GREEN SYNTHESIS OF SILVER NANOPARTICLE USING *MUNTINGIACALABURA LEAF* AND STUDY IT'S ANTIFUNGAL ACTIVITY

Submitted in partial fulfillment of the requirements for the award of

Bachelor of science degree in Biotechnology

By

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DEPARTMENT OF BIOTECHNOLOGY

SCHOOL OF BIO AND 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 Sriban V (40770050) and Rahul R (40770042) who carried out the project entitled "**Green Synthesis of Silver Nanoparticle Using *Muntingia Calabura Leaf* and Study It's Antifungal Activity"** under my supervision from September 2022 to May 2023.

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I **RAHUL.R (40770042)** hereby declare that the Project Report entitled "**Green Synthesis of Silver Nanoparticle Using *Muntingia Calabura Leaf* and Study It's Antifungal Activity"** was done by us under the guidance of **DR. SUDHA S** and **DR. JAYASHREE S** as internal at Sathyabhama institute of Science and Technology, submitted in partial fulfillment of the requirements for the award of Bachelor of Science inBiotechnology.

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INDEX

ABSTRACT

The present work aims to investigate a green synthesis of AgNPs using Muntingia calabura leaf extract as reducing and stabilizing agents. The AgNPs formation was monitored using a UV-Vis spectrophotometer. Characterisations of AgNPs size and shape were observed by SEM. The elemental analysis was analyzed using XDS. The maximum surface Plasmon resonance for AgNPs was detected at 425-430 nm. This study revealed that the AgNPs were polydisperse and polycrystalline in nature. The microbial inhibition test against Escherichia coli and Bacillus cereus showed that the muntingia leafmediated AgNPs had inhibited the growth of these bacteria, as indicated by the formation of the inhibition zone. The average inhibition zone for Escherichia coli was 10.3±0.5 mm and for Bacillus cereus at 9.5±0.6 mm. SEM results showed that the synthesis AgNPs have spherical form with the sizes ranging from 22 to 37 nm. Hence, the synthesis AgNPs can potentially be applied for water treatment and medicinal purposes.

LIST OF ABBREVIATIONS

% - Percentage

AgNPs – Silver Nanoparticles

DLS - Dynamic light scattering

EDX - Energy Dispersive X-ray analysis

FTIR – Fourier transform infrared spectroscopy

mg - Milligram

mS – Milli second

SEM - Scanning Electron Microscope

TEM – Transmission electron microscopy

UV-V – Ultraviolet visible spectroscopy

XRD – X-ray diffraction

µS – Microsecond

nm – nanometer

GC/MS – Gas Chromatography Mass Spectrometry

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: INTRODUCTION

1.1. NANO TECHNOLOGY

Nanotechnology is a field of science and technology that deals with the design, creation, manipulation and application of materials and devices with dimension in the nanometer scale range (typically 1 to 100 nanometers). It involves the use of techniques and tools to control and manipulate matter at the atomic, molecular and supramolecular levels. These particles occur in several shapes such as nanospheres (Fig 1) (Agam M *et al*., 2007)

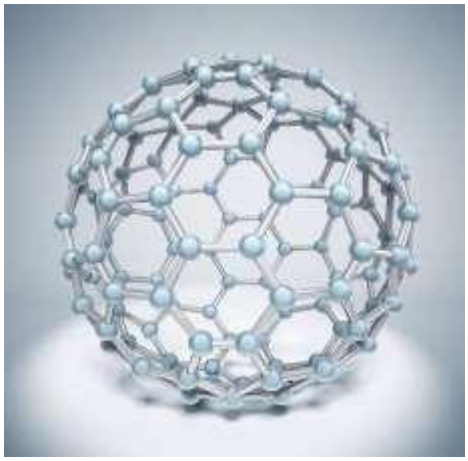


Fig 1.1 Nanosphere

Nanotechnology has numerous applications in various fields such as medicine, electronics, energy and materials science. For example, in medicine, nanotechnology has led to the development of targeted drug delivery systems, which can deliver drugs directly to diseased cells, minimizing the impact on healthy cells. In electronics, the use of nanotechnology has led to the development of faster and more efficient computer chips, and in material science, it has led to the creation of stronger and lighter materials.

Nanotechnology is a rapidly growing field with new developments and discoveries being made every day. However, it also raises concerns about the potential risks associated with the use of nanoparticles, including their toxicity and impact on the environment. Therefore, the study of nanotechnology requires careful consideration of both its potential benefits and risks.

1.2 NANOPARTICLES

Nanoparticles are tiny particles with at least one dimension between 1 and 100 nanometers. They have unique properties that differ from the same material at larger scales, such as increased surface area to volume ratio and altered optical, electronic and magnetic properties. These unique properties make nanoparticles attractive for a wide range of applications in fields such as biomedicine, electronics, energy and environmental remediation.

There are many different types of nanoparticles, each with their unique properties and applications. Some common types of nanoparticles include:

1. METAL NANOPARTICLES: These are nanoparticles made of metals such as gold, silver, platinum and iron. They ae commonly used in biomedical applications, catalysis and electronics.

2. SEMICONDUCTOR NANOPARTICLES: Also known as quantum dots, these are nanoparticles made of semiconducting materials such as cadmium selenide and indium phosphide. They have unique optical and electronic properties and are used in applications such as solar cells, biological imaging, and light-emitting diodes (LEDs).

3. MAGNETIC NANOPARTICLES: These are nanoparticles made of magnetic materials such as iron oxide and nickel. They are used in applications such as magnetic resonance imaging (MRI), targeted drug delivery and environmental remediation.

4. CARBON-BASED NANOPARTICLES: Carbon-based nanoparticles include fullerenes, carbon nanotubes and graphene. They have unique mechanical, electrical and thermal properties and are used in a wide range of applications, including energy storage, electronics and biomedicine.

