GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING *Pterocarpus marsupium* Roxb. AND ASSESSMENT OF ITS *IN-VITRO* ANTI DIABETIC ACTIVITY

A Dissertation submitted to
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OCTOBER 2017

CERTIFICATE

This is to certify that the M.Pharm dissertation entitled **"Green Synthesis and Characterization of Silver Nanoparticles using *Pterocarpus marsupium* Roxb. and Assessment of its *in-vitro* Anti Diabetic Activity"** being submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai was carried out by **Haritha.H (Reg. 261510152)** in the Department of Pharmaceutics, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, under my direct supervision, guidance and to my fullest satisfaction.

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CONTENTS

INTRODUCTION

Growth and applications of nano science and nanoparticles have a great footprint in recent years. There is increasing optimism that nanotechnology, as applied to medicine, will bring significant advances in the diagnosis and treatment of disease. Working with these extremely small structures is very much interesting due to its unique properties.

ADVANTAGES OF NANOPARTICLES

- Nanoparticles deliver drugs through oral, nasal, parenteral, intra-ocular routes etc.
- Ability to control and sustain the drug release before reaching the specific site of action
- Protects the drug from rapid degradation and maintains the drug at specific site
- Lower doses of drug shows high therapeutic efficacy and reduced side-effects
- Drugs can be incorporated without any chemical reaction resulting in the preservation of pharmacological activity of the drug **(Sriharitha* and Preethi. J, 2016)**

Based on their importance and unique features of nano particles, such as surface to mass ratio of nanoparticles is much larger than that of other particles, their quantum properties and their ability to adsorb and carry other compounds, nanoparticles (NPs) are more attractive. NPs have large surface area which is able to bind, adsorb and carry other compounds such as drugs, probes and proteins. Although the definition identifies nanoparticles as having dimensions below 0.1μm or 100 nm, especially in the area of drug delivery relatively large (size >100 nm) sufficient amount of drug loaded onto the particles.

NANOPARTICLES AND DRUG DELIVERY

Drug delivery and related pharmaceutical development in the context of nano medicine should be viewed as science and technology of nano meter scale complex systems (10–1000 nm), consisting of at least two components, one of them is a pharmaceutically active ingredient. The whole system leads to a special function related to treating, preventing or diagnosing diseases sometimes called smart-drugs .The primary goals for research of nano-bio-technologies in drug delivery include:

- More specific drug targeting and delivery,
- Reduction in toxicity while maintaining therapeutic effects,
- Greater safety and biocompatibility,
- Faster development of new safe medicines. **(Wim H De Jong and Paul JA Borm, 2008)**

SYNTHESIS OF SILVER NANOPARTICLES

Silver nanoparticles (AgNPs) are increasingly used due to their unique physical and chemical properties in various fields such as medical, food, health care, consumer, and industrial purposes. These include optical, electrical, and thermal, high electrical conductivity and biological properties. Due to their peculiar properties, they have been used for several applications.

Syntheses of silver nanoparticles are carried out using three methods physical, chemical, and biological methods **(Xi-Feng Zhang et.al, 2016).**

Physical methods:

Nanoparticles are prepared by evaporation-condensation using a tube furnace at atmospheric pressure. Conventional physical methods including spark discharging and pyrolysis were used for the synthesis of AgNPs. The advantages of physical methods are speed, radiation used as reducing agents and no hazardous chemicals involved, but the downsides are low yield and high energy

consumption, solvent contamination, and lack of uniform distribution.

Chemical methods:

This method uses water or organic solvents to prepare the silver nanoparticles. This process usually employs three main components, such **as metal precursors, reducing agents, and stabilizing/capping agents**. Basically, the reduction of silver salts involves two stages

(1) Nucleation

(2) Subsequent growth

In general, "top-down" and "bottom-up" are the two methods by which Nano particles can be prepared.

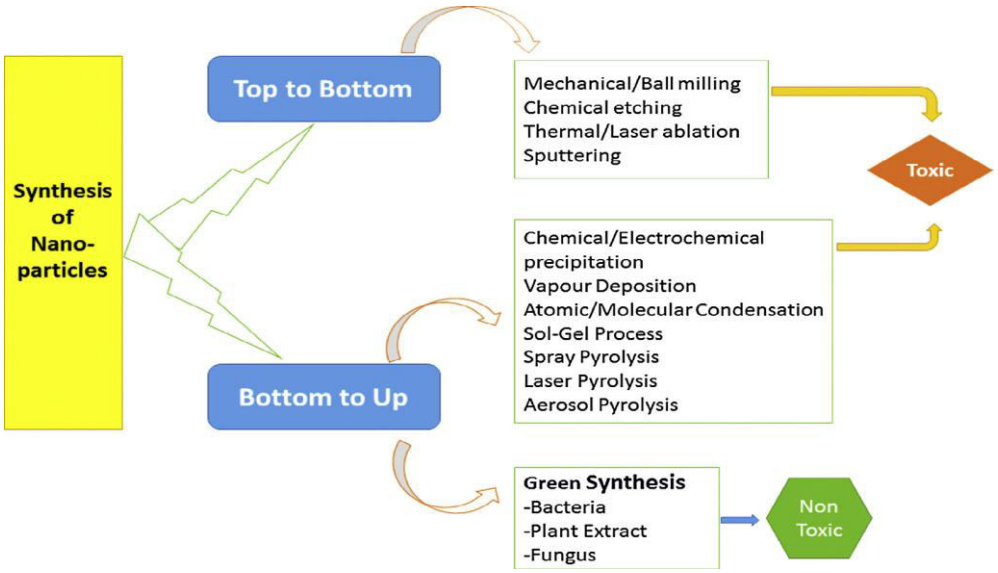


Figure 1 : Synthesis of Nanoparticles

In "top-down" method mechanical grinding of bulk metals with subsequent stabilization using colloidal protecting agents occurs. "Bottom-up"

methods include chemical reduction, electrochemical methods, and sono decomposition. High yield is the major advantage of chemical method compare to physical method. These methods are extremely expensive and the chemical reagents used are Thio-glycerol, 2-mercaptoethanol, citrate, borohydride are toxic and hazardous. And also the manufactured particles are not of expected purity, as their surfaces were found to be settled with chemicals. Preparation of silver nano particles with definite size along with the prevention of agglomeration is very difficult as well as in chemical method toxic and hazardous chemicals are excised out as byproducts **(Shakeel Ahmedet.al, 2016).**

Biological methods:

Biological methods have emerged to overcome all the problems related to physical and chemical methods. Silver nanoparticles are synthesised with definite size using different biological systems including bacteria, fungi, plant extracts, and small bio molecules like vitamins and amino acids as an alternative method to chemical methods not only for AgNPs, but also for the synthesis of several other nanoparticles

In recent years, the development of efficient green chemistry methods employing natural reducing, capping, and stabilizing agents to prepare silver nanoparticles with desired morphology and size have become a major focus of researchers. Biological methods can be used to synthesize silver nanoparticles without the use of any harsh, toxic and expensive chemical substances. The bio reduction of metal ions by combinations of bio molecules found in the extracts of certain organisms (*e.g.,* enzymes/proteins, amino acids, alkaloids, alcohol, polysaccharides, and vitamins) is eco friendly **(Iravani. S, 2013).**

Biologically-mediated synthesis of nanoparticles has been shown to be simple, cost effective, dependable, and environmentally friendly approaches and much attention has been given to the high yield production of AgNPs

ADVANTAGES OF SILVER NANOPARTICLES

- High scale production possibility.
- Long stability
- Freeze dried to form powder formulation (**Khalid Alaqad and Tawfik A Saleh, 2016**)

GREEN SYNTHESIS OF NANOPARTICLES

Green chemistry introduces new approach to the synthesis of nano particle for reducing threads to environment and health (**Bhosale et. al, 2014**). These new approaches are known as

- Environmentally Benign Chemistry
- Clean Chemistry
- Atom Economy
- Benign-by-design Chemistry

The basic idea of green synthesis is to protect the environment from pollution. Thus, the goal of green synthesis is to create better, safer chemicals while choosing the safest and most efficient ways to synthesize them and reduce wastes.

Benefits of green synthesis

- Economical
- Energy efficient
- Lower cost of production and regulation
- Lesser waste
- Fewer accidents
- Safer products
- Protects human health, environment, and compatible for pharmaceutical and other biomedical applications.

NEED FOR GREEN SYNTHESIS

Green synthesis of nanoparticles is a kind of bottom up approach where

the main reaction occurring is reduction/oxidation. The biosynthesis of nanoparticles by physical and chemical processes was costly. Often, chemical synthesis method leads to presence of some of the toxic chemical absorbed on the surface that may have adverse effect in the medical application. Green synthesis method did not use any chemicals for reducing metal ions. So the resultant product is more compatible and cost effective and no need to use high temperature, energy and pressure **(Shanker Kalakotla et.al, 2015).**

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING PLANT EXTRACT

The plant extract has been used in the production of silver nanoparticles had drawn attention due to its rapid, eco friendly, non pathogenic and providing a single step technique for the biosynthetic processes.

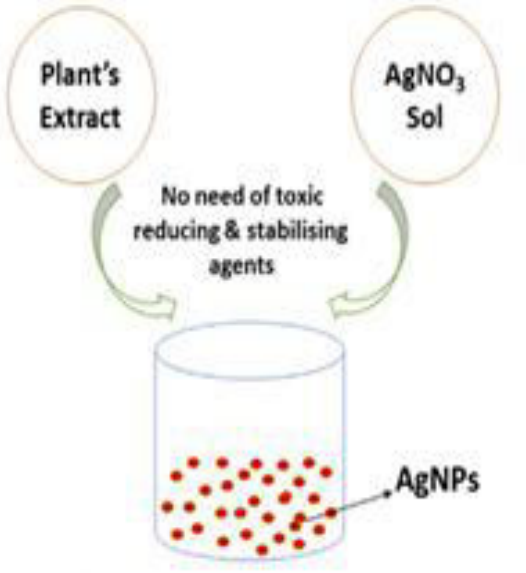


Figure 2: Synthesis of Silver Nanoparticles using plant extract

The bio molecules present in the plant extract such as proteins, aminoacids, enzyme, polysaccharides, alkaloids, tannins, phenolics, saponins, terpenoids, and vitamins are responsible for reduction and stabilization of silver

ions. A large number of plants are reported to facilitate silver nanoparticles syntheses they are **(Shakeel Ahmed et.al, 2016)**

Table: 1 Different plants used for the synthesis of silver nanoparticles

Plants	Plant part used	SIZE (nm)	Shape
Alternanthera dentate	Leaves	50- 100	Spherical
Acorus calamus	Rhizome	31.83	Spherical
Boerhaavia diffusa	Whole plant	25	Spherical
Tea extract	Leaves	20- 90	Spherical
Tribulus terrestris	Fruit	16-28	Spherical
Psoralea corylifolia	Seed	100-110	Spherical
Hevetia peruviana	Latex	10-30	Spherical
Aloe vera	Leaves	50-350	Spherical, Triangular
Nelumbo nucifera	Leaves	25- 80	Spherical, Triangular
Citrus sinensis	Peel	10-35	Spherical
Melia dubia	Leaves	35	Spherical
Centella asiatica	Leaves	30-50	Spherical
Abutilon indicum	Leaves	7-17	Spherical
Premna herbacea	Leaves	10-30	Spherical

ADVANTAGES OF HERBAL DRUG AS NANOPARTICLE (Anupam Kumar Sachan & Ankita Gupta, 2015).

- Herbal nanoparticles were used to target specific organs which improve

the drug delivery, safety, effectiveness, selectivity.

- High concentration drugs can be delivered to target site due to its unique size and loading capacity.
- Drug dissolution rate in blood increases because of small particle size and large surface area of nanoparticles.
- Decrease side effects.
- Widespread availability
- Lower cost
- Exhibits passive targeting to the disease site of action without the addition of any particular ligand moiety

DIABETES MELLITUS

Diabetes mellitus is a chronic metabolic disorder characterised by a high blood glucose concentration – hyperglycaemia caused by insulin deficiency, often combined with insulin resistance. Hyperglycaemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose by skeletal muscle with reduced glycogen synthesis. When the renal threshold for glucose reabsorption is exceeded, glucose spills over into the urine (glycosuria) and causes an osmotic diuresis (polyuria) which, in turn, results in dehydration, thirst and increased drinking (polydipsia) **(Rang and Dale).**

There are three main types of diabetes mellitus:

Diabetes mellitus type 1 (also known as **type 1 diabetes**) is a form of diabetes mellitus in which enough insulin is not produced. This results in high blood sugar levels in the body. The classical symptoms are frequent urination, increased thirst, increased hunger and weight loss. Additional symptoms may include blurry vision, tired, and poor healing. Symptoms typically develop over a short period of time.

The cause of type 1 diabetes is unknown. However, it is believed to

involve a combination of genetic and environmental factors. Risk factors include having a family member with the diabetic condition. The underlying mechanism involves an autoimmune destruction of the insulin-producing beta cells in the pancreas.

There is no known way to prevent type1 diabetes. Treatment with insulin is typically required for survival. Insulin therapy is usually given by injection just under the skin but can also be delivered by an insulin pump. A diabetic diet and exercise are an important part of management. Untreated, diabetes can cause many complications.

Diabetes mellitus type 2 (also known as **type 2 diabetes**) is a long term metabolic disorder that is characterized by high blood sugar, insulin resistance, and relative lack of insulin. Common symptoms include increased thirst, frequent urination, and unexplained weight loss. Symptoms may also include increased hunger, feeling tired, and sores that do not heal. Often symptoms come on slowly. Long-term complications from high blood sugar include heart disease, strokes, diabetic retinopathy which can result in blindness, kidney failure, and poor blood flow in the limbs which may lead to amputations. The sudden onset of hyperosmolar hyperglycemic state may occur; however, ketoacidosis is uncommon.

Type 2 diabetes primarily occurs as a result of obesity and lack of exercise. Some people are more genetically at risk than others. Type 2 diabetes makes up about 90% of cases of diabetes, with the other 10% due primarily to diabetes mellitus type 1and gestational diabetes. In diabetes mellitus type 1 there is a lower total level of insulin to control blood glucose, due to an autoimmune induced loss of insulin-producing beta cells in the pancreas. Diagnosis of diabetes is by blood tests such as fasting plasma glucose, oral glucose tolerance test, or glycated hemoglobin.

Gestational diabetes is a condition in which a woman without diabetes

develops high blood sugar levels during pregnancy. Gestational diabetes generally results in few symptoms; however, it does increase the risk of pre-eclampsia, depression, and requiring a Caesarean section. Babies born to mothers with poorly treated gestational diabetes are at increased risk of being too large, having low blood sugar after birth, and jaundice. If untreated, it can also result in a stillbirth. Long term, children are at higher risk of being overweight and developing type 2 diabetes.

Gestational diabetes is caused by not enough insulin in the setting of insulin resistance. Risk factors include being overweight, previously having gestational diabetes, a family history of type 2 diabetes, and having polycystic ovarian syndrome. Diagnosis is by blood tests. For those at normal risk screening is recommended between 24 and 28 weeks gestation. For those at high risk testing may occur at the first prenatal visit.

Prevention is by maintaining a healthy weight and exercising before pregnancy. Gestational diabetes is a treated with a diabetic diet, exercise, and possibly insulin injections. Most women are able to manage their blood sugar with a diet and exercise. Blood sugar testing among those who are affected is often recommended four times a day. Breastfeeding is recommended as soon as possible after birth **(https://en.wikipedia.org)**

Among these type 2 diabetes mellitus is the most common form of the disease for 90 – 95 % of the cases.

Herbal medicine has a long history to treat diseases Medicinal plants are very useful in a number of ways in combating diseases. In the last few years there has been an exponential growth in the use of herbal drugs. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. Herbs are also effective in prevention and treatment of the toxicity induced by

other drugs or toxins. Certain bioactive substances, flavanoids, antioxidants and phenolic substances are rich in medicinal plants which are regarded as a vital source in developing drugs for variety of diseases like Type II diabetes Mellitus, cancer, atherosclerosis and cardiovascular diseases. These medicinal plants are also used for the treatment of various infectious diseases **(Anamika Mubayi1 et.al, 2012).** According to World Health Organization (WHO) there are about 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are found in India. India is called as botanical garden of the world because of the rich herbal medicine resources. Very recently, two exhaustive reviews have been published based on global literature survey on 150 plants and 343 plants from different parts of the world.some plants like *Allium cepa* (Onion, piyaj), *Allium sativum* (garlic, lasun), *Syzygiumcumini* (Syn. *Eugenia jambolana*; (black plum; jamun), *Momordica charantia* (bitter gourd; karela) *Gymema sylvestre* (Gurmar), *Pterocarpus marsupium* (Vijay-) sar) etc. are well noticed by scientists as well as laymen, in recent years.

Pterocarpus marsupium **Roxb** is a medicinal plant widely distributed in India. *Pterocarpus marsupium* Roxb (pm) is also known as Indian kino, Bijasal, Vijaysar, belongs to the family Leguminosae. *Pterocarpus marsupium* Roxb is grown in central, western and southern regions of India. The heart wood, leaves, flowers and bark have useful medicial properties **(Maneesha Tiwari et .al, 2015).** The plant is traditionally used for various disease like diabetics, angina, cancer, and used as cardiotonic, brain tonic, etc **(Bala Chandra prathap et.al, 2012).**

The chemical constituents present in the plant are pterostilbene 4-5%, alkalois 0.4%, tannins, protein pentosan, pterosupin, pseudobaptigenin, liquiritigenin, isoliquiritigenin, garbanzol, 5-de-oxykaempferol, epicatechin, marsupol, carsupin, marsupinol. Aqueous extract of the heartwood of PM contains five new flavanoids c-glucosides namely 6- hydroxyl-2-(4-hydroxybenzyl)-benzo-

furan-7-c-a-D- glucopyranoside, 3-(a-methoxy-4-hydroxybenzylidene)-6-hydroxybenzo-2(3H)-furanonE-7-C-a-D-glucopyranoside, 2-glucopyranoside, 8-(C-a-D-glucopyranosyl)-7,3,4-trihydroxyflavone and 1,2-bis (2,4-dihydroxy, 3-C-glucopyranosy) – ethanedione and two known compounds C-a-D-glucopyranosyl-2, 6-dihydroxyl benzene and sesquiterpene were isolated **(Maneesha Tiwari et al, 2015)**

Figure 3 *Pterocarpus marsupium roxb* tree

Hypoglycaemic activity of *Pterocarpus marsupium* Roxb bark and wood is popular in traditional medicine. Water kept overnight in the tumblers of pterocarpus marsupium heart wood is used to redeuce blood glucose level. Many studies were conducted with pterocarpus marsupium for diabetic activity and found the following effects.

