

**A STUDY ON THE GREEN SYNTHESIS OF SILVER AND
COPPER NANOPARTICLES FROM *SWIETENIA MACROPHYLLA*
AND ITS THERAPEUTIC APPLICATIONS**

Submitted in partial fulfilment of the requirements for the award of Master
of Technology degree in biotechnology

by

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**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO & CHEMICAL ENGINEERING**

SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of P.Rahul Prakash (41810015) who carried out the project entitled "**A Study on the green synthesis of Silver and Copper nanoparticles from *Swietenia macrophylla* and its therapeutic application**" under my supervision from January 2023 to May 2023.



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DECLARATION

I, P.Rahul Prakash hereby declare that the Project Report entitled “ **A study on the green synthesis of silver and copper nanoparticle from *Swietenia macrophylla* and its therapeutic application**” done by me under the guidance of Dr. Sudha.S at the Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai, is submitted in partial fulfillment of the requirements for the award of Master of Technology degree in Biotechnology.

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SIGNATURE OF THE CANDIDATE

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ABSTRACT

Swietenia macrophylla, commonly known as mahogany or skyfruit, is a species of plant in the *Meliaceae* family which have great variety of pharmacological activities. Preliminary pharmacological assay revealed that *Swietenia macrophylla* seeds possess significant hypoglycemic activity in experimental diabetic rats. Further methanolic extract of the seed was prepared for the synthesis of silver and copper nanoparticles. The silver nanoparticles were characterized using UV-Vis spectroscopy which showed a peak at 435nm and FTIR analysis was carried out to identify the functional groups and bonding affinities.

The average particle size was found to be 18nm with agglomerate structure which was characterized using Scanning Electron Microscope. Identification of bioactive compounds in the seed extract was done using GC/MS. Further, anti-bacterial, anti-fungal and antidiabetic activities were carried out for the methanolic extract. Anti-diabetic activity using alpha amylase inhibition was done and the maximum α -amylase enzyme inhibition was 84.81 ± 0.81 % at 200 $\mu\text{g/mL}$ concentration with the IC_{50} of 65.65 $\mu\text{g/mL}$ concentration. The MTT assay against MCF-7 cells showed a higher toxicity of 72% at 100 $\mu\text{g/mL}$ of AgNP while 1ng/mL was 16%. These experiments showed that *S.macrophylla* has greater potency against anti-fungal and anti-diabetic activity which can be used as efficient drug and as a carrier for other therapeutic applications.

Key words: green synthesis, silver nanoparticles, *S.macrophylla*.

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LIST OF ABBREVIATIONS

- | | |
|------------------|------------------------------|
| 1. NP | - Nanoparticle |
| 2. UV | - Ultraviolet |
| 3. MNP | - Metal nanoparticle |
| 4. AgNPs | - Silver nanoparticles |
| 5. MCF-7 | - Michigan Cancer Foundation |
| 6. Au | - Gold |
| 7. S.macrophylla | - Swietenia macrophylla |
| 8. AgNP | - Silver Nanoparticle |

1. INTRODUCTION

The nanotechnology is one of the enthusiastic fields in modern materials science. Nanotechnology is based on synthesise of nanomaterials (1-100 nm) and its applications in various fields especially for industrial revolution. Nanotechnology has been emerging as a cutting-edge technology convergence with physics, chemistry, biology, material science and medicine. The primary concept of Nanotechnology was presented by Richard Feynman in 1959 in a lecture titled “There is plenty of room at the bottom”. In 2006, American Society for Testing and Materials (ASTM) defined the term ‘nanoparticles’ as particles containing at least two or more dimensions ranging from 1 to 100 nm in size (Alanazi *et al.*, 2010). Therefore, Nanotechnology is the application of science that controls matter at molecular level. According to the British Standards Institution, nanoparticles are the particles having one or more dimensions at nanoscale (Wang *et al.*, 2007). At this scale, the properties of particles are quite different from their properties at atomic or molecular levels. Therefore, the size of a nanoparticle (1-100 nm) serves as an intermediate between the atomic and molecular (bulk) states (Ahmad *et al.*, 2017). This intermediate state plays a key role in deciding the fate of nanomaterial and also provides unexpected physicochemical properties. These changes contribute the characteristic thermal conductivity, catalytic, melting point, biological, sterical, mechanical, electrical and optical properties of nanoparticles (Bogunia-Kubik and Sugisaka, 2002; Daniel and Astruc, 2004; Zharov *et al.*, 2005).

In recent years, nanoparticles have been shown to have great potential in biomaterials research (Capek, 2017). For instance, Durán *et al.* (2016) have shown the potentiality of metallic nanoparticles for treating various viral infections. Wang *et al.* (2017) have anticipated that silver nanoparticles can emerge as viable alternative to existing antibiotics and/ or antibacterial drugs and hold immense promise to solve the problem of the emergence of multi drug resistant pathogens. It is also possible to use nanotechnology to control neglected diseases that are currently ignored by the government and pharmaceutical industries (Islan *et al.*, 2017). Owing to their low or no toxicity and biocompatibility, gold nanoparticles can be explored as ideal candidates for advanced clinical research (Ahmad *et al.*, 2003a). Nowadays, research on targeted nanoparticles is growing at a rapid pace, leading to the development of

effective multifunctional gold nanoparticles for treating many diseases, for example, cancer (Capek, 2017).

1.1 METAL NANOPARTICLES

There are many chemical methods available to synthesis nanoparticles which are costly. The strong toxic and hazardous chemicals are associated with biological risks. Therefore, Green synthesis is the most commonly adopted method for the synthesis of nanoparticles, in which the extracts of various parts of the plant body are employed. Green synthesis method has special advantage over other methods such as easy availability, much safer to handle, widely distributed and plants are the source with several medicinally valuable metabolites (Saima Gul *et al.* 2016). The surface area of the nanoparticles is indirectly proportional to the particle size. The surface area of the nanoparticle makes the difference in the physical and chemical properties from the bulk material. Nanoparticles play an important role in the development of nanotechnology. It also pays attention to various applications in the field of life sciences, physics, energy, environment, information technology and new materials to make large scale production with miniature of huge equipments (Girilal 2013).

1.2 SILVER NANOPARTICLE

One of the foremost and natural questions is that why we attracted towards silver nanoparticles (AgNPs), and investigated their biological and physico-chemical properties. The metal nanoparticles are synthesized from different types of metal salts such as copper (CuSO₄), iron (FeCl₃), gold (AuCl₃) cerium (CeNO₃), Zinc (ZnCl₂), Cobalt (CoCl₂) and silver (AgNO₃), etc. Among the various metal nanoparticles, Ag nanoparticles (AgNPs) have several advantages. In the recent decade, AgNPs have been used in a wide range of electronic and medical devices, surgical instruments, bone cementing, surgical masks, etc (Prabhu and Poulouse 2012; Nisha *et al.*, 2015; Slane *et al.*, 2015; Li *et al.*, 2006).

Furthermore, the specific quantity of ionic silver is used for treating wounds and burns (Lansdown, 2006; Atiyeh *et al.*, 2007; Lansdown, 2007). Therefore, AgNPs are being used in post-surgical treatment as antiseptic agents. In fact, AgNPs are also used as molecular labelling due to having huge scattering cross-section and surface

plasmon resonance (Schultz and Nicholas 2000). Thus, AgNPs are presently being recognized for their enormous applications (Gan and Li 2012; Rafique *et al.*, 2017; Kumar *et al.*, 2016)

Silver is a safe inorganic non-toxic element. Silver nanoparticles is used as a drug for about 650 types of diseases against microorganisms. The colloidal form of silver is used as a medicine for bacterial contagion in open wounds, bandages, preparation of ointments, wound dressings, contraceptive and water disinfectant. Silver nanoparticles (AgNPs) is a more interesting material, this is due to its broad applications in various fields as catalysis, thin film formation, herbicide detection, nanotechnology, photocatalysis, optics, antimicrobials, anticancer studies and biomaterial production. Silver nanoparticles are cheapest material; thus, it is used as goods (that is, plastics, clothing, creams and soaps) and also increases their market demand due to their antimicrobial property. Silver nanoparticles implanted into socks, to reduce the odour, it can leach from anti-fouling membrane in water purification. Further, it is used for the treatment of contaminated ground water. A study on toxic effect of silver nanoparticles was examined on Zebra fish, due to its rapid progress and translucent body structure. The biocompatibility and toxicity of silver nanoparticles were revealed by examining silver nanoparticle inside the embryos at each stage. The abnormalities were observed in Zebra fish were strongly depends upon the dosage of silver nanoparticles. Silver nanoparticles are good antibiotic and preservative and thus it can easily contact with human beings. It makes more interest to study the effect that silver nanoparticles could have on human beings.

1.2.1 IMPORTANCE OF SILVER NANOPARTICLES

- Purification and quality management of air, imaging, biosensing and drug delivery system.
- It is used as a coating for solar energy absorption, batteries, optical receptors, catalysts, biolabeling, antimicrobials.
- Silver nanoparticles are used in high sensitivity, biomolecular detection, diagnostics, antimicrobials, therapeutics and catalysis.
- It has some potential application like biological implants (like heart valves) wound dressings, surgical instruments and bone prostheses.

1.3 METHODS FOR SYNTHESIS OF NANOPARTICLE

The nanoparticles are synthesized by mainly two processes, (i) Top-down, and (ii) Bottom-up approach. In the Top-down approach, bulk materials are converted to fine nanoparticles by using milling, grinding and sputtering technic, whereas, in the Bottom-up approach, nanoparticles are synthesized by the chemical and biological processes.

1.3.1 PHYSICAL METHODS

The physical approach is one of the common methods, where nanoparticles are synthesized by high energy ball-milling (HEBM) (Salah *et al.*, 2011), laser pyrolysis (Bouhadoun *et al.*, 2015), electrospraying (Foroutan *et al.*, 2015), laser ablation (Scaramuzza *et al.*, 2015), and evaporation-condensation (Abbasi *et al.*, 2016). Some other physical techniques, also used in the synthesis of nanoparticles are metal sputtering (Tsuji *et al.*, 2003), annealing (Nakade *et al.*, 2002) arc discharge technic (Tsuji *et al.*, 2002) and atomization (Okuyama and Lenggoro 2003). The physical approaches have more recommendations than the chemical approaches due to lack of solvent use (Hornyak *et al.*, 2008).

1.3.2 CHEMICAL METHODS

The hydrothermal, sol-gel method, micro emulsion technique, chemical vapour synthesis and polyol synthesis techniques are mainly used in chemical approaches. In the chemical mediated procedures, some reducing agents are used for reduction, which may be organic or inorganic compounds such as sodium citrate, NaBH₄, hydroquinone, elemental hydrogen and garlic acid, etc. In general, chemical methods are performed in the solution stage and the products are found in precipitate form, therefore, these reactions are called as co-precipitation (Cushing *et al.*, 2004).

1.3.3 BIOLOGICAL METHODS

In the biological methods, the nanoparticles are synthesized by using plants, animal products, fungi, bacteria, and their metabolites. The main advantages of the biological method are that there is no requirement of costly and hazardous chemicals/solvents. In most of the cases, the constituents of the biological system may act as capping, reducing and stabilization agents. AgNPs have been synthesized by different types of organisms such as bacteria, protists, plants, and fungi as described in literature review (Mohanpuria *et al.*, 2008).

1.4 GREEN SYNTHESIS OF SILVER NANOPARTICLES (AgNPs)

1.4.1 FUNGI MEDIATED

Various groups of Fungi like moulds, yeasts, mushrooms, etc. have been employed for the synthesis of NPs. *Fusarium* sp. (Mukherjee *et al.*, 2002), *Phaenerocheate*, *Chrysosporium* (Vigneshwaran *et al.*, 2006) and *Colletotrichum* sp. (Shankar *et al.*, 2003) were used for synthesis of nanoparticles owing that the fungi have high binding and metal-accumulation ability (Ahmad *et al.*, 2003a; Ahmad *et al.*, 2003b; Ahmad *et al.*, 2003c). The nanoparticles synthesizing from fungi are more advantageous than using other microorganisms due to their easy culture in the laboratory than that of the bacteria.

1.4.2 BACTERIA MEDIATED

The nanoparticles of silver, copper and gold are being synthesized from bacteria. Different gram-negative or gram-positive bacterial strains namely *Acinetobacter calcoaceticus*, *Staphylococcus aureus*, *Bacillus fexus* and *Bacillus amyloliquefaciens* are used for synthesis of the silver nanoparticles (Nanda and Saravana 2009; Reddy *et al.*, 2010; Wei *et al.*, 2012; Saravanan *et al.*, 2011; Priyadarshini *et al.*, 2013; Das *et al.*, 2014). The bacteria mediated Ag-NPs have been found in different shapes like discoidal, spherical, triangular, hexagonal and cuboidal. Saifuddin and his co-workers have synthesized AgNPs from *Bacillus subtilis* in a microwave condition in the presence of water (Saifuddin *et al.*, 2009). Shahverdi group

of researchers have synthesised AgNPs within 5 minutes by using *Escherichia coli*, *Enterobacter cloacae* and *Klebsiella pneumonia* (Shahverdi *et al.*, 2007).

1.4.3 ALGAE MEDIATED

The nanoparticles are also being synthesized by algae. The gold nanoparticles are synthesized by using *Spirulina platensis* and a blue alga (Suganya *et al.*, 2015). Singaravelu and co-workers have synthesized stable gold nanoparticles within 8-12 nm size by using *Sargassum wightii* (Singaravelu *et al.*, 2007). AgNPs synthesized by *Sargassum wightii* were investigated for their antibacterial potential (Govindaraju *et al.*, 2009).

