ANTIBACTERIAL STUDY OF MICROWAVE ASSISTED GREEN SYNTHESIS OF SILVER NANOPARTICLE FROM BIOPHYTUM SENSITIVUM PLANT EXTRACT

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In partial fulfilment of the requirements for the award of the degree of

BACHELOR OF SCIENCE IN CHEMISTRY

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CERTIFICATE

This is to certify that the project report entitled "**ANTIBACTERIAL STUDY OF MICROWAVE ASSISTED GREEN SYNTHESIS OF SILVER NANOPARTICLE FROM BIOPHYTUM SENSITIVUM PLANT EXTRACT**" is the bonafide work carried out by **ROHITH K SUKUMARAN (Reg. No.: 200021026027)** for the partial fulfilment of the requirement for the award of degree **BACHELOR OF SCIENCE IN CHEMISTRY** through the Department of Chemistry, Bharata Mata College, Thrikkakara, affiliated to Mahatma Gandhi University, Kottayam, Kerala.

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DECLARATION

I, **PAVITHRA P PRAKASH,** hereby declare that this project report entitled "**ANTIBACTERIAL STUDY OF MICROWAVE ASSISTED GREEN SYNTHESIS OF SILVER NANOPARTICLE FROM BIOPHYTUM SENSITIVUM PLANT EXTRACT"** is an authentic work carried out during my course of study under the guidance of Mr. Baiju K P, Assistant Professor, Department of Chemistry, Bharata Mata College, Thrikkakara.

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ROHITH K SUKUMARAN

CONTENTS

ANTIBACTERIAL STUDY OF MICROWAVE ASSISTED GREEN SYNTHESIS OF SILVER NANOPARTICLE FROM BIOPHYTUM SENSITIVUM PLANT EXTRACT

ABSTRACT

We have successfully synthesized silver nanoparticles using Biophytum sensitivum from silver nitrate solutions in the presence of microwave irradiation.

First, we prepared Biophytum sensitivum leaf extract. To do this, 25 g of washed and dried leaves were placed in an RB flask, boiled in 200 ml of distilled water for 20 minutes, cooled and filtered. 10 ml of this extract are poured into 90 ml of 1 mM silver nitrate solution and stirred well. Microwave irradiated this mixture for 5 minutes on medium-high power. Then the powder is cooled, washed and centrifuged. Characterizations such as UV-Vis spectroscopy, FTIR spectroscopy, particle size analyzer and SEM microscopy are performed. The presence of silver nanoparticles was confirmed by the optical absorption peak at 418 nm obtained from the UV-Vis absorption spectrum. C-H, C-C, C-N and O-H vibrational bands are indicated from FTIR spectroscopy. Biofunctional groups help stabilize silver nanoparticles. A rod-like microstructure with a grain size of 715 nm is confirmed by SEM analysis and particle size analyzer.

Antibacterial activity of silver nanoparticles (AgNPs) against Staphylococci, Pseudomonas, Bacillus and E. coli was tested and compared with azithromycin as an antibiotic. Silver nanoparticles have a 7 mm zone of inhibition against Staphylococcus sp. and thus may have potential as antibacterial agents against the tested bacteria. 9 mm for Pseudomonas species and Bacillus species comparable to azithromycin. However, azithromycin and silver nanoparticles showed no activity against the Gram-negative bacterium Escherichia coli.

CHAPTER - 1

INTRODUCTION

OBJECTIVES

- To synthesize silver nanoparticles using Biophytum Sensitivum under the microwave assisted method.
- Characterize the as-synthesized nanoparticles using UV-Visible spectroscopy, FTIR spectroscopy, Scanning electron microscopy and particle size analyzer.
- Investigate the antibacterial characteristics of the nanoparticles that have been created.

1.1: NANOPARTICLES

A nanoparticle is a particle with at least one nanoscale dimension. The nanoscale is characterized as having dimensions ranging from 1 nm to 100 nm. They exist in nature as well as a result of human action. Because of their submicroscopic size, they have unique material properties, and manufactured nanoparticles may find practical application in a variety of fields such as medicine, engineering, catalysis, and environmental remediation. Nanoparticles are classified according to their size, shape, and material properties, such as organic, inorganic, and so on. Nanoparticles have different physical, chemical, and mechanical properties that differ from those of the same materials in bulk form due to their high surface-to-volume ratio. A nanoparticle is an aggregate of a few hundred atoms. Because they are made up of only a few atoms, smaller nanoparticles are referred to as clusters. Nanoparticles are objects that exist between atom domains. Nanoparticle-based technologies are aimed at increasing the efficiency, sustainability, and speed of existing operations. The nanoparticle-based method uses less material, much of which is already highly

reactive. Nanoparticles have enormous potential for new or enhanced forms of health care, spawning a new discipline of science known as nanomedicine.