- Beta cell regeneration
- Inhibition of amylase and glucosidase
- Reduce TNF- α
- Increase peroxisome proliferator activator receptors
- Insulin release
- Antiglycation effects

Beta cell regeneration:

The chemical constituent epicatechin is effective in beta cell regeneration. Studies found that epicatechin given for 4 to 5 days resulted in regeneration of β cell population which are previously necrosed due to alloxan in alloxan induced diabetic rats.

Inhibition of amylase and glucosidase:

α-Amylase and α-glucosidase are principle enzymes involved in digesting dietary carbohydrates into absorbable molecules. Inhibition of these enzymes helps to reduce the rise in blood sugar level

Reduce TNF-α:

Cytokines are small soluble peptides release by cells of immune system. The inflammatory cytokine tumor necrosis factor TNF- α is elevated in type 2 diabetes and it is known to cause insulin resistance. Reports suggests that aqueous extract of PM heart wood (100 and 200 mg/kg/day) given orally for 4 weeks decreased TNF- α to the normal levels in diabetic rats.

Increase peroxisome proliferator activator receptors:

PPARs are nuclear receptors that regulate metabolic events of cells. It has been revealed that pterostilbene (100 μm) activates PPAR- α activity by 8 fold.

Antiglycation effects:

Excess glucose is converted to glycogen with the action of insulin resulting in lowering blood glucose. Aqueous extract of PM (1g/kg/day) altered the glycogen content occurred in insulin dependent tissues such as liver and skeletal muscle of diabetic rats.

Insulin release:

The studies revealed that anti diabetic constituents in the aqueous extract of PM heartwood given stimulated the insulin secretion from the mouse pancreas in a concentration manner *in vitro*. Epicatechin in the water extract of PM increased the cAMP content of the islets of pancreas in rats with increase in insulin, release and conversion of pro insulin to insulin **(H.K.I. Perera, 2016).**

SIGNIFICANCE OF SILVER NANOPARTICLES (Nicholaos Kakouros et.al, 2011).

Silver is the basic element which can exists in three different forms Ag^0, Ag^{2+}, Ag^{3+}. Pure silver has the highest electrical, optical and thermal conductivity and has lowest contact resistance.

Patients with diabetes mellitus have an increased prevalence of vascular disease. Pathologic thrombosis associated with atherosclerotic plaque rupture is a major cause of morbidity and mortality. Platelets are intimately involved in the initiation and propagation of thrombosis. Evidence suggests that platelets from patients with type 2 diabetes have increased reactivity and baseline activation compared to healthy controls. This is associated with biochemical factors such as hyperglycemia, hyperlipidemia, insulin resistance. So anti platelet should be prescribed to DM patients at >10% 10-year risk of cardiovascular disease and considered in patients at intermediate (5%–10%) risk according to the recent position statement of the American Diabetes Association. Silver nanoparticles inhibit thrombin-induced platelet aggregation in a concentration-dependent manner. Clumping of blood platelets is often a serious problem for diabetic patients which may lead to heart problem **(Nicholaos Kakouros et.al, 2011).**

Silver nanoparticles inhibit the aggregation of platelets obtained from patients with noninsulin-dependent diabetes mellitus by nearly 50%. Moreover, intravenous administration of nanoparticles (218 mg/kg body weight) in two different mice strains led to significant inhibition of platelet aggregation in mouse

whole blood (studied by electronic impedance) in a dose-dependent manner. Studies found that aggregation was significantly reduced in platelets exposed to silver nanoparticles **(Siddhartha Shrivastava et.al, 2009).**

REVIEW OF LITERATURE

Halawani (2017) The researcher developed an ecofriendly and rapid method for the first time to synthesize silver nanoparticles using *Zizyphus spina christi L* aqueous leaves extract (ZSE) and their antibacterial properties. The extract was found to have the potential to form silver nanoparticles at room temperature within few minutes. The green synthesized silver nanoparticles were characterized by using different techniques. The UV-visible spectrum of the solution containing AgNPs showed a peak at 414 nm corresponding to the plasmon absorbance of silver nanoparticles. The transmission electron microscopy (TEM) showed that the formed particles were hexagonal in shape with appreciable Nano size ranging from 21.5 to 59.67 nm. Fourier Transform Infrared Spectroscopy analysis (FTIR) of biosynthesized AgNPs confirmed the role of ZSE as reducing and capping agent of Ag+ ions to AgNPs, and X-Ray Diffraction patterns (XRD) showed that they could be indexed as face-centered cubic structure of silver. Antibacterial activity of AgNPs was determined by well diffusion and micro plate assay methods, showing maximum inhibition zones of 24 mm, 23 mm, 15 mm and 17 mm against *Staphylococcus aureus*, *Acinetobacter sp , Pseudomonas aeruginosa and Escherichia coli* respectively.

Akkiraju et.al (2017) Green synthesis of nanoparticles was done from *Punica granatum L.* by using Silver nitrate (AgNO3) from the exocarp, mesocarp and the juice of Aarakta, a local variety of Maharashtra, Silver nanoparticles were synthesised by using the peel (exocarp and mesocarp) and the seed juice of pomegranates. The procedures for development of stable silver nanoparticles were characterized. The synthesis of nanoparticles was confirmed through UV-Visible spectroscopy. The interaction between nanoparticles with functional groups was confirmed by using FT-IR and PCA was performed. The antimicrobial activity of these Pomegranates synthesised silver nanoparticles was evaluated against different microorganisms viz. *Escherichia coli, Pseudomonas aeruginosa and*

Proteus vulgaris. Out of the three microorganisms, Pseudomonas aeruginosa was found to be sensitive for the extractions of pomegranate and formed the antibiotic zones nearer to standard antibiotic, Ampicillin at a quantity of 40 µl per sample. From this study it is clear that, pomegranate nanoparticles can be used as potential antibacterial agents in future therapeutics. And the study proved that, the pomegranate exocarp, mesocarp and seed juice are capable of producing nanoparticles in association with silver nitrate. These nanoparticles showed a tremendous antimicrobial activity against *E. coli*, *P. aeruginosa* and *P. vulgaris*. However, their antimicrobial activities were comparatively less with the standard antibiotic Ampicillin. This study gives a path for those aspirants who are willing to work with pomegranate nanoparticles and their antimicrobial activities.

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) results showed that AgNPs had MIC, MBC of 45, 57 µg/mL, 49, 61µg/mL, 63, 90 µg/mL and 59, 82 µg/mL against *S. aureus* , *Acinetobacter sp*., *P. aeruginosa* and *E. coli* respectively. Furthermore, the green synthesized AgNPs were loaded on band-aids and screened for antibacterial activity. The AgNPs loaded on band-aids exhibited strong antibacterial effect against multi drug resistant bacteria. These nanoparticles could be used for treating wounds and preparing wound dressing. Such researches are crucial in the demonstration of therapeutic importance of silver nanoparticles in medical application.

Anandalakshmi K et.al (2016) made a research on characterization of silver nanoparticles by green synthesis method using *Pedalium murex* leaf extract and their antibacterial activity. An aqueous extract of fresh leaves of *Pedalium murex* was used for the synthesis of silver (Ag) nanoparticles. Different biological methods are gaining recognition for the production of silver nanoparticles (AgNPs) due to their multiple applications. The use of plants in the green synthesis of nanoparticles emerges as a cost-effective and eco-friendly approach. Characterization of nanoparticles was done using different methods, which

include; ultraviolet–visible spectroscopy (UV–Vis), Fourier transform infrared (FTIR), powder X-ray diffraction (XRD), field emission scanning electron microscope (FESEM), energy dispersive X-ray analysis (EDAX), fluorescence emission spectroscopy, transmission electron microscope (TEM), dynamic light scattering (DLS), zeta potential and antibacterial activity. UV–visible spectrum of the aqueous medium containing silver nanoparticles showed absorption peak at around 430 nm. Fourier transform infrared spectra had shown that the biomolecule compounds were responsible for the reduction and capping material of silver nanoparticles. XRD study showed the particles to be crystalline in nature, with a face-centered cubic (fcc) structure. The size and stability were detected using DLS and zeta potential analysis. The biosynthesized AgNPs were found to have a pronounced antibacterial activity against *E. coli*, *K. pneumonia, M. flavus, P. aeruginosa, B. subtilis, B. pumilus and S. aureus.* In this present study, proteins and flavonoids in the *Pedalium murex* leaf extract play an important role in the formation of silver nanoparticles.

Bheemesh vangalapati et.al (2016), The Study was designed to assess the *Pterocarpus marsupium* heartwood aqueous extract effect on diabetes induced cognitive impairment. Diabetes mellitus was induced by Streptozotocin (STZ) & Nicotinamide (NA) intraperitoneal route injection. Animals were divided into 7 groups for comparing the activity of *Pterocarpus marsupium* at two doses 250 mg/kg & 500 mg/kg body weight against standard (Glibenclamide) & controls groups. Rats having blood glucose above 250 mg/dL were considered as diabetic. Learning & memory was tested using Morris water maze test. Time taken to reach the platform (escape latencies) by animals was noted from day 1 to 8 and probe trial was conducted on day 9 to record the time spent in the different quadrants.

Blood glucose levels were significantly ($p<0.001$) reduced in plant treated and glibenclamide groups when compared to diabetic controls. Also both the treated groups had decreased escape latencies in learning phase. During probe

trial, test and standard treated groups spent significantly more time in target quadrant with less entries into other quadrants compared to untreated diabetic controls.

Thus the study was concluded that aqueous extract of *Pterocarpus marsupium* heartwood (both doses i.e. 250 mg & 500 mg) along with its blood glucose lowering effects in diabetic rats, displayed beneficial effects in diabetes induced cognitive impairment by restoring the learning & memory activities.

Kirti Barde et.al (2016), In this study the diabetic potential is investigated through the use of different chemicals and an exotic plant. This study belongs to the use of typical Leguminosae or Fabaceae family medicinal plant *Pterocarpus marsupium Roxb*. This is mainly impacted to elicit anti diabetic activity. Increment in the blood glucose level is known as diabetic because Islets of Langerhans are not able to produce sufficient insulin which levels the blood glucose barrier.

Here different *In vitro* anti diabetic study was carried out such as In vitro assay for α-amyalse inhibition, Inhibition assay for α- glucosidase activity, Glucose uptake in Yeast cells. The results were found be as follows in case of α-amyalse inhibition study the IC_{50} value for *pterocarpus marsupium* were found to be 147.23±0.15, α-Glucosidase inhibition IC50 value for *pterocarpus marsupium* is 169.42±0.28 .The comparative % increase in glucose uptake by yeast cell due to the effect of ethanolic extract of *P. marsupium* and reference standard drug metronidazole at lowest concentration 50 µg/ml were 57.82±1.17 and 61.52±0.32 and for higher concentration 2000µg/ml were found to be 76.14±0.78 and 67.21±1.12.

In this study alcoholic extract of *Pterocarpus marsupium* elicit more dynamic activity in compensation with aqueous extract. So the plant is favourable to investigate with the levels of therapy produced by marketed drug metronidazole in this case the levels of affectivity of anti diabetic action produced by alcoholic

extracts of *Pterocarpus marsupium* is compared with the drug Metronidazole to investigate acquired action.

Koyagura narendar et.al (2016) made a comparison on the effect of *pterocarpus marsupium* with pioglitazone in dexamethasone-induced insulin resistance. The aim of this study was to evaluate the preventive effect of heartwood of *Pterocarpus marsupium* in dexamethasone-induced hyperinsulinemia and hyperglycemia and compare it with that of Pioglitazone.

Male albino wistar rats were divided into five groups (n=6). Plain control group received gum acacia (2%) orally from d 1 to d 12. Dexa control group received gum acacia (2%) orally for d 1 to d 12 and Dexa (8 mg/kg) intraperitoneal (i.p.) from d 7 to d 12, during the study period. Two test groups received ethanolic extract of *Pterocarpus marsupium heartwood* (PME) (1 and 2 g/kg/) per oral (PO) and standard control group received pioglitazone (60 mg/kg/PO) from day 1 to day 12. During the 12 day study period, the two test groups and standard control group received Dexa (8 mg/kg/i.p.) from day 7 to day 12. On last day of the study, the blood samples were collected by retro-orbital sinus puncture and used for estimation of serum insulin and glucose levels. Homeostatic model assessment (HOMA) method was employed to calculate the degree of insulin resistance (IR). Results were analyzed by using one-way analysis of variance followed by Scheffe's multiple comparison test ($p<0.05$).

Treatment with ethanolic extract of *Pterocarpus marsupium* and pioglitazone significantly ($p<0.05$) reduced the elevated insulin and glucose levels as well as HOMA-IR and HOMA-IS values in dexa treated animals. Hence the study concluded that the heartwood of *Pterocarpus marsupium* has more effective insulin-sensitizing property compared with that of pioglitazone.

Wickramaratne et.al, (2016) Diabetes has caused a major burden to the health sector in the developing countries and has shown an increasing trend among the urban population. It is estimated that most patients are with type II diabetes which could be easily treated with dietary changes, exercise, and

medication. Sri Lanka carries a long history ayurvedic medicine where it uses the plant for treating many diseases. Therefore it is important to screen medicinal plants scientifically so they could be used safely and effectively in the traditional medical system and also be used for further investigations. *Adenanthera pavonina* is a plant used in the Ayurvedic medical system in Sri Lanka for treating many diseases including diabetics. We evaluated the anti-diabetic properties and the antioxidant properties of *Adenanthera pavonina* leaves.

The methanolic extract of the leaves was sequentially extracted with petroleum ether and thereafter was partitioned between Ethyl acetate and water. The α-amylase inhibition assay was performed using the 3,5- dinitrosalicylic acid method. The antioxidant activities were measured using the DPPH free radical scavenging activity and the total phenolic content using Folin-Ciocalteu's reagent. The cytotoxicity of the extract was evaluated using the Brine shrimp bioassay. The IC50 values of α amylase inhibitory activity of Methanol, Ethyl acetate, Petroleum ether, and water extracts of *Adenanthera pavonina* were 16.16 ± 2.23, 59.93 ± 0.25, 145.49 ± 4.86 and 214.85 ± 9.72 µg/ml respectively and was similar to that of Acarbose (18.63 ± 1.21 (µg/ml). Antioxidant activities were also determined and the Ethyl acetate fraction showed the highest total phenolic content (34. 62 ± 1.14 mg/g extract) and the highest DPPH scavenging activity with an IC50 of 249.92 ± 3.35 µg/ml.

The leaf extracts of *Adenanthera pavonina* exhibit remarkable α-amylase inhibitory activity in the crude methanolic extract. Hence leaves of *Adenanthera pavonina* has a potential to be used as a regular green vegetable and also be investigated further in isolating pure compounds with anti-diabetic activity.

Ajithadas Aruna et.al (2015), Synthesised silver nanoparticles from methanolic extract of Insulin plant (*Costus pictus*) leaves using 1mM AgNO3 solution and incubates 5hr at room temperature. Characterization of synthesized nanoparticles was done by UV–Vis absorption spectroscopy, SEM, poly dispersity index and zeta value. The surface Plasmon band of silver nano particles remain

close to 420nm which is analysed by uv visible absorption spectroscopy analysis. The average particle size (z-average) was found to be 132.6nm, its polydispersity index was 0.248 and zeta values were measured and it was found to be -25.1mV with the peak area of 100% intensity. This indicates that the formed silver nano particles are stable. A SEM images showed that the silver nanoparticles formed were spherical in shape, with an average size of around 100nm. SEM showed uniformly distributed silver nanoparticles on the surface of the cells was observed. The Biomedical application of silver nanoparticles can be rendered more effective by using biologically synthesized nanoparticles that are found to be exceptionally stable and also toxicity and cost.

Johnson I and Joy Prabu H (2015), Introduced a method for the biosynthesis of silver nanoparticles. The main reaction occurring is reduction. Since silver nanoparticles (AgNPs) has been used for infection prevention in medical field. This method is good for anti-microbial activity against bacteria, viruses and other microorganisms and hence clearly enhances the medicinal usage of AgNPs. This type of green biosynthesis of nanoparticles has received increasing attention. In the process of synthesizing AgNPs, it was observed that a rapid reduction of silver ions leading to the formation of stable crystalline AgNPs in the solution. Plant extracts from *Cycas circinalis*, *Ficus amplissima, Commelina benghalensis* and *Lippia nodiflora* were used for the synthesis of AgNPs from silver nitrate solution. AgNPs were characterized by different techniques. Uv spectral analysis gives primary conformation of formation silver nanoparticles by a visual colour change of solution, FTIR measurement was carried out to identify the possible bio molecules responsible for capping and efficient stabilization of Ag nanoparticles synthesized using plant extracts Scanning electron microscopy provided further insight into the morphology and size details of the AgNPs. The XRD spectra analysis used to confirm the crystalline nature of the AgNPs synthesized. In this study it is found that the leaves of medicinal plants were good source for the synthesis of AgNPs and have

many medicinal advantages.

Sneha Paul et.al (2015) The present study involves the aqueous extraction of root (*Pongamia Pinnata*) and analyzing the phytochemical compounds and functional groups present in plant root by FTIR. The synthesis of silver nanoparticles was carried out by root extract (*Pongamia pinnata*); confirmation and characterization of synthesized silver nanoparticles was done by UV-VIS Spectroscopy, SEM and EDAX. Preparation of nano gel was done by varying the concentrations of silver nano particle and paraffin wax. The best of 3 were selected for the further studies like *in-Vitro* anti-inflammatory, drug release and degradation studies; in respective of results observed *in vitro* drug release method showed that the nano gel was dispersed from dialysis membrane according to the time interval, *in vitro* degradation rate varies according to the concentration of nano gel preparation (Gel 1-40%,Gel 2-56% & Gel 362%),*in vitro* anti-inflammatory studies of membrane stabilization and protein denaturation showed no much of significant difference between standard drug aspirin along with prepared nano gel. The prominent results show that it can be further taken to drug delivery system analysis.