1.4.4 PLANT MEDIATED

The syntheses of silver nanoparticles from the plants and their isolated metabolites are one of the most attractive research areas due to its economic protocol, rapid mode, eco-friendly and non-pathogenic procedure. The biomolecules like amino acids, proteins, terpenes, polysaccharides, vitamins, alkaloids, saponins and phenolic compounds present in the plant extracts act as reducing and stabilising agents in the green synthesis of NPs (Roy and Das 2015).

1.5 SWIETENIA MACROPHYLLA

Swietenia macrophylla, commonly known as mahogany or skyfruit, is a species of plant in the Meliaceae family. In Tamil it is generally called as THENKANI whose seeds are used as a cure for diabetes. There are several data in the literature indicating a great variety of pharmacological activities of *S. macrophylla*, which exhibits antimicrobial, anti-inflammatory, antioxidant effects, antimutagenic, anticancer, antitumor and antidiabetic activities. Preliminary pharmacological assay revealed that *Swietenia macrophylla* seeds possess significant hypoglycemic activity in experimental diabetic rats. Petroleum ether extract of *Swietenia macrophylla* seeds has been reported to produce significant antidiarrheal activity and its aqueous extract has been reported to be effective against *Plasmodium falciparum*.

The methanol seed extract was found to have anti-diabetic and anti-fungal activity towards Diabetes and also against fungal wounds.



Fig 1.1: Dry seed picture of *S. macrophylla*

1.5.1 CHARACTERISTICS AND USES OF *S. MACROPHYLLA*:

The fruits are called as “Sky fruits” because of its upward growth towards the sky. The fruits are measured to be 40cm in length, in a brown capsule. Each capsule could contain 71 winged seeds. In Tamil it is generally called as THENKANI. The sky fruit concentrate and pure seeds contains antihyperglycemic activity, improve blood circulation and skin condition and also helps in anti-erectile dysfunction problems. However, there are reports stating liver injury or hepatotoxicity due to consumption of these seeds where their mechanism is unknown. Stopping the consumption restores normal liver functionality. These injuries are caused due to ingestion of over dosage of the seeds as it is recommended that an intake of less than 325 mg/kg of body weight is prescribed as a required dosage per person. The sky fruit is used as a medicine due to its major anti-diabetic activity which act as a cure for Type-II diabetes (Diabetes mellitus). Diabetes mellitus, commonly known as diabetes, is a group of metabolic disorders characterized by a high blood sugar level over a prolonged period of time. Symptoms often include frequent urination, increase thirst, and increased appetite. If

left untreated, diabetes can cause many complications. Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death. Serious long-term complications include cardiovascular-disease, stroke, chronic kidney disease, foot ulcers, damage to the nerves, damage to the eyes and cognitive impairment.

1.5.2 CHEMICAL CONSTITUENTS

Swietenia macrophylla contained various chemical compounds. A number of limonoids have been reported from the genus *Swietenia* with structures assigned on the basis of spectral data. Hence, limonoids and their derivatives have been identified as major constituents of this plant. Limonoids are derived from tetracyclic triterpenes similar to euphol (H-20b) by a series of oxidative changes at their side chain to a β -substituted furan ring by the loss of four carbon atoms, along with some molecular rearrangements. Therefore, an alternative name, tetranortriterpenoids, with a 4,4,8-trimethyl-17-furanylsteroidal skeleton is used to recognize this compound.

Also, there are presence of some other compounds, which isolated from the seeds included 5-Methyl-2,4-diisopropylphenol (13.38%), methyl ester (22.82%), Cyclohexane (5.62%), oleic acid (19.43%), Eugenol (12.53%), 3- Buten-1-ol (15.63%), Flavone (17.07%), Isoquinoline-4-carbonitrile (17.78%), Morin (18.8%), 9-Octadecenoic acid (20.87%), Ketogestin (21.12%), and Coumarine (22.25%), linolenic acid (19.03%). The abundance of polyunsaturated fatty acid especially linolenic acid shows that *S. macrophylla* seed oil has the ability to provide benefit to human health. The seeds of *S. macrophylla* were also rich in fat, thus the composition of this seed fat has enhanced the possibility of using this oil.

1.6 OPTIMIZATION OF GREEN SYNTHESIS OF SILVER NANOPARTICLES

Synthesis processes of Ag-NPs are depending upon various factors such as temperature, pH, reaction time, concentration of metal ions and concentration of plant extracts.

1.6.1 EFFECT OF REACTION TIME

The quality, morphology and properties of the silver nanoparticles depend on the reaction time (Darroudi *et al.*, 2011). Aggregation and shrinkage of NPs may occur for long incubation time (Baer 2011).

1.6.2 EFFECT OF PLANT EXTRACTS CONCENTRATION

The concentration of plant extracts plays a vital role to determine the size and shape of silver nanoparticles. Dubey *et al.* have synthesized AgNP and AuNPs by using different concentrations (0.5 mL, 1.0mL, 2.8mL and 4.8mL) of fruit extract (*Tanacetum vulgare*) (Dubey *et al.*, 2010). The absorption peaks of both NPs were increased by increasing the concentration of plant extracts (Murugan *et al.*, 2015). AgNPs and AuNPs were also synthesized from the leaf extract of *Chenopodium album*, and the particle size was found to be decreased with increasing concentration of plant extracts (Dwivedi and Gopal, 2010).

1.6.3 EFFECT OF SILVER NITRATE CONCENTRATION

The concentration of metal ions plays an important role to determine the shape, rate of reduction process and size of NPs. AgNPs and AuNPs were synthesized by using tansy fruit extract with varying concentration of metal ion from 1 to 3mM (Dubey *et al.*, 2010). They observed that the large sized nanoparticles were formed at a higher concentration of the metal ion. When the Silver and gold nanoparticles were synthesized by leaf extract of *Chenopodium album*, the absorption peaks and particle size were increased with increasing the metal ion concentration. The yields of the synthesized nanoparticles were also increased by increasing the concentration of metal ion (Dubey *et al.*, 2010).

1.6.4 EFFECT OF pH

The formation of nanoparticles also depends on the pH of the reaction medium (Gericke and Pinches 2006). From the literature review, it was revealed that lower pH values favour larger size of the nanoparticles (Dubey *et al.*, 2010; Sathishkumar *et al.*,

2010). The larger rod shapes AuNPs were synthesized using *Avena sativa* at pH 2, whereas, smaller size NPs were synthesized at comparatively high pH that is 3 and 4 (Armendariz *et al.*, 2004). Spherical shape of AgNPs was reported by using Cinnamon zeylanicum at pH 5 and above (Sathishkumar *et al.*, 2009).

1.6.5 EFFECT OF TEMPERATURE

The shapes and sizes of the nanoparticles are affected by the temperature of the reaction medium. The AuNPs were reported using the leaf extract of *cymbopogon flexosus*, where nanotriangles synthesized at low temperature, whereas spherical shapes of nanoparticles were formed at high reaction temperature (Raju *et al.*, 2011).

1.7 APPLICATION OF GREEN SYNTHESIZED SILVER NANOPARTICLES

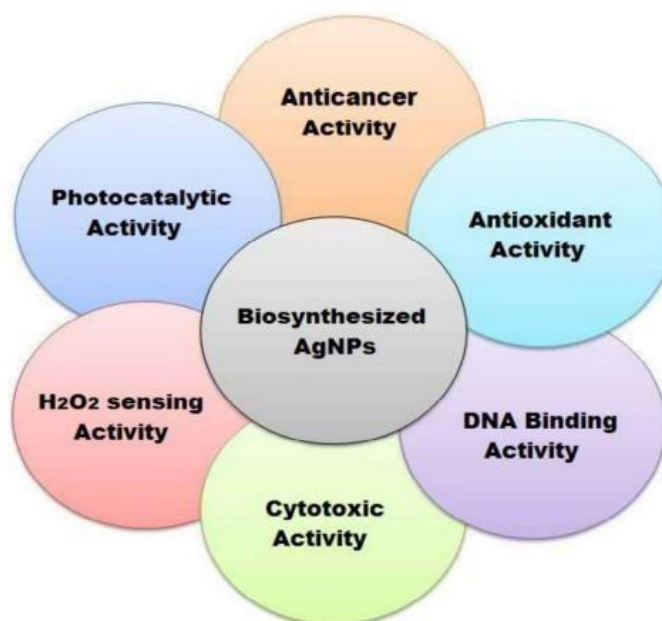


Figure 1.2: Applications of the silver nanoparticles

1.7.1 USED AS A PHOTOCATALYST FOR DEGRADATION OF SYNTHETIC DYES

The catalytic activity of AgNPs has been investigated with different types of pollutant dyes such as Methylene blue (MB), Methyl orange (MO), Naphthol orange

(NO) and Methyl red (MR), etc. in presence of sunlight. Jyoti *et al.* synthesized AgNPs using leaf extracts of *Zanthoxylum armatum* and investigated their photocatalytic activity against methylene blue (MB), methyl orange (MO), methyl red (MR) and safranin-O (Jyoti and Singh 2016). Kolya *et al.* synthesized AgNPs from leaf extract of *Amaranthus gangeticus* and studied their photocatalytic activity against congo red (CR) dye (Kolya *et al.*, 2015). Arya *et al.*, synthesized AgNPs using leaf extract of *Cicer arietinum* and evaluated the photocatalytic activity against 4-nitrophenol, congo red (MR) and methylene blue (Arya *et al.*, 2017).

1.7.2 USED AS HYDROGEN PEROXIDE (H₂O₂) SENSOR

Hydrogen peroxide (H₂O₂) is a toxic compound and little amount of contaminating food with H₂O₂ is harmful to the living organism. AgNPs are capable of sensing very little amount of hydrogen peroxide. Raja *et al.* reported photosynthesis of AgNPs using leaf extract of *Calliandra haematocephala* and investigated their sensing properties against hydrogen peroxide (H₂O₂) (Raja *et al.*, 2017). Aadil *et al.* reported AgNPs from *Acacia lignin* and used them as a sensor for the detection of hydrogen peroxide (H₂O₂) (Aadil *et al.*, 2016). Kumar *et al.* detected H₂O₂ using AgNPs synthesized from *Euphorbia hirta* (Kumar *et al.*, 2016a).

1.7.3 USED IN HEAVY METAL SENSING

Heavy metals are unquestionably toxic substances, and their detection in food and water bodies are very important. Therefore, the heavy metals are being detected by AgNPs from contaminated systems. Silver and gold nanoparticles synthesised from *L.Tyrosine*, were investigated for their metal sensing property against heavy toxic metals like Mn²⁺, Hg²⁺ and Pb²⁺ (Annadhasan *et al.*, 2014). Firdaus *et al.* synthesized AgNPs from papaya fruit and used them as a sensor against Hg²⁺ (Firdaus *et al.*, 2017). Tagad group of researchers reported AgNPs using the root extract of *Panax Ginseng* and investigated the sensing property against several heavy metals (Tagad *et al.*, 2017).

1.7.4 BIOLOGICAL APPLICATIONS

1.7.4.1 DNA INTERACTION

Silver nanoparticles have been reported to bind with calf-thymus DNA (CT-DNA) in vitro which stopped the normal functioning of DNA and resulted into damaged cell. Ribeiro *et al.* synthesized silver nanoparticles (AgNPs) using the extract of black tea, and investigated their CT-DNA binding capacity using the electronic absorption and fluorescence spectroscopies (Ribeiro *et al.*, 2018). Rahban and co-workers reported the interaction between AgNPs and CT-DNA using the electronic absorption spectroscopy (Rahban *et al.*, 2010). Roy *et al.* have also reported the interaction of biosynthesized AgNPs with CT-DNA (Roy *et al.*, 2015b).

1.7.4.2 CYTOTOXIC EFFECT

Silver nanoparticles are used as an antimitotic agent. Jacob and co-workers reported AgNPs from leaf extract of *Piper longum* and studied their cytotoxic activity (Jacob *et al.*, 2012). AgNPs were synthesized using leaf extract of *Rauvolfia tetraphylla* by Vinay *et al.* who studied their cytotoxic effect on the root cells of *Allium cepa* (Vinay *et al.*, 2019). Suman *et al.* reported AgNPs using the root of *Morinda citrifolia* and investigated their cytotoxic activity on the HeLa cell (Suman *et al.*, 2013).

1.7.4.3 ANTIMICROBIAL EFFECT

Silver metal ions are well known for their antimicrobial effects and some of the plant extracts have also been reported to possess similar properties. Some of the plant mediated silver nanoparticles have been reported with better antimicrobial activity than their source materials (AgNO₃ and plant extracts). Silver nanoparticles were synthesized from the leaf extract of *Chenopodium murale* and investigated for their antibacterial activity against *Staphylococcus aureus* (Abdel-Aziz *et al.*, 2014). Silver nanoparticle synthesized from the plant extract of *Eclipta alba* was reported by Premasudha *et al.* for their antimicrobial activity against gram-positive and gram-negative bacteria (Premasudha *et al.*, 2015).

1.7.4.4 ANTI-CANCER EFFECT

Now days, silver nanoparticles are highly valuable for their medical applications. AgNPs are used as anti-cancer drugs either alone or in combination with standard drugs as facilitators. Kuppusamy *et al.*, synthesized and characterized silver and gold nanoparticles from the plant extract of *Commelina nudiflora* and further investigated the anti-cancer activity against Colon cancer cell lines (HCT-116) (Kuppusamy *et al.*, 2016). The silver nanoparticles were synthesized by the plant extract of *Plantago major* and their anti-cancer activity was reported against human breast cancer cell lines (MCF-7) (Poor *et al.*, 2017). Kathiravan *et al.* have synthesized Ag-NPs from the *Melia dubai* extract and characterized by XRD, UV-visible spectroscopy and SEM-EDX. They evaluated the anti-cancer properties of the nanoparticles against human breast cancer (KB) cell lines (Kathiravan *et al.*, 2014).