1.2: CLASSIFICATION

I. Classification based on dimensions

The nanostructured materials were categorised by Pokropivyn and skorokhod based on their dimensions. There are four types of nanoparticles: 1) zero dimensional nanoparticles (0D-NPs), 2) one dimensional nanoparticles (1D-NPs), 3) two dimensional nanoparticles (2D-NPs), and 4) three dimensional nanoparticles (3D-NPs).

a) Zero dimensional nanoparticles (0D-NPs)

All dimensions of zero-dimensional nanoparticles (ZD-NPs) are measured on the nanoscale; (0D-NPs) comprise atomic clusters, filaments, and cluster assemblies. These materials' characteristics and dimensions are all less than 100 nm. 0D-NPs can be 1) amorphous or crystalline, 2) single crystalline or polycrystalline, 3) include one or more chemical elements, and 4) come in a range of shapes and configurations.

b) One - dimensional nanoparticles (1D-NPs)

One dimension in 1 NPs is outside the nanoscale. For example, nanorods, nanowires, and nanotubes. Because electrons are limited in two dimensions in this system, they cannot flow freely. 1D nanostructures, like 0 NPs, can be 1) amorphous or crystalline. 2) monocrystalline or polycrystalline 3)Metallic ceramics or polymeric materials. Monolayers are thin films of 1D nanostructures that can be deposited in a controlled manner and should be only one atom thick.

c) Two dimensional nanoparticles (2D-NPs)

Two dimensions lie outside the nanoscale in 2NPs nanoparticles. For example, nanosheets, nonofilms, and nanoribbons. Electrons are limited within one dimension in this system, and they cannot move freely within the associated dimension. 2 D can also be amorphous or crystalline, and made up of metallic, ceramic, or polymeric matrixes. Because of their atomic thickness, they have excellent mechanical flexibility and optical transparency.

II. Classification based on materials

a) Organic-based nanoparticles

Organic nanoparticles or polymers include dendrimers, micelles, liposomes, and others. They are biodegradable and harmless, as well as sensitive to thermal and electromagnetic radiation like heat and light. They are commonly utilised for targeted medication delivery in the biomedical industry.

b) Inorganic - based nanoparticles

It is divided into two categories. Metal-based nanoparticles and metal oxide-based nanoparticles are the two types.

- **Metal based nanoparticles**

They are created from metals using either destructive or constructive methods. Metals used in the manufacture of nanoparticles include Al, Cd, Co, Au, and Ag. They have distinct qualities such as diameters ranging from 10 to 100 nm. Surface properties include a large surface area to volume ratio, pore size, surface charge, and surface charge density. Structure might be crystalline or amorphous. Spherical and cylindrical in shape.

• Metal oxide based nanoparticles.

Because of their improved reactivity and efficiency, metal oxide based nanoparticles are synthesized in order to change the properties of the corresponding metal based nanoparticles. Examples include aluminium oxide, iron oxide, titanium oxide, and zinc oxide, among others.

c) Carbon - based nanoparticles

They are known as carbon-based nanoparticles since they are formed of carbon. Fullerenes, graphene, carbon nanotubes (CNT), carbon nanofibers, and carbon black are the various types.

•Fullerenes

Example Fullerenes (C60) are carbon atoms that are bound together by sp2 hybridization. The shape is spherical. A spherical structure composed of 28 to 1500 carbon atoms has a diameter of 8.2 nm for single layer fullerenes and 4 to 36 nm for multi-layered fullerenes.

•Graphene

A carbon allotrope. It is a two-dimensional planar hexagonal honeycomb lattice network. The thickness is around 1nm.

•Carbon Nano Tube (CNT)

A honeycomb lattice graphene nanofoil is wound into a hollow cylinder to create nanotubes with diameters as small as 0.7nm in single layered CNTs and 100 nm in multilayered CNTs. The length varies from a few micrometers and many centimeters.

• Carbon Nanofiber

Carbon nanofiber is made from graphene nanofoil, which is coiled into a cone or cup shape.

•Carbon Black

It is an amorphous substance with spherical sizes ranging from 20 to 70 nm.

1.3: SYNTHESIS OF NANOPARTICLES

1. **Microwave synthesis** has been employed in the synthesis of nanoparticles as it combines the advantage of speed and homogeneous heating of the precursor materials. Microwave irradiation has a penetration characteristic, which makes it possible to homogeneously heat up the reaction solution.

2. **Sol–gel synthesis** is one of the simple, fastest and economically less expensive method, and has its own advantages like low processing temperature, homogeneity of the produced material and formation of the complex structures or composite materials.

3. The **hydrothermal method** is one of the most commonly employed techniques for synthesis of metal oxides, metals, and metal composites with different crystalline structures and morphologies, that are in the form of fine particles. The hydrothermal synthesis of nanoparticles involves hydrolysis of metal salt and condensation of metal hydroxide to produce ultrafine metal or metal oxide particles.

4. **Chemical vapour deposition** (CVD) is the technique in which substances that are in vapour phase are condensed to generate solid phase material. This method changes optical, electrical, and mechanical attributes as well as corrosion resistance of different substances.

5. **Spray pyrolysis** is a process in which a nanostructure is obtained when a solution containing a precursor is sprayed or injected using a nanoporous nebulizer onto the hot substrate in the furnace, leading to the decomposition of the precursor to form the final desired material on the substrate.

1.4: CHARACTERIZATION

• UV- Visible spectroscopy:

In UV-Visible spectroscopy, a beam of light with a range of wavelengths is passed through a sample, and the amount of light absorbed by the sample is measured. The amount of light absorbed by the sample is related to the energy required to excite electrons in the sample from one energy level to another. This energy is related to the wavelength of the light, and thus, the absorption spectrum of a sample provides information about the electronic structure of the molecules in the sample. The resulting UV-Visible absorption spectrum is a plot of the amount of light absorbed by the sample as a function of wavelength or frequency. The spectrum contains characteristic peaks or bands that correspond to the electronic transitions within the sample. These peaks or bands can be used to identify and quantify the concentration of the compounds in the sample.

It has unique optical properties that are sensitive to the size, shape, concentration, agglomeration state, and refractive index near the nanoparticle surface, which makes UV-Vis a valuable tool for identifying, characterizing, and studying nanomaterials. UV/Visible spectroscopy is a technique used to quantify the light that is absorbed and scattered by a sample.