Stephina Wilson et.al (2015) The present investigation focuses on the green synthesis of silver nanoparticles (CANPs) by utilizing the reducing activity of *Centella asiatica* and exploring the anti-diabetic property exhibited by CANPs carried out by in vitro antidiabetic tests such as glucose Uptake by Yeast Cells, non enzymatic glycosylation of hemoglobin assay, Inhibition of alpha amylase enzyme assay, followed by UV-Visible spectrophotometry, X-Ray Diffraction (XRD) analysis and Scanning electron microscope (SEM) for characterization. The shape and size of CANPs were studied using Transmission electron microscopy (TEM).UV-Visible Spectrophotometry showed the Plasmon resonance peak at 430nm. The antioxidant and anti diabetic property of CANPs was determined. These results indicate that CANPs possess effective anti-oxidant and anti-diabetic properties. In entirety, the silver nanoparticles prepared are safe

to be free in the environment and perhaps utilized in industrial and remedial purpose.

Ali Alkaladi et.al, (2014), studied zinc oxide and silver nanoparticles for their anti diabetic activity. For this study fifty male albino rats with weight 120 ± 20 and age 6 months were used. The animals were grouped as follows: control group; did not receive any type of treatment, diabetic group ; received a single intra peritoneal dose of streptozotocin (100 mg/kg), diabetic + zinc oxide nanoparticles (ZnONPs), received single daily oral dose of 10 mg/kg ZnONPs in suspension, diabetic + silver nanoparticles (SNPs); received a single daily oral dose of SNP of 10 mg/kg in suspension and diabetic + insulin; received a single subcutaneous dose of 0.6 units/50 g body weight.

ZnONPs and SNPs were elucidated as anti diabetic agents. The results showed a great reduction in blood glucose level in diabetic groups treated with ZnONPs, SNPs and insulin (75.8%, 68.2% and 84.2%) respectively. Although the study concluded that ZnONPs are more powerful in their effect than silver nanoparticles, both zinc oxide and silver nanoparticles lead to reduction of blood glucose, increased insulin level and expression, increased GK activity and expression and improved expression level of IRA, GLUT-2 in diabetic rats.

Umoren et.al (2014), The research work is based on simple and effective ecofriendly approach for the synthesis of silver nanoparticles (AgNPs) from silver nitrate using *Malus domestica* (red apple) fruit. The fruit extract act as both reducing and capping agents. The synthesized AgNPs were characterized using various instrumental techniques including ultraviolet-visible spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD), scanning electron microscope (SEM), energy dispersive spectroscopy (EDS) and dynamic light scattering (DLS). Surface Plasmon Resonance (SPR) for AgNPs was observed at 422 nm. FTIR spectroscopy is useful for identifying the chemical composition present on the surface of silver nanoparticles and to identify the capping agents. The experiment was continuously observed the colour change as well as with UV–vis spectrophotometer. The effects of different parameters such

as (i) concentration of silver nitrate, (ii) concentration of *Malus domestica* extract and (iii) reaction time on nanoparticle formation were examined. The SEM images showed flower-like shaped structures .The zeta potential of the synthesized AgNPs was determined in water as dispersant. The zeta potential was found to be −65.07 mV. The high value confirms the repulsion among the particles and there by increases in stability of the formulation and average size of silver nanoparticles is 150 nm. The crystalline nature of AgNPs was confirmed by the analysis of XRD pattern the synthesized silver nanoparticles were found to be stable at room temperature as revealed by the negative value of zeta potential due to the presence of natural products.

Kandarp Mavani & Mihir Shah (2013), Developed a research on Chemical reduction of silver nanoparticles $AgNO_3$ using $NaBH_4$ .Chemical reduction of silver nanoparticles involves the reduction of a silver salt such as silver nitrate with a reducing agent like sodium borohydride in the presence of colloidal stabilizer. Sodium borohydride has been used with Poly vinyl alcohol (PVA), Poly vinyl pyrrolidone (PVP), Albumin (BSA), citrate and cellulose which are used as stabilizing agents. The Ag solution became yellowish in colour because of the absorption wavelength at 386nm. Uv visible spectral analysis is the primary confirmation for the formation of silver nanoparticles. The silver nanoparticles were estimated to be 10 to 20nm in diameter. Thus Silver nanoparticles absorption and scattering properties can be tuned by controlling the particle size, shape, and the local refractive index near the particle surface. As Silver nanoparticles have unique optical, electrical and thermal properties, they are been incorporated into products that range from photovoltaics to biological and chemical sensors. The study also deals with various applications of silver nano particles like diagnostic applications, antibacterial application, conductive application and optical application.

Khot Uttamkumar Vitthal et.al (2013) Solid lipid nanoparticles (SLN) loaded with Bacoside were prepared by micro emulsion probe sonicator method.

Solid lipid nanoparticles (SLNs) have been proposed as suitable colloidal carriers for delivery of drugs with limited solubility. Bacoside as a model drug which was incorporated into SLNs prepared from stearic acid using Tween 80 emulsifiers. SLNs in the rage of 33.3-257nm with mean particle size of 56 nm was obtained. The characteristics of the SLNs with various lipid and surfactant composition were investigated. The mean particle size of drug loaded SLNs decreased upon mixing with Tween 80 as well as upon increasing total surfactant concentration. The zeta potential of these SLNs varied in the range of –25 to –26 (mV), suggesting the presence of similar interface properties. High drug entrapment efficiency of 74.1% revealed the ability of SLNs to incorporate a poorly water-soluble drug such as bacoside. In vitro drug release study showed up to 84.68% drug release from Solid lipid nanoparticles. The drug release from Solid lipid nanoparticles follows zero order kinetics of drug release.

Anamika Mubayi1 et.al (2012),Made a study on, non-toxic as well as eco-friendly procedure for synthesizing AgNPs. The capping around each particle provides regular chemical environment formed by the bio-organic compound present in the *Moringa oleifera* leaf broth, which may be chiefly responsible for the particles to become stabilized. The fixed ratio of plant extract and silver ions were mixed and kept at room temperature for reduction. The color change from yellow to reddish brown confirmed the formation of nanoparticles. Further, the synthesized nanoparticles were characterized by UV, EPMA, XRD and FTIR data. The antimicrobial activity of synthesized nanoparticle has also been examined in gram positive and gram negative bacteria and encouraging results are in hand.

Sakey Ravindra et.al (2012), The present work involves the development of curcumin loaded silver hydrogel nanocomposites based on acrylamide and 2-acrylamido-2-methyl propane sulfonic acid, as a template by redox co-polymerization in the presence of hydrophilic cross linker N,N1-

methylenebisacrylamide. Silver nitrate was taken as the metal precursor and sodium borohydride as a reducing agent. The formation of silver nanoparticles was monitored using UV–Vis absorption spectroscopy. The developed hydrogel silver nanocomposites (HSNC) were characterized by FTIR, UV–Vis, thermogravimetric analysis, scanning electron microscopy and transmission electron microscopy. The curcumin loading and release characteristics were performed for different hydrogel systems. The developed HSNCs were evaluated for preliminary antibacterial applications. Curcumin suppresses growth of the bacteria and or the release of silver nanoparticles from hydrogel networks Therefore, From these analysis it is conclude that the curcumin loaded HSNC's are excellent antibacterial materials.

V. Vats et.al (2012) developed a research on the hypoglycemic effect of the aqueous (Aq) extract of the bark of *Pterocarpus marsupium* (PM) and alcoholic (Alc) extract of seeds of *Trigonella foenum-graecum* (FG) and leaves of *Ocimum sanctum* (OS) was investigated in both normal and alloxan-induced diabetic rats. The aqueous extract of PM (1 g/kg PO) significantly (P< 0.001) reduced the blood sugar levels from 72.32± 5.6 to 61.35± 1.2 mg% 2 h after oral administration of the extract and also significantly lowered the blood glucose in alloxan diabetic rats from 202.91± 5.44 to 85.22± 11.28 mg% 21 days after daily oral administration of the extract (P< 0.001). Similarly, reduction was seen with alcoholic extract of FG (74.33± 4.77 to 60.56± 1.9 in normal rats and 201.25± 7.69 to 121.25± 6.25 in diabetic rats) (P< 0.001) and OS (204.48± 11.0 to 131.43± 7.86 in normal rats and 73.54± 3.7 to 61.44± 2.3 in diabetic rats (P< 0.001. Although the study was concluded that the anti-hyperglycemic effect with OS and FG plateaued at the end of the second week and was less pronounced than PM.

K. Vijayaraghavan et.al (2012), Developed a research on eco-friendly and novel drug delivery route for preparing silver nanoparticles. The study was carried out

by using *Syzygium aromaticum* extract as a reducing and capping agent. The reaction process was simple and convenient to handle, and monitored by ultraviolet– visible spectroscopy (UV–Vis). The results were promising and rapid in the production of silver nanoparticles with a surface plasmon resonance occurring at 430 nm. The reduction of silver ions and stabilization of the AgNPs occur through the participation of proteins. The morphology of the NPs was determined by scanning electron microscopy (SEM), and the elements present in the nanoparticles were determined by using EDAX. The particle size of formed nanoparticles ranges from 20 to 149 nm which is almost spherical in shape.

V.R. Mohan et.al (2011), Developed a research on ethanolic extracts of *Pterocarpus marsupium* wood, bark and combined extract of wood and bark for its anti diabetic effect in wistar albino rats. Diabetes was induced in Albino rats by administration of alloxan monohydrate (150mg/kg,i.p). The ethanol extracts of *Pterocarpus marsupium* wood and bark at a dose of 150mg/kg of body weight respectively and combined extracts of wood and bark at a dose of 150 +150mg/kg of body weight were administered at single dose per day to diabetes induced rats for a period of 14 days. The effect of ethanol extracts of *Pterocarpus marsupium* on blood glucose, plasma insulin, glycosylated haemoglobin, serum lipid profile [total cholesterol, triglycerides, low density lipoprotein - cholesterol (LDL-C), very low density lipoprotein - cholesterol (VLDL-C), and high density lipoprotein cholesterol(HDL-C)] serum protein, albumin, globulin, A/G ratio, serum enzymes [Serum glutamate pyruvate transaminases (SGPT), serum glutamate oxaloacetate transaminases (SGOT) and alkaline phosphatase (ALP)], antioxidant enzymes lipoprotein peroxidation (LPO), reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), Erythrocytes (catalase (CAT) and superoxide dismutase (SOD) were measured in the diabetic rats. The ethanolic extracts of *Pterocarpus marsupium* resulted significant reductions of blood glucose ($p<0.01$), lipid parameters except HDL-C, serum enzymes and significantly increased HDL-C and antioxidant enzymes.The extracts also caused

significant increase in plasma insulin ($p<0.01$) in the diabetic rats.

In conclusion, the present study has shown that the ethanol extract of *P. marsupium* wood, bark and combined extracts have antidiabetic and anti hyperlipidaemic and antioxidant effects. And the phytochemical analysis has shown the presence of potent phytochemicals like flavonoids, terpenoids, tannins, glycosides, sterols, phenols and saponin. Hence the antidiabetic effect of ethanolic extract of *Pterocarpus marsupium* may be due to the presence of more than one anti hyperglycemic principles and their synergistic effects.

Wolfgang mehnert et.al (2001) This research paper presents an overview about the selection of the ingredients, different ways of SLN production and SLN applications. Aspects of SLN stability and possibilities of SLN stabilization by lyophilization and spray drying are discussed. Special attention is paid to the relation between drug incorporation and the complexity of SLN dispersions, which includes the presence of alternative colloidal structures (liposomes, micelles, drug nano suspensions, mixed micelles, liquid crystals) and the physical state of the lipid (super cooled melts, different lipid modifications). Appropriate analytical methods are needed for the characterization of SLN. The use of several analytical techniques is a necessity. Alternative structures and dynamic phenomena on the molecular level have to be considered. Aspects of SLN administration and the in vivo fate of the carrier are discussed. In summary, SLN are very complex systems with clear advantages and disadvantages to other colloidal carriers.

RESEARCH OBJECTIVE

Pterocarpus marsupium Roxb is a herb used for reducing blood glucose level from ancient time. The drug is presently given as an oral formulation, tablet in the dose of 100 and 450 mg twice daily and also in the form of capsule 400 mg. In traditional medicine people used to keep the bark in water for overnight and drink it in early morning to reduce blood glucose level **(Perera H.K.I, 2016).** This may lead to patient non-compliance. Therefore to overcome the drawbacks of conventional dosage form, trials for a novel approach was performed to enhance the delivery of drug. Hence the main objective of this study is to formulate *Pterocarpus marsupium* Roxb as silver nanopariticles by green synthesis method and to evaluate its antidiabetic activity.

Selection criteria for the herbal drug - *Pterocarpus marsupium* Roxb

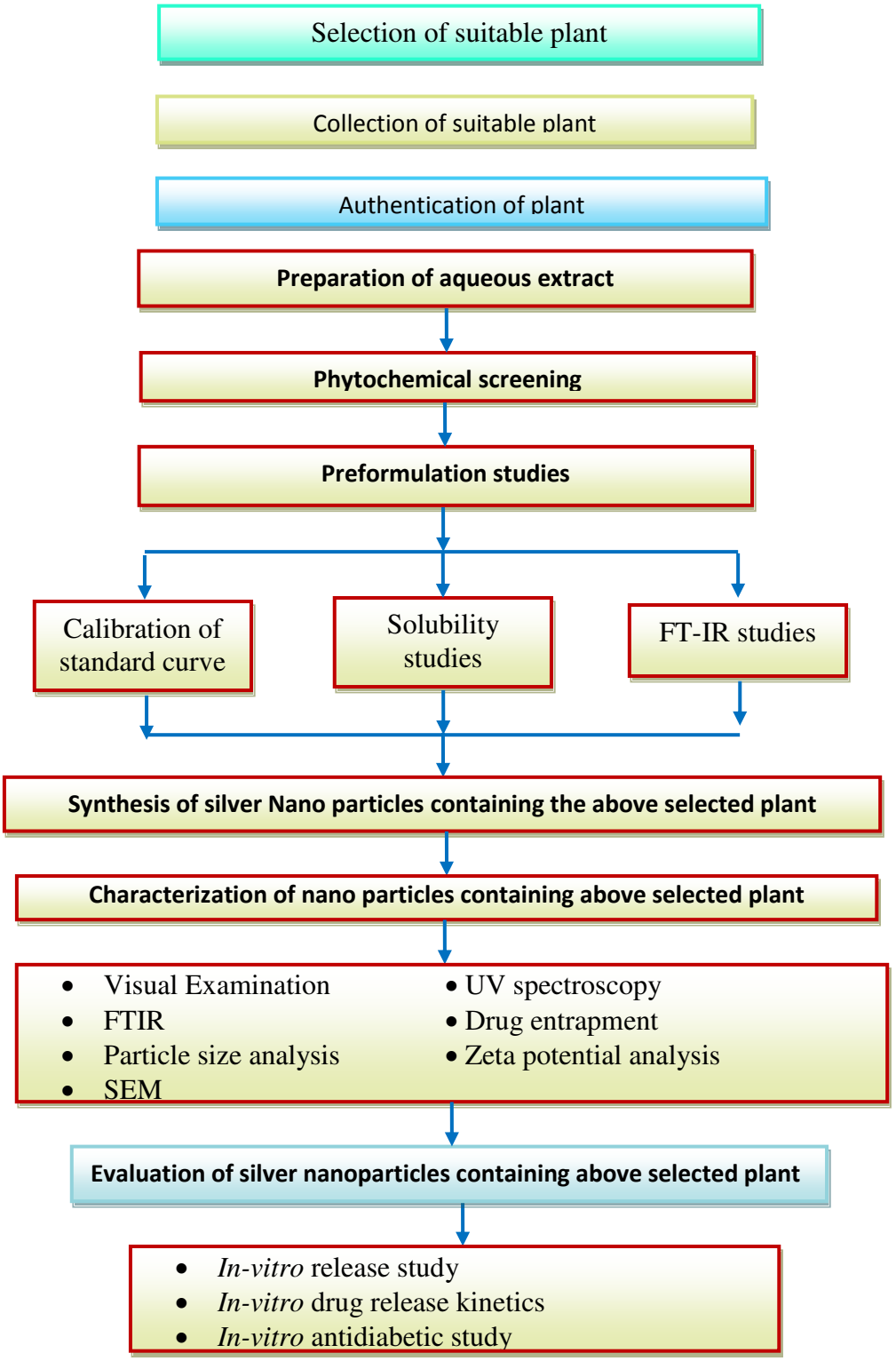
- It is mainly used to lower blood glucose level.
- It is having better water soluble property.
- It does not have any toxic or side effects

OBJECTIVE OF THE STUDY

An attempt has been made to develop the above selected herbal drug as herbal silver nanoparticles to evaluate its anti diabetic activity. Green synthesis technique was adopted to the herbal silver nanoparticles. The Silver nanoparticles synthesised by using bark and wood extract were subjected to characterization and also evaluated for its anti diabetic activity. Also the research work includes the following areas

- Development of cost effective and ecofriendly method.
- Reduce toxicity of chemical reagents
- Reduce side effects of drug
- Enhancement of pharmacological activity
- Enhancement of stability

PLAN OF WORK


Selection of suitable plant
Collection of suitable plant
Authentication of plant
Preparation of aqueous extract
Phytochemical screening
Preformulation studies
Calibration of standard curve
Solubility studies
FT-IR studies
Synthesis of silver Nano particles containing the above selected plant
Characterization of nano particles containing above selected plant
Visual Examination
FTIR
Particle size analysis
SEM
UV spectroscopy
Drug entrapment
Zeta potential analysis
Evaluation of silver nanoparticles containing above selected plant
In-vitro release study
In-vitro drug release kinetics
In-vitro antidiabetic study

MATERIALS AND EQUIPMENT'SS

MATERIALS USED	SOURCE
1. Silver nitrate	Qualigens fine chemicals, Mumbai
2. *Pterocarpus marsupium* Roxb	
Bark and wood	Natural Source., Palakkad
3. Sodium potassium tartrate	Merck
4. Starch	SD chemical limited
5. α amylase enzyme	Himedia, Mumbai
6. 3,5- Dinitrosalicylic acid	Himedia, Mumbai
7. Sodium hydroxide	Sigma
8. Sodium chloride	SD chemical limited
9. Sodium phosphate monobasic	Sigma

EQUIPMENT'S USED	SOURCE
1. Magnetic Stirrer	REMI – 2MLH
2. UV Spectrophotometer	JASCO V-530
3. FT-IR Spectrometer	FTIR JASCO – 4100
4. pH meter	pH TESTER 1,2 (EUTECH)
5. Zeta Sizer	MALVERN
6. SEM	Hitachi X650, Tokyo, Japan
7. Dialysis membrane 50	Himedia, Mumbai

PLANT PROFILE

Plant name	:	*Pterocarpus marsupium Roxb*
Synonym	:	Indian kino, Bijasal, Vijayasar, Bibla, Malabar kino tree, Gammalu, Kino, Indian Kino Tree, Bastard teak, East Indian Kino, Vaengai, Ponnai, Karavenga, Venga,
Family	:	Leguminosae

Taxonomical classification

Kingdom	:	Plantae
Phylum	:	Tracheophyta
Class	:	Magnoliopsida
Order	:	Fabales
Family	:	Leguminosae

Distribution:

It is native to India, Nepal, and Sri Lanka, where it occurs in parts of the Western Ghats in the Karnataka-Kerala region and also in the forests of Central India.