1.7.4.5 ANTIOXIDANT EFFECT

The antioxidant activities of the silver nanoparticles have been investigated with the scavenging capacity of the stable free radicals that is 2, 2 -diphenyl-1-picryl hydrazyl (DPPH). Kharat *et al.*, synthesized and characterized the AgNPs using the leaf extract of *Elephantopus scaber* and evaluated the antioxidant activity of ES-AgNPs (Kharat and Mendhulkar 2016). Silver nanoparticles were synthesized using the root extract of *Helicteres isora* and investigated for their antioxidant activity (Bhakya *et al.*, 2016). Ravichandran *et al.* synthesized NPs using leaf extract of *Artocarpus altalis* and studied their antimicrobial and antioxidant activity (Ravichandran *et al.*, 2016).

1.7.4.6 ANTI-DIABETIC EFFECT

There are several data in the literature indicating a great variety of pharmacological activities of *S. macrophylla*, which exhibits antimicrobial, anti-inflammatory, antioxidant effects, antimutagenic, anticancer, antitumor and antidiabetic activities.

Preliminary pharmacological assay revealed that *Swietenia macrophylla* seeds possess significant hypoglycemic activity in experimental diabetic rats. (Dewanji *et al.*,2007). Petroleum ether extract of *Swietenia macrophylla* seeds has been reported to produce significant antidiarrheal activity and its aqueous extract has been reported to be effective against *Plasmodium falciparum*. (Maiti *et al.*,2019).

The effect of the anti-Diabetic activity of *Swietenia macrophylla* was evaluated in Streptozotocin and Nicotinamide induced type II diabetic rats. Methanol extract of *Swietenia macrophylla* seeds was administered orally at doses of 150 and 300 mg/kg body weight for 12 consecutive days. Fasting blood sugar (FBG) was estimated on overnight fasted rats on day 1, 5 and 12 days. Lipid profiles, liver glycogen levels and changes in body weight were also measured. The extract was found more effective at the dose of 300 mg/kg body weight and it lowered FBG levels was statistically significant $P < 0.01$ (32.78%) in diabetic rats at day 12. Extract at the same dose also significantly reduced $P < 0.01$ the elevated level of serum total cholesterol (18.56%) and triglyceride (10.41%), and increased $P < 0.01$ (46.27%) the reduced liver glycogen level. Though statistically non-signify the extract at both the doses was found to improve the body weight of diabetic rats. This observation concludes that the methanol extract of seeds of *Swietenia macrophylla* has hypoglycemic as well as hypolipidemic effect.

1.8 SAMPLE CHARACTERIZATION AND ANALYSIS TECHNIQUES

Characterization of the nanoparticles is one of the important parts of the material research work. Without characterization we not confirm the formation of nanoparticles. In our research work, different techniques such as UV-visible spectroscopy, X-ray Diffraction (XRD), Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electronic Microscopy (SEM), Transmission Electronic Microscopy (TEM) are used for the characterizations of green synthesized nanoparticles.

1.8.1 UV-VISIBLE SPECTROSCOPY

Ultraviolet-visible (UV-Vis) spectrum is the count of minimization of light beams after passing through a sample or after refraction from the surface of the sample. UV-Visible spectrophotometer is used in the characterization of the organic and inorganic molecules. In this research work, the formations of the nanoparticles are confirmed characteristic spectra produced by the UV-visible spectroscopy. Moreover, CT-DNA binding, dye degradation, antioxidant activity, hydrogen peroxide sensing, and heavy metal sensing were evaluated by the UV-Visible spectrophotometer (Shimadzu, UV-1800) in IGNTU, Amarkantak, MP, India.

1.8.2 SCANNING ELECTRON MICROSCOPY (SEM)

Scanning Electron Microscope (SEM) is a technique that scans the surface of a sample with an electron beam and creates images (Stokes 2008). SEM can generate the surface topography and compositional information of the nanoparticles by interacting electron and atoms of the nanoparticles (McMullan 1995). In the present study, Phyto fabricated nanoparticles were characterized by SEM to observe the surface topography and compositions. The average size of the nanoparticles was also calculated from the SEM images. The fine structure of the prepared nanoparticles is analysed by Scanning Electronic Microscopy (SEM) (Carl Zeiss Germany, Model Supra-40). SEM and energy dispersive X-ray (EDX) of the samples were measured from NIT Raipur, CG, India.

1.8.3 FOURIER TRANSFORM INFRA-RED (FTIR) SPECTROSCOPY

In Fourier Transform Infrared (FT-IR) spectroscopy, an infrared spectrum is obtained from emission and absorption of samples like gas, liquid or solid (Griffiths and De Haseth 2007). Each functional groups and chemical bond have a specific range of frequency that they can absorb. Therefore, the characteristic peaks are observed for each functional group or part of the molecule. In the present investigation,

assigned peak for each functional group of green synthesized nanoparticles obtained using FT-IR (400-4000 cm⁻¹ range) of Thermo Scientific and Bruker-Germany.

2. LITERATURE SURVEY

Plants have been used as natural source and medicine. Many plants are used in Tibetan and Unani medicine. Humans are using numerous plants and plant derived products to cure and relief from various physical and mental illness. Ancient literatures such as Rigveda and Yajurveda use plants for the treatment of various health problems.

Phytochemical composition, nutritional value, pharmacological studies, antioxidant activity, antimicrobial activity, anti-diarrheal activity, anti-diabetic activity, anti-proliferative activity, anti-inflammatory activity and toxic activity are some of the studies have been reviewed from the articles.

Swietenia macrophylla King, commonly known as big leaf mahogany or “skyfruit,” is a very slow-growing, evergreen, monoecious tree indigenous to tropical and subtropical regions of the World. *S. macrophylla* is a commercially important woody plant, which has been regarded as the finest tropical timber for high-class furniture and other appliances. In addition, it is a medicinally important species with ethnomedicinal significance (Dewanjee *et al.*, 2020)

The seed of *S. macrophylla* has been reported to have anti-inflammatory, antimutagenicity, and antitumor activity. During our investigation of folk usage of *S. macrophylla* seeds, we discovered that it was effective against diabetic rats. Subsequent work was carried out to isolate novel antidiabetic molecule from *S. macrophylla* seeds (Dewanjee *et al.*, 2009).

There are several data in the literature indicating a great variety of pharmacological activities of *S. macrophylla*, which exhibits antimicrobial, anti-inflammatory, antioxidant effects, antimutagenic, anticancer, antitumor and antidiabetic activities. Various other activities like anti-nociceptive, hypolipidemic, antidiarrheal, anti-infective, antiviral, antimalarial, acaricidal, antifeedant and heavy metal phytoremediation activity have also been reported (moghadamtousi *et al.*, 2013).

Traditionally, raw seeds of *S. macrophylla* are chewed to treat diabetes. In Malaysia, these seeds are chewed or pounded and swallowed to treat high blood pressure and in India, they are used to treat diabetes and hypertension

The green synthesis method using plant extracts has increased attention than physical, chemical, and even microorganism mediated synthesis of AgNPs. Synthesis of metallic NPs using plant extracts is cost-effective, and therefore it can be used as an inexpensive and useful substitute for large scale metallic NPs synthesis. Aqueous extract of plants contained a vast number of phytochemicals. They act as reducing, capping, and stabilizing agents. NPs have attracted considerable attention owing to their various applications as narrated in the last section.

Roy *et al.*, (2015a) have reported the microbial synthesis of AgNPs from yeast extract of *Saccharomyces cerevisiae*. AgNPs were confirmed by UVvis spectra, and absorbance peak was found at 450 nm. TEM and XRD analysis showed spherical shape with an average size distribution of 10 nm having fcc crystal lattice. Photocatalytic activity of *S. molesta* extract mediated AgNPs was studied using Methylene blue (MB) under the sunlight, and these NPs showed efficiency in degrading the dye within a few hours of exposure.

Ravichandran *et al.*, (2019) have studied the green synthesis of AgNPs from aqueous leaf extract of *Parkia speciosa* by bioreduction of AgNO_3 . Green synthesis was confirmed by UV Visual spectroscopy with SPR peak at 410.5 nm. In this study, different physicochemical parameters like pH, time, temperature, AgNO_3 conc. and the volume of leaf extract was optimized spectrophotometrically. The average size of AgNPs was found 31 nm, 35 nm, and 155.3 d.nm by SEM, TEM, and DLS analysis, respectively. The presence of Ag in green synthesized NPs was confirmed through XRD and EDX analysis. Green synthesized AgNPs exhibited significant photocatalytic activity against azo dye MB under solar irradiation, antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*, and antioxidant activity against DPPH free radical.

Green synthesis of AgNPs was also reported by Kadam *et al.*, (2020) using the waste extract of *Brassica oleracea* var. botrytis. The whole experiments were optimized by pH, temperature, extract, and AgNO_3 concentration by UV-Visual spectroscopy. Green synthesized AgNPs were characterized by TEM, SEM, XRD, FT-

IR, SAED, XPS, and BET. The results of those analyses confirmed the formation of spherical AgNPs in the range of 5-50 nm. FTIR analysis revealed that functional groups present in the extract of *B. oleracea* are responsible for the green synthesis of AgNPs by acting as capping and reducing agents. AgNPs were also investigated for degradation of MB dye under solar irradiation and sensing for mercury ions. MB did not show its characteristic absorption maxima at 664 nm after being degraded by AgNPs as determined by using UV-vis. spectroscopy. MB dye was degraded 97.57 % within 150 min. suggesting prominent photocatalytic activity of AgNPs. Such NPs were found exceptionally capable of sensing up to 0.1 mg / l Hg^{2+} ions.

Sumitha *et al.*, (2018) have studied *Durio zibethinus* aqueous seed extract mediated green synthesis procedure of AgNPs and find its photocatalytic, cytotoxic effects, and antibacterial properties. SPR peak at 420 nm confirmed green synthesis of AgNPs. The characterization results of NPs revealed the spherical and rod-shaped nature. *zibethinus* AgNPs showed photocatalytic activity against MB, significant antibacterial activity, and showed cytotoxicity against brine shrimp.

Aqueous leaf extract of *Mangifera indica* (MILE) was used for the green synthesis AgNPs (Samari *et al.*, 2018). MILE reported as reducing, stabilizing, and capping agents during the synthesis of AgNPs. The green synthesis was governed under varying pH, leaf extract and silver nitrate concentration, various temperature, and different reaction time. The optimized AgNPs were characterized by XRD, TEM and FTIR to confirm the crystalline nature, morphology and surface biomolecules of green synthesized NPs. MI-AgNPs degraded MB and Rh-B dyes under the sunlight in the presence of sodium borohydride. Furthermore, the green synthesized AgNPs was reported as a potential chemical sensor for the detection of toxic Hg_2^+ ion in water bodies following colorimetric studies. Its potential application was recognised after MTT assay against HCT116 and SW480 human colon cancer cell lines and antioxidant capacity by the ferric reducing antioxidant power (FRAP) assay.

Kumar *et al.*, (2017) used *Murraya koenigi* leaf extract as a reducing and capping agent for photo-induced green synthesis of AgNPs. The green synthesized AgNPs were spherical with 8.6 nm (TEM), and 1.8 nm (AFM) in size. XRD study where Bragg's diffraction pattern at (111), (200), (220) and (311) agreed to a metallic Ag Fcc lattice.

The functional groups which are present in the surface of AgNPs come from aqueous *M. koenigi* leaf extract are analysed by FTIR in between 4000 and 400 cm⁻¹.

The metal nanoparticles which are mentioned above is synthesized using various methods such as hard-template method, bio-reduction method (Canizal, G., *et al.*, 2001; Mouxing, F. U., *et al.*, 2006) and solution phase synthesis method (Sun, Yugang, *et al.*, 2003) In correspondence to the synthesis and construction procedure of nanoparticles and nanomaterials, precursors of various elemental origin are used such as liquids, solids or gases enlisting physical, chemical and biological approaches. The construction techniques fall under two approaches: 'Bottom-up' and 'Top-down' methods. (Leela, Arangasamy *et al.*, 2008)

The process of drug delivery using metal nanoparticles is an effective method in treatment of various diseases (Sankar, Renu, *et al.*, 2015). To obtain higher efficiency in drug delivery, the drugs are released slowly towards their respective target sites (De Jong *et al.*, 2008). This optimization can be obtained by using active or passive drug delivery system (De Jong *et al.*, 2008). The latest literature studies have demonstrated the effectiveness of metal nanoparticles for drug delivery purposes (Torchilin, 2010). The study of silver nanoparticles has provided greater affinity towards cancer therapy and treatment (Kotcherlakota, Rajesh, *et al.*, 20187).

There have been only very few reports at present talking about drug delivery potential of silver nanoparticles. A cancer drug delivery system was devised which consists of silver nanoparticles that are obtained from *Butea monosperma* plant extract. These silver nanoparticles were administered with chemotherapeutic drug *doxorubicin* to develop a drug delivery system (DDS) and identify its anticancer efficacy in various cancer cells (Kotcherlakota, Rajesh, *et al.*, 20187). The DDS system showed higher cytotoxicity than other existing drugs by executing *doxorubicin* into the cancer cells. This study helped to understand that biosynthesized AgNPs can be utilized for drug delivery procedure in cancer therapies. The delivery of antifungal drug into cells was demonstrated by kumar *et al* by using AgNPs obtained from the supernatant of *Delftia sp.* strain KCM-006 culture. The experiment was a success which inhibited ergosterol biosynthesis and biofilm formation of fungus (Patra, Sujata, *et al.*, 2015) and also the antifungal drug miconazole was successfully administered.

The implementation of chemotherapeutic agents has various limitations like increasing drug resistance, poor bio-availability and non-specific toxic nature. This resulted in fabrication of alternative treatment methodologies for cancer therapy and cure. The experiment of silver nanoparticle synthesis from *Bacillus funiculus* culture supernatant displayed antiproliferative properties in MDA-MB-231 (human breast cancer) cells through generation of ROS (reactive oxygen species) thereby progressing to apoptosis (Mirza, Salma, *et al.*, 2020).