1.2.1 METALNANOPARTICLES

Metal nanoparticles are nanoparticles made of metals such as gold, silver, copper, platinum and iron, which have unique physical and chemical properties due to their small

size. Metal nanoparticles are used in a wide range of applications, including biomedical, environmental and industrial applications.

Metal nanoparticles can be synthesized using various methods such as chemical reduction, electrochemical deposition and laser ablation. The properties of metal nanoparticles can be tuned by controlling their size, shape and surface chemistry.

2. SILVER NANOPARTICLES

Silver nanoparticles have been the subject of much research in recent years due to their unique physical, chemical and biological properties. Silver nanoparticles have a high surface area to volume ratio, which makes them highly efficient in various applications such as antimicrobial agents, drug delivery, biosensing and imaging.

The antimicrobial properties of silver nanoparticles are of particular intrest as they have the potential to combat antibiotic-resistant bacterial infections. Silver nanoparticles can damage the bacterial cell membrane by generating reactive oxygen species, leading to cell death. The property has been exploited in various applications such as wound dressings, water treatment and food packaging.

Silver nanoparticles have also been investigated for their drug delivery applications as they can improve the solubility and stability of drugs and enhance their therapeutic efficacy. Silver nanoparticles have been shown to accumulate in tumor tissues making them a promising candidate for targeted drug delivery and cancer therapy

3. CHARACTERISTIC FEATURE OF SILVER NANOPARTICLES

Silver nanoparticles have unique physical, chemical and biological properties due to their small size and high surface area to volume ratio. Here are some characteristic features of silver nanoparticles.

1. SIZE AND SHAPE: Silver nanoparticles can range in size from a few to hundreds of nanometers, with a typical size range of 1-100 nm. The shape of silver nanoparticles can vary, including spherical, rod-shaped, triangular, and cubic.
2. OPTICAL PROPERTIES: Silver nanoparticles exhibit strong absorption and scattering of light due to the excitation of surface plasmon resonances, which

depends on their size, shape and surrounding medium. This property is used in various applications such as biosensing, imaging and photothermal therapy.

3. ANTIBACTERIAL PROPERTIES: Silver nanoparticles have been shown to exhibit potent antimicrobial activity against a broad spectrum of bacteria, viruses and fungi. They can damage the bacterial cell membrane and inhibit the growth of microorganisms, making them a promising candidate for various applications such as wound dressings, water treatment and food packaging.
4. BIOCOMPATIBILITY: Silver nanoparticles have been shown to be compatible and non-toxic at low concentrations, making them suitable for various biomedical applications such as drug delivery tissue engineering and imaging.
5. CHEMICAL STABILITY: Silver nanoparticles are stable in various chemical environments, including acidic and basic conditions. However, their stability can be affected by factors such as temperature, pH and ionic strength.
6. SURFACE CHEMISTRY: The surface of silver nanoparticles can be functionalized with various chemical groups such as thiol, amine and carboxyl, which can modulate their physiochemical properties and enhance their stability, biocompatibility and targeting ability.

1.3 muntingia calabura

Muntingia calabura, also known as jamaica cherry, is a small, fast growing tree that belongs to the family *muntingiaceae.* It is native to central and south america but has been widely introduced to other parts of the world including southeast asia, where it is known as ‘aratiles’.

The jamaica cherry tree can reach up to 15 meters in height and has a spreading canopy. It produces small, edible, red or yellow cherry-like fruits that are sweet and juicy, with a slightly tangy flavor. The fruit is commonly used in desserts, jams and jellies and is also consumed fresh.

In addition to its culinary uses, *muntingia calabura* has been used in traditional medicine for its various medicinal properties. The leaves, bark, and fruits of the tree have been

used to treat various ailments such as fever, cough, diarrhea and skin infections. The tree also has antimicrobial, anti-inflammatory and antioxidant properties.

Muntingia calabura is also considered a beneficial plant for the environment, as it is fast-growing and can help prevent soil erosion. The tree is also used as a shade tre in coffee and cocoa plantations, as it provides shade for the crops and improves soil fertility.

4. SYNTHESIS OF SILVER NANOPARTICLES

Silver nanoparticles can be synthesized by various methods, including physical, chemical and biological methods. Here are some common methods used for the synthesis of silver nanoparticles.

1. CHEMICAL REDUCTION METHOD: In this method, silver ions are reduced to silver nanoparticles using a reducing agent such as sodium borohydride, hydrazine or citrate. The reduction reaction is typically carried out in the presence of a stabilizing agent such as polyvinylpyrrolidone (PVP) or sodium dodecyl sulfate (SDS) to prevent aggregation of the nanoparticles.
2. GREEN SYNTHESIS METHOD: Green synthesis involves the use of natural or biological sources such as plant extracts, fungi or bacteria to synthesize silver nanoparticles. In this method, the reducing and capping agents are provided by the natural source. For example, plant extracts contain various phytochemicals such as flavonoids, phenolics and terpenoids, which can reduce and stabilize silverions to form nanoparticles.
3. PHOTOCHEMICAL SYNTHESIS METHOD: In this method silver nanoparticles are synthesized by exposing a silver ion solution to light of a specific wavelength. The light induces the reduction of silver ions to form nanoparticles. This method is relatively simple and does not require a reducing agent or stabilizing agent.
4. ELECTROCHEMICAL SYNTHESIS METHOD: In this method, silver nanoparticles are synthesized by applying an electric current to a silver electrode in a silver ion solution. The electric current induces the reduction of silver ions to form nanoparticles. This method allows for precise control over the size and shape of the nanoparticles.

CHAPTER 2: LITERATURE AND SURVEY

Silver nanoparticles (AgNPs) have gained significant attention due to their unique properties and potential applications in various fields, including medicine, electronics, and agriculture. The green synthesis of AgNPs using plant extracts is an environmentally friendly and sustainable approach, which eliminates the use of toxic chemicals and reduces the environmental impact compared to conventional methods.