Parts used: Bark and wood

Figure 4 Bark of *Pterocarpus marsupium roxb*

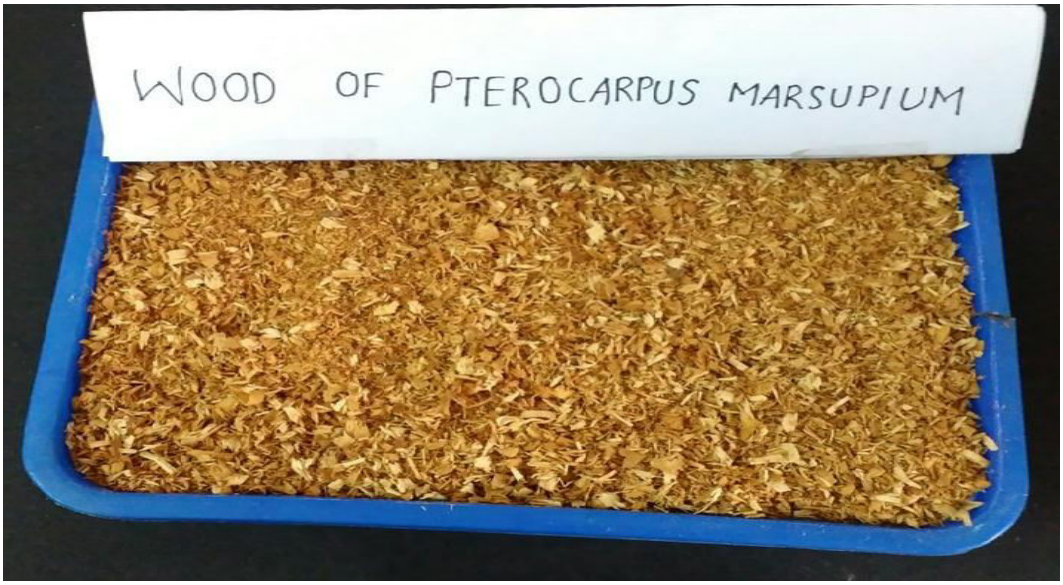


Figure 5 Wood of *Pterocarpus marsupium* roxb

Description: It is a decidous tree about 90 feet or more high.

Bark: stem bark is grey brown. Heart wood is yellow and bark yields a reddish gum.

Leaves: 3-5 inches long have 5-7 leafts long, margin wavy and obtuse. The petioles are round, smooth and waved from leaftlet, 5 or 6 inches long and no stipules.

Flower: 1.5 c.m. long, very numerous, white, with a small tinge of yellow, stamens are 10. United near the base but soon dividing into two parcels of 5 each; anthers are gllobose and 2 lobed**.**

Chemical Constituents: pterostilbene 4-5%, alkalois 0.4%, tannins, protein pentosan, pterosupin, pseudobaptigenin, liquiritigenin, isoliquiritigenin, garbanzol, 5-de-oxykaempferol, epicatechin, marsupol, carsupin, marsupinol. Aqueous extract of the heartwood of PM contains five new flavanoids c-glucosides namely 6- hydroxyl-2-(4-hydroxybenzyl)-benzo-furan-7-c-a-D-glucopyranoside, 3-(a-methoxy-4-hydroxybenzylidene)-6-hydroxybenzo-2(3H)-furanonE-7-C-a-D-glucopyranoside, 2-glucopyranoside, 8-(C-a-D-glucopyranosyl)-7,3,4-trihydroxyflavone and 1,2-bis (2,4-dihydroxy, 3-C-glucopyranosy) – ethanedione and two known compounds C-a-D-glucopyranosyl-2, 6-dihydroxyl benzene and sesquiterpene.

Ethnomedicinal Uses: Useful parts of the herb are heartwood, leaves, flowers, gum. The phloem of stem contains red astringent fluid present in secretory cell, which exudes after given incision. Kino is odourless but has astringent taste and sticks in the teeth, colouring the saliva red in colour. As astringent it is used in diarrhoea, dysentery etc.

Bruised leaves are applied on fractures, leprosy, leucoderma, skin deseases, sores and boils, Constipation, depurative, rectalgia, opthamology,

hemorrhages and Rheumatoid arthritis. Bark is used as diuretic in Gabon and fresh leaves are used as food in Nizeria. Leaves are used in GIT disorders. Stem in the treatment of neurological problems.

Bark and wood have been used to treat diabetics marsupin and Pterostilbene significantly lower the blood glucose levels useful in NIDDM.

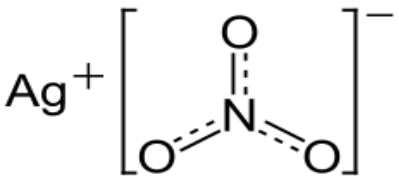
Traditionally the plant has been used for Angina, Cancer, and used as Cardiotonic and brain tonic

EXCIPIENT PROFILE

SILVER NITRATE

IUPAC name	:	Silver nitrate
Synonyms	:	Nitric acid silver (1+) salt
Molecular Formula	:	$Ag\ No_3$
Molecular Weight	:	169.872
Colour	:	White
Odour	:	Odour less solid
Density	:	4.35 g/cm^3
Melting point	:	209.7 °C
Boiling point	:	440 °C
pH	:	5.4-6.4 (100g/l, H2O, 20℃)
Storage temperature	:	2- 8^oc

Structure of silver nitrate



Solubility

Soluble in Water, Acetone, Ammonia, Ether, Glycerol,

Uses :

Anti-Infective Agents, Disinfection, destruction of cutaneous warts, Precursor to other silver compounds, Halide abstraction, Organic synthesis

Safety

As an oxidant, silver nitrate should be properly stored away from organic compounds. Despite its common usage in extremely low concentrations to prevent gonorrhea and control nose bleeds, silver nitrate is still very much toxic and corrosive. Brief exposure will not produce any immediate side effects other than the purple, brown or black stains on the skin, but upon constant exposure to high concentrations, side effects will be noticeable, which include burns. Long-term exposure may cause eye damage. Silver nitrate is known to be a skin and eye irritant (**https:/wiki/Silver_nitrate**).

EXPERIMENTAL METHODOLOGY

SAMPLE COLLECTION

Pterocarpus marsupium Roxb bark and wood were collected from Palakkad, Kerala. Then it was cleaned properly with water.

1) Authentication

The plant specimen was identified and authenticated by Botanical survey of India, Southern regional centre, Coimbatore.

2) Drying and Pulverizing

The bark and wood were collected and shade dried at temperature not exceeding 40°C. It was grounded into fine coarse powder with mixer and passed through the sieve number 16 and kept in a well closed container in a dry place.

3) Preparation of Aqueous Extract of *Pterocarpus marsupium* Roxb

Aqueous extract of *Pterocarpus marsupium* Roxb was prepared by cold maceration method. The air-dried coarse powder (50g) of *Pterocarpus marsupium* Roxb accurately weighed and macerated with 500 ml of solvent that is distilled water. And then allowed to stand for 24 hrs and filtered rapidly with muslin cloth. The filtrate was evaporated to dryness at a temperature not exceeding 40 ^{0}c for 2 hours **(Perera, H.K.I. 2016).**

4) Phytochemical Screening

5(1) Preparation of Test Solution

The filtered aqueous extract of *Pterocarpus marsupium* Roxb was used as a test solution for preliminary screening of phytochemical constituents.

PRELIMINARY QUALITATIVE PHYTOCHEMICAL ANALYSIS (C. K.Kokate et.al)

5(2) Test for Alkaloids

a) Dragendorff's Reagent: To the test solution 1 ml of dragendroff's reagent (potassium bismuth iodide solution) was added.

b) Wagner's Reagent: To the test solution 1ml of Wagner's reagent (Iodine potassium iodide solution) was added.

c) Hager's Reagent: To the test solution add Hager's reagent (saturated solution of picric acid).

d) Tannic Acid Test: To the test solutions add Tannic acid solution.

5(3) Test for Amino Acids

Millon's Test : To the test solutions add 2 ml of Millon's reagent.

5(4) Test for Carbohydrates

Molisch's Test: To the test solution add few drops of alcoholic a-naphthol, then add few drops of concentrated sulphuric acid through sides of test tube.

5(5) Test for Flavonoids

Alkaline Reagent Test: To the test solution add few drops of sodium hydroxide solution, and then add few drops of dilute acid.

5(6) Test for Glycosides

a) Legal's Test: Treat the test solution with pyridine and add alkaline sodium nitroprusside solution.

b) Baljet's Test: Treat the test solution with picric acid or sodium picrate, orange colour formed indicates the presence of glycosides.

5(7) Test for Tannins (Phenolic Compounds)

a) Ferric chloride Test: Treat the extract with few drops of ferric chloride.

b) Test for Chlorogenic Acid: Treat the test solution with aqueous ammonia and expose to air

5(8) Test for Starch

To the aqueous extract add weak aqueous Iodine solution.

5(9) Test for Proteins

Warming Test: Heat the test solution in a boiling water bath.

5(10) Test for Steroids

Salkowski Test: Treat the extract with few drops of concentrated sulphuric acid.

6 PREFORMULATION STUDY

6(1) Solubility Test

Pterocarpus marsupium Roxb. bark and wood extract powder about 1mg was taken in a test tube and solubility in ethanol, water, chloroform and diethyl ether were checked.

6(2) UV- VIS spectral analysis of *Pterocarpus marsupium* Roxb Wood and Bark

0.5ml of *Pterocarpus marsupium* Roxb wood extract and 0.5ml of bark extract were taken in a 10 ml standard flask and diluted with distilled water. And then Uv- visible spectra were taken in the range of 200- 400 nm using phosphate buffer at pH 7.4 as blank (**I. Johnson. H. Joy prabu, 2015).**

6(3) Preparation Of Calibration Curve Of *Pterocarpus marsupium* Roxb Bark and Wood Extract Using UV- Visible Absorption Spectroscopy using phospate buffer at pH 7.4

10 mg of *Pterocarpus marsupium* Roxb extract was dissolved in 10ml phosphate buffer at pH 7.4 to give a concentration of 1 mg/ml. From this solution 2.5ml was diluted with 25ml of phosphate buffer. Further dilutions are made to get the concentration (4-20 µg/ml). Then absorbance of each solution were measured at 279 nm using phosphate buffer as blank **(Mukesh S. Sikarwar et.al, 2008).**

6(4) FTIR Spectroscopy of *Pterocarpus marsupium* Roxb Wood and Bark

50 mg each of dried *Pterocarpus marsupium* Roxb bark and wood were mixed with 100 mg of spectral grade KBr and pressed into disc under hydraulic pressure. Then FTIR spectra were recorded in the 4000- 400cm-1 range **(Holler, Skoog, Crouch).**

6(5) FTIR spectroscopy of silver nitrate

100mg of Silver nitrate was mixed with 100 mg of spectral grade KBr and pressed into disc under hydraulic pressure. Then FTIR spectra were recorded in the 4000- 400cm-1 range **(Holler, Skoog, Crouch).**

7) GREEN SYNTHESIS OF SILVER NANOPARTICLES

7(1) Preparation of Stock Solution

1 mg of aqueous extract was weighed and diluted to 10 ml with distilled water.

7(2) Preparation of 1mM silver nitrate aqueous solution

0.017g of silver nitrate was dissolved in 100 ml of distilled water and stored in amber coloured bottle until further use **(Ajithadas aruna et al, 2014).**

7(3) Synthesis Of *Pterocarpus marsupium* Roxb Silver Nanoparticles

5 ml of *Pterocarpus marsupium Roxb* aqueous extract was taken in a beaker and paced on a magnetic stirrer with hot plate. To this 50 ml of 1Mm silver nitrate solution was added drop wise with constant stirring at120 rpm .The colour change of the solution was checked periodically. **(Ajithadas aruna et al, 2014).**

7(4) Separation of Silver Nanoparticles

The synthesised *Pterocarpus marsupium* Roxb silver nanoparticles were separated by centrifugation using REMI centrifuge at 5000 rpm for 15 minutes. The supernatant liquid was discarded and the pellets were collected stored. **(Ajithadas aruna et al, 2014).**

8) CHARACTERIZATION OF SYNTHESIZED *Pterocarpus marsupium* Roxb SILVER NANOPARTICLES

Characterization of *pterocarpus marsupium* Roxb silver nanoparticles was carried out using the following parameters.

8(1) Visual Examination

The primary confirmation of the synthesised PM silver nanoparticles is done by visual basis. The colour change of *Pterocarpus marsupium* Roxb extract and silver nitrate solution with respect to time was observed **(I. Johnson. H. joy prabu, 2015).**

8(2) UV- Visible Spectroscopy

Uv- visible spectral analysis characterizes the formation and completion of PM silver nanoparticles. The reduction of silver ions were monitored by measuring UV-Vis spectrum of reaction medium from the wavelength 400-800

nm by using distilled water as blank. Periodic sampling at time intervals of 30min, 60 min,90 min,120 min, and 26 h was carried out **(Ajithadas aruna et al, 2014).**

8(3) Fourier Transform Infrared Spectroscopy

Dried samples (PM silver nanoparticles) of about 100 mg were mixed with 100 mg of spectral grade KBr and pressed into discs under hydraulic pressure. FTIR spectra were recorded in the range 4000-400 cm-1. FTIR measurements were carried out to identify the bio molecules responsible for capping and stabilization of metal nanoparticles synthesised **(Holler, Skoog, Crouch).**

8(4) Drug Entrapment

10 mg of *Pterocarpus marsupium* Roxb silver nanoparticles were dissolved in phosphate buffer at pH 7.4. After centrifugation, amount of drug present in supernatant was determined by UV Spectrophotometry **(Renu Tiruwa, 2015).**

8(5) Determination of Particle Size

The dried powders of *Pterocarpus marsupium* Roxb silver nanoparticles dispersed in water to obtain proper scattering intensity of *Pterocarpus marsupium* Roxb silver nanoparticles. The particle size was determined by Malvern zeta size analyser **(Khot Uttamkumar Vitthal, 2013).**

8(6) Determination of Zeta Potential

The zeta potential was measured by using ZetaSizer (Malvern Instruments) having zeta cells, polycarbonate cell with gold-plated electrodes and using water as medium for sample preparation. Zeta potential determines the surface potential of silver nanoparticles and it is essential for the characterization of stability of nanoparticles **(Khot Uttamkumar Vitthal, 2013).**

8(7) Scanning Electron Microscopy

SEM analysis of *Pterocarpus marsupium* Roxb. silver nanoparticles were performed to evaluate the surface morphology of nanoparticles. Silver

nanoparticles were prepared and dried well to remove the moisture content and images were taken by using Hitachi X650, Tokyo, Japan**. (GOPI .G, 2015)**

IN-VITRO ANTI DIABETIC STUDY

9) α- AMYLASE INHIBITION ASSAY

From 1 mg/ ml stock solution different concentrations of plant extracts were prepared in phosphate buffer. 500µl of test/ standard was added to 500µl of α- amylase (0.5mg/ml) was incubated for 10 minutes at room temperature. Then added 500 µl of 1% starch solution and incubated for another 10 minutes. After that 1ml of colouring reagent was added to reaction mixture it was prepared by mixing Sodium potassium tartrate solution (12g dissolved in 8ml of 2M NaoH) and 96Mm 3,5-Dinitrosalicylic acid and heated in boiling water bath for 15 minutes after cooling, 10 ml of distilled water was added. To measure the absorbance of coloured extracts blank was prepared for each set of concentration of test sample by replacing the enzyme with buffer. Control incubations representing 100% enzyme activity was prepared by replacing test drug with buffer. Absorbance measured at 540 nm **(G. Dhivya & M.Rajasimman, 2015).**

Inhibition activity (%) = $\frac{Abs(control)-Abs(\ extract)}{Abs\ (\ control)}$× 1oo

POSITIVE CONTROL

Acarbose is an anti-diabetic drug used to treat Type- 2 diabetes mellitus. It was used as a positive control. **(G. Dhivya and M.Rajasimman, 2015).**

10) *IN -VITRO* DRUG RELEASE STUDY

In-vitro release kinetics of *pterocarpus marsupium* Roxb silver nanoparticles were determined by dialysis bag diffusion method. In this method Dialysis Membrane – 50 (Himedia) having molecular weight cut off 12000 to 14000 were used. Dialysis membrane was activated in boiling water for about 30

minutes. Then prepared pterocarpus marsupium silver nanoparticles were placed inside the dialysis membrane which is sealed at both the ends. Then it was placed in a beaker containing 100 ml phosphate buffer at pH 7.4. Then the beaker was placed over a magnetic Stirrer and rpm was maintained at 100. Samples (2 ml) were withdrawn at a definite time intervals and replaced with equal amounts of fresh pH 7.4 phosphate buffer. After suitable dilutions the samples were analyzed using UV–Visible spectrophotometer at 431 nm. **(Bohrey et.al, 2016)**

Release kinetics

In order to understand the release kinetics of a drug, the results of *in vitro* drug release studies of nanoparticles were fit to various kinetic equations such as zero order (Time vs Cumulative percentage release), first order (Time vs log cumulative percentage), and Higuchi's model (Square root of time vs Cumulative percentage release), Korsmayer Peppas(Log time vs log cumulative percentage)). **(Priyanka K and Abdul Hasan Sathali).**

Zero-Order Kinetics: Cumulative amount of drug released was plotted against time ($C = K_0t$) where K_0 is the zero-order rate constant expressed in units of concentration/time and *t* is the time in hours. A graph of concentration Vs time would yield a straight line with a slope equal to K_0 and intercept the origin of the axis. This kinetics describes concentration independent drug release from the formulations.