The generation of protein-capped silver nanoparticle from *Penicillium shearii* AJP05 fungus displayed effective anticancer properties in epithelial (hepatoma) and mesenchymal (osteosarcoma) cells. The cytotoxic effectiveness of these biosynthesized silver nanoparticle was viewed due to their production of ROS (reactive oxygen species). These biosynthesized silver nanoparticles modify the cancer cells as cisplatin resistant by sensitizing them (Gurunathan, Sangiliyandi, *et al.*, 2013).

Firdhouse *et al* reported that AgNPs produced by using *Alternanthera sessilis* plant extract showed high amount of cytotoxic activity towards prostate cancer cells (PC-3) (Fageria, Leena, *et al.*, 2008).

3. AIM AND SCOPE

3.1 AIM

To synthesis Silver and Copper nanoparticles by green synthesis method using *Swietenia macrophylla* seed extract and their therapeutic applications.

3.2 SCOPE

- To synthesize silver and copper nanoparticles
- Characterization of these nanoparticles
- To evaluate the pharmacological activities
- To study their effectiveness in therapeutic applications

4. MATERIALS AND METHODS

4.1 COLLECTION OF SEEDS

The seed samples were collected from Ennore, Tamilnadu, India. The seeds were primarily left to dry for a period of 3 days. The seed kernels were grounded into a coarse powder as reduction in particle size leads to increased extraction. This was submerged in methanol for 2 days of time. This was done for the extraction of the components of the seed into the ethanol.

4.2 Biomass extraction from seed using methanol

The apparatus shown in Fig.4 consists of a base layer called the mortar and a grinding pestle. Mortar and pestle are implements used since ancient times to prepare ingredients or substances by crushing and grinding them into a fine paste or powder in the kitchen, laboratory, and pharmacy. The mortar is a bowl, typically made of hard wood, metal, ceramic, or hard stone, such as granite. The pestle is a heavy and blunt club-shaped object. The substance to be ground, which may be wet or dry, is placed in the mortar, where the pestle is pressed and rotated onto it until the desired texture is achieved.

The seeds are grinding and made into a fine powder with the help of mortar and pestle. The fine powder is then administered with 50ml of methanol where the methanol comes in contact with the powdered seed kernel and performs lipid extraction. This results in the extraction of the components of the fine powder in the methanol.



FIG 4.1: Mortar and pestle

4.3 SAMPLE PREPARATION

The sample was prepared by mixing 1.5ml of the seed extract in 2ml of methanol. The methanol comes in contact with the powdered seed kernel and performs lipid extraction.

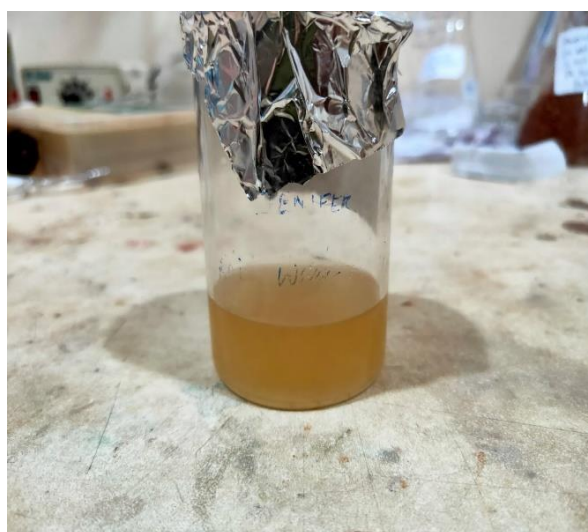


FIG 4.2: Methanolic seed extract of *S. macrophylla*

4.4 SYNTHESIS OF AGNPs

The aqueous solution of 1 mM silver nitrate was prepared in 250 ml Erlenmeyer flask. 180 ml of methanolic seed extract was mixed with 90 ml of 1mM silver nitrate solution in 1:1 ratio. The reaction mixture was kept at room temperature in the dark to reduce photoactivation of silver nitrate. Reduction of Ag⁺ to Ag was visually inferred by colour change of the solution from colourless to brown.

4.5 CHARACTERIZATIONS OF AGNPs

4.5.1 UV-VIS SPECTROSCOPY

An analytical technique suitable for a large class of organic chemicals as well as certain inorganic species, UV-vis spectroscopy is affordable, straight forward, adaptable, and non-destructive (Pons *et al.*, 2004). The absorbance or transmittance of light flowing through a material as a function of wavelength is measured using UVvis spectrophotometers. UV-vis detectors are integrated into high-performance liquid chromatography and ultra-high-performance liquid chromatography to detect and measure the concentration of chemicals in liquid streams (Rocha *et al.*, 2018). All species identification is facilitated by combining these methods with mass spectrometry. A variation that has improved scattering capabilities for measuring the characteristics of solids and powders is UV-vis diffuse reflectance spectroscopy. The reduced silver nanoparticles that we created using a green synthesis method was quantified for this experiment using a Shimadzu UV-1800 purchased from Japan (Piovesan *et al.*, 2018).

The progress of the reaction and absorption spectra were recorded on UV visible spectrophotometer at 400-500nm range.

4.5.2 FTIR ANALYSIS

The measurement of a sample's infrared radiation transmission or absorption using FTIR spectroscopy is a method often used to characterize nanoparticles in terms of their chemical makeup and functional groups (Petit and Puskar, 2018). An expert tool, such as an FTIR spectrophotometer, is needed to conduct FTIR spectroscopy on

nanoparticle samples. The procedures listed below may be used to conduct FTIR spectroscopy on samples of powdered nanoparticles using an FTIR spectrophotometer .

4.5.2.1 PREPARE THE SAMPLE

The sample of powdered nanoparticles has to be combined with an appropriate infrared-transparent matrix, such KBr or Nujol. A hydraulic press is then used to shape the mixture into a thin pellet.

4.5.2.2 PLACE THE PELLETT IN THE SAMPLE HOLDER

The Shimadzu UV-1800 spectrophotometer's sample container is filled with the pellet, and the device is then mounted with the sample holder inside.

4.5.2.3 COLLECT THE SPECTRUM

The FTIR spectrophotometer is set up to scan the sample across several wavelengths to obtain the spectrum. In transmission mode, when infrared light passes through the sample and is detected on the opposite side, the spectrum is normally gathered.

4.5.2.4 ANALYZE THE SPECTRUM

The functional groups present in the sample and the nanoparticles' chemical makeup are both determined by analyzing the acquired FTIR spectrum.

The Fourier Transform Infrared Spectrometer (FTIR) readings were recorded using AgNPs pellets at 400-4000 cm^{-1} range.

4.5.3 SCANNING ELECTRONIC MICROSCOPY

The surface morphology, size, and structure of the NPs were analyzed by Scanning Electronic Microscopy (SEM; EVO 18, Carl Zeiss, Germany). SEM analysis was employed to visualize the morphology and size of AgNPs. Different magnifications of SEM images are shown, which confirms green synthesis of silver nanoparticles using methanolic seed extract of *Swietenia macrophylla*. The formation of homogenous and relatively spherical AgNPs. The elemental composition of NPs was analyzed through energy dispersive X-ray analysis (EDX). Figure, represents the EDX spectrum showing the major elemental peak which is specified for metallic silver. Other small peaks were also arisen due to the capping of AgNPs by biomolecules of *S. macrophylla*. Quantitative estimation reveals elemental Ag with the higher weight percentage of 51.18%, whereas, O, C, Cl, K, and S having weight percentages of 20.93%, 14.02%, 12.09%, 1.74% and 0.04%, respectively.

4.6 ANTIDIABETIC ACTIVITY

Antidiabetic drugs are medicines developed to stabilise and control blood glucose levels amongst people with diabetes. Antidiabetic drugs are commonly used to manage diabetes.

There are a number of different types of antidiabetic drug including: Insulin. A substance that helps a person with diabetes control their level of glucose (sugar) in the blood. The digestive enzyme (α -amylase) is responsible for hydrolyzing dietary starch (maltose), which breaks down into glucose prior to absorption.

The sample components which were added greatly influences and inhibits the activity of this enzyme which prevents hydrolyzing the starch and hence the glucose concentration remains unchanged.

Thus, by the activity of the sample over the enzyme the breakdown of starch into glucose is prevented.

Here, the antidiabetic activity of *S. macrophylla* is analysed by Alpha amylase enzyme inhibition assay method.

4.6.1 ALPHA AMYLASE ENZYME INHIBITION

α - amylase enzyme inhibition assay was carried out based on the starchiodine test. The total assay mixture was composed of various concentration (20- 120 $\mu\text{g/mL}$) of leaves extract of *S. macrophylla*, 10 μL of alpha amylase enzyme prepared in 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM 28 sodium chloride) and was incubated at 37°C for 10 min. Then soluble starch (1%, w/v) was added to each reaction set and incubated at 37°C for 60 min. One hundred μL of 1 M HCl was added to stop the enzymatic reaction and followed by 200 μL of iodine reagent (5 mM I₂ and 5 mM KI) was added.

The colour change was noted and the absorbance was read at 595 nm. The control reaction representing 100% enzyme activity did not contain any plant extract. A dark-blue colour indicates the presence of starch; a yellow colour indicates the absence of starch, while a brownish colour indicates partially degraded starch in the reaction mixture.

In the presence of inhibitors from the extracts, the starch added to the enzyme assay mixture is not degraded and gives a dark-blue colour complex, whereas no colour complex is developed in the absence of the inhibitor, indicating that starch is completely hydrolyzed by α -amylase.

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) * 100}{(\text{Absorbance of control})}$$

4.7 GAS CHROMATOGRAPHY/MASS SPECTROMETRY

For GC-MS analysis, the samples were injected into a HP-5 column (30 m $\times$ 0.25 mm i.d with 0.25 μm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Following chromatographic conditions were used: Helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV; ion source

temperature of 250°C; interface temperature of 250°C; mass range of 50-600 mass units

4.7.1 IDENTIFICATION OF COMPOUNDS

The database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for the interpretation on mass spectrum of GC-MS.

The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

4.8 ISOLATING AND SUBCULTURING THE PHYTOPATHOGENS

4.8.1 IDENTIFYING AND ISOLATING THE FUNGAL PHYTOPATHOGENS

Twenty grams of soil sample was collected from the Banana field in Embalam, Puducherry was collected at a depth of 15 cm. It was then shade dried. One gram from the twenty grams of soil collected was weighed using an electronic weighing machine and was transferred to 9 ml of distilled water which is marked as 10^{-1} , this marks the beginning of the serial dilution process (Cao *et al.*, 2004; Nel *et al.*, 2006). The serial dilution was performed where 1ml from a 10^{-1} test tube was transferred using a micropipette to a 10^{-2} test tube and the contents were mixed uniformly using a vortex. Then the process was further continued in the same way until it reached 10^{-10} test tubes. Now, 1ml using micropipettes from 10^{-3} , 10^{-4} , and 10^{-5} was poured aseptically onto sterile Petri plates (90mm). Then 10 mL of Potato Dextrose Agar medium was poured into those Petri plates and after solidification, the plates were kept for 5 days during the incubation period (Chang *et al.*, 2016).

The symptomatic banana leaf and trunk samples were collected from the field at Embalam, Puducherry. Then the sample isolation technique was adopted where the infected parts were cut using a sterile blade and washed with running tap water and blot dried. Then they were surface sterilized with 0.1% mercuric chloride for 1 min and again soaked immediately in sterile distilled water for 30 secs. The surface-sterilized

infected parts of the root were placed near the edge of the Petri plates containing sterilized and solidified Potato Dextrose Agar (PDA) medium under a laminar flow hood. Then it was placed on the solidified potato dextrose agar medium and was left for incubation (García-Bastidas *et al.*, 2014). A similar process was carried out for other fungal phytopathogens

4.8.2 SUBCULTURING THE IDENTIFIED FUNGAL PHYTOPATHOGENS

The fungal phytopathogens that were isolated from the incubated soil sample collection plate which was used for confirming the presence of pathogen were transferred to the Petri plate with solidified potato dextrose agar using a cork-borer under the laminar flow hood. Then this was kept for incubation for the proper growth of the pathogen that can be used for further studies (Yassin *et al.*, 2021).

4.9 ANTIFUNGAL ASSAY

The fungal culture was taken for three different fungi viz; *Athelia*, *Fusarium* and *Trichoderma sp.* These are cultured in test tubes for a period of 24-48 hours. The PDB plates were prepared for three different fungi and were inoculated by spread plate method. Three holes were punched on each plate for control, methanolic seed extract and green synthesised silver nanoparticles respectively. They were left to react for 2days, later the inhibition zone was noted and measured.

4.10 ANTIBACTERIAL ACTIVITY

4.10.1 MICROBIAL STRAINS

The microorganisms of Gram-positive strains such as *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus* as well as Gram negative strains such *Escherichia coli*, *Shigella flexneri* and *Proteus vulgaris* were used for the evaluation of antibacterial activity.

4.10.2 REFERENCE AND CONTROL

Tetracycline was used as the standard for bacteria. The controls consist of solidifying agar onto which was solvent, and the extract was soluble in it.

4.10.3 ASEPTIC CONDITIONS

The aseptic chamber which consists of a wooden box (1.3m x 1.6m x 0.6m) with a door, was cleaned with 70% ethanol and irradiated with short wave UV light (from lamp).

4.10.4 NUTRIENT BROTH AGAR MEDIUM

Nutrient broth agar medium was prepared (peptone-5 g; yeast extract-3 g; NaCl-5 g; distilled water-1000 mL; pH-7.0±0.2; Agar-20 g) according to the standard method and required amount was weighed, suspended in required volume of 200 mL distilled water in a 500 mL conical flask, stirred and then autoclaved at 15 lbs and at 121°C for 15 min . The hot medium was poured in sterile petriplates which were kept in the aseptic Laminar chamber. The medium was allowed to solidify for 15 min.