Muntingia calabura, commonly known as Jamaican cherry or Panama berry, is a tropical plant that is known for its various medicinal properties. The leaf extract of M. calabura contains a wide range of bioactive compounds, including flavonoids, alkaloids, and phenolics, which have been reported to possess antifungal properties. Therefore, utilizing M. calabura leaf extract for the green synthesis of AgNPs could potentially lead to the production of AgNPs with enhanced antifungal activities.

Several studies have reported the green synthesis of AgNPs using various plant extracts, including M. calabura leaf extract. For instance, a study by Gupta *et al*. (2018) demonstrated the synthesis of AgNPs using M. calabura leaf extract and characterized the synthesized nanoparticles using various analytical techniques such as UV-Vis spectroscopy, X-ray diffraction (XRD), and transmission electron microscopy (TEM). The study found that the synthesized AgNPs were stable, spherical, and ranged in size from 10 to 50 nm.

Another study by Sathishkumar *et al*. (2016) reported the green synthesis of AgNPs using M. calabura leaf extract and investigated their antifungal activities against various fungal pathogens. The study found that the synthesized AgNPs showed potent antifungal activities against Candida albicans, Aspergillus niger, and Fusarium oxysporum, indicating their potential as a natural antifungal agent.

Furthermore, a study by Sharma *et al*. (2017) reported the green synthesis of AgNPs using M. calabura leaf extract and evaluated their antifungal activities against clinical isolates of Candida species. The study found that the synthesized AgNPs exhibited significant antifungal activities against various Candida species, including Candida

albicans, Candida glabrata, and Candida tropicalis, suggesting their potential as an alternative antifungal agent.

One example is a study by Shankar *et al*. (2014), which reported the green synthesis of AgNPs using Aloe vera leaf extract and characterized the nanoparticles using UV-Vis spectroscopy, XRD, and TEM. The study found that the synthesized AgNPs were stable, spherical, and ranged in size from 10 to 50 nm, and exhibited potential antimicrobial activities against various pathogens.

Another study by Huang *et al*. (2017) demonstrated the green synthesis of AgNPs using green tea extract from Camellia sinensis and evaluated their catalytic activities for the reduction of 4-nitrophenol. The study found that the synthesized AgNPs exhibited excellent catalytic activities, indicating their potential as a green catalyst in chemical reactions.

Moreover, a study by Gopinath *et al*. (2015) reported the green synthesis of AgNPs using neem leaf extract from Azadirachta indica and evaluated their antibacterial activities against various bacterial strains. The study found that the synthesized AgNPs showed potent antibacterial activities, suggesting their potential as a natural antibacterial agent.

In addition to plant extracts, microorganisms, such as bacteria and fungi, have also been used for the green synthesis of AgNPs. For example, a study by Durán *et al.,* (2016) reported the green synthesis of AgNPs using a fungus called Fusarium oxysporum and investigated their antimicrobial activities. The study found that the synthesized AgNPs showed strong antimicrobial activities against various bacteria and fungi, indicating their potential as a natural antimicrobial agent.

Furthermore, biomolecules, such as proteins, enzymes, and polysaccharides, have also been employed for the green synthesis of AgNPs. For instance, a study by Sastry *et al.* (2003) reported the green synthesis of AgNPs using proteins extracted from the seeds of Moringa oleifera and studied their properties. The study found that the synthesized AgNPs were stable, well-dispersed, and exhibited unique optical properties, suggesting their potential in various applications.

Green synthesis of AgNPs has been reported using various natural materials, including plant extracts, microorganisms, and biomolecules. Plant extracts have been widely used as reducing and stabilizing agents in the synthesis of AgNPs. For instance, a study by Mittal *et al*., (2018) reported the green synthesis of AgNPs using leaf extract of Ocimum sanctum and evaluated their antimicrobial activities. The study found that the synthesized AgNPs exhibited potent antimicrobial activities against various bacteria and fungi, indicating their potential as natural antimicrobial agents.

Similarly, microorganisms, such as bacteria and fungi, have been used for the green synthesis of AgNPs. For example, a study by Shah *et al*., (2017) reported the green synthesis of AgNPs using a bacterium called Bacillus licheniformis and investigated their catalytic activities. The study found that the synthesized AgNPs showed excellent catalytic activities in the reduction of 4-nitrophenol, suggesting their potential as a green catalyst in chemical reactions.

Moreover, biomolecules, such as proteins, enzymes, and polysaccharides, have also been employed for the green synthesis of AgNPs. For instance, a study by Iravani (2014) reported the green synthesis of AgNPs using chitosan, a natural polysaccharide, and studied their properties. The study found that the synthesized AgNPs were stable, well-dispersed, and exhibited unique optical properties, indicating their potential in various applications.

Characterization of the synthesized AgNPs is also a crucial aspect of green synthesis. Various characterization techniques, such as UV-Vis spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM), dynamic light scattering (DLS), and Fourier-transform infrared spectroscopy (FTIR), among others, have been used to study the size, shape, stability, and surface properties of the synthesized AgNPs.

CHAPTER 3: AIM AND SCOPE

1. AIM:

- To synthesis silver nanoparticles by green synthesis method using *muntingia calabura* leaf extract and to study their antifungal activity.

2. SCOPE:

- To synthesis of silver nanoparticles.
- To characterize these nanoparticles.
- To evaluate the pharmacological activities.
- To study their effectiveness in antifungal applications.

CHAPTER 4: MATERIALS AND METHODS

1. MATERIALS:

- *Mutingia calabura* leaf extract
- Test tubes
- UV spectrophotometer
- Mortar and pestle
- Methanol
- Ethanol
- Centrifuge
- Distilled water
- Deionized water
- Test tube stand
- Water bath
- Silver nitrate
- Shaker incubator
- Incubator
- Electronic balance
- Whatman filter paper
- Cotton
- Aluminum foil
- Beaker
- Micropipette
- Magnetic stirrer

2. METHODS:

1. Biomass extraction from leaf 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 leaves 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 leaf 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

4.2.2 SAMPLEPREPARATION

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

FIG 4.2: Methanolic leaf extract of muntingia calabura

4.3 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. CHARACTERIZATIONS OF AGNPs

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.