First order kinetics: First order as log cumulative percentage of drug remaining vs time. This kinetics describes concentration dependent drug release from the formulations. $LogC = LogC_0 - kt / 2.303$ where C_0 is the initial concentration of drug, *k* is the first order constant, and *t* is the time.

Higuchi's Model: Higuchi's model as cumulative percentage of drug released vs. square root of time. $Q = Kt_{1/2}$ where *K* is the constant reflecting the design variables of the system and *t* is the time in hours. This model describes the

release of drug on the basis of Fickian diffusion as a square root of time dependent process from swellable matrix.

Korsmayer Peppas (Sadiq and Rassol, 2014): To evaluate the mechanism of drug release, the first 60% of drug release were plotted in Korsmeyer et al's equation log cumulative percentage of drug released vs log time, and the exponent *n* was calculated through the slope of the straight line, $Mt / M\infty = Ktn$**.** Where $Mt/M\infty$ is the fractional solute release, t is the release time, K is a kinetic constant characteristic of the drug/polymer system, and n is an exponent that characterizes the mechanism of release of tracers. This type of drug release is controlled by combination of polymer swelling, erosion and diffusion through the hydrated matrix.

- The value of $n< 0.5$ or $n= 0.5$ indicating fickian diffusion
- The value of n between 0.5 to 1 ($0.5<n<1$) indicating non fickian diffusion
- The value of $n=1$, indicating the zero release or case2 transport
- The value of $n>1$, indicating the super case2 transport.

RESULT AND DISCUSSION

1) SAMPLE COLLECTION

Pterocarpus marsupium Roxb bark and wood were collected from Palakkad, and this was cleaned with water to remove the mud and other substances present on the surface of bark and wood.

2) AUTHENTICATION

The plant specimen was identified and authenticated by botanical survey of India, southern region, Coimbatore. The authentication letter was attached in the certificate section.

3) DRYING AND PULVERIZING

The collected bark and wood were shade dried at temperature not exceeding 40^{o}c. After the removal of moisture content it was grounded into coarse powder with the help of mixer and then passed through the sieve number 16 .Then the powder obtained was stored in well closed container and kept in dry place.

4) PREPARATION OF AQUEOUS EXTRACT OF *Pterocarpus marsupium* Roxb

Aqueous extract of *Pterocarpus marsupium* Roxb was prepared by cold maceration method. And the maceration for bark and wood has been done separately. To the beaker containing 500ml of distilled water 50g of *Pterocarpus marsupium* Roxb bark and wood was added separately and stirred .Then it was allowed to stand for 24 hrs. After 24 hrs macerated *Pterocarpus marsupium* Roxb bark and wood were filtered with muslin cloth and the filtrate was evaporated to get concentrated dry powder at a temperature not exceeding 40^{o}c.

5) PHYTOCHEMICAL SCREENING

The sample containing bark and wood aqueous extract of *Pterocarpus marsupium* Roxb has been chemically subjected to various tests to check the presence of different chemical constituents like alkaloids, amino acids, carbohydrates, flavanoids, glycosides, tannins (phenolic compounds), starch and proteins and the results were shown in the table: 2(**C. K.Kokate et.al**)..

Table: 2 Phytochemical screening of *Pterocarpus marsupium* Roxb aqueous bark and wood extract

Sl. No	Constituent	Test	*Pterocarpus marsupium* Roxb	
			Bark	Wood
1	Alkaloid	Dragendroffs	+++	++
		Wagner's	+++	-
		Hager's	+	+
		Tannic acid	+	+
2	Aminoacid	Millon's	-	-
3	Carbohydrates	Molisch's	+++	+++
4	Flavonoids	Alkaline reagent	+++	+++
5	Glycosides	Legal's	+++	++
		Baljet's	++	++
6	Tannins (Phenolic compounds)	Ferric chloride	+++	++
		Chlorogenic acid	-	+
7	Starch	Iodine	+++	++
8	Proteins	Warming test	-	-

High: +++, Medium: ++, Low: +, Absence: -

The various tests like Dradendroffs, Wagner's, Hager's and Tannic acid test have been carried out to check the presence of Alkaloid **(C. K.Kokate et.al)**. In Dragendroffs test intense reddish brown precipitate was observed for aqueous extract of bark and slight reddish brown precipitate for aqueous extract wood. Wagner's test shows reddish brown precipitate for aqueous extract of bark and there is absence of reddish brown precipitate for wood extract. In Hager's test slight yellow precipitate was observed for both bark and wood extract .Tannic acid test shows that slight buff colour precipitate for both bark and wood extract of *Pterocarpus marsupium* Roxb. From the phytochemical screening tests it was found that aqueous extract of bark is having more Alkaloids than that of wood extract.

Millons test was carried out to identify the presence of Amino acids but no white precipitate was observed therefore it was concluded that aminoacids were absent in aqueous bark and wood extract of *Pterocarpus marsupium* Roxb.

Presence of carbohydrate was identified by Molisch's test. Violet colour ring was observed for both bark and wood aqueous extract of *Pterocarpus marsupium* Roxb hence strong presence of Carbohydrate was confirmed for both bark and wood.

Alkaline reagent test was carried out to identify chemical constituent flavanoids; yellow colour solution formed turns colourless on addition of dilute acid for bath bark and wood aqueous extract of *Pterocarpus marsupium* Roxb. and hence identified the presence of flavanoids in both bark and wood aqueous extract.

Legal's test identifies the presence of glycosides. Intense blood red colour was observed for bark aqueous extract of *Pterocarpus marsupium* Roxb. and slight red colour for wood aqueous extract. So the presence of glycosides in bark is more as that of wood aqueous extract of *Pterocarpus marsupium* Roxb.

Baljet's test also identifies the presence of glycosides in this test slight orange colour was observed for both bark and wood aqueous extract of *Pterocarpus marsupium* Roxb. Hence presence of glycoside was identified for both bark and wood.

Ferric chloride test identifies the presence of phenolic compounds. Intense blue colour was observed for aqueous extract of bark and slight blue colour for aqueous extract of wood. And hence it was observed that aqueous extract of bark is having more phenolic compounds than that of aqueous wood extract of *Pterocarpus marsupium* Roxb.

Chlorogenic acid test identifies the presence of tannins, absence green colour was observed for aqueous extract of bark and slight green colour was observed for aqueous wood extract of *Pterocarpus marsupium* Roxb. From analysis it was observed that chlorogenic acid test tannic acid was absent in

aqueous extract and slightly present in aqueous wood extract of *Pterocarpus marsupium* Roxb.

Iodine test identifies the presence of starch. Intense blue colour was observed for aqueous extract of bark and slight blue colour for wood extract of *Pterocarpus marsupium* Roxb and hence it was found that bark extract is having more starch content than that of wood extract of *Pterocarpus marsupium* Roxb.

Warming test identifies the presence of proteins. In warming test no proteins gets coagulated for both bark and wood aqueous extract of *Pterocarpus marsupium* Roxb. Hence proteins are absent in both aqueous extract of bark and wood.

6) PREFORMULATION STUDY

6(1) Solubility Test

Solubility test for powdered bark and wood extract of *Pterocarpus marsupium* Roxb. were carried out in different solvents such as Ethanol, Water, Chloroform and Diethyl ether were given in table: 3

Table: 3 solubility of bark and wood extracts of *pterocarpus marsupium Roxb.* in different solvents

Solvent	Soluble	Sparingly soluble	Insoluble
Ethanol	✓	-	-
Water	✓	-	-
Chloroform	-	✓	-
Diethyl ether	-	✓	-

From the solubility test analysis it was found that powdered bark and wood extract of *Pterocarpus marsupium* Roxb. are soluble in Ethanol and Water and sparingly soluble in Chloroform and Diethyl ether. Hence the powdered *Pterocarpus marsupium* Roxb bark and wood extract is more soluble in polar solvents and sparingly soluble in non polar solvents.

6(2) UV- VISIBLE SPECTRAL ANALYSIS OF *Pterocarpus marsupium* Roxb WOOD AND BARK

Pterocarpus marsupium Roxb 0.05 mg of both bark and wood were taken in a 10 ml standard flask and diluted with distilled water. Then UV-Visible absorption spectra for the sample were taken in the range of 200-800nm.The absorption peak obtained was shown in the figure: 6

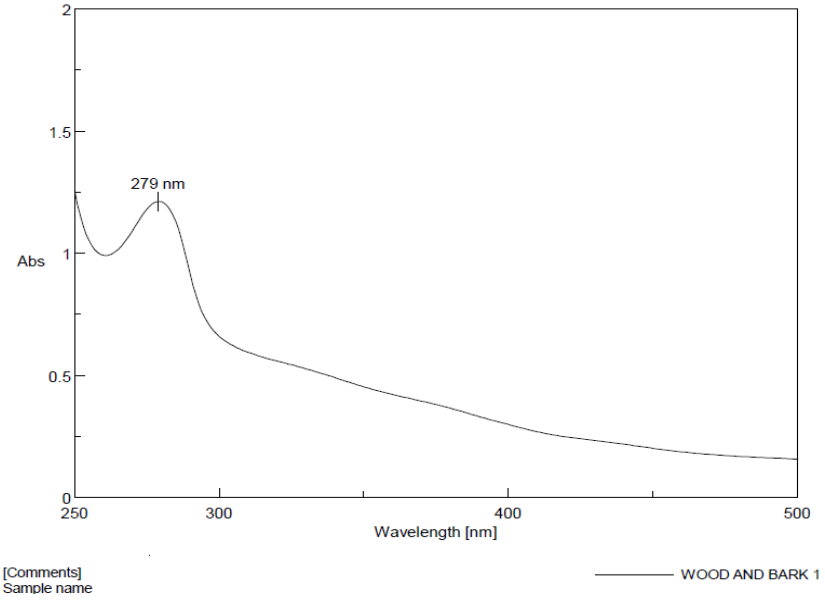


Figure: 6 Uv-Visible absorption spectra of *pterocarpus marsupium* Roxb wood and bark extract

The maximum absorption of *pterocarpus marsupium* Roxb wood and bark extract was found at 279 nm and hence selected as the wavelength for further studies.

6(3) Preparation of Calibration Curve for *Pterocarpus marsupium* Roxb Bark and Wood Extract using UV- visible absorption spectroscopy AT 279 nm

In the standard curve, linearity was obtained between the concentrations of 4- 20µg/ml and the regression value was found to be R^2 = 0.999. Hence the sample *Pterocarpus marsupium* Roxb bark and wood aqueous extract at the concentration between 4- 20µg/ml obeys the beer lamberts law. Concentration versus absorbance values were given in table: 4

Table:4 Calibration curve of *Pterocarpus marsupium* Roxb bark and wood extract using uv- Visible absorption spectroscopy at 279nm

Concentration (µg/ml)	Absorbance
4	0.0313
8	0.0517
12	0.0713
16	0.0912
20	0.112

In the standard curve concentration vs absorbance were plotted and given in the figure: 7

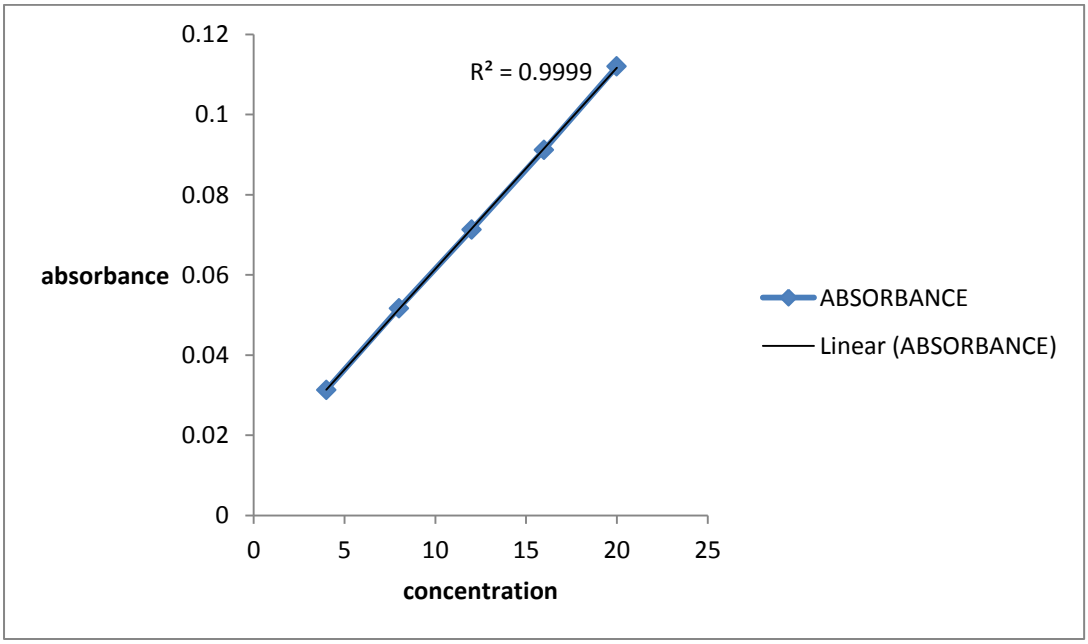


Figure: 7 Calibration curve of *pterocarpus marsupium* Roxb. bark and wood extract and its regression value

6(4) FTIR Spectroscopy OF *Pterocarpus marsupium* Rox. Bark and Wood

In the *Pterocarpus marsupium roxb* bark and wood FTIR spectrum strong

absorption peaks at 3693.01 and 3413 indicates OH stretching due to the presence of alcohol and phenol. Peaks at 1619.91 and 1530.24 indicates C=C stretching of α,β,unsaturated ketone and N-O stretching of nitro compounds absorption peaks at 1384.64, 821,527 and 786.815 and 727.996 indicates the presence of alkanes, alkenes, and aromatic rings. FTIR wave numbers of sample and standard wave numbers range along with interpretation is given in the table: 5. **(Robinson et.al).**

Table: 5 FTIR interpretations of *pterocarpus marsupium* Roxb. Bark and Wood

Material	Standard wave number Range	Test wave number	Inference
Pterocarpus marsupium roxb bark and wood	3700-3584	3693.01	O-H Stretching Alcohol
	3550-3200	3413	O-H Stretching Alcohol
	1620-1610	1619.91	C=C stretching
	1550-1500	1530.24	N-O stretching
	1390-1310	1384.64	Alkanes
	810 ±20	821	C-H bending
	550-490	527	Alkanes
	690-900	786.815	Alkenes
	675-995	727.996	Aromatic rings

FTIR interpretation results are given in the above table: 5 and the spectrum of *Pterocarpus marsupium* Roxb. bark and wood were shown in the figure: 8.

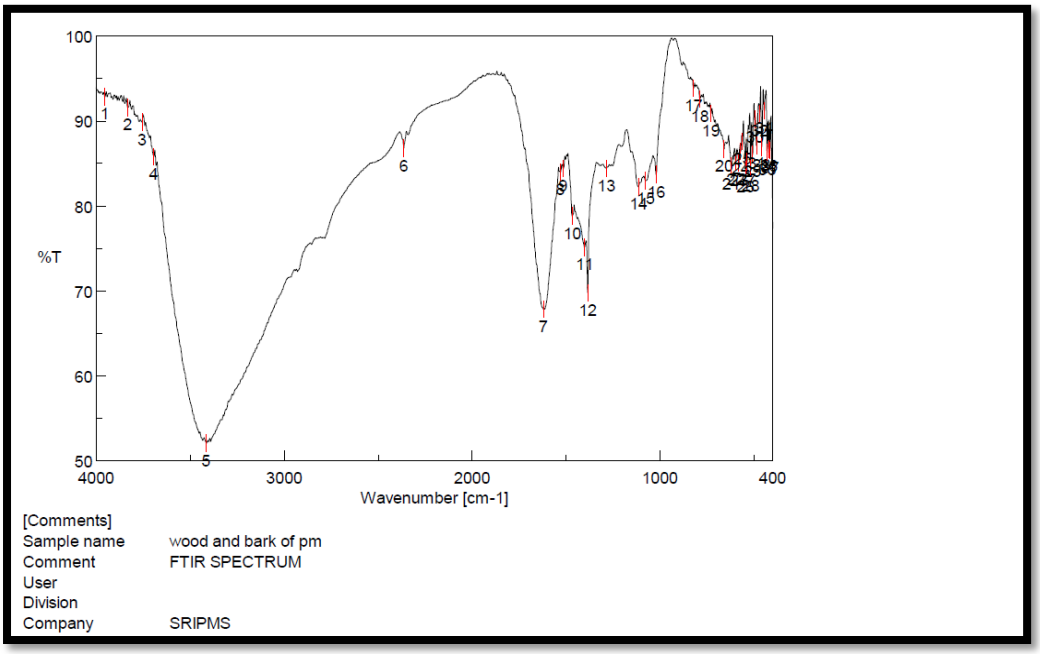


Figure: 8 FTIR Spectra of *pterocarpus marsupium* Roxb. bark and wood

6(5) FTIR Spectroscopy of Silver Nitrate

In the FTIR spectrum of silver nitrate strong absorption peaks at 3648.66, 3446.17, 2925.48 were resulted from the OH groups due to the presence of alcohols and phenols and strong absorption peak at 1541.81was resulted from stretching of N-O (nitro compounds) FTIR wave numbers of sample and standard wave number range along with interpretation is given in the table:6. **(Holler, Skoog, Crouch).**

Table: 6 FTIR Interpretation of Silver Nitrate

Material	Standard wave number Range	Test wave number	Inference
Silver nitrate	3700-3584	3648.66	O-H Alcohol free
	3550-3200	3446.17	O-H Alcohol
	3200-2700	2925.48	O-H Alcohol
	1550-1500	1541.81	N-O Nitro compound

FTIR interpretation results were given in the above table: 6 and the FTIR Spectrum of silver nitrate is shown in the figure: 9

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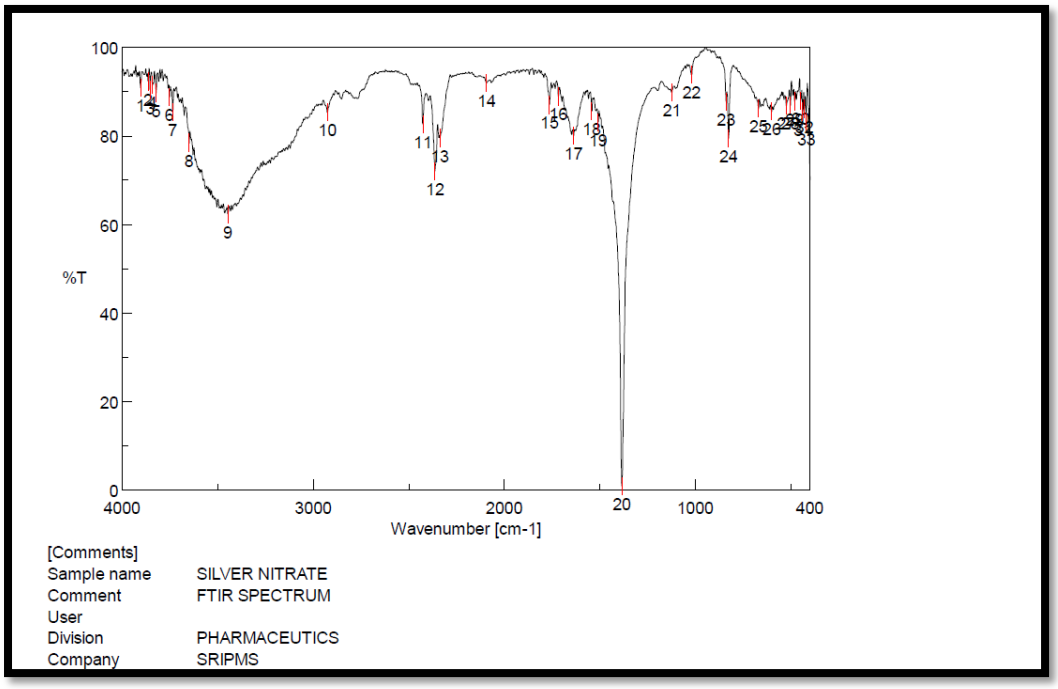

Figure: 9 FTIR Spectrum of silver nitrate

7) GREEN SYNTHESIS OF SILVER NANOPARTICLES

Pterocarpus marsupium Roxb. silver nanoparticles were prepared by drop wise addition of silver nitrate solution to the *Pterocarpus marsupium* Roxb bark and wood extract which is placed on the magnetic stirrer with hot plate at 120 rpm. Formation of silver nanoparticles is indicated by the colour change from yellow to brown colour.

