post prandial hyperglycemia in diabetic condition. The *in vitro* anti-diabetic activity of, *Gymnema sylvestre* leaves in all the two extracts (ethanol & water) were determined by estimating the inhibitory activity of the enzyme α -amylase which involves the breakdown of starch to produce maltose and then to glucose by modified method of Bern Feld.

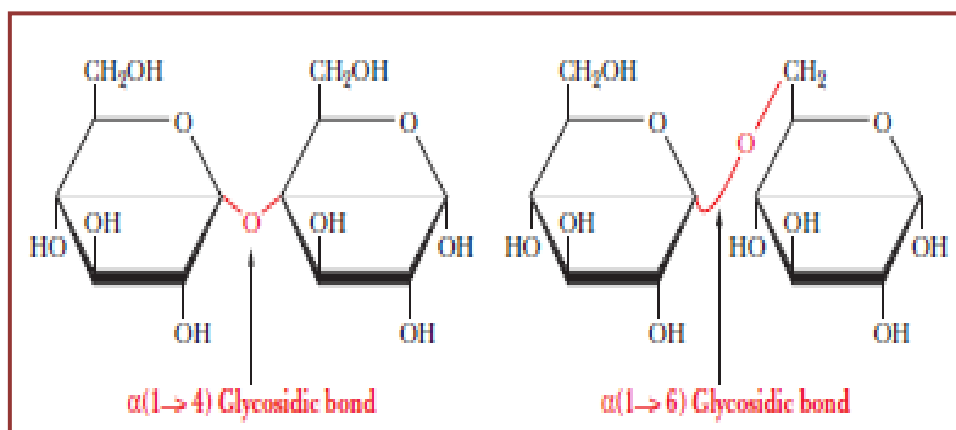


Figure 5.7. Structure of Glycosidic Bond

5.3.5.b. Experimental Procedure for α -Amylase Inhibition Assay

Anti-diabetic activity of plant extract was studied via the α -Amylase inhibition technique. In-vitro α -amylase inhibition was studied by DNS method. In brief, 500 μ L of the test compound (10, 20, 40, 60, 80 & 100 μ g/ml) was allowed to react with

500µL of 0.1M phosphate buffer pH 6.9 containing α-amylase enzyme (fungal diastase (0.5%). After 10-minute incubation at 250°C, 500 µL of 1% starch solution in 0.1M phosphate buffer pH6.8 was added and incubated at 250°C for 10 min.

The same was performed for the controls where 500µL of the enzyme was replaced by the buffer. After incubation, 1000µL of dinitro salicylic acid reagent was added to both control and test. They were kept in a boiling water bath for 10 min and cooled. The blank was prepared by replacing the enzyme with buffer for each set of concentrations of a test sample.

The control was maintained without the addition of a sample that represented 100% enzyme activity. The absorbance was recorded at 540nm using a spectrophotometer and the percentage inhibition of α-amylase enzyme was calculated using the formula. Acarbose was used as the positive control. The silver nanoparticles synthesized by *Gymnema sylvestre* indicated a higher level of α-amylase inhibitory activity than acarbose at all concentrations tested, as shown in Tables 5.4.a and 5.4.b Since the concentration of silver nanoparticles, the percentage of inhibition is also increased by dose- dependent manner. Similarly, silver nanoparticles synthesized by other medicinal plants have been exposed to the inhibitory activity of α-amylase. The current work focused on *Gymnema sylvestre* green synthesis of silver nanoparticles and the evaluation of their anti-diabetic effectiveness by blocking carbohydrate- hydrolyzing enzymes. By blocking carbohydrate metabolizing enzymes such α- amylase, synthesized silver nanoparticles provide the strongest anti-diabetic effect. As a result, synthesized nanoparticles could be a useful therapeutic treatment for diabetes management by blocking carbohydrate hydrolyzing enzymes.

α-amylase enzyme inhibition assay of silver nanoparticles developed from the Ethanolic Extract

Table 5.4.a. silver nanoparticles developed from the Ethanolic Extract

SAMPLE	CONCENTRATION	ABSORBANCE AT 540nm	PERCENTAGE INHIBITION
Standard Acarbose	100	0.21	58%
Ethanolic Extract of	10	0.30	37%
	40	0.25	45%

Silver Nanoparticles	100	0.21	51%
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α -amylase enzyme inhibition assay of silver nanoparticles developed from the Aqueous Extract

Table 5.4.b. silver nanoparticles developed from the Aqueous Extract

SAMPLE	CONCENTRATION	ABSORBANCE AT 540nm	PERCENTAGE INHIBITION
Standard Acarbose	100	0.21	58%
Aqueous Extract of Silver Nanoparticles	10	0.34	34%
	40	0.29	42%
	100	0.24	48%

The silver nanoparticles synthesized by *Gymnema sylvestre* indicated a higher-level of α -amylase inhibitory activity than acarbose at all concentrations tested, as shown in Tables 1 and 2. Since the concentration of silver nanoparticles, the percentage of inhibition is also increased by dose-dependent manner. Similarly, silver nanoparticles synthesized by other medicinal plants have been exposed to the inhibitory activity of α -amylase.

The current work focused on *Gymnema sylvestre* green synthesis of silver nanoparticles and the evaluation of their anti-diabetic effectiveness by blocking carbohydrate-hydrolyzing enzymes. By blocking carbohydrate metabolizing enzymes such α -amylase, synthesized silver nanoparticles provide the strongest anti-diabetic effect. As a result, synthesized nanoparticles could be a useful therapeutic treatment for diabetes management by blocking carbohydrate hydrolyzing enzymes.

Discussion:

The plant was selected and authenticated by the biologist. The extraction was prepared for both Ethanolic and Aqueous extract. Among these solvents ethanolic extract show better results. Phytochemical Screening was done for both the Ethanolic and Aqueous extract. It was concluded that these extracts have saponins,

glycosides, alkaloids, steroids, phenols and flavonoids. Synthesis of silver nanoparticle confirmed by UV the color change to reddish brown and faint yellow color. For silver nanoparticles the characterization are UV, FTIR, Particle size analysis and SEM analysis were done.

CHAPTER-VI

CONCLUSION

In conclusion, the silver nanoparticles were biologically synthesized from *Gymnema sylvestre* was affordable, harmless and environmentally benign.

The current work concentrated on the green synthesis of silver nanoparticles from *Gymnema sylvestre* and the evaluation of their antidiabetic efficacy by blocking carbohydrate hydrolyzing enzymes. The phytoconstituents in *Gymnema sylvestre* leaf extract caused biological reduction of AgNO_3 produced silver nanoparticles. Due to the reducing and capping nature of the bioactive phytochemicals, present in the aqueous extracts. Synthesized silver nanoparticles have the most potent anti-diabetic effect by inhibiting carbohydrate metabolizing enzymes such as alpha-amylase. Hence as a result, manufactured nanoparticles might be an effective therapeutic therapy for the control of diabetes management.