• Infrared spectroscopy (IR):

Infrared spectroscopy (IR) is a technique used to identify and analyze chemical compounds based on the absorption or transmission of infrared radiation. IR spectroscopy is based on the principle that molecules absorb specific frequencies of infrared radiation, which correspond to the vibrational modes of the chemical bonds within the molecule. By measuring the frequencies of radiation that are absorbed or transmitted by a sample, IR spectroscopy can provide information about the functional groups and chemical bonds present in the sample. In an IR spectroscopy experiment, a sample is exposed to a range of infrared radiation, typically in the range of 4000-400 cm^{-1}. The sample is usually prepared as a thin film or a liquid and is placed in a sample holder that allows it to be exposed to the infrared radiation. The radiation that is transmitted through the sample is detected by a detector, and a spectrum of the sample is generated based on the intensity of the radiation that is absorbed or transmitted by the sample at different frequencies. The resulting IR spectrum is a plot of the intensity of the transmitted radiation as a function of the frequency of the radiation. The spectrum contains characteristic peaks or bands that correspond to the vibrational modes of the chemical bonds within the molecule. The positions and intensities of these peaks can be used to identify the functional groups and chemical bonds present in the sample.

IR spectroscopy provides highly discriminatory information due to the excitation of inherently specific fundamental vibrational transitions characteristic of molecular

species. Infrared spectroscopy is commonly used as a technique for the characterization of nanoparticles of diverse nature including metallic NPs and carbon nanomaterials, as well as core-shell and hybrid nanoparticles. A multitude of publications describe the use of IR techniques – mainly Fourier transform infrared spectroscopy (FTIR) – for evaluating the functional groups present within a colloidal suspension of NPs.

• Particle size analyzer:

Dynamic light scattering (DLS) is used to determine particle size. Particles suspended in a liquid are always moving at random, and the speed of this motion is determined by particle size: smaller particles move faster than larger particles. Light is dispersed by the sample in DLS, and the scattering is then detected and recorded many times. A comparison of those records reveals how far the particles have travelled in the time between them (and thus how rapidly they are travelling). The average particle size and size distribution can be estimated using this information.

Particle size distribution and morphology are the most important parameters of characterization of nanoparticles. Morphology and size are measured by electron microscopy. The major application of nanoparticles is in drug release and drug targeting. It has been found that particle size affects the drug release. Smaller particle offer large surface area. As a result, most of the drug loaded onto them will be exposed to the particle surface leading to faster drug release.

• Scanning Electron Microscopy:

The scanning electron microscope (SEM) is an imaging tool that uses a beam of high-energy electrons to scan a sample surface and produce high-resolution images of its topography, morphology, and composition. The SEM operates on the principle of interaction between a focused electron beam and the atoms of a sample, which

generates signals that can be detected and used to produce an image. The SEM consists of a high-voltage electron gun, which generates a beam of electrons, and a series of electromagnetic lenses, which focus the beam onto a small spot on the sample surface. The electron beam interacts with the sample surface, and several types of signals are generated as a result. The most commonly used signals in SEM imaging are secondary electrons (SE), backscattered electrons (BSE), and X-rays. When the primary electron beam hits the sample surface, some of the electrons in the sample are excited and ejected from the surface, generating SE. These SE are detected by a special detector and used to produce a high-resolution image of the sample surface topography. Since the SE are generated only from the surface of the sample, SEM imaging provides high-resolution images of the sample surface features.

Scanning electron microscopy (SEM) is giving morphological examination with direct visualization. The techniques based on electron microscopy offer several advantages in morphological and sizing analysis; however, they provide limited information about the size distribution and true population average. For SEM characterization, nanoparticles solution should be first converted into a dry powder, which is then mounted on a sample holder followed by coating with a conductive metal, such as gold, using a sputter coater. The sample is then scanned with a focused fine beam of electrons. The surface characteristics of the sample are obtained from the secondary electrons emitted from the sample surface.

• Transmission Electron Microscope:

TEM operates on different principle than SEM, yet it often brings same type of data. The sample preparation for TEM is complex and time consuming because of its requirement to be ultra-thin for the electron transmittance. The nanoparticles dispersion is deposited onto support grids or films. To make nanoparticles withstand

the instrument vacuum and facilitate handling, they are fixed using either a negative staining material, such as phosphotungstic acid or derivatives, uranyl acetate, etc, or by plastic embedding. Alternate method is to expose the sample to liquid nitrogen temperatures after embedding in vitreous ice. The surface characteristics of the sample are obtained when a beam of electrons is transmitted through an ultra-thin sample, interacting with the sample as it passes through.

• X-ray diffraction - XRD

It is a versatile technique used commonly in the field of nanotechnology to characterize and acquire accurate information regarding the composition, crystal structure, and crystalline grain size of nanoparticles. Nanoparticles characterization from XRD begins with the identification of the sample material's phase; the crystal type in the sample is determined through a search-match process, which is done in the regions where the peaks of high intensities are observed.

1.5: SILVER NANOPARTICLE

Bacteria, viruses, and fungus come into contact with living things. Because of their outstanding capabilities, silver nanoparticles are regarded as a forerunner in the fight against pathogenic microbial activities. Silver has significant areas of strength for slowing down their movement. The greater surface area of silver nanoparticles in comparison to the solid form of silver is responsible for their behaviour in this regard. As a result, biocidal activity increases and interaction with microorganisms improves. Silver nanoparticles are effective against a wide variety of Gram-negative and Gram-positive bacteria, including a few antibiotic-resistant species. Silver nanoparticles have been proven to be biocidal against Gram-negative bacteria such as Pseudomonas, Salmonella, Acinetobacter, Escherichia, and others. Silver

nanoparticles were also found to be viable against Gram-positive microscopic organisms such as Bacillus, Enterococcus, Listeria, Staphylococcus, and Streptococcus. Recent research have demonstrated that silver nanoparticles and medicines such as penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin work synergistically to attack Escherichia coli and Staphylococcus aureus. Silver nanoparticles have been found to limit virus reproduction, making them a potent antiviral weapon. Their efficiency has been established even against the HIV-1 virus and the influenza virus.