8) CHARACTERIZATION OF SILVER NANOPARTICLS CONTAING *Pterocarpus marsupium* Roxb.

8(1) Visual Examination

Initial colour of *Pterocarpus marsupium* Roxb. extract was yellow and it turns to dark brown colour after the addition of silver nitrate solution. Beyond 90 minutes there is no significant change in colour indicating the completion of the reduction reaction **(I. Johnson. H. joy prabu, 2015).**

Pterocarpus marsupium Roxb. bark and wood extract is shown in figure:10. The change in colour of the reaction mixture after 2 hours is presented in Figure: 11 which indicated the formation of AgNPs. This formation indicates that silver ions in reaction medium have been converted to elemental silver having the size of nanometric range.

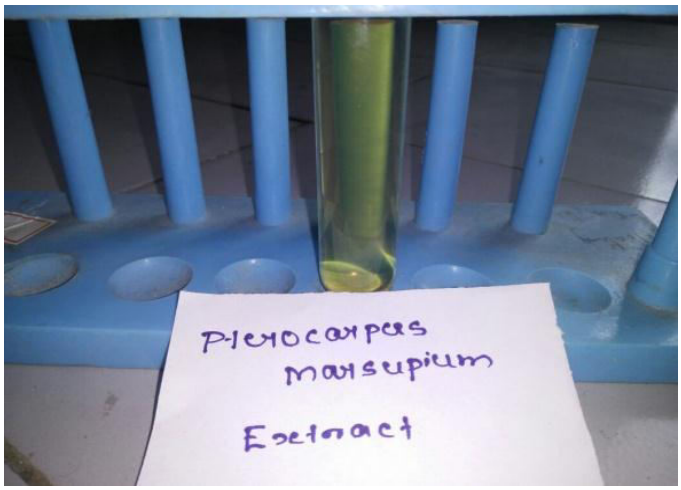

Figure: 10 *Preocarpus marsupium* Roxb. extract

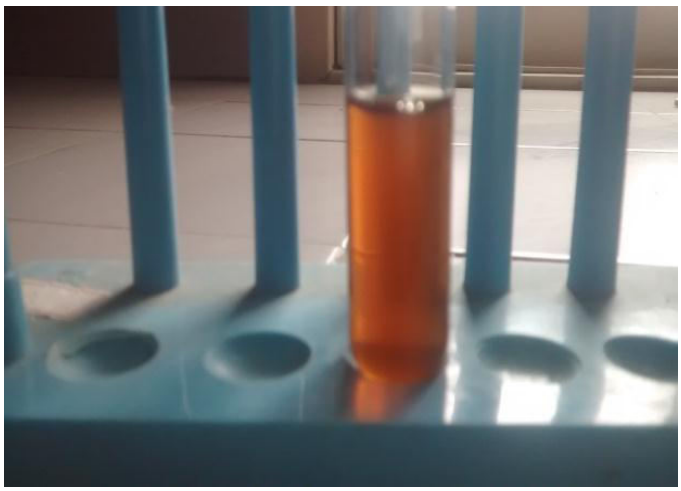

Figure: 11 *Pterocarpus marsupium* Roxb. silver nanoparticles formed after 2h

8(2) UV Visible Spectral Analysis

Uv- Visible spectral analysis characterizes the formation and completion of silver nanoparticles. The reduction of silver ions was monitored by measuring Uv- Vis spectrum of reaction medium from the wavelength of 200- 800nm by using distilled water as blank. Periodic sampling at the time interval of 30 min, 90 min, 210 min and 24 hr was carried out and depicted in the figure: 12.

The reduction of Ag+ to Ag^0 via the active bio molecules present in the bark and wood extract of *Pterocarpus marsupium* Roxb. was indicated by a colour change from yellow to brown colour. Silver nanoparticles exhibit Plasmon absorption band in the visible region. The metal nanoparticles have free electrons which gives the surface Plasmon resonance absorption band due to the combined vibrations of electrons of metal nanoparticles in resonance with light wave.

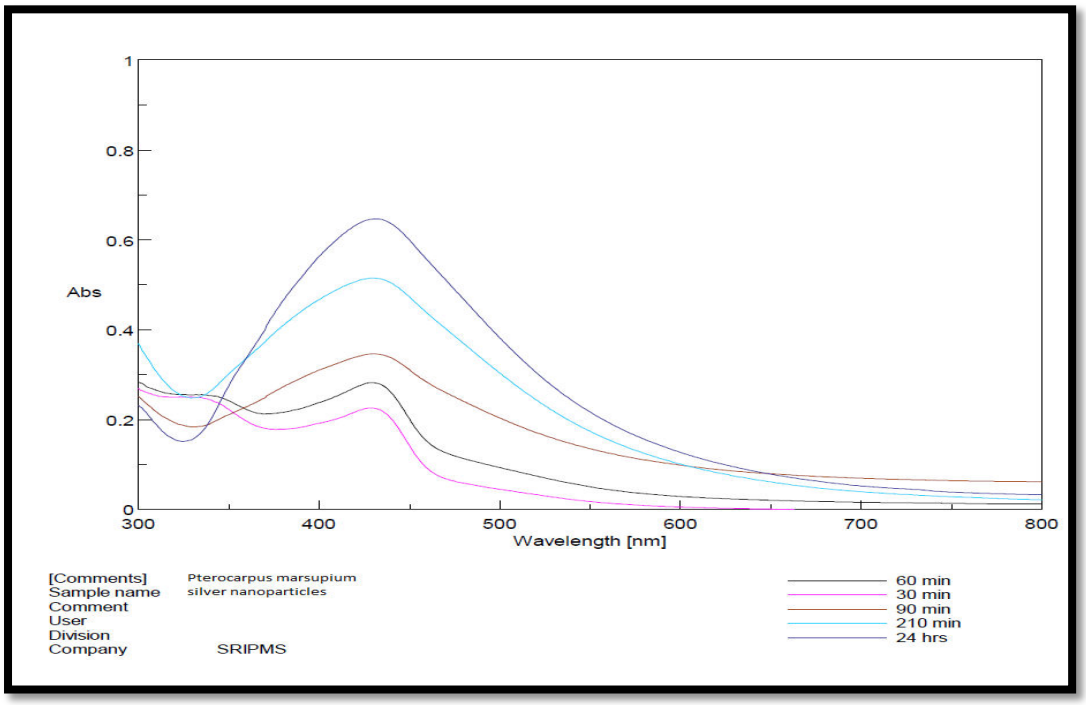

Figure: 12 Uv- visible absorption spectra of *pterocarpus marsupium* Roxb. silver nanoparticles at different time intervals

Silver nanoparticles are known to exhibit Uv – Visible absorption in the range of 400- 500nm. The sharp absorption bands of *Pterocarpus marsupium* Roxb. silver nanoparticles were observed at around 431nm.

8(3) FTIR Spectroscopy OF *Pterocarpus marsupium* Roxb. Silver Nanoparticles

FTIR measurements were carried out to identify the biomolecules responsible for capping and stabilization of metal nanoparticles synthesised. The IR spectrum of *Pterocarpus marsupium* Roxb. silver nanoparticles have following interpretations.

In the FTIR spectra of silver nanoparticles band between 3688.19 corresponds to OH stretching of free alcohol, 3437.49 corresponds to hydrogen bonded alcohols and phenols, 2789.53 corresponds to CH stretching in aldehyde, 1771.3 C=O stretching in carboxylic acid, 1541corresponds to nitro compounds **(Robert M. Silverstein).**

From the FTIR spectral analysis it was concluded that hydroxyl and carboxyl groups present may act as reducing and stabilizing agent and phenolic group present may act as capping agent. FTIR spectrum is shown in the figure: 13

Table: 7 FTIR Interpretations of *Pterocarpus marsupium* Roxb. silver nanoparticles

Material	Standard wave number Range	Test wave number	Inference
Pterocarpus marsupium Roxb silver nanoparticles	3700-3584	3688.19	O-H Alcohol
	3600-3200	3437.49	Hydrogen bonded alcohols and phenols
	2830- 2695	2789.53	CH stretching in aldehyde
	1720-1706	1771.3	C=O stretching in carboxylic acid
	1550- 1500	1541	N-O Nitro compound

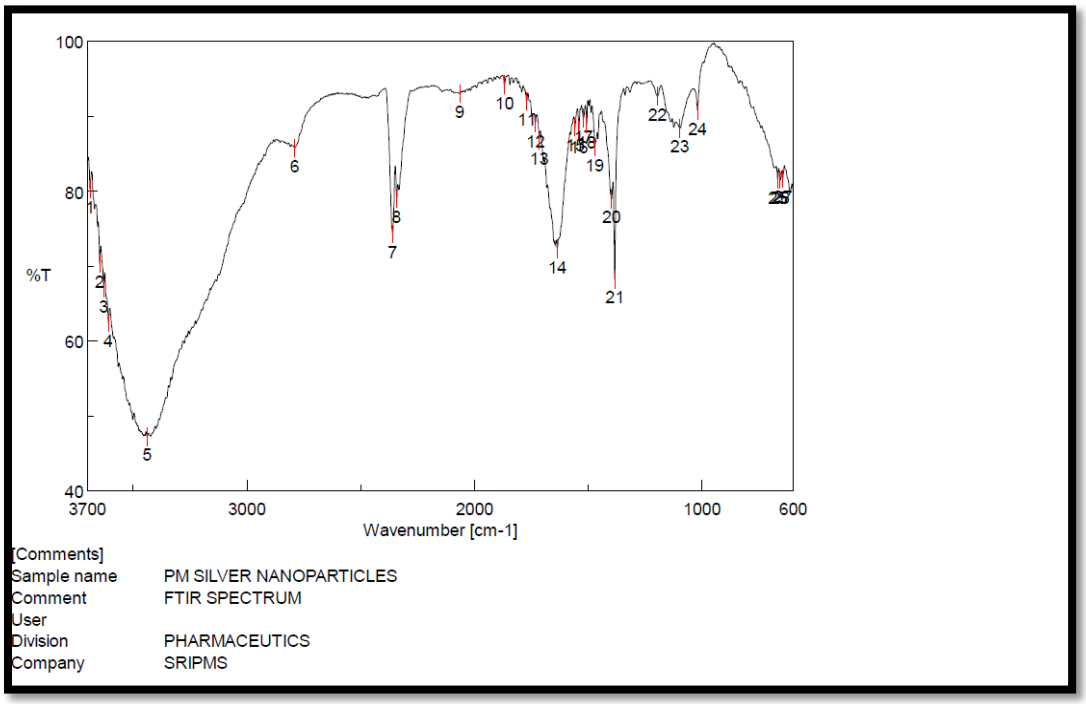

Figure 13: FTIR spectrum of *pterocarpus marsupium* Roxb. silver nanoparticles

8(4) Drug Entrapment

Drug entrapment can be determined from the supernatant liquid of *pterocarpus marsupium* Roxb. silver nanoparticles after centrifugation by uv Spectrophotometry at 431 nm. A calibration curve was plotted between concentration vs absorbance. From this standard curve amount of drug present in supernatant liquid was determined.

Amount of drug present in the supernatant obtained from standard calibration curve is subtracted (w) from the total amount used in the preparation of nanoparticles (W). (W-w) is the amount of drug entrapped. Percentage entrapment is calculated by **(Renu Tiruwa, 2015)**

$$\% \text{ drug entrapment} = \frac{W-w}{W} \times 100$$

The % entrapment of drug or drug content of *Pterocarpus marsupium* Roxb. silver nanoparticles were found to be 93.5%

8(5) Particle Size Measurement

The particle size is one of the most important parameter for characterization nanoparticles. The average particle size of *Pterocarpus marsupium* Roxb was found to be 148.5nm. Particle size analysis showed the presence of nanoparticles with polydispersity indices PDI value 0.336 with intercept 0.963. Percentage intensity of particle size distribution of biosynthesised *pterocarpus marsupium* Roxb silver nanoparticles were depicted in the figure: 14

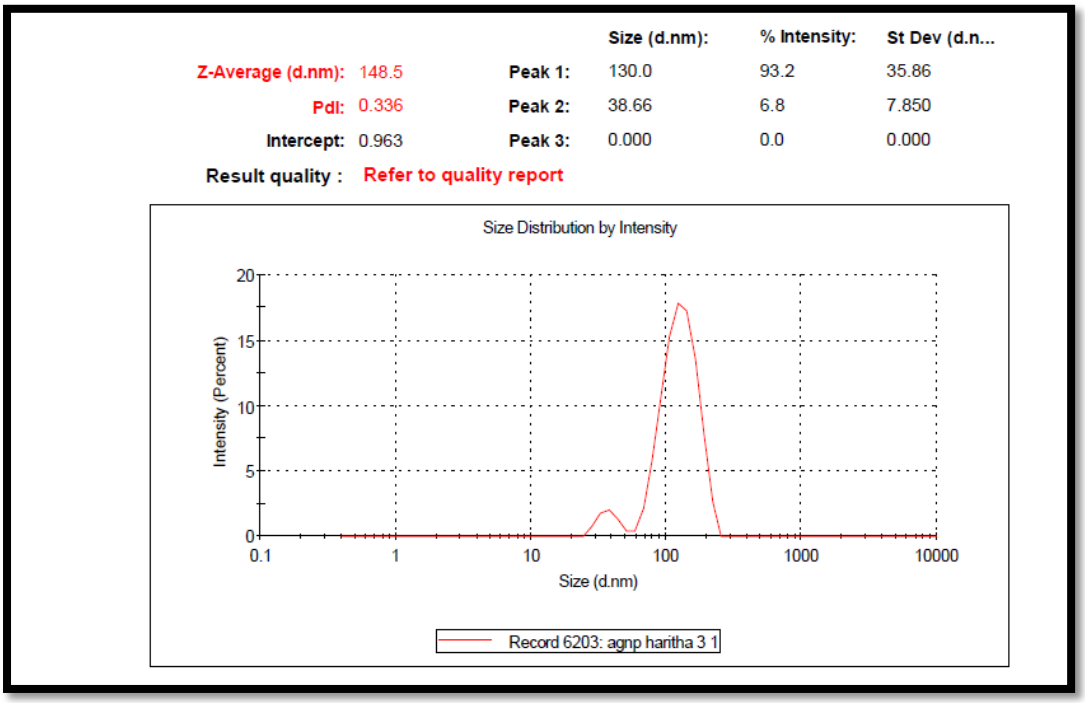

Figure: 14 Percentage intensity of particle size distribution of biosynthesised *pterocarpus marsupium* Roxb silver nanoparticles

8(6) Zetapotential Measurement

Zeta potential is a key indicator for determining the stability of aqueous silver nanoparticles. For *Pterocarpus marsupium* silver nanoparticles zeta potential measured was found to be -28 mV with peak area of 100% intensity. These values indicate the full stabilization of nanoparticles. Zeta potential distribution of *pterocarpus marsupium* Roxb. silver nanoparticles were depicted in the figure:15

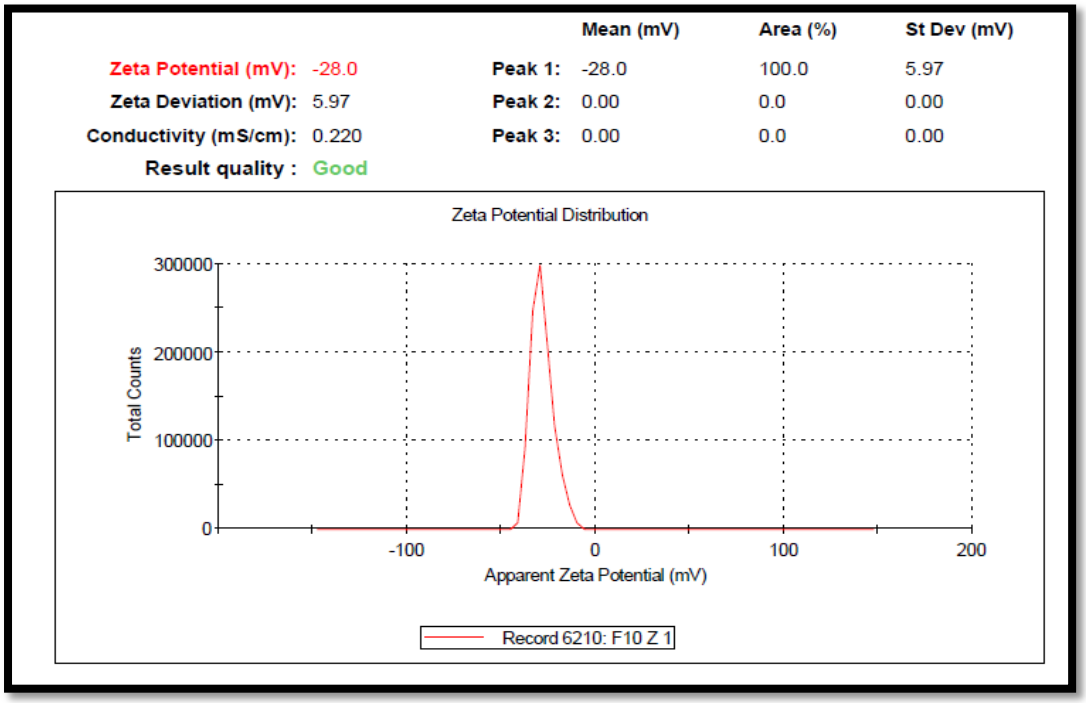

Figure: 15 Zeta potential distribution of *pterocarpus marsupium* Roxb. silver nanoparticles

8(7) Scanning Eletron Microscopy

SEM analysis of the synthesised *Pterocarpus marsupium* Roxb *silver* nanoparticle were performed to evaluate the surface morphology of nanoparticles **(GOPI G, 2015).** Silver nanoparticles were prepared and dried well to remove the moisture content and images were taken by using Hitachi X650, Tokyo, Japan. The SEM images were taken in different magnification such as 1000 X , 3000X, 10,000 X and 30,000 X and shown in the figures: 16-18 respectively.