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.

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.

2. PLACE THE PELLET 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.

4.4.2.3 COLLECT THESPECTRUM

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.

4.4.2.4 ANALYZE THESPECTRUM

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.

4.4.3 SCANNING ELECTRONICMICROSCOPY

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 leaf extract of *muntingia calabura*. 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 *muntingia calabura*. 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.

4.5 GAS CHROMATOGRAPHY/MASS SPECTROMETRY

For GC–MS analysis, the samples were injected into a HP-5 column (30 m × 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.

4.5.1 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.

6. ISOLATING AND SUBCULTURING THE PHYTOPATHOGENS

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. 20 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 was 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.

4.6.2 SUBCULTURING THE IDENTIFIED FUNGALPHYTOPATHOGENS

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).

4.7 ANTIFUNGAL ASSAY

The fungal culture was taken for three different fungi wiz; *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 2 days, later the inhibition zone was noted and measured.

CHAPTER 5 : RESULT AND DISCUSSION

5.1 METHANOLIC LEAF EXTRACT

The leaf of *muntingia calabura 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 Leaf 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.

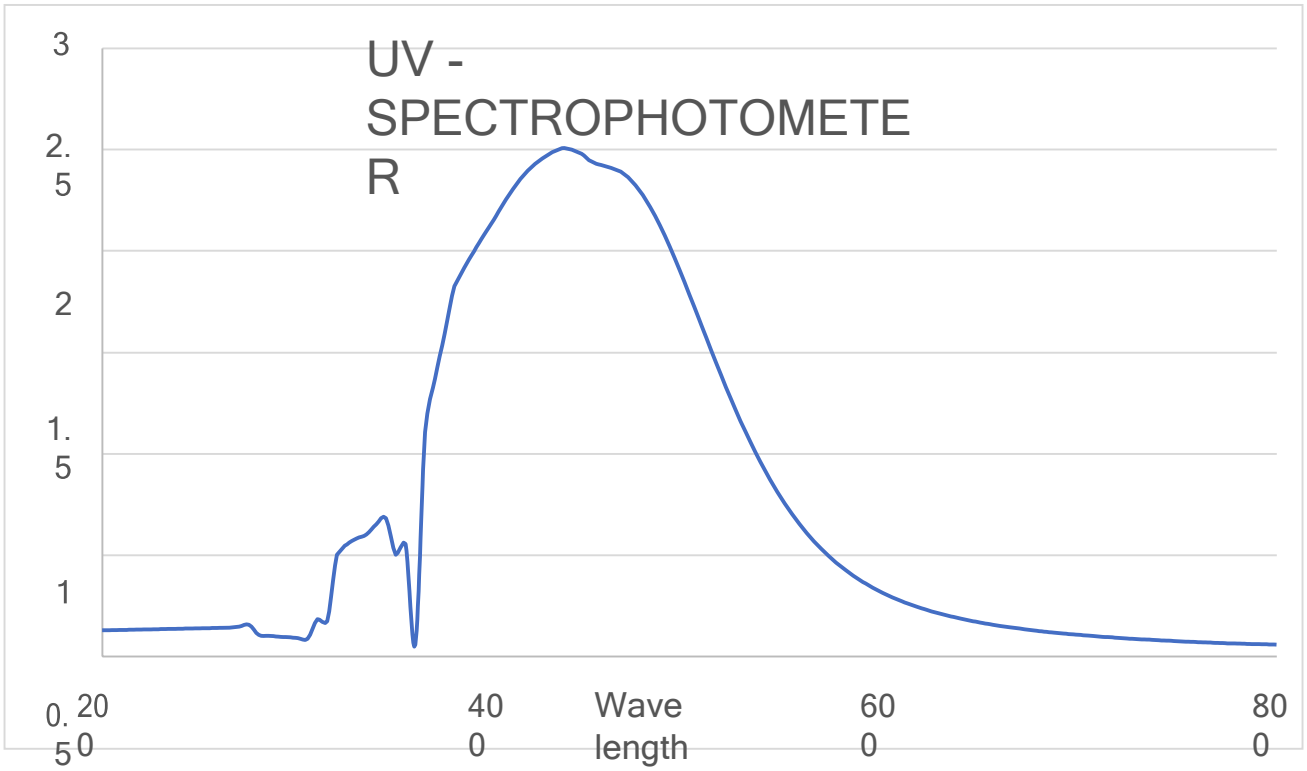


FIG 5.2: UV-VIS SPECTROSCOPY OF* AgNPs *synthesis

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 dark reaction

Fig 5.4 Day 2 dark reaction

Fig 5.5 light reaction

5.3 GAS CHROMATOGRAPHY –MASS SPECTROMETRY(GC-MS)

The methanolic leaf extract of *muntingia calabura* was subjected to GC-MS to elucidatethe compounds present in the analyte. The results obtained for GC-MS were listed and thepeaks 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 leaf extract.