The shape and size of nanoparticles are absolutely necessary for the effectiveness of processes that lead to the destruction of viruses. Additionally, certain fungi are not indifferent to silver nanoparticles. Aspergillus, Candida, and Saccharomyces are just a few examples of the fungi they have been shown to kill quickly and effectively.

Like a coin has two sides, it has some negative effects also. The number of products that contain nanoparticles, particularly metallic silver, has significantly increased in recent years. Skin, respiratory, and digestive systems are all channels through which nanoparticles can enter the human body. Silver nanoparticles typically build up in a variety of organs, particularly the lungs, kidneys, and liver. The liver's presence of silver nanoparticles may pose a particular threat. Silver nanoparticles' alleged accumulation in the lungs may also result in negative effects that are likely to manifest in the future.

There is no doubt that additional research on the toxicity of silver nanoparticles to living things is required. Due to the incompletion of developed conclusions, complete scientific information is not yet available. In order to determine the actual amount of silver nanoparticles entering the environment, it is necessary to carry out the entire research cycle.

1.6: APPLICATIONS OF SILVER NANOPARTICLES

•Antibacterial Activity of Silver Nanoparticle

Silver Nanoparticles have shown profoundly antibacterial activity against various Gram-positive and Gram-negative microbes. In any case, the specific system by which they apply inhibitory development or bactericidal action has not been completely clarified at this point. Different mechanisms that take into account Silver Nanoparticle physicochemical properties, such as size and surface, which enable them to interact or even pass through cell walls or membranes and directly affect intracellular components are supported by the existing experimental evidence.

The first postulate that Silver Nanoparticles act at the membrane level because they can penetrate the outer membrane and accumulate in the inner membrane, where the adhesion of the nanoparticles to the cell causes them to destabilize and damage the cell, increasing membrane permeability, causing leakage of cellular contents, and ultimately the cell's death. Silver Nanoparticles have also been shown to be able to interact with sulfur-containing proteins in bacteria's cell walls, potentially causing structural damage and cell wall rupture.

The subsequent instrument suggests that nanoparticles not exclusively can break and cross the cell film, changing its construction and porousness yet can likewise enter the cell where it. That's what has been proposed, because of its properties, Silver Nanoparticles will have a fondness to associate with sulfur or phosphorus gatherings, present in intracellular substance like DNA and proteins adjusting their design and works. By interacting with thiol groups in the enzymes that induce free radicals and reactive oxygen species, causing damage to intracellular machinery, and activating the apoptosis pathway, they may also alter the respiratory chain in the inner membrane. A third component that is proposed to happen in lined up with the two

others is the arrival of silver particles from the nanoparticles, which due to their size and charge, can collaborate with cell parts modifying metabolic pathways, layers, and, surprisingly, hereditary material.

•Antifungal activity of Silver Nanoparticles

Immunosuppressed patients are more likely to get fungal infections, and there are only a few antifungal medications on the market today, making it difficult to treat fungi-mediated diseases . As a result, it is necessary and urgent to develop biocompatible, non-toxic, and environmentally friendly antifungal agents. At this point Silver Nanoparticle play a crucial role as anti-fungal agents against a variety of fungi-caused diseases. Clinical isolates and ATCC strains of Trichophyton mentagrophytes and Candida species were effectively inhibited by nano-Ag.

By inhibiting conidial germination, the biologically synthesized Silver Nanoparticle demonstrated potent antifungal activity against Bipolaris sorokiniana. It is interesting to note that in addition to inhibiting pathogenic fungi that are harmful to humans and plants, Silver Nanoparticle also inhibit indoor fungal species like Penicillium brevicompactum, Aspergillus fumigatus, Cladosporium cladosporoides, Chaetomium globosum, Stachybotrys chartarum, and Mortierella alpina.

•Sensoring activity of Silver Nanoparticle

The colorimetric detecting property of Silver Nanoparticle can recognize the weighty metal particles Ni, Co, and Hg (II) and sulfide anions. Silver nanoplates shaped like triangles exhibit a lightning rod effect and higher anisotropy. They have been used in plasmon sensors to detect Hg2+ ions in the solution because of this; the ordinary blue shift apparently is expanding the centralization of the Hg2+ particles. Ag NPs doped with tris(4,7-diphenyl-1,10-phenanthroline) ruthenium (II) dichloride complex (Ru(dpp)3Cl2) epitomised in plasticized polymethyl methacrylate

(PMMA) has been utilised to set up the film, which goes about as ratiometric sensors for the estimation of disintegrated oxygen in the watery arrangements. The normal herbicide atrazine (Atz) has been distinguished through an electrochemical sensor created by Ag NPs. SERS sensors, which were created through the in situ growth of Ag nanoparticles on polydopamine (PDA) templated filter papers (FPs), are extremely adaptable and have the ability to quickly collect and detect malachite green residues.