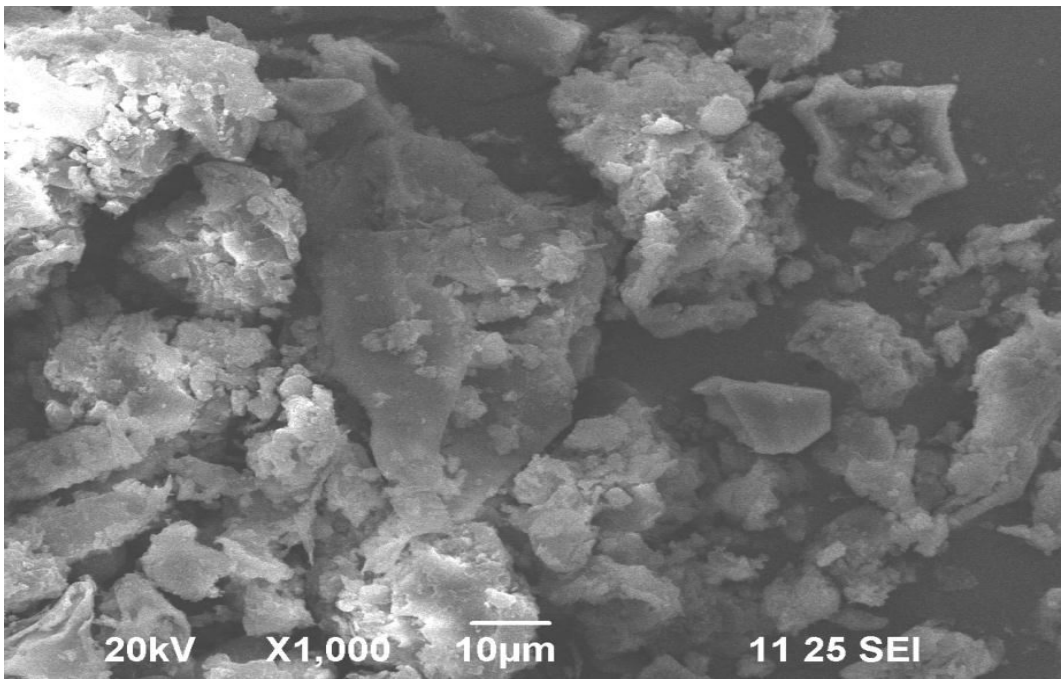

Figure: 16 SEM analysis of *Pterocarpus marsupium* Roxb. Silver nanoparticles at 1000X magnification

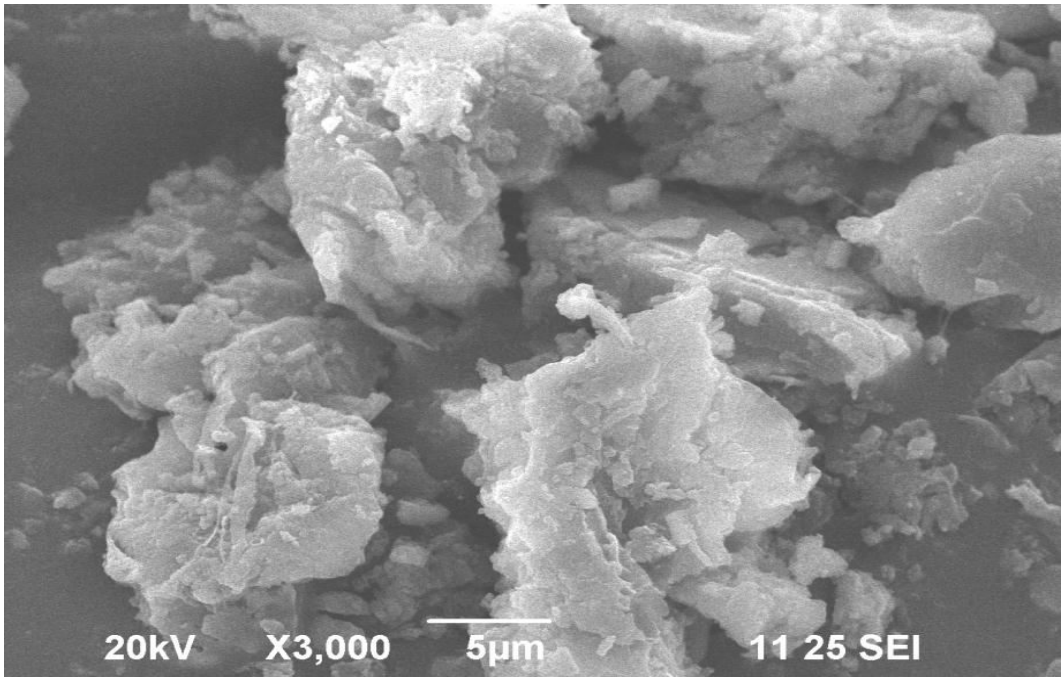

Figure: 17 SEM analysis of *Pterocarpus marsupium* Roxb. Silver nanoparticles at 3000X magnification

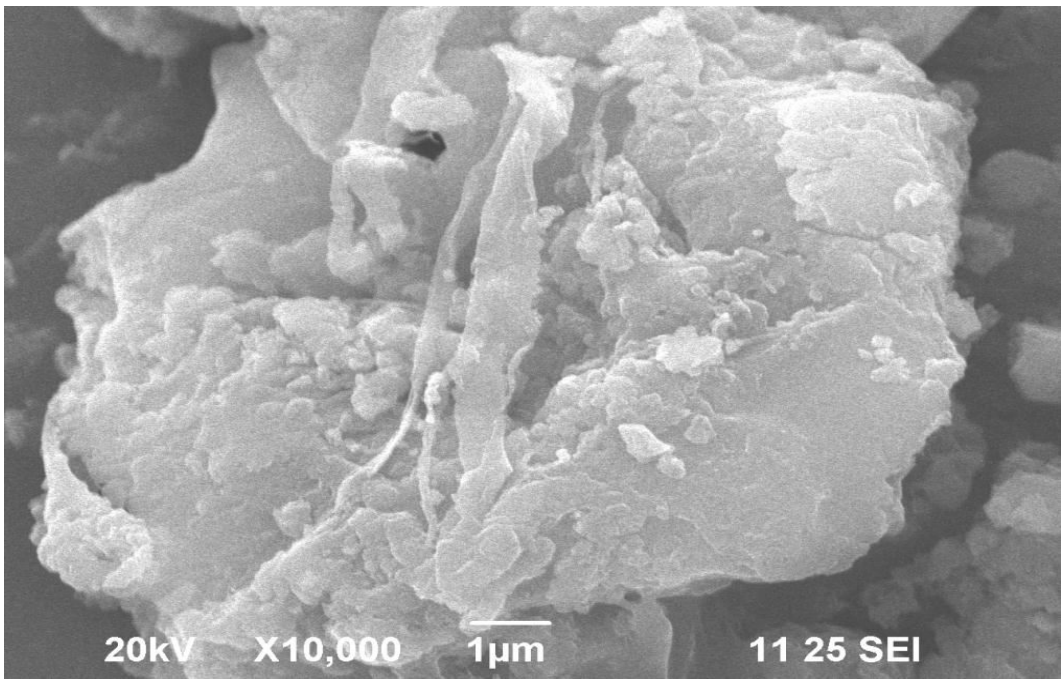

Figure: 18 SEM analysis of *Pterocarpus marsupium* Roxb. Silver nanoparticles at 10,000X magnification

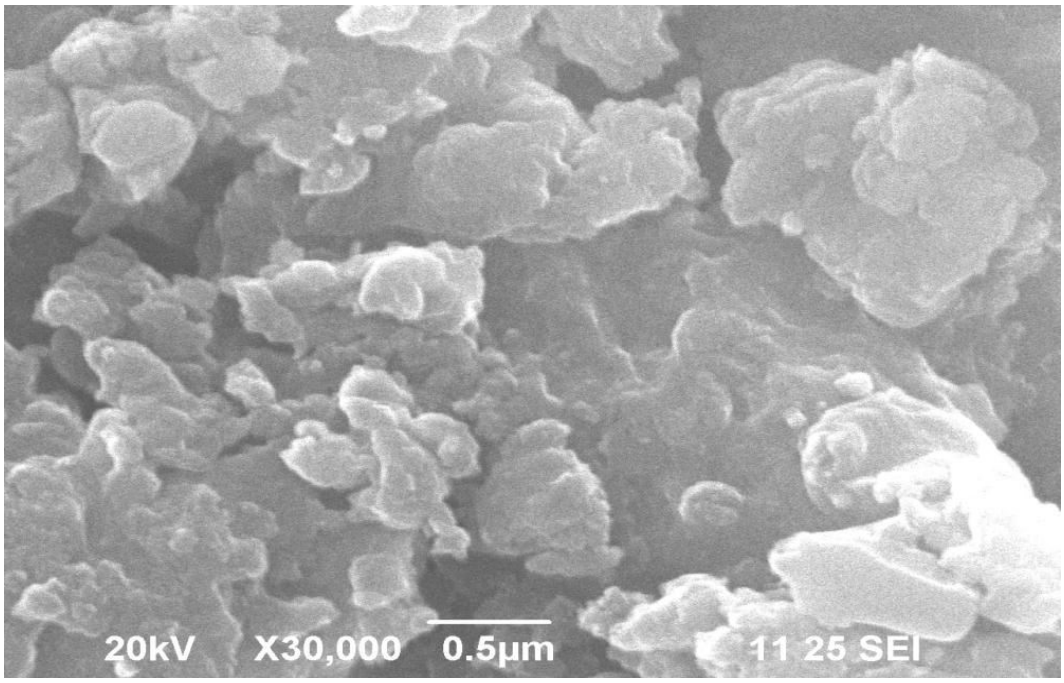

Figure: 19 SEM analysis of *Pterocarpus marsupium* Roxb. Silver nanoparticles at 30,000X magnification

The electrostatic interactions such as hydrogen bond, bio- organic bond and capping molecules are the reason for biosynthesis of silver nanoparticles**.** Scanning electron microscopy provides further insight into morphology. From the SEM analysis it was found that *Pterocarpus marsupium* Roxb.silver nanoparticles have spherical shape and the assembling of silver nanoparticles on the surface.

IN-VITRO ANTIDIABETIC STUDY

9) α- Amylase Inhibition Study

The glucose obtained from the indigestible carbohydrate food which contains starch is readily absorbed from the gastrointestinal tract into the blood stream after the hydrolysis of glycosidic bonds by the enzyme α-amylase. In diabetics, the inhibition of this enzyme reduced the high postprandial blood glucose level. In the present study, an *in vitro* alpha-amylase inhibition model was used to screen the *Pterocarpus marsupium* Roxb. silver nanoparticles to evaluate the hypoglycemic effects **(Karthik. V. P et.al, 2016).** The alpha-amylase inhibitors obstruct the absorption and the digestion of carbohydrates. Acarbose, a synthetic alpha-amylase inhibitor delays the digestion of carbohydrates and inhibits the action of pancreatic amylase in the break down of starch, which leads to side effects such as abdominal pain, diarrhoea and soft faeces in the colon.

The result suggests that *Pterocarpus marsupium* Roxb. exhibits good α-amylase inhibition under *in vitro* conditions. α- Amylase inhibitory effects of positive control Acarbose were shown in table: 8 and in table: 9 shows the α-Amylase inhibitory effects of *Pterocarpus marsupium* Roxb silver nanoparticles

Table 8: α- Amylase inhibitory effects of positive control Acarbose

Concentration (µG/ML)	Absorbance	Percentage Inhibition
0	0.832	41.44%
100	0.703	50.52%
200	0.625	56.01%
400	0.426	70.02%
800	0.226	84.09%

Percentage inhibition of α amylase for the positive control Acarbose was found to be 41.44% at concentration 50µg/ml. When the concentration is increased to 100 µg/ml percentage inhibition is increased by 1.2 fold then the concentration was increased to200 µg/ml so the percentage inhibition is increased by 1.1 fold, further the concentration was increased to 400µg/ml then the percentage inhibition is increased by 1.2 fold again the concentration was increased to 800µg/ml which resulted in the increase of percentage inhibition by 1.2 fold.

Table 9: α- Amylase inhibitory effects of *Pterocarpus marsupium* Roxb. silver nanoparticles

Concentration (µg/ml)	Absorbance	Percentage inhibition
50	1.110	21.88%
100	0.951	33.07%
200	0.642	54.82%
400	0.531	62.63%
800	0.410	71.14%

Percentage inhibition of α amylase for the *pterocarpus marsupium* Roxb. silver nanoparticles was found to be 21.88% for the concentration 50µg/ml. When the concentration is increased to 100 µg/ml percentage inhibition is increased by

1.5 fold then the concentration was increased to200 µg/ml so the percentage inhibition was also increased by 1.6 fold, further the concentration was increased to 400µg/ml then the percentage inhibition is increased by 1.1 fold again the concentration was increased to 800µg/ml which resulted in the increase of percentage inhibition by 1.1 fold.

Comparison of α- alpha amylase inhibition of Acarbose vs *Pterocarpus marsupium* Roxb. silver nanoparticles were shown in the figure: 20. From the figure we can compare the percentage inhibition of both positive control and test drug.

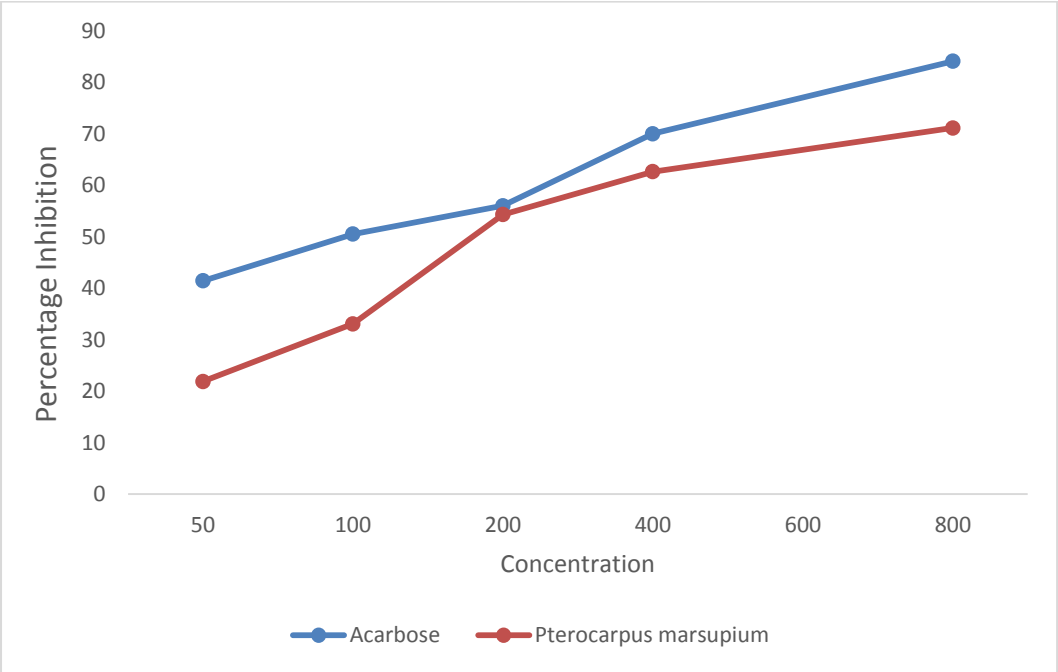

Figure: 20 Comparison of α alpha amylase inhibition of Acarbose vs *Pterocarpus marsupium* Roxb. silver nanoparticles

The percentage α amylase inhibition of positive control Acarbose at lower and higher concentration was found to be 41.44% and 84.09% and for test *pterocarpus marsupium* Roxb. silver nanoparticles percentage α amylase inhibition at lowest and highest concentration was found to be 21.88% and 71.14% respectively.

10) IN-VITRO DRUG RELEASE STUDY

In- vitro drug release study was carried out using dialysis bag diffusion method. Amount of drug released at different time intervals (1h, 2h, 3h, 4h, 5, 6h, and 24 h) were found out. Figure : 21 shows the dialysis diffusion method.