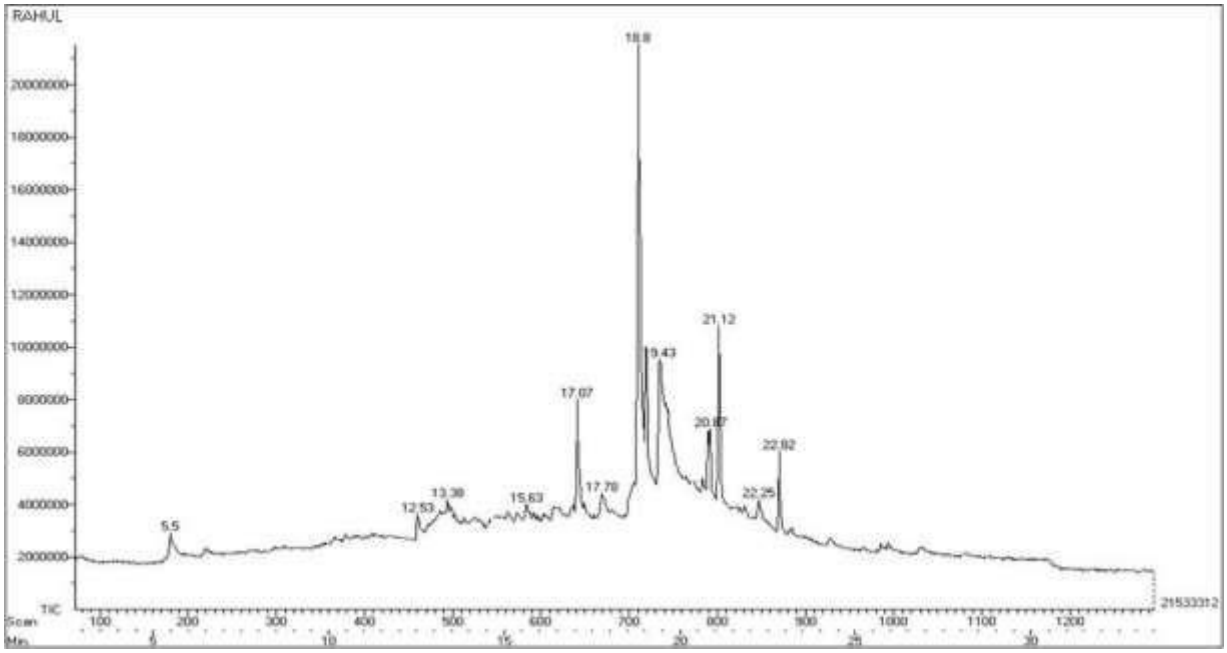
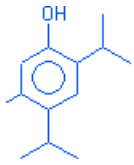
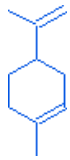
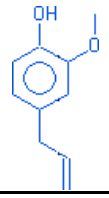
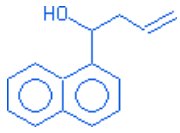
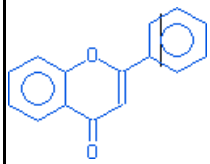
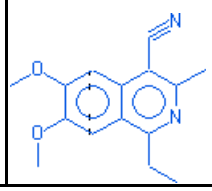
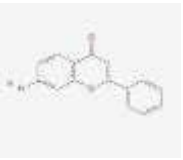
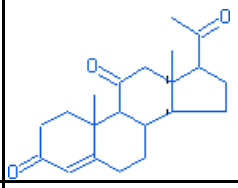
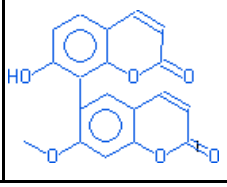


Fig 5.6 GC/MS spectrum

Table 5.1: Compound found by using GC/MS

S. No	RT	Name	Structure	Mol. Wt. g/mol	Mol. formula
1.	13.38	5-Methyl-2,4-diisopropylphenol		192.3	C13H20O
2.	22.82	Docosanoic acid, methyl ester		354.6	C23H46O2
3.	5.62	Cyclohexane		136.23	C10H16
4.	12.53	Eugenol		164	C10H12O2
5.	15.63	3-Buten-1-ol,1-(naphthyl)-		198.26	C14H14O
6.	17.07	Flavone		222.24	C15H10O2
7.	17.78	Isoquinoline -4- carbonitrile		256	C10H6N2

8.	18.8	7- hydroxy flavanol		238.24	C15H10O3
9.	19.43	Oleic Acid		282.5	C18H34O2
10.	20.87	9-Octadecenoic acid		296.5	C19H36O2
11.	21.12	Ketogestin		328	C21H28O3
12.	22.25	Coumarine		336.3	C19H12O6