•Water Treatment activity of Silver Nanoparticle

The bactericidal film produced for water sanitization by impregnating 1 mg/L of biosynthesized Silver Nanoparticles on nitrocellulose layer channels showed the total restraint of the microbial local area of E. coli, Enterococcus faecalis, Pseudomona aeruginosa, and S. aureus suspensions, and the inactivation and evacuation of E. coli and S. aureus arrived at up to 6 and 5.2 significant degrees. Silver Nanoparticles, alongside zwitterionic sulfobetaine methacrylate (SBMA) joined on the polyimide (PI) film, worked on the antifouling and antimicrobial properties of the layer. The Silver Nanoparticles can be handily eliminated from the dots and repress organisms' development in a genuine water test. Multifunctional nanocomposites made out of multiwalled carbon nanotubes (MWCNTs) with installed iron oxide and silver nanoparticles have intense antimicrobial effectiveness. In water treatment, the polyacrylonitrile (PAN) sorbent stores pathogenic microorganisms on its surface. On the other hand, surface biofilm formation was not observed when treated with Ag NPs. Water contaminated by bacteria could be effectively treated in an emergency by passing through silver nanoparticle-enhanced paper.

CHAPTER - 2

MATERIALS AND METHODS

2.1. Materials

Biophytum sensitivum (leaves):-25g

Distilled water:-500 ml

Wattman no:1 filter paper :-1

Silver nitrate:- 4.259g

2.2. Preparation of Biophytum sensitive leaf extract

The leaves of the biophytum sensitivum were collected and properly cleaned with tap water, followed by distilled water, to remove dust. The leaves were then dried and sliced into little pieces before being placed in a 500 ml RB flask and boiled for 20 minutes with 200 ml distilled water. Following the boiling. The solution was cooled to room temperature before being filtered using Whatman paper and maintained at room temperature.

2.3. Preparation of 1millimolar silver nitrate solution.

Take 0.084g of silver nitrate and place it in a 500 ml standard flask. Shake thoroughly before adding the solution to the burette. Pour 90 ml of the burette solution into a beaker. Use a 10 ml pipette to transfer the 10 ml leaf extract solution into the beaker. Silver nitrate solution and leaf extract solution should be thoroughly combined.

2.4. Synthesis of silver nanoparticles (microwave method)

Warm up the microwave. 800 watts of medium-high power and a 5-minute timer were selected as the settings. The solution is placed in a beaker, covered with a watch glass, and microwaved. After 5 minutes, remove the beaker and let it aside to cool.

2.5. Characterization of Silver nanoparticles

Characterized the as-synthesized nanoparticles using UV-Visible spectroscopy, FTIR spectroscopy, Scanning electron microscopy and particle size analyser.

2.6. Antibacterial Study of Silver Nanoparticles

Bacteria Used

1. Staphylococcus sp. - Gram positive- bacteria
2. Pseudomonas - Gram negative -bacteria
3. Bacillus sp. - Gram positive -bacteria
4. Escherichia coli - Gram negative- bacteria

Procedure

Prepare some paper discs to coat the metal on. Autoclave sterilize all instruments used in the experiment with aluminum foil after covering them. Cultures and bacterial coatings were prepared in a laminar flow chamber. Sterilized instruments and other substances are only opened in a laminar flow chamber. Place it in the laminar flow chamber for a few minutes, then wash your hands with a small amount of alcohol.

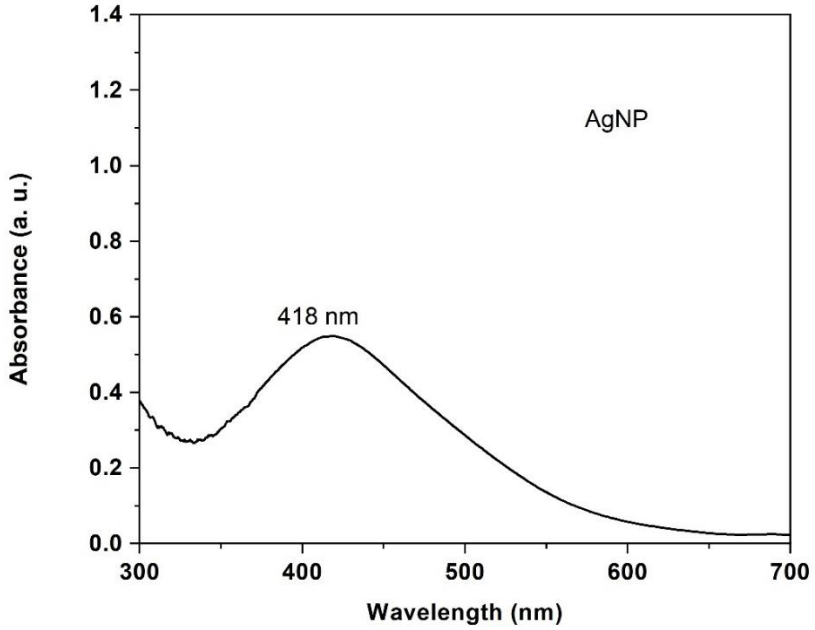
Open the sterilized material by opening the chamber slightly and placing the handle into the chamber. Pour the nutrient agar into the petri dish and wait a few minutes

for the liquid agar to gel. After the agar has set, spread the bacteria onto each Petri dish by gently tapping with a cotton swab. Between uses, the swab should be sterilized by heating it with an alcohol lamp. Bacteria slowly coat the agar plate without breaking the nutrient agar. Place a paper disc soaked with silver nanoparticles on the culture area and store safely in the incubator for 24 hours. Observe the bacterial culture in the laminar flow chamber after 24 hours. Observe the antimicrobial activity exhibited and measure the radius of the halozone formed.