Figure: 21 Dialysis bag method

The release data obtained from the *in-vitro* dialysis bag diffusion method were fitted into various mathematical models. The kinetic models included zero order, First order, Higuchi equation (matrix system) and Korsmeyer -Peppas models. Table: 9 summarize the release study of *Pterocarpus marsupium roxb* silver nanoparticles. Table: 10 shows their R^2 values and n exponential value .The overall curve fitting showed that the drug release from the sustained release matrix tablets followed either zero order or korsmeyer –Peppas model. The values of the exponential factor 'n' were found to be in between 0.83-0.95 indicating the non-fickian dissolution controlled drug release. **(Bohrey et.al, 2016)**

Table: 10 *In vitro* drug release study of *Pterocarpus marsupium* Roxb silver nanoparticles

Time	Log time	Square root of time	Cumulative amount of drug released	Cumulative % of drug released	Log cumulative amount of drug released
1	0	1	1.535	15.35%	1.1861
2	0.3010	1.4142	2.1325	21.32%	1.3288
3	0.4771	1.7320	2.6425	26.42%	1.4219
4	0.6020	2	2.9175	29.17%	1.4649
5	0.6989	2.2360	3.323	33.23%	1.5215
6	0.778	2.4494	3.8237	38.23%	1.5824
24	1.3802	4.8989	8.9287	89.28%	1.9507

In vitro drug release of *Pterocarpus marsupium* Roxb silver nanoparticles were studied using dialysis bag diffusion method and carried out at different time intervals. After 1h the cumulative percentage of drug released was found to be 15.35%, 21.32% for 2 h, 26.42% for 3 h, 29.17% for 4h, 33.23% for 5, 38.23% for 6 h and 89.28% for 24h.

Drug release data fitted to various kinetic models

Zero order:

Time vs Cumulative percentage release were plotted and shown in the figure: 22. From the the graph R^2 value were found to be 0.7723.

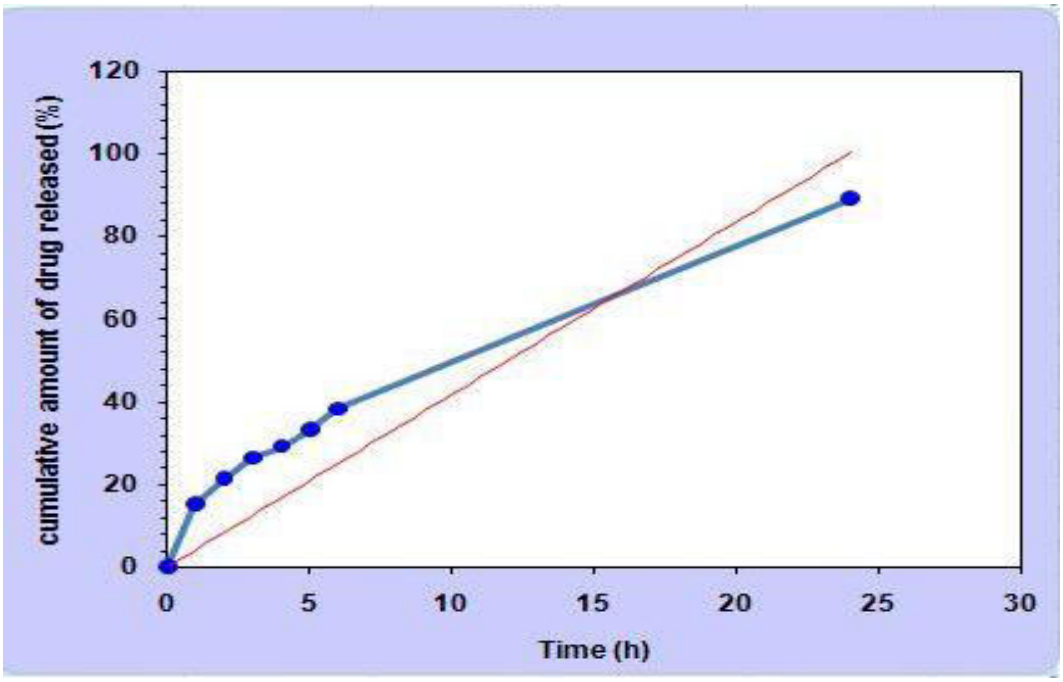

Figure: 22 Drug release kinetic data of *Pterocarpus marsupium* Roxb silver nanoparticles fitted into zero order.

First order:

Time vs log cumulative percentage were plotted and shown in the figure: 23. From the the graph R^2 value found to be 0.5168

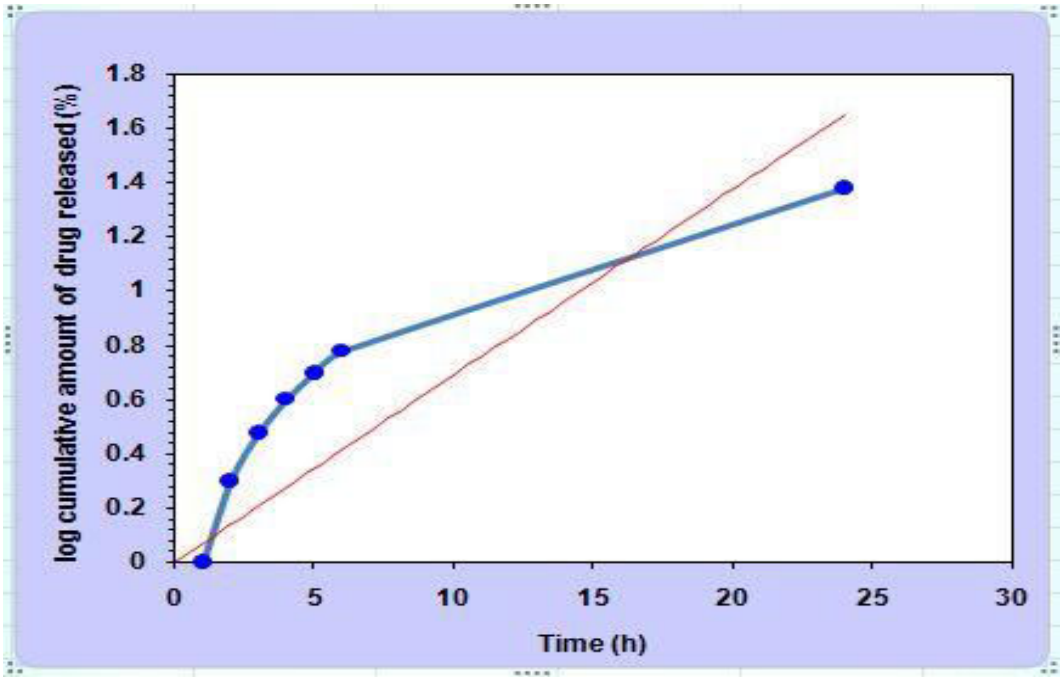

Figure: 23 Drug release kinetic data of Pterocarpus marsupium silver nanoparticles fitted into first order

Higuchi's:

Square root of time vs Cumulative percentage release and shown in the figure: 24 and the R^2 value found to be 1.000

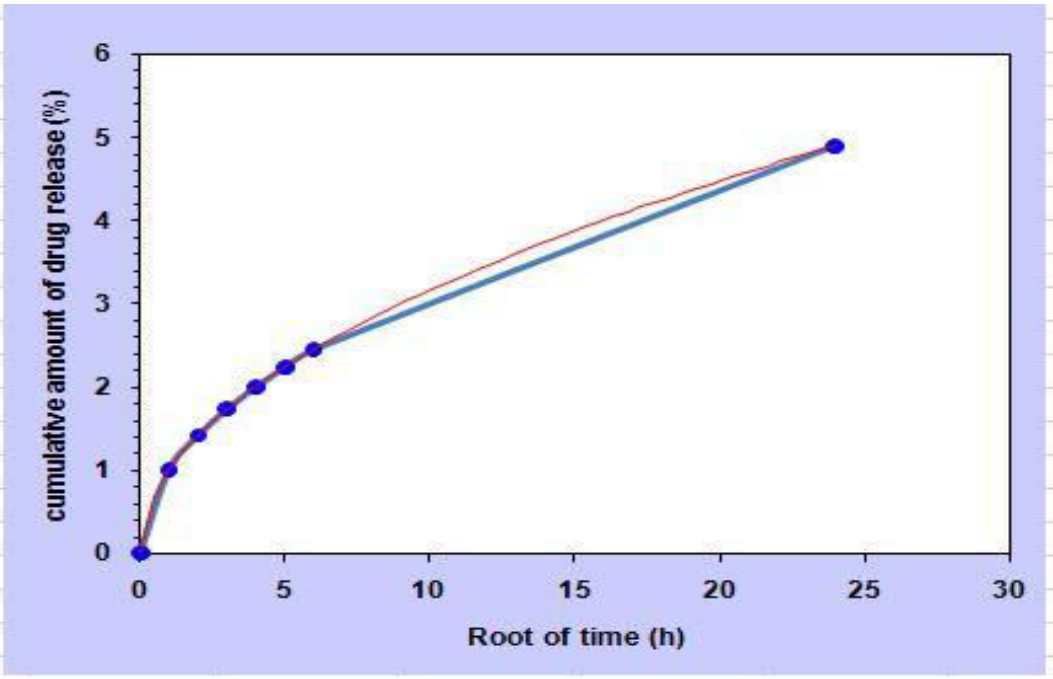

Figure: 24 Drug release kinetic data of Pterocarpus marsupium silver nanoparticles fitted into Higuchi's model

Korsmayer Peppas:

Log time vs log cumulative percentage and shown in the figure: 25 and R^2 value found be 0.9999 and the n value is 0.156

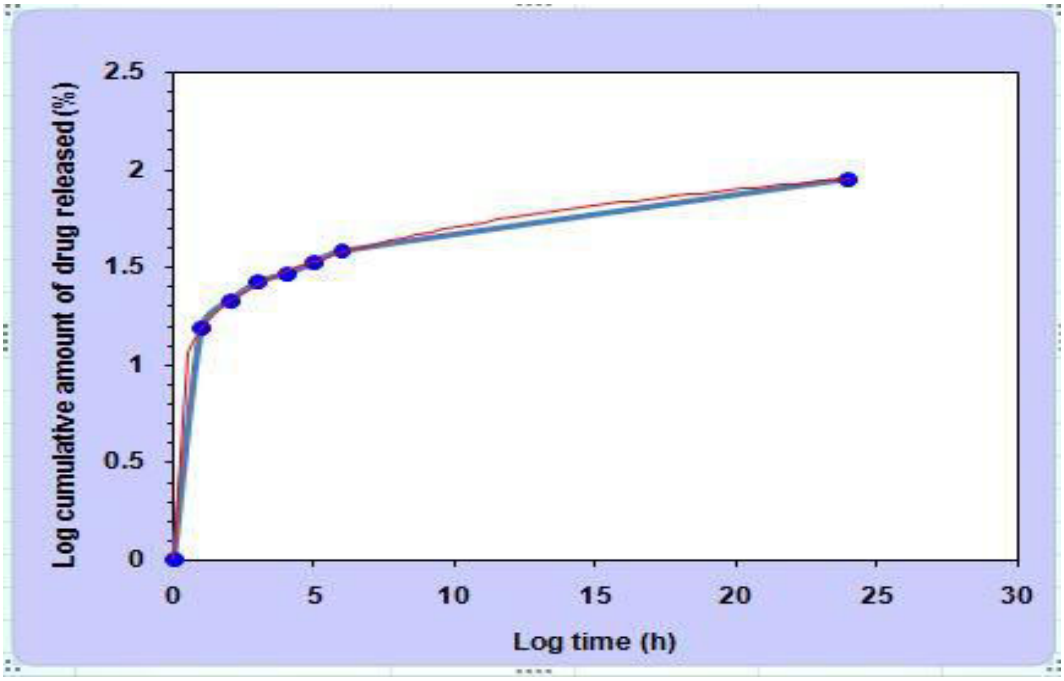

Figure: 25 Drug release kinetic data of *Pterocarpus marsupium* Roxb. silver nanoparticles fitted into Korsmayer Peppas

Table: 11 R^2 value obtained for various kinetic release models

Formulation	Zero order kinetics R^2	First order kinetics R^2	Higuchi kinetics R^2	Korsmeyer peppas kinetics	
				R^2	n
Pterocarpus marsupium roxb silver nanoparticles	0.7723	0.5168	1.000	0.9999	0.156

The release of *Pterocarpus marsupium* Roxb. silver nanoparticles were best fitted into Higuchi equation. The Higuchi equation describes the diffusion of drug from homogenous and granular matrix systems. Furthermore Korsmeyer-Peppas model describes the drug release kinetics in a most befitting manner, where the values of the diffusion exponent (n) was 0.156, confirming that the formulation followed Fickian diffusion kinetics (**Sadiq and Rassol, 2014**).

From the release kinetic data it can be concluded that the formulation *Pterocarpus marsupium* Roxb. silver nanoparticles fit with the highest correlation coefficient (R^2) was resulted with Higuchi and followed by Korsmeyer peppas, zero order, first order.

SUMMARY AND CONCLUSION

Nanotechnology is developing rapidly and more methods to obtain nanoscale particles are emerging continuously. Eco-friendly processes for the synthesis of nanoparticles attracted huge attention especially because conventional chemical methods produce hazardous by products. Organisms ranging from simple bacteria to more complex eukaryotes and numerous plant extracts are used for the biosynthesis of silver nanoparticles with different sizes and morphologies.

Silver nanoparticles were prepared by green synthesis method using *Pterocarpus marsupium* Roxb. bark and wood extract. Biosynthesis of nanoparticles is a kind of bottom- up approach where the main reaction occurring is reduction. The reduction of silver ions leads to the formation of stable silver nanoparticles. This work aimed to synthesis a novel drug delivery system with herbal drug in order to reduce possible side effects.

Herbal drug extract were prepared by cold maceration method and phytochemical screening tests were carried out to identify the chemical constituents such as Alkaloids, Amino acid, Carbohydrate, Flavonoids, Glycosides, Phenolic compounds, Starch, Proteins present in the *Pterocarpus marsupium* Roxb. bark and wood extract.

Preformulation studies were carried out before synthesis of silver nanoparticles by green synthesis method. Solubility tests give the idea that the *Pterocarpus marsupium* Roxb bark and wood is soluble in polar solvents and sparingly soluble in non polar solvents.

Uv- Visible absorption spectra for *Pterocarpus marsupium* Roxb bark and wood extracts were taken. And the maximum absorption peak was observed at 279 nm.

Then the calibration curve of *Pterocarpus marsupium* Roxb. bark and wood extracts were plotted and R^2 value was found to be 0.999 for the concentration between 4- 20 µg/ml.

FTIR measurements for *Pterocarpus marsupium* Roxb. bark and wood as well as for Silver nitrate were carried out to identify the chemical constituents present in the extract and the functional groups present in the silver nitrate solution.

Silver nanoparticles were prepared by green synthesis method using *Pterocarpus marsupium* Roxb. bark and wood extract along with silver nitrate solution. Then following characterizations were carried out.

Visual examination of synthesised *Pterocarpus marsupium* Roxb. silver nanoparticles for the colour change from yellow to brown indicates the formation of silver nanoparticles.

This was further confirmed by Uv- visible spectral analysis of the sample at different time intervals and found to 431 nm.

FTIR measurements of *Pterocarpus marsupium* Roxb. silver nanoparticles were carried out to identify the bio molecules responsible for capping and stabilization of metal nanoparticles synthesised and found to be hydroxyl and carboxyl groups present act as reducing and stabilizing agents and phenolic group act as capping agent.

Drug entrapment test was carried out and the percentage drug entrapment was found to be 93.5%

Particle size is analysed by using zeta sizer and the average particle size was found to be 148.5 nm with polydispersity index 0.336 and intercept 0.963

Zeta potential measured was found to be -28 mV with peak area of 100 % intensity. These value indicates the stabilization of silver nanoparticles.

SEM analysis was carried out to study the morphology of synthesised *Pterocarpus marsupium* Roxb silver nanoparticles and found to be spherical shape.

In vitro α amylase inhibition test suggests that *Pterocarpus marsupium* Roxb. silver nanoparticles exhibit good α amylase inhibition . Percentage inhibition of positive control Acarbose at lower and higher concentration was found to be 41.44% and 84.09% and that of *Pterocarpus marsupium* Roxb. silver nanoparticle were 21.88% and 71.14%.

In vitro drug release kinetics were evaluated by substituting all the data in zero order, first order, Higuchi, Korsmeyer-Peppasmodels . The R^2 values for zero order and first order is0.7723 and 0.5168 and that of Higuchi and Korsmeyer-Peppasmodels is 1.0000 and 0.9999 and the diffusion exponent n value is 0.15. From the release kinetics it was found that *Pterocarpus marsupium* Roxb. silver nanoparticles follows fickian diffusion transport mechanism.

The association of plant Phytochemistry and nanotechnology will assure a secured manner of curing diversified diseases in nearby future. The current study proved that, the *Pterocarpus marsupium* Roxb. bark and wood extracts were capable of producing silver nanoparticles using green synthesis technique in association with silver nitrate. These nanoparticles were characterized and evaluated for various tests such as visual examination, uv- visible spectral analysis, FTIR spectroscopy, drug entrapment, determination of particle size, determination of zeta potentional, SEM analysis ,i*n- vitro* anti diabetic study and *in vitro* drug release and kinetic study. The results of the above tests were showing promising results which can be a lead to develop *Pterocarpus marsupium* Roxb. as silver nanoparticles so as to enhance its anti diabetic activity.

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MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA

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सं. भा.व.स./द.क्षे.के./No.: BSIS/RC/5/23/2016/Tech. 165

दिनांक/Date: 19th April 2017

सेवा में / To

Ms. Haritha. H.
II Year M. Pharmacy
Department of Pharmaceutics
College of Pharmacy- Sri Ramakrishna Institute of
Paramedical Sciences
Coimbatore – 641 044

महोदया/Madam,

The plant specimen brought by you for authentication is identified as *Pterocarpus marsupium* Roxb. - LEGUMINOSAE. The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

(डॉ. एम. पलनिसामी/Dr. M. Palanisamy)
वैज्ञानिक ' डी ' प्रभारी/Scientist 'D'-In-Charge
वैज्ञानिक 'सी' / SCIENTIST 'C'
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