5.4 FTIR ANALYSIS

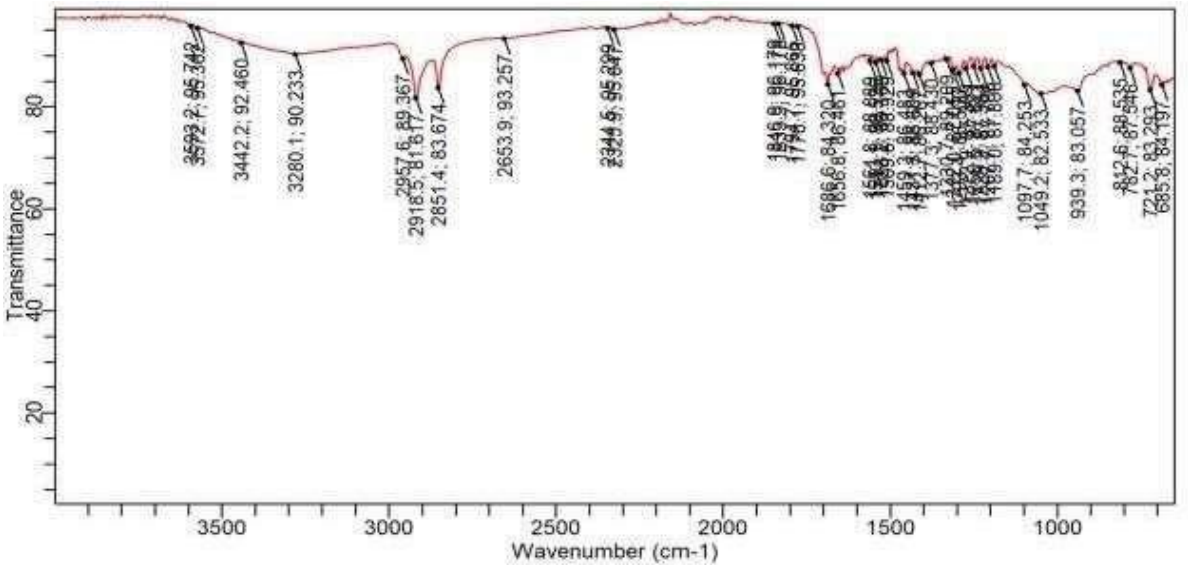


Fig 5.7 FTIR Analysis result

Table 5.2: FTIR compound interpretation

Peak number	Wavenumber (cm^{-1})	Intensity
1	685.83016	84.19656
2	721.23987	83.29337
3	782.74095	87.54584
4	812.55965	88.53546
5	939.28913	83.05665
6	1049.24560	82.53285
7	1097.70099	84.25289
8	1189.02077	87.80603
9	1207.65746	87.70624
10	1228.15782	87.38443

11	1250.52184	87.82112
12	1272.88587	87.36864
13	1293.38623	86.50033
14	1312.02292	87.05525
15	1330.65961	89.20916
16	1377.25133	88.42989
17	1412.66104	86.26743
18	1431.29773	86.60914
19	1459.25276	86.48289
20	1509.57182	88.92887
21	1528.20851	89.16887
22	1543.11786	88.53612
23	1561.75455	88.88881
24	1656.80167	86.46110
25	1686.62037	84.31999
26	1776.07648	95.63795
27	1794.71317	95.86501
28	1833.85022	96.17783
29	1846.89590	96.17851

30	2325.85881	95.04741
31	2344.49550	95.29898
32	2653.86454	93.25712
33	2851.41345	83.67446
34	2918.50553	81.61675
35	2957.64257	89.36744
36	3280.05730	90.23303
37	3442.19649	92.46045
38	3572.65332	95.36163
39	3593.15368	95.74188