CHAPTER - 3

RESULTS AND DISCUSSION

3.1. UV-Vis Spectroscopy



The most practical tool for confirming the creation of silver nanoparticles is UV-Visible spectroscopy. It is based on an optical phenomenon known as Surface Plasmon Resonance (SPR). Silver nanoparticles have distinct optical absorption spectra in the UV-Visible range. According to the literature, the optical absorption peak of nanoparticles ranges from 400 to 470 nm, depending on their size, structure, stability, and aggregation. The sample's absorption peak is a single, strong band visible at 418 nm. It is a confirmation of silver nanoparticles, suggesting that they are isotopic and uniform in size.

3.2. FTIR Spectroscopy

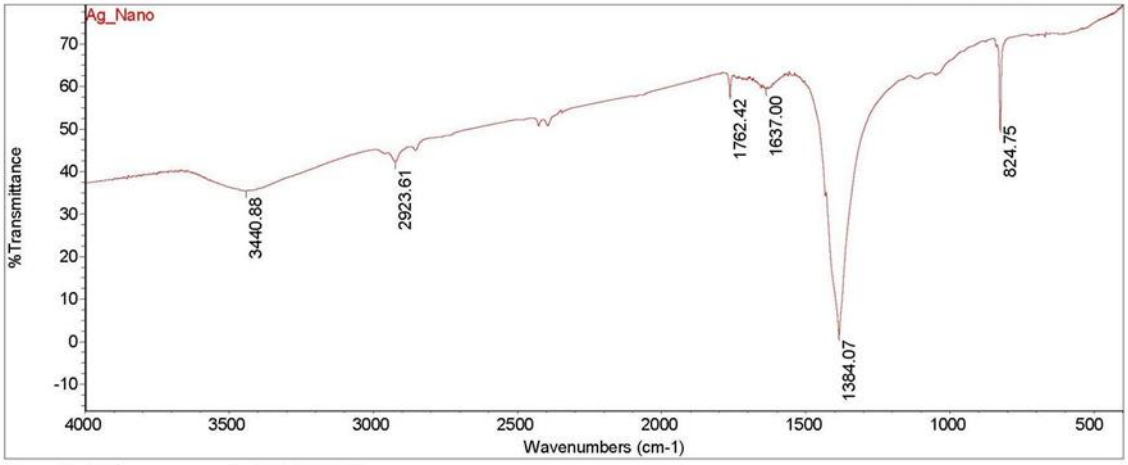
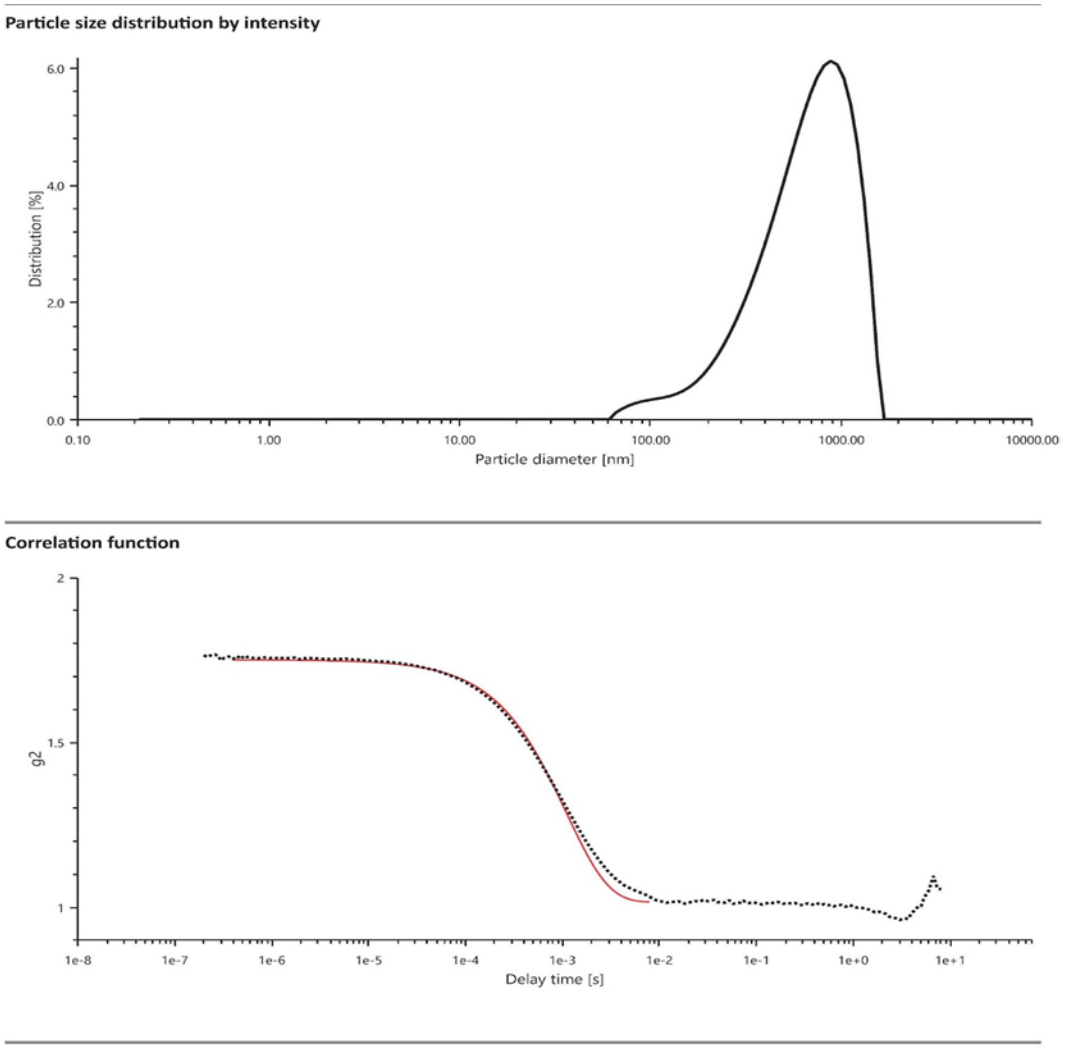