4.10.5 AGAR WELL-DIFFUSION METHOD

The potential antibacterial activity of leaves extract was analyzed by agar well diffusion method . The solidified nutrient agar in the petriplates was inoculated by dispensing the inoculums using sterilized cotton swabs and spread evenly onto the solidified agar medium. Five wells were created in each plate with the help of a sterile well-borer of 8 mm diameter. The extract was then poured into each well to get desirable concentrations. Tetracycline was used as the standard with the concentration of 25 µg. All the plates containing sample loaded wells were incubated for 24 h at 37°C. After the incubation period, zone of inhibition in each plate, for each concentration of extract and standard were measured by calculating the diameter of zone of inhibition.

4.11 MTT Assay

DMEM powdered medium was dissolved in 900 ml of autoclaved distilled water in a conical flask under sterile conditions. 3.7 g sodium bicarbonate was added and stirred until completely dissolved and the pH of the medium was adjusted to 7.2 by adding 3 ml of 1N HCl. Following this antibiotic such as penicillin 120U/ml, streptomycin 75 µg/ml, gentamycin 160 µg/ml, amphotericin B 3µg/ml were added. Finally, 10% FCS were added and the medium was sterilized using 0.2-micron filter under pressure and stored at 4°C.

The following solutions are prepared: 10X salt: Dissolve 8g of NaCl, 0.4g of KCl, 1.0g of D-glucose and 0.35g of NaHCO₃ in 100ml of water. Vernese: 90ml of distilled water is added with 1.0g of EDTA and completely dissolved by adding 5N NaOH in drops. For STV preparation, 100 mg of trypsin, 10 ml of 10X saline and 2.5 ml of versene were added and was made upto 100 ml using double distilled water. The solutions prepared were filter sterilized and stored at 4°C.

DMEM as the growth medium the Human breast cancer cell (MCF 7) lines were grown on polystyrene coated flasks. After the attainment of confluence, the cell line was passaged every two days. The culture medium was removed and the traces of the medium were washed using STV solution. 2.5ml STV was added to the flask and incubated at 37°C for several minutes until the cells began to separate from the surface. STV action was neutralized using DMEM containing serum after complete detachment. The cells were centrifuged and re-suspended in a fresh medium. This was seeded into a flask and stored at 37°C.

The MCF-7 cell lines were cultured in DMEM with 2 mM Glutamine, 10% FBS. On reach of confluence, the cells were subjected to differentiate. The cultures were fed fresh medium every other day and incubated in an incubator moistened at 37°C with 5% CO₂ and 95% air. The medium was replaced again with a fresh culture medium (DMEM with 10% FBS) after 3 days by monitoring the formation of multi-nucleation in the cell, and the extent of differentiation was measured.

The L6 cells were seeded in a 96-well tissue culture plate with 5×10^3 cells in 100 μ L medium per well, and after 24 h the cells were treated with different concentrations of lyophilized samples prepared 100 ng, 10 ng, 1 ng and 1 μ g. After 24 h incubation, cells were treated with MTT reagent and incubated for 2 to 4 h. The reaction was terminated by aspirating the medium, the formazan crystals formed were dissolved in DMSO and the absorbance was read at 570 nm, which was proportional to the cell viability. Based on cell viability, % cytotoxicity of the extracts was calculated.

The cytotoxicity of the synthesized nanoparticles was screened against Human breast cancer cells (MCF-7) by means of MTT Assay. After incubation, the silver nanoparticles synthesized were found to be cytotoxic to the MCF-7 cells. The result obtained with this colorimetric assay demonstrates a significant concentration-dependent increase in cytotoxicology against breast cancer cells. The viability of cells decreases as the nanoparticle concentration increases. Prolonged exposure will end in additional toxicity to the cells %. As compared to the control group, the highest toxicity 72% was found at 100 μ g/mL of AgNPs while at 1ng/mL the toxicity is 16%.

4.12 Cell Line Study

3T3-L1 is a cell line derived from (mouse) 3T3 cells that is used in biological research on adipose tissue. 3T3-L1 cells have a fibroblast-like morphology, but, under appropriate conditions, the cells differentiate into an adipocyte-like phenotype. It is mostly used for invitro studies of diabetic medicines because of its adipogenic properties. Here we compare cells grown in controlled environment against cells grown in increasing concentration of our extract. When the concentration of extract increases it inhibits the glucose formation so that the cells starve for glucose and then die at a point.

5. RESULT AND DISCUSSION

5.1 METHANOLIC SEED EXTRACT

The seeds of *Swietenia macrophylla* were broken down into a fine or coarse powder with the help of mortar and pestle. The powder was then soaked in methanol for the extraction of bioactive components. The separation was undergone by using a separating funnel where the bioactive components were separated from the crude sample and were stored in a beaker.



FIG 5.1: SEED EXTRACT

5.2 UV-VIS SPECTROSCOPY

For the confirmation of the synthesized nanoparticle, the synthesized silver nanoparticle was subjected to UV-visible spectroscopy. There was a peak identified at 435 nm. This result confirms that the particle synthesized is silver nanoparticle.

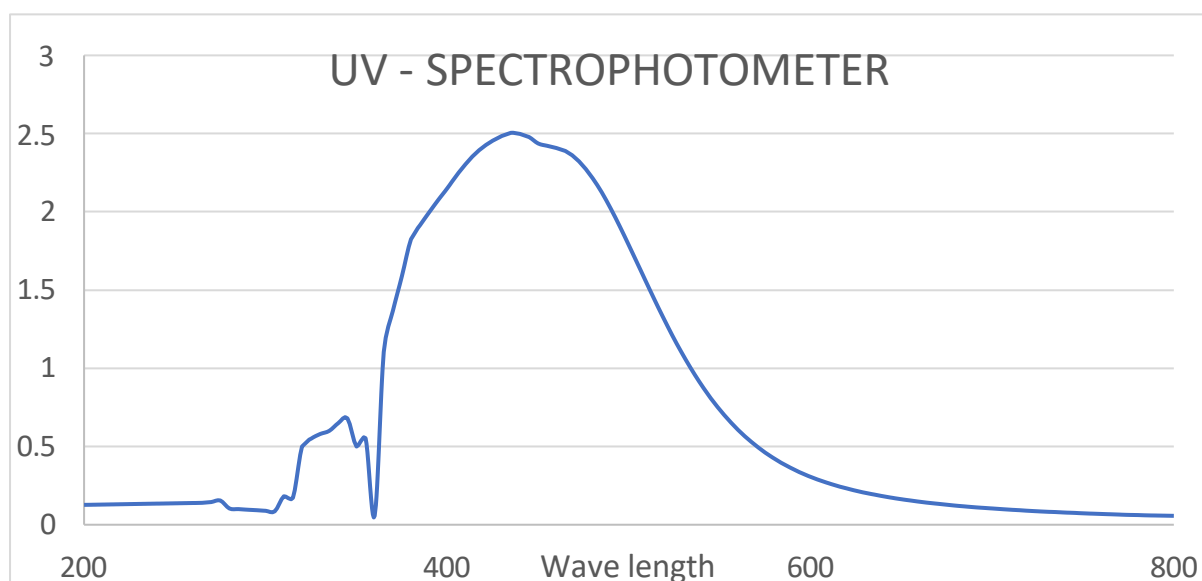


FIG 5.2: UV-VIS SPECTROSCOPY OF SILVER NANOPARTICLE

The synthesis process for AgNP was undergone in both light and dark reaction methods. Here, comparatively the results obtained from dark reaction had higher AgNP formation rates rather than light-based reaction. This is because the silver nanoparticle loses their reactivity on exposure to sunlight.

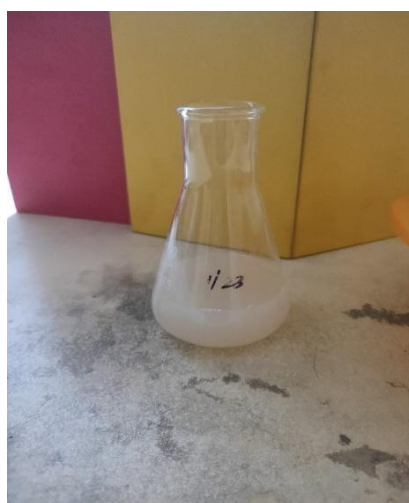


FIG 5.3: DAY 1 AgNP dark reaction

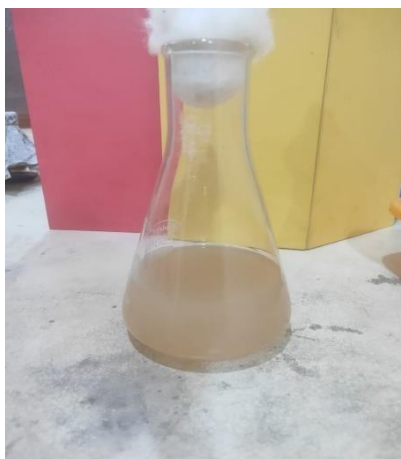


FIG 5.4: DAY 2 AgNP dark reaction



FIG 5.5: DAY 3 AgNP dark reaction

The copper nanoparticle synthesis was also undergone and the uv-vis reading was taken which showed a negative result that showed the inability of the seed sample to synthesis copper nanoparticles.

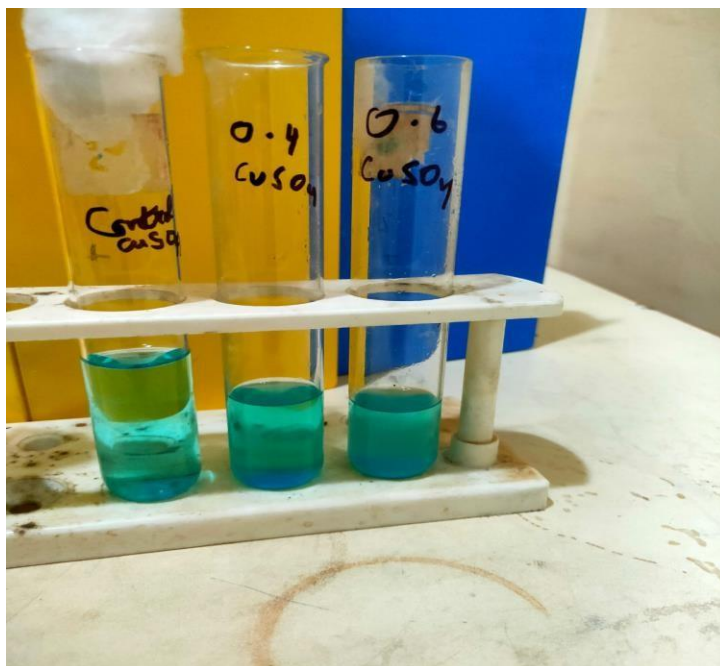


FIG 5.6: COPPER NANOPARTICLE SYNTHESIS

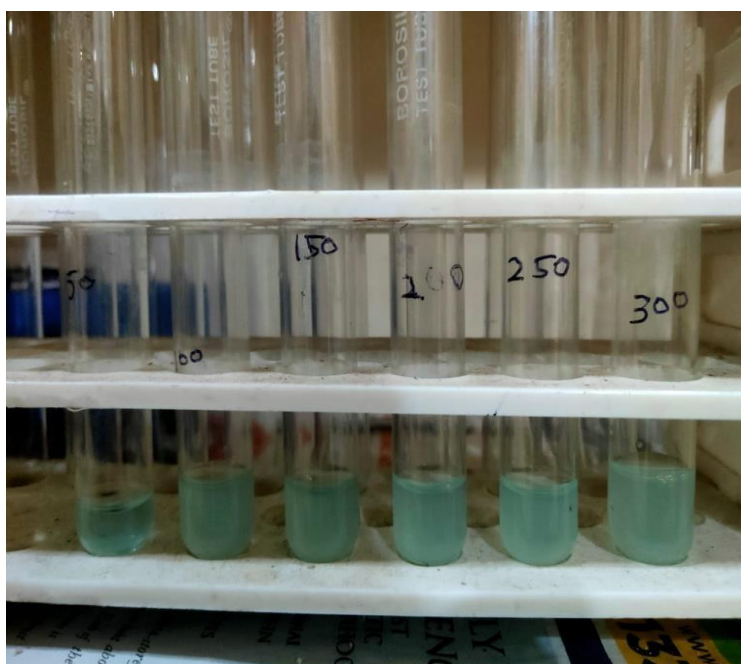


FIG 5.7: DIFFERENT CONC OF CuNP synthesis

The results for copper nanoparticle synthesis showed no characteristic color change from blueish green to brown. Hence there is no formation of CuNPs.

The UV-visible spectrum was recorded in the range of 200- 800 nm. The peak of absorbance was observed at 226 nm. The required wavelength is around 450-

550nm. This indicated that there is no synthesis of **copper nanoparticle** in the solution.