5.5 Antifungal 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 are represented below.

Fig 5.8: Fusariumorxysporum

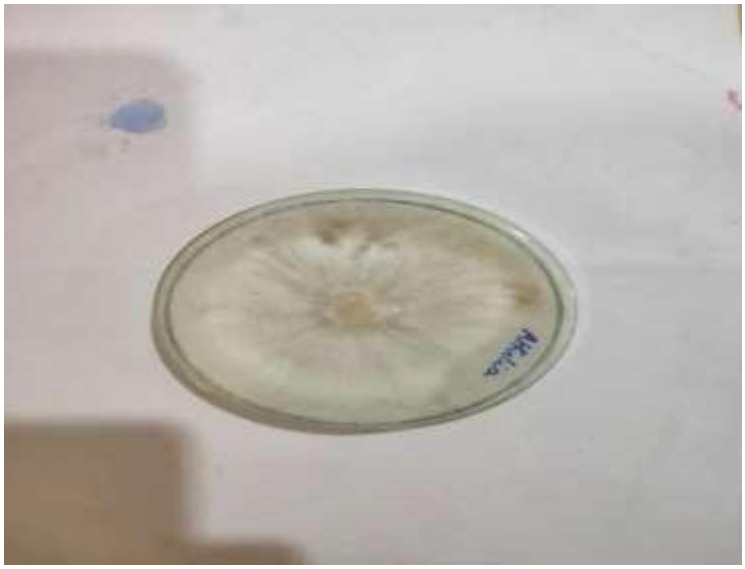


Fig 5.9 athelia rolfsii

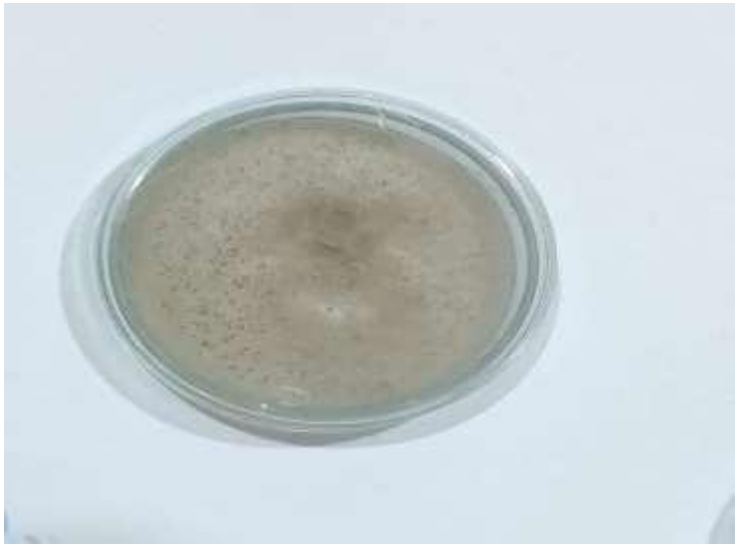


Fig 5.10 Trichoderma sp

5.5.1 antifungal activityestimation

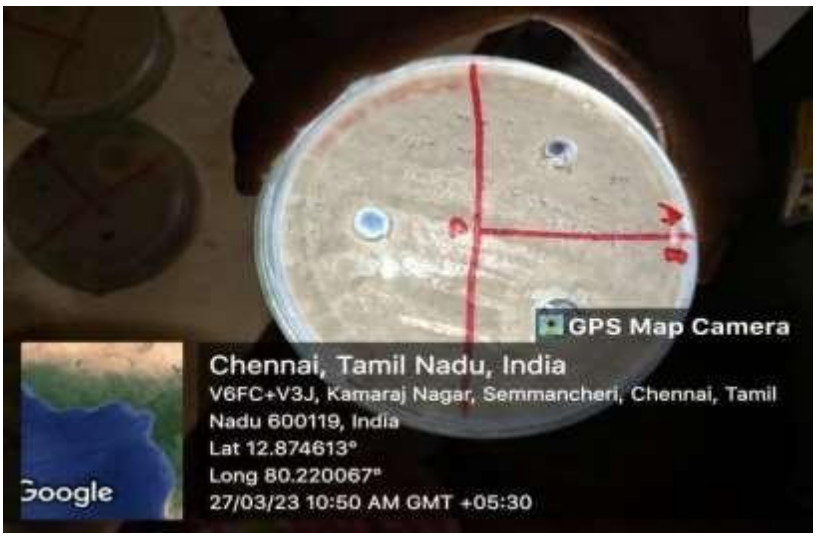


Fig 5.11 Fusarium orxysprorum zone inhibition

Fig 5.12 athelia rolfsii zone inhibition

Fig 5.13 trichoderma sp zone inbition

Table 5.3: Anti Fungal activity estimation

Fungal Pathogen	Zone of Inhibition (mm)		
	Control	Sample A	Sample B
Athelia rolfsii	10	-	15
Fusarium orxysporum	7.5	-	16

Trichoderma sp.	10	-	20

5.6 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±0.81% at 200 µg/mL concentration with the IC50 of 65.65 µg/mL concentration.

Fig 5.14 Alpha Amylase inhibition

Table 5.4 Alpha Amylase Inhibition

Concentration (µg/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

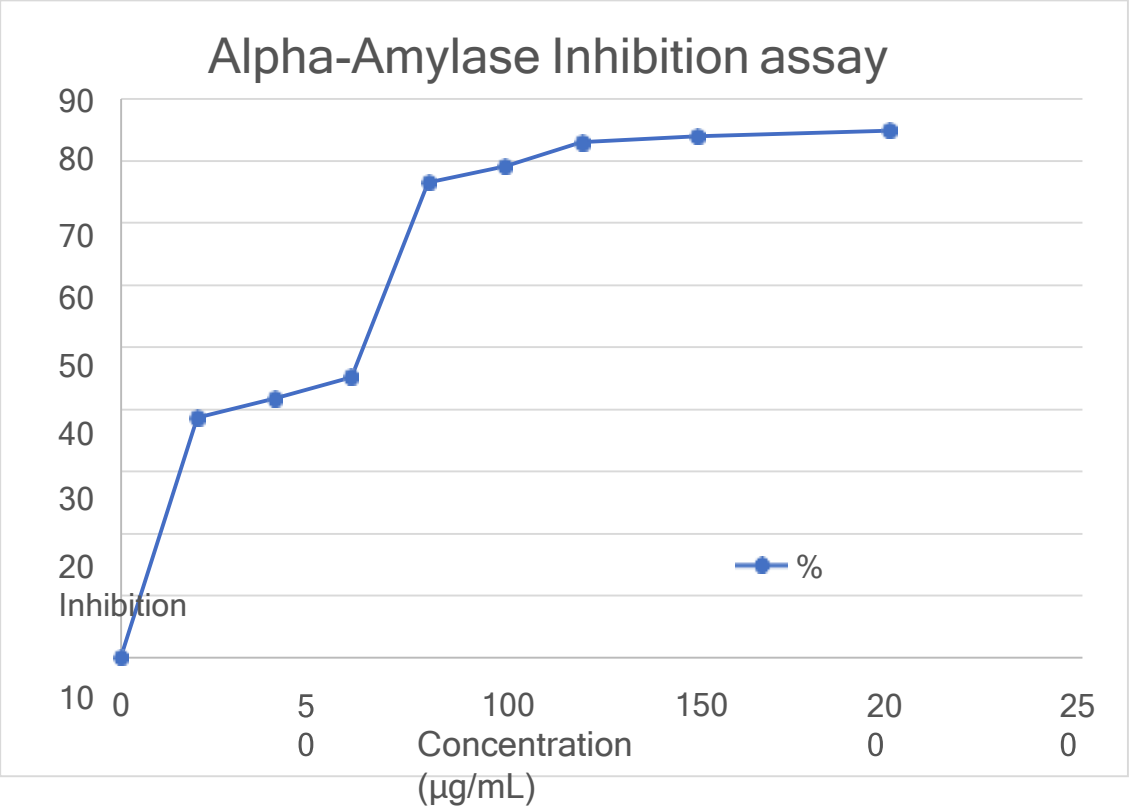


Fig 5.15 graph of alpha amylase inhibition

5.7 SEM

The particles were poly dispersed spherical shape.