Figure depicts the FTIR spectrum of the prepared sample. The band at 3440.88 cm-1 was found due to water, phenol, and alcohol O-H stretching vibrations. At 2923.61 cm-1, the distinctive peak of aliphatic C-H stretching vibrations is observed. Carbonyl stretching vibrations of acid are responsible for the band at 1762.42 cm1. C-C and C-N stretching may be seen in the band recorded at 1637 cm-1. O-H bending vibrations cause the largest peak at 1384.07 cm-1. Furthermore, the signal at 824.75 cm-1 is caused by C-H stretching in alkenes or aromatic rings.

The FTIR spectrum of our sample shows that the soluble elements present in Biophytum Sensitivum extract contain a higher percentage of functional bio molecules such as hydroxyl, carboxylic, phenol, and amine groups, which are involved in the reduction of silver nitrate into silver nanoparticles and contribute to nanoparticle stabilization.

3.3. Particle Size Analyzer

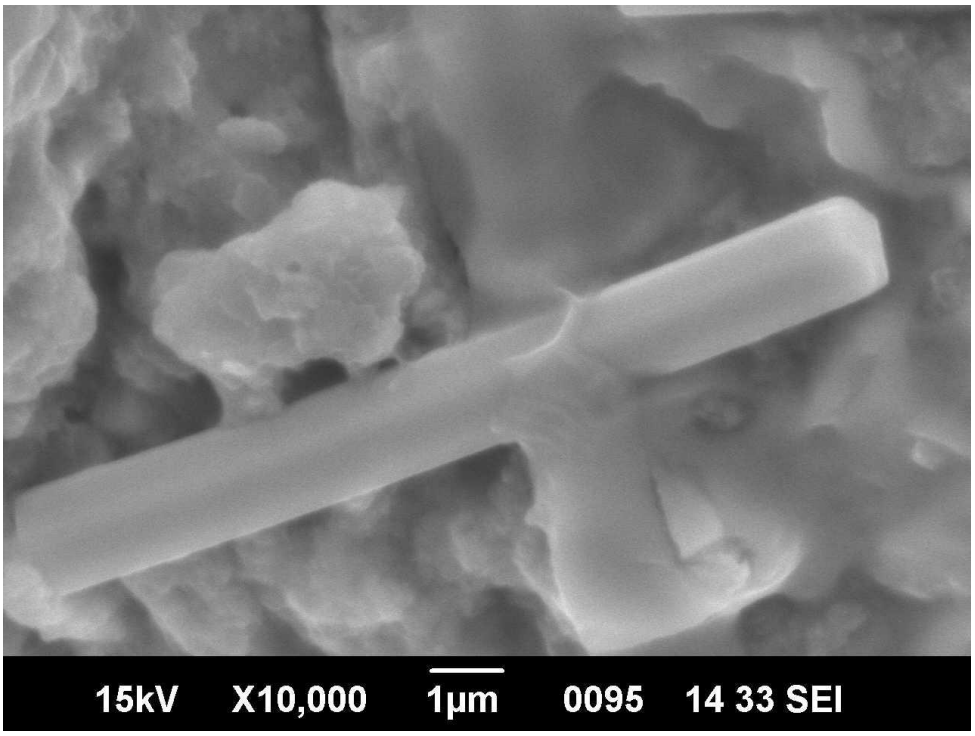
The particle size of the synthesized silver nanoparticles is determined using the particle size analyzer depicted in the figure.



The particle size is found to be 715.6 nm which is actually the size of the solvated nanoparticle.

Particle size distribution peaks (intensity)			
	Size [nm]	Area [%]	Standard deviation [nm]
Peak 1	715.6	100.00	568.2
Peak 2	-	-	-
Peak 3	-	-	-

3.4. Scanning Electron Microscopy (SEM)



The microstructure of the silver nanoparticles is observed from SEM images. The silver nanorods are clearly seen in the image.

3.5. Antibacterial Study

a) Antibacterial activity against Staphylococcus sp.

Staphylococcus is a genus of **Gram-positive bacteria**. Staphylococcus includes at least 43 species. Of these, nine have two subspecies. Some of which cause suppurative disease processes in animals and humans.

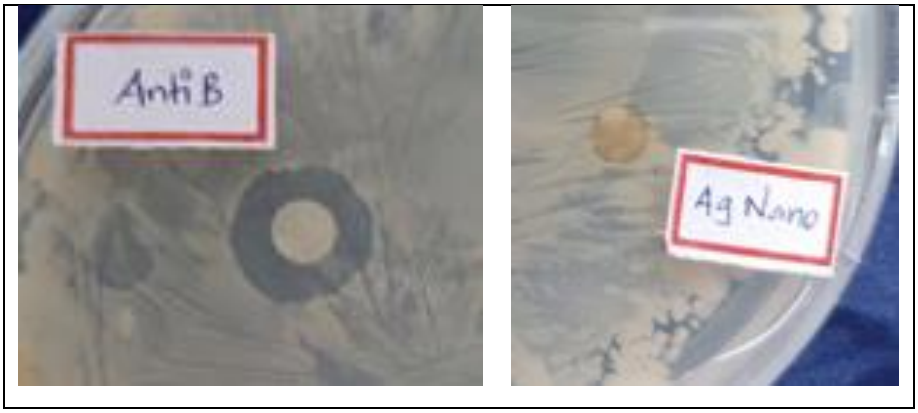


Plate description:

Antibiotic used – Azithromycin – 14mm
AgNano- 7 mm
Mm represents the zone of inhibition

b) Antibacterial activity against Pseudomonas sp.