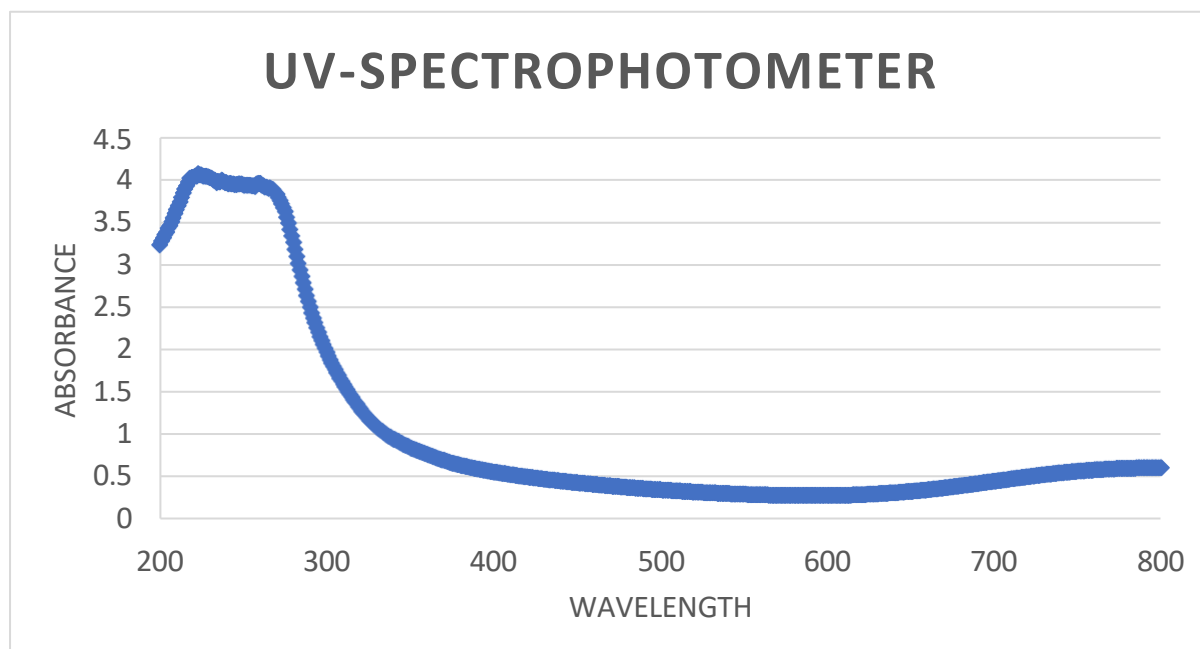


FIG 5.8: UV-VIS reading of CuNPs Synthesis

5.3 GAS CHROMATOGRAPHY –MASS SPECTROMETRY(GC-MS)

The methanolic seed extract of *S. macrophylla* were subjected to GC-MS to elucidate the compounds present in the analyte. The results obtained for GC-MS was listed and the peaks obtained in the mass spectrum and their retention time. The mass spectrum obtained from the analyte were interpreted and compared with known spectrum components which were stored in NIST library and the structure were identified in PUBCHEM database with their molecular formulae and corresponding retention time which could provide further analysis of the soluble seed extract.

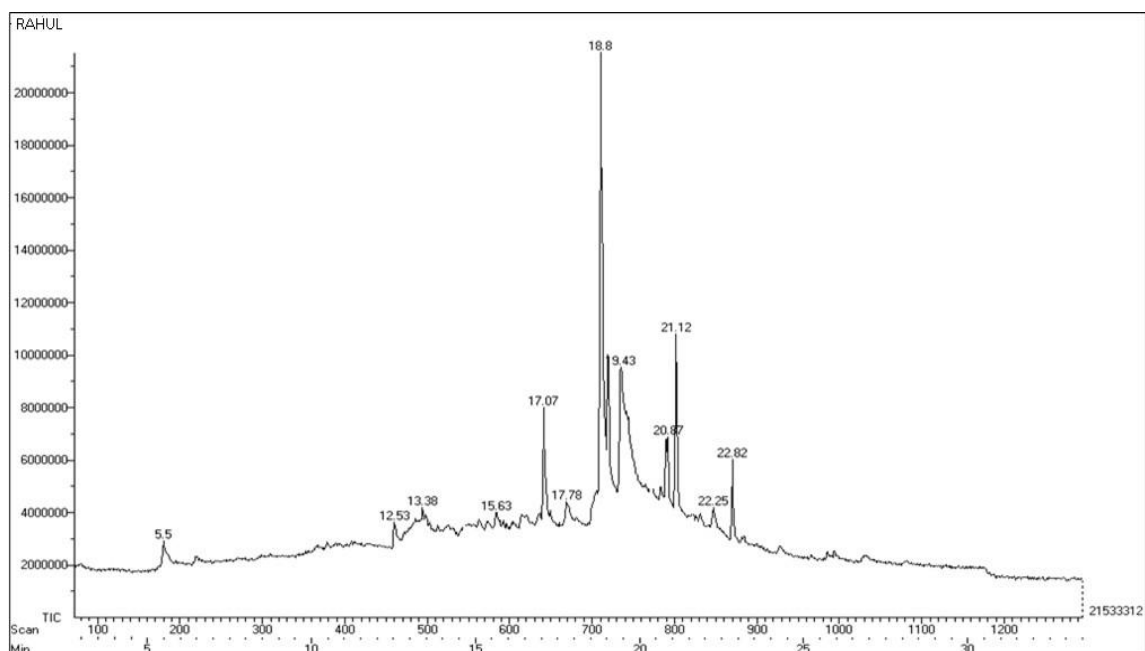


FIG 5.9: GC/MS spectrum

S. No	RT	Name	Structure	Mol. Wt. g/mol	Mol. formula
1.	13.38	5-Methyl-2,4-diisopropylphenol		192.3	$C_{13}H_{20}O$
2.	22.82	Docosanoic acid, methyl ester		354.6	$C_{23}H_{46}O_2$
3.	5.62	Cyclohexane		136.23	$C_{10}H_{16}$

4.	12.53	Eugenol		164	$C_{10}H_{12}O_2$
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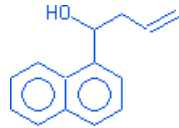
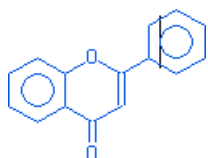
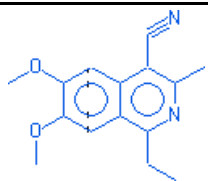
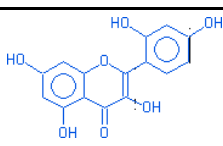
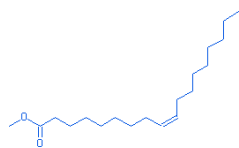
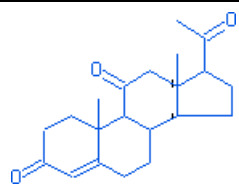
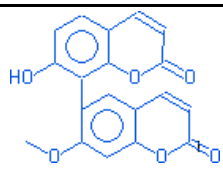
5.	15.63	3-Buten-1-ol,1-(naphthyl)-		198.26	C ₁₄ H ₁₄ O
6.	17.07	Flavone		222.24	C ₁₅ H ₁₀ O ₂
7.	17.78	Isoquinoline-4-carbonitrile		256	C ₁₀ H ₆ N ₂
8.	18.8	Morin		302.23	C ₁₅ H ₁₀ O ₇
9.	19.43	Oleic Acid		282.5	C ₁₈ H ₃₄ O ₂
10.	20.87	9-Octadecenoic acid		296.5	C ₁₉ H ₃₆ O ₂
11.	21.12	Ketogestin		328	C ₂₁ H ₂₈ O ₃
12.	22.25	Coumarine		336.3	C ₁₉ H ₁₂ O ₆

TABLE 5.1: Compounds found by using GC/MS

5.4 FTIR ANALYSIS

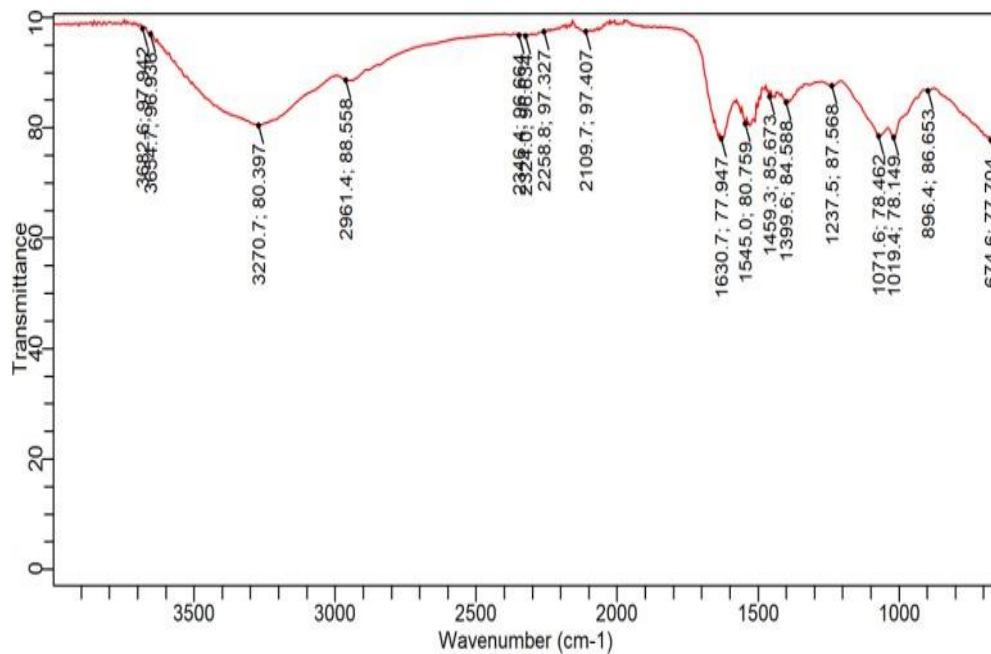


FIG 5.10: FTIR ANALYSIS RESULT

TABLE 5.2: FTIR COMPOUND INTERPRETATION

S. No	Absorption (cm ⁻¹)	Vibration	Bond	Compound
1	3270.73	Stretching	O-H	Alcohol
2	2961.4	Stretching	N-H	Amine Salt

3	2346.3	Stretching	O=C=O	Carbondioxide
4	2258.7	Stretching	C≡C	Alkyne
5	2109.6	Bending	N-H	Amine group
6	1544.9	Bending	N-O	Nitro Compound
7	1399.6	Bending	O-H	Carboxylic acid
8	1237.47	Stretching	C-O	Alkyl Aryl ether
9	1071.6	Stretching	C-N	Amine
10	896.4	Bending	C=C	Alkene
11	674.64	Stretching	C-Br	Halo Compounds

5.5 Antibacterial activity by agar well diffusion method

The antibacterial activity was carried out for methanol leaves extract of *S. macrophylla* against Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*). The antibacterial sensitivity of the crude extract and their potency were assessed quantitatively by measuring the diameter of clear zone in

cultures in petriplates. The antibacterial activity of these extracts could be correlated to the presence of secondary metabolites such as alkaloids, terpenoids and phenolic compounds that adversely affect the growth and metabolism of microbes.

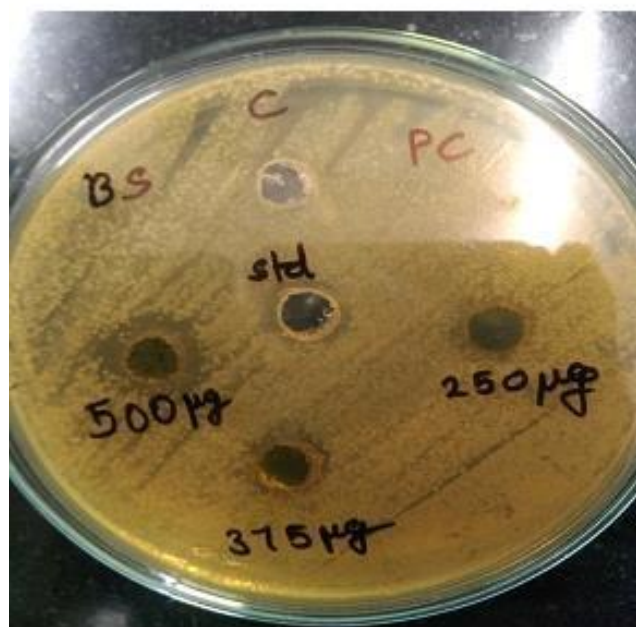


FIG 5.11: *Bacillus subtilis*



FIG 5.12: *Staphylococcus aureus*

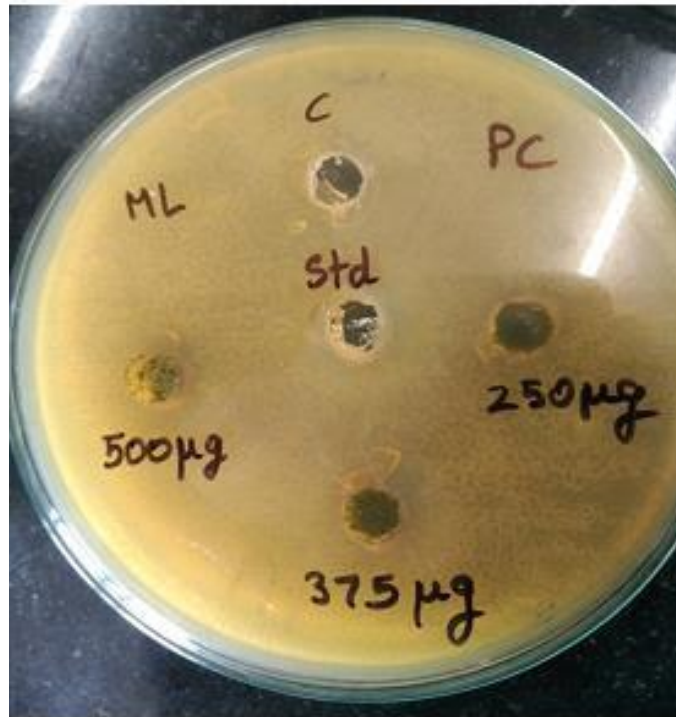


FIG 5.13: *Micrococcus luteus*

Table 5.3: Antibacterial activity

Bacterial Pathogen	<i>Zone of Inhibition (mm)</i>			
	Standard (Tetracycline)	250 $\mu\text{g/mL}$	375$\mu\text{g/mL}$	500$\mu\text{g/mL}$
<i>Bacillus subtilis</i>	11	11	12	13
<i>Staphylococcus aureus</i>	-	10	11	-
<i>Micrococcus luteus</i>	11	10	11	12

5.6 Anti-fungal activity

Anti-fungal assay was tested against three different fungal phytopathogens, by uniformly mixing the prepared nanoparticle sample in different concentrations in the sterilized PDA media. The results obtained on the 7th day after subculturing the fungus is represented below.