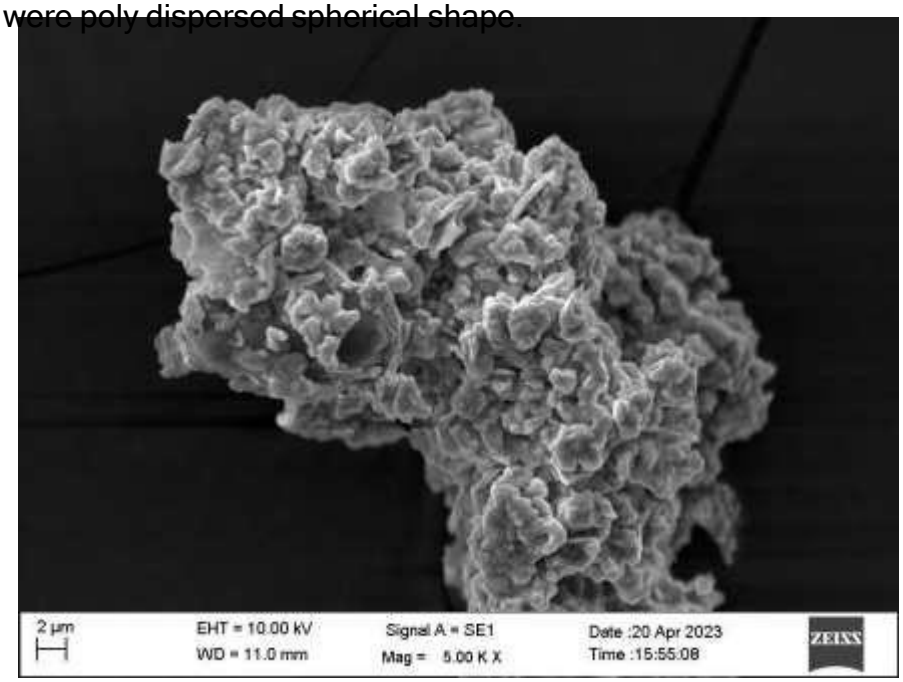


Fig 5.16 SEM image

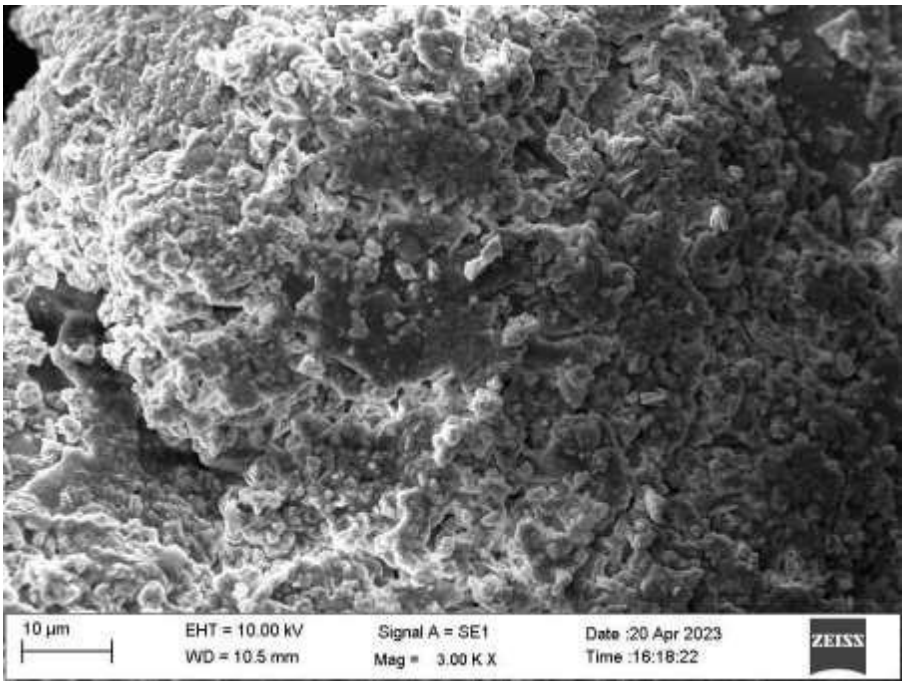


Fig 5.17 SEM image

5.8 SEM EDX

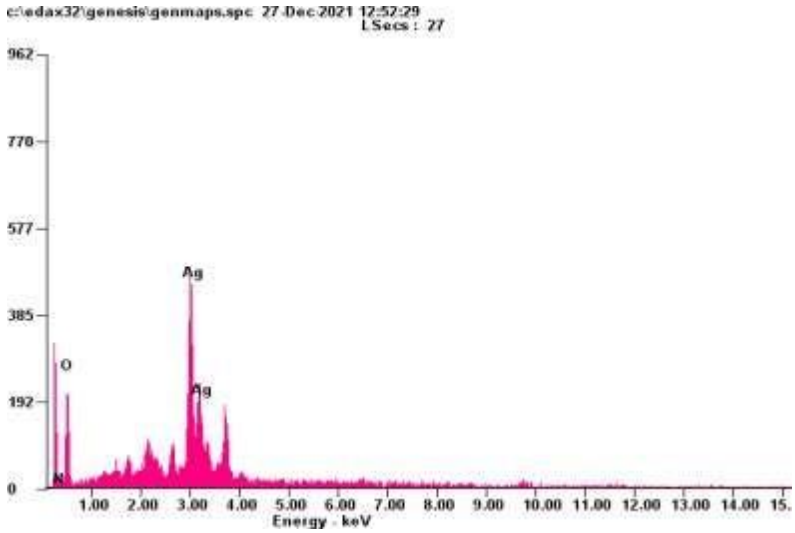


Fig 5.18 SEM EDX result

Table 5.5: SEM-EDX components

ELEMENT	Wt % (weight)
Nitrogen	2.04
Oxygen	55.77
Silver	42.19

CHAPTER 6 CONCLUSION

Muntingia calabura has antioxidant and anti-inflammatory characteristics. It is a plant that is abundant in polyphenols, flavonoids, and carotenoids, all which aid to shield the body from oxidative stress and inflammation. As a result, it can be used to treat a variety of inflammatory disorders like diabetes, asthma, and arthritis. *Muntingia calabura* leaves havebeen used for centuries to aid in the healing of wounds. To lessen swelling and speed up healing, the leaves can be crushed and used directly to wounds. Studies have revealed that*Muntingia calabura* has anti-diabetic qualities that can assist diabetics control their blood sugar levels. This is because it can boost the body's absorption of glucose and improve insulin sensitivity.

Treatment for candidiasis: Candidiasis is a fungal infection that can affect the skin, mouth, throat, and genitalia and is brought on by the Candida fungus. A promising natural remedy for candidiasis, *Muntingia calabura* extract has been proven in studies to have antifungal action against Candida species.

A fungal condition that affects the skin, scalp, and nails, ringworm can be treated. It has been demonstrated that *Muntingia calabura* possesses antifungal action against dermatophyte fungus, the most typical cause of ringworm.

The synthesized silver nanoparticle from methanolic leaf extract of Muntingia calabura 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 portential.

Historically, fungus infections have been treated with *Muntingia calabura*. It may be used as a natural treatment for fungal infections because studies have revealed that it possesses antifungal qualities.

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