Pseudomonas is a **gram-negative type** of bacteria (germ) that is found commonly in the environment, like in soil and in water.

Plate description:

Antibiotic used – Azithromycin – 7 mm
AgNano- 9 mm
Mm represents the zone of inhibition

c) Antibacterial activity against Bacillus sp.

Bacillus sp. is a **gram-positive**, soil-dwelling bacterium, the most commonly used biological pesticide worldwide.

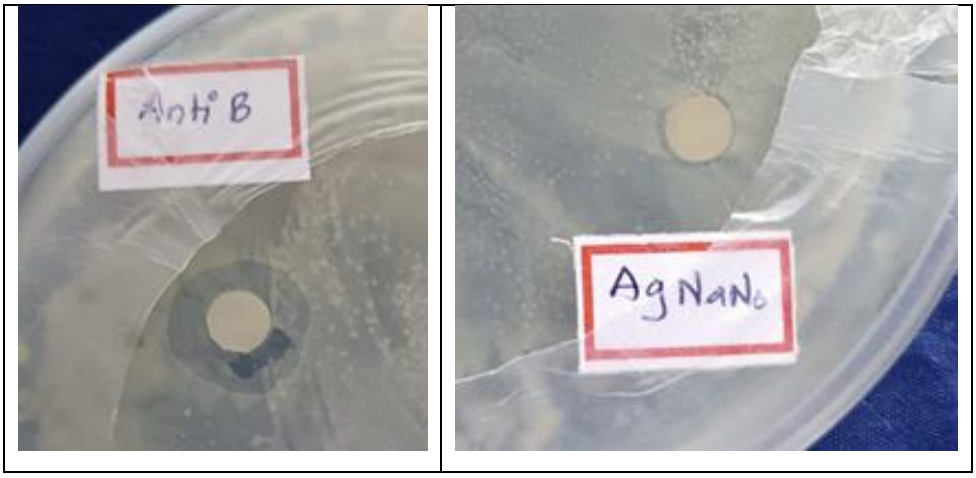


Plate description:
Antibiotic used – Azithromycin – 13 mm
AgNano- 9 mm
Mm represents the zone of inhibition

d) Antibacterial activity against Escherichia coli

Escherichia coli, also known as E. coli, is a **Gram-negative**, facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms.

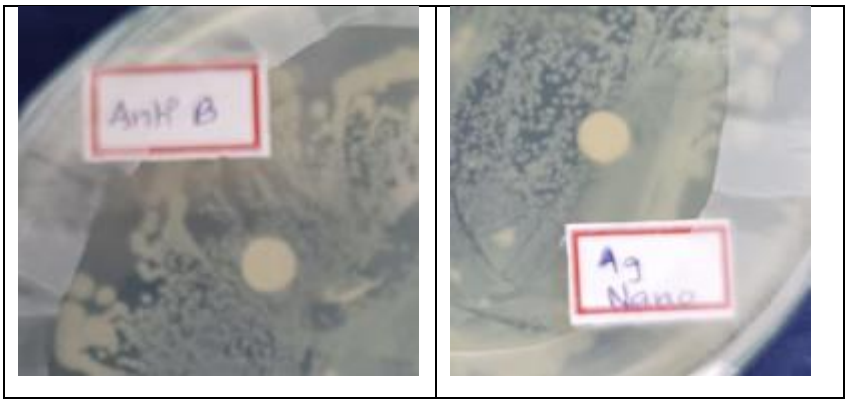


Plate description:
Antibiotic used – Azithromycin – no activity
AgNano- no activity
Mm represents the zone of inhibition

The antibacterial activity of silver nanoparticles (AgNPs) against Staphylococcus sp., Pseudomonas sp., Bacillus sp., and Escherichia coli was tested and compared with azithromycin as an antibiotic.

Sl. No.	Bacteria	gram-positive/ Gram-negative	Antibiotic used	Zone of inhibition
1	Staphylococcus sp.	Gram-positive	Azithromycin – 14 mm	7mm
2	Pseudomonas sp.	Gram-negative	Azithromycin – 7 mm	9 mm
3	Bacillus sp.	Gram-positive	Azithromycin – 13 mm	9 mm
4	Escherichia coli	Gram-negative	Azithromycin -0 mm	no activity

Comparing the results of the antibacterial study using silver nanoparticles against the tested bacteria with the base antibiotic azithromycin, we have:

Staphylococcus sp. (Gram-positive): The zone of inhibition for silver nanoparticles is 7mm, which is lower than that of the base antibiotic azithromycin

(14mm). Pseudomonas sp. (Gram-negative): The zone of inhibition for silver nanoparticles is 9mm, which is higher than that of the base antibiotic azithromycin (7mm). Bacillus sp. (Gram-positive): The zone of inhibition for silver nanoparticles is 9mm, which is lower than that of the base antibiotic azithromycin (13mm). Escherichia coli (Gram-negative): The silver nanoparticles did not show any activity against Escherichia coli, while the base antibiotic azithromycin also did not show any activity.

Overall, it can be seen that the antibacterial activity of silver nanoparticles against the tested bacteria is comparable to that of