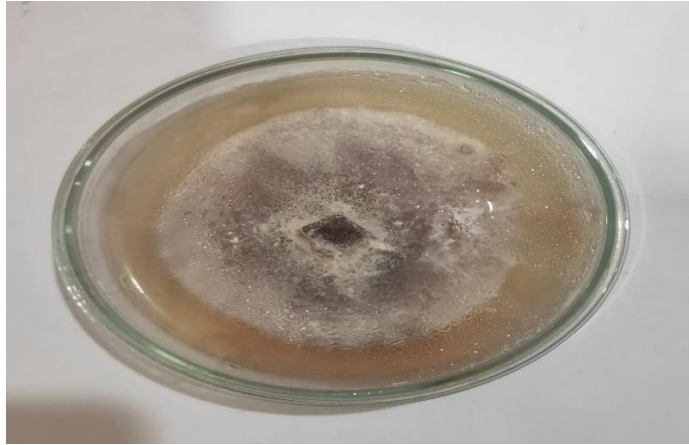


FIG 5.14: *Fusarium oryzae*



FIG 5.15: *Athelia rolfsii*



FIG 5.16: *Trichoderma* sp

5.6.1 Anti-Fungal activity estimation

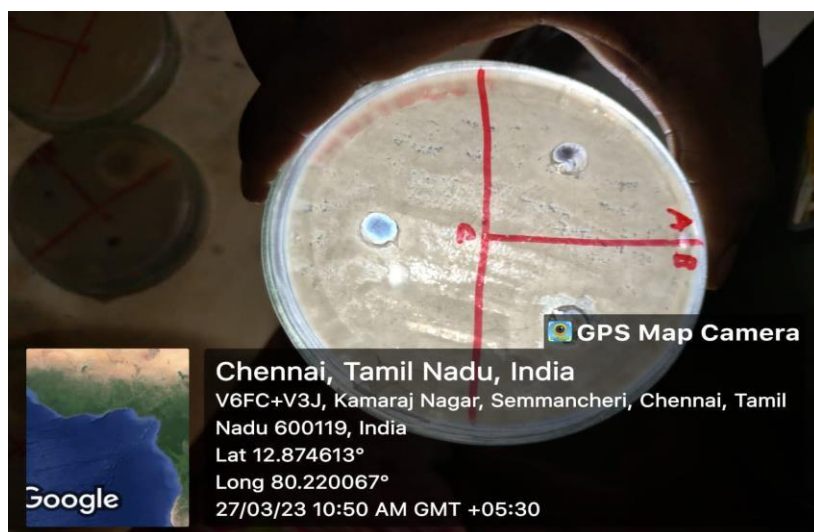


FIG 5.17: *Fusarium oryzae* zone inhibition

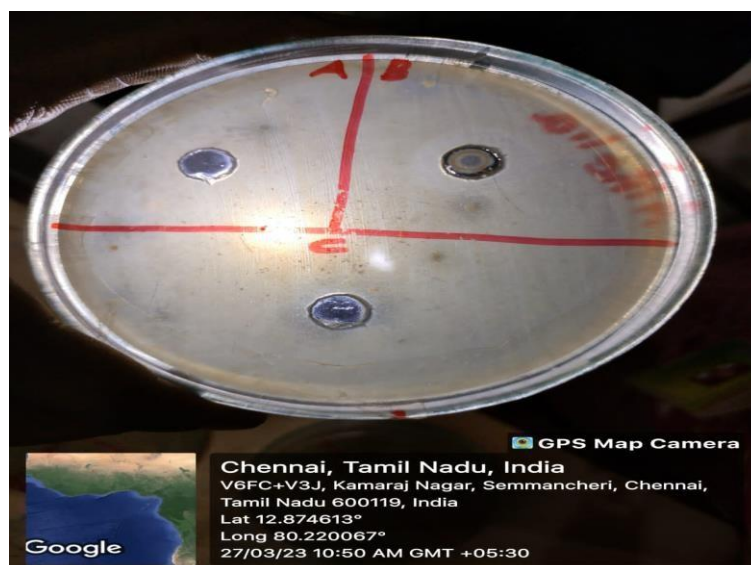


FIG 5.18: *Athelia rolfsii* zone inhibition



FIG 5.19: Trichoderma sp zone inhibition

Table 5.4: Anti-fungal activity estimation

Fungal Pathogen	Zone of Inhibition (mm)		
	Control	Sample A	Sample B
Athelia rolfsii	10	-	15
Fusarium oryzae	7.5	-	16
Trichoderma sp.	10	-	20

5.7 Alpha-Amylase Inhibition Assay

The inhibition of carbohydrate hydrolyzing enzymes such as α - amylase can be an important strategy to lower postprandial blood glucose levels. The α -amylase and α - glucosidase enzymes are responsible for the breakdown of oligo and/or disaccharide to monosaccharides. Inhibitors of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time causing a marked decrease in the rate of glucose absorption thereby blunting the post prandial plasma glucose rise. The maximum Alpha amylase inhibition activity was $84.81 \pm 0.81\%$ at $200 \mu\text{g/mL}$ concentration with the IC_{50} of $65.65 \mu\text{g/mL}$ concentration. The standard inhibitory range for alpha amylase inhibition is around 55-93.4%. Thus, the obtained result shows great activity towards inhibition of diabetes.

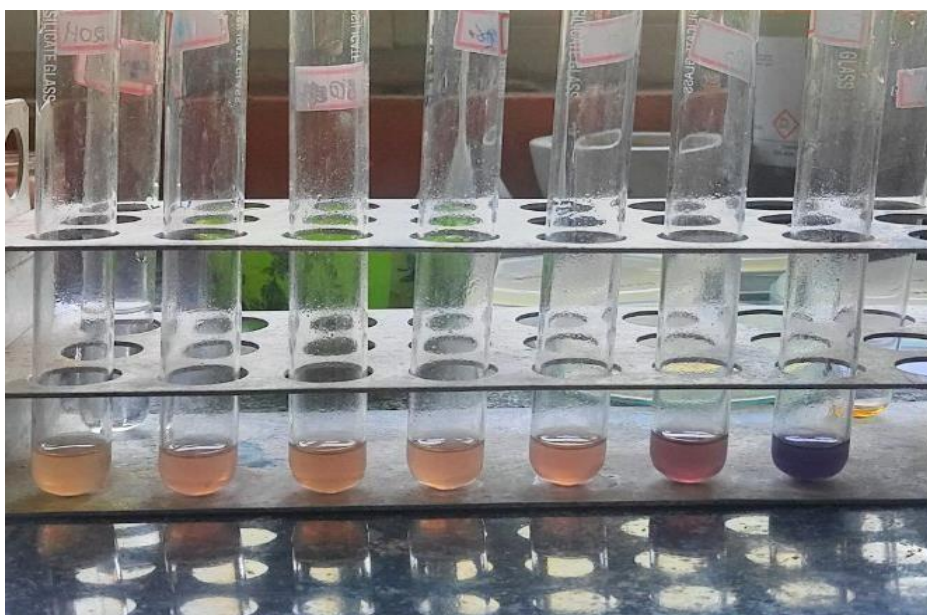


Fig 5.20: Alpha-amylase inhibition

Concentration ($\mu\text{g/mL}$)	%Inhibition at 595nm
20	38.03 ± 0.54
40	41.27 ± 0.37

60	45.69±0.53
80	77.40±0.94
100	79.67±0.63
120	82.73±0.83
150	83.94±0.82
200	84.81±0.81

Table 5.5: Alpha Amylase Inhibition

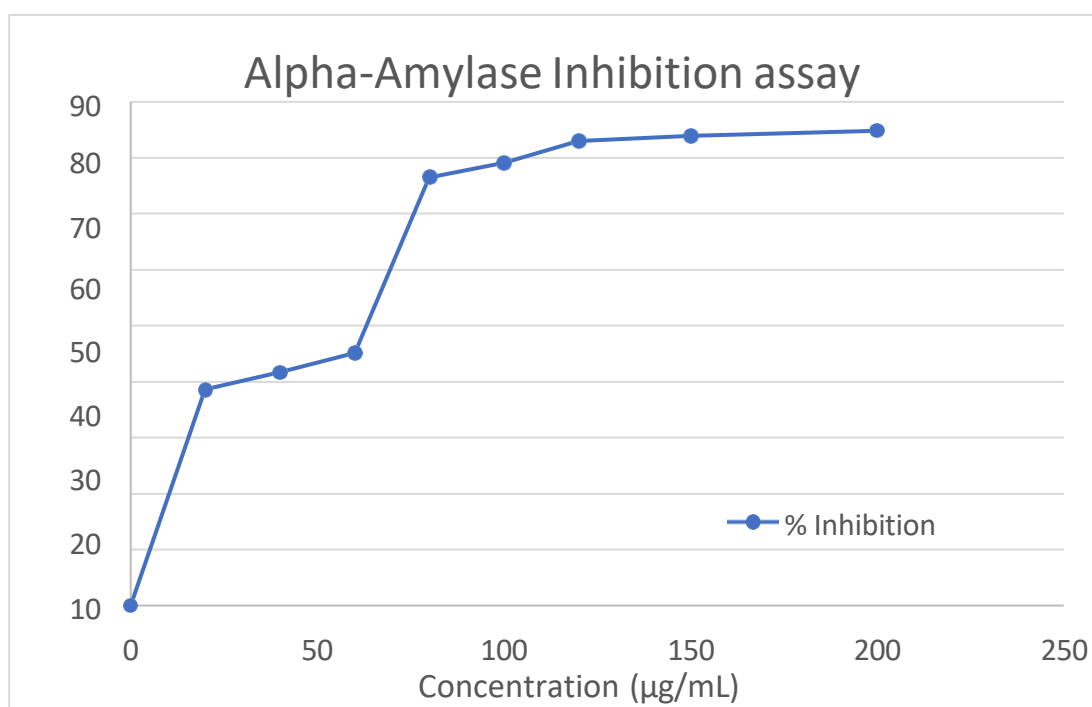


FIG 5.21: Graph of Alpha Amylase Inhibition

5.8 SEM-EDX

The SEM results showed that the nanoparticles were been evenly coated over seed particles and formed into a agglomerate complex.

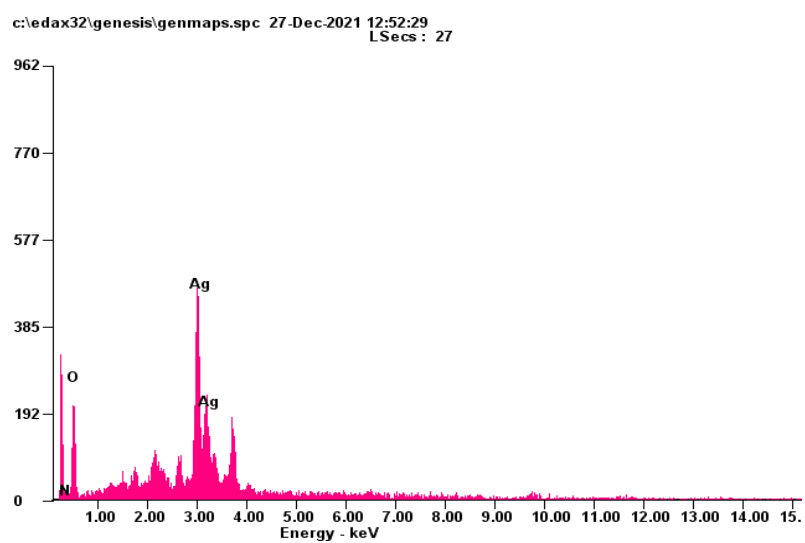
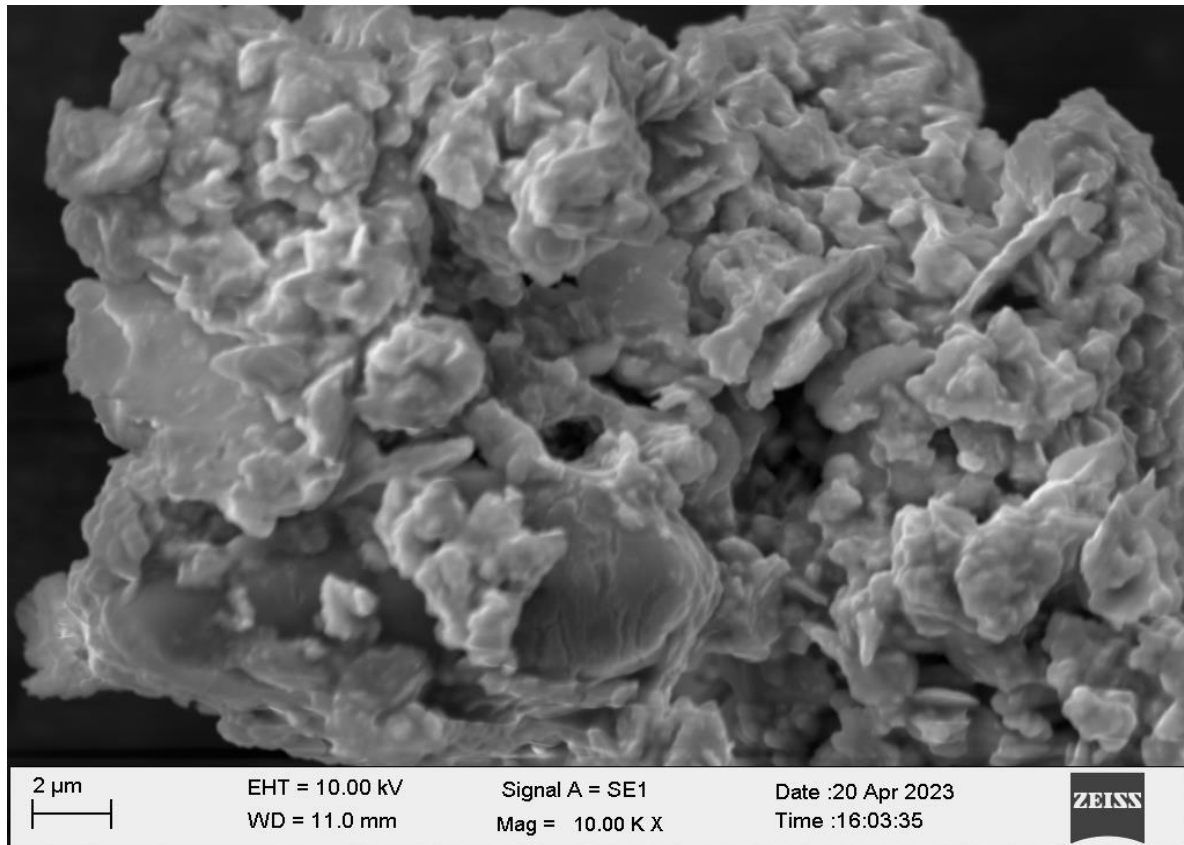
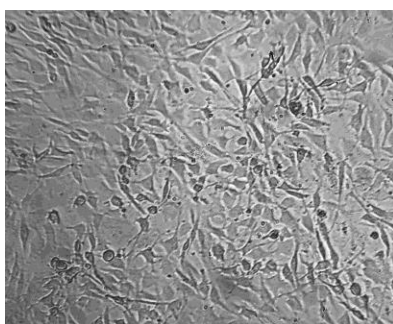


FIG 5.22: SEM-EDX result

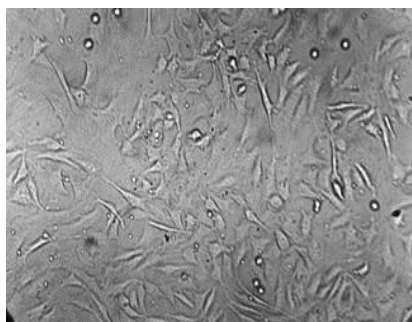
ELEMENT	Wt%
Nitrogen	2.04
Oxygen	55.77
Silver	42.19

Table 5.6: SEM-EDX components

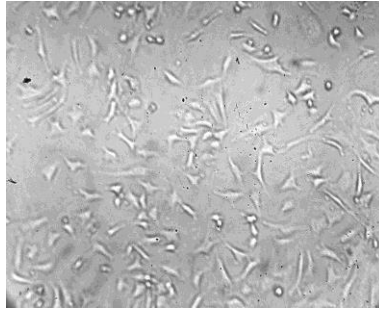
5.9 MTT Assay of Silver Nanoparticle



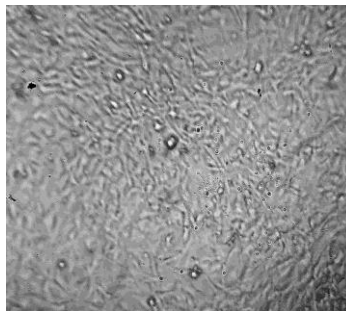
Control MCF-7



MCF-7 (low drug concentration-1ng)



MCF-7 (Mid drug concentration-1µg)



MCF-7 (High drug concentration-100µg)

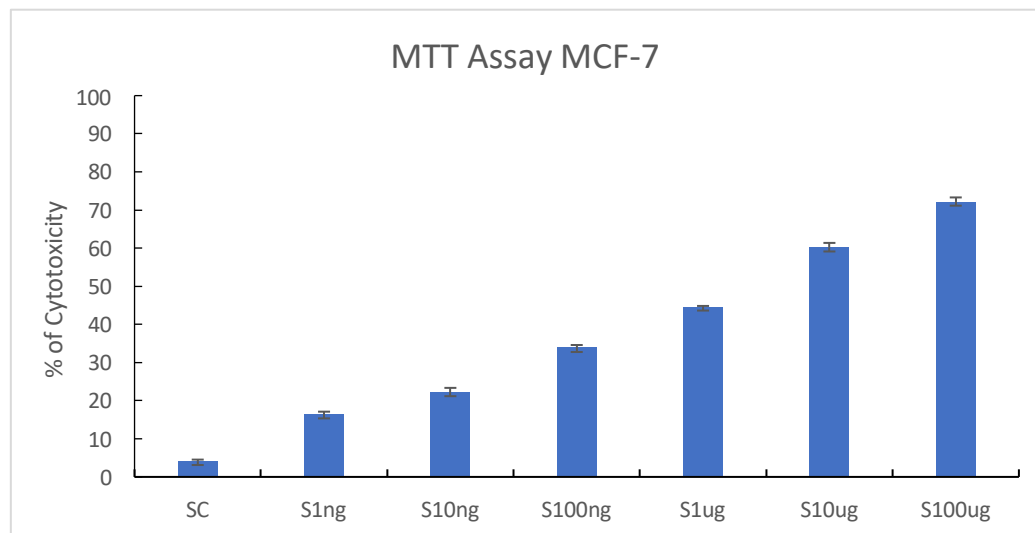


Fig 5.23: Graphical representation of Nanoparticles cytotoxicity

The cytotoxicity of the synthesized nanoparticles was screened against Human breast cancer cells (MCF-7) by means of MTT Assay. After incubation, the silver nanoparticles synthesized were found to be cytotoxic to the MCF-7 cells. The result obtained with this colorimetric assay demonstrates a significant concentration-dependent increase in cytotoxicology against breast cancer cells. The viability of cells decreases as the nanoparticle concentration increases. Prolonged exposure will end in additional toxicity to the cells %. As compared to the control group, the highest toxicity 72% was found at 100µg/mL of AgNPs while at 1ng/mL the toxicity is 16%.

5.10 CELL LINE STUDIES

3T3-L1 is a cell line derived from (mouse) 3T3 cells that is used in biological research on adipose tissue. 3T3-L1 cells have a fibroblast-like morphology, but, under appropriate conditions, the cells differentiate into an adipocyte-like phenotype. It is mostly used for invitro studies of diabetic medicines because of its adipogenic properties. Here we compare cells grown in controlled environment against cells grown in increasing concentration of our extract. When the concentration of extract increases it inhibits the glucose formation so that the cells starve for glucose and then die at a point.

Here the % of cell death denotes the cutting of glucose transport into cells by the action of our extract which stops the breakdown of starch by inhibiting the starch hydrolyzing enzymes. The maximum cell death percentage was noted to be $80.09 \pm 3.5\%$ of concentration 100µg/ml. The 50% cell death at the concentration of 6.25 µg/ml.

Control:

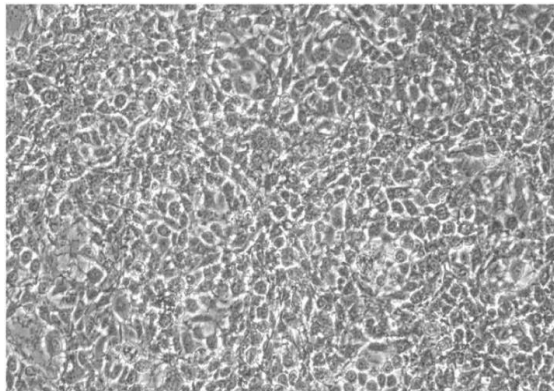


FIG 5.24

1.562 μ g/mL:

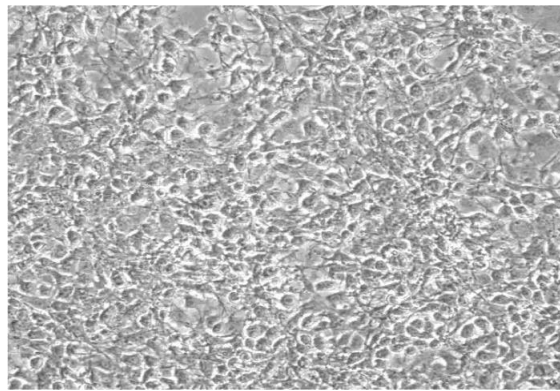


FIG 5.25

6.25 μ g/mL:

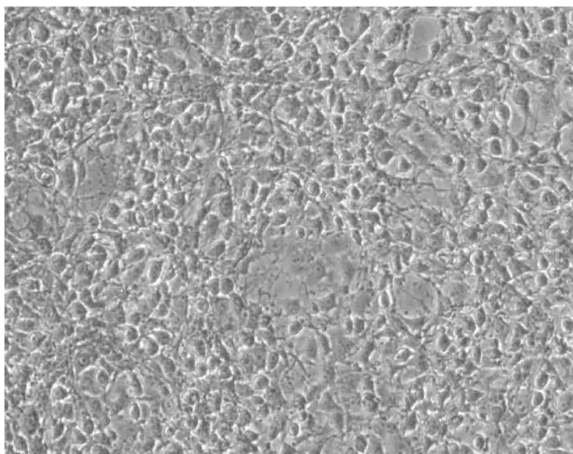


FIG 5.26

12.5 μ g/mL:

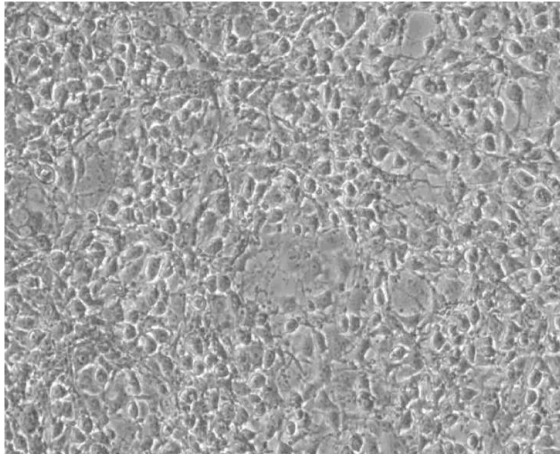


FIG 5.27

50µg/mL:

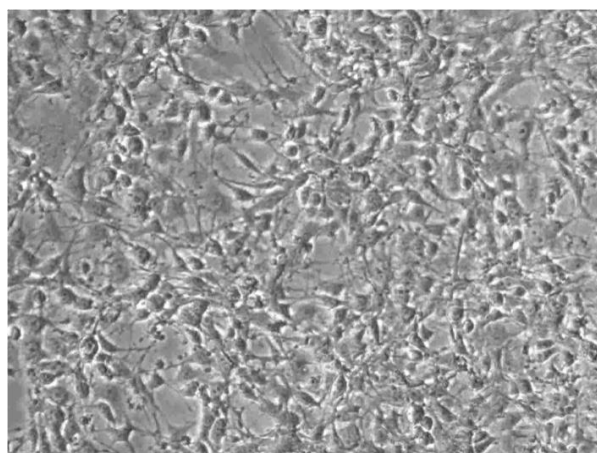


FIG 5.28

100µg/mL:

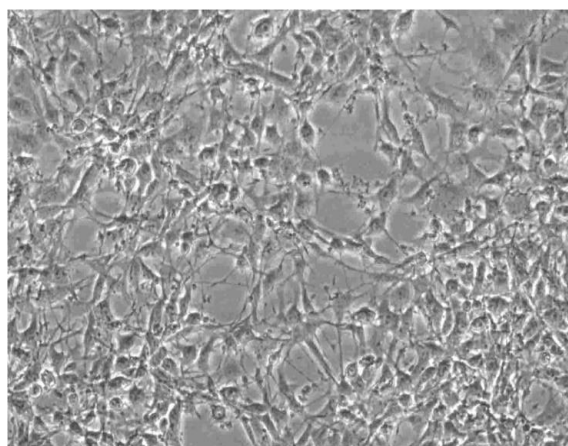


FIG 5.29

Table 5.7: Cell Line Studies

CONCENTRATION (µg/ml)	OD 1	OD 2	OD 3	% OF CELL DEATH		
100	0.718	0.751	0.822	82.76	81.59	75.92
50	0.814	0.807	0.801	76.45	76.91	77.3
12.5	0.981	0.952	1.091	65.46	67.37	58.22
6.25	1.232	1.213	1.203	48.95	50.2	50.86
1.562	1.443	1.461	1.473	20.07	12.88	12.09
Control	1.502	1.487	1.492	10.18	12.17	10.84

Table 5.8: Cell Line Conc & Mean values

Concentration (µg/mL)	Mean value
100	80.09±3.5
50	76.89±0.43
12.5	63.68±4.82
6.25	50±0.97
1.562	15.01±0.99
control	11.10±0.5

6. CONCLUSION

Swietenia macrophylla seeds have been mentioned in different folk medicines as a phyto-therapeutic agent in diabetes mellitus and hypertension. Several preclinical studies supported the folklore claims of *S. macrophylla* seeds as antidiabetic agent, anti-fungal agent which have been discussed in this chapter. In addition to the hypoglycemic effects, *S. macrophylla* seeds have been found to be effective against various pathological conditions having mechanistic resemblance with diabetic complications. *S. macrophylla* seeds have been reported to possess antioxidant, hypotensive, anti-inflammatory, antihyperlipidemic, and wound healing properties.

The synthesized silver nanoparticle from methanolic seed extract of *S. macrophylla* has shown great activity in antifungal, antidiabetic and antioxidant properties. The SEM-EDX analysis showed that the nanoparticles are fully coated and can act with their full potential. The MTT assay against MCF-7 cells resulted in a 16% toxicity at 1 µg concentration which is the half-life concentration of these cells. Thus, as a result the use of seed extract for the green synthesis of AgNPs have proven significantly advantageous over other methods of synthesis and the obtained nanoparticles can be further implemented in therapeutic applications in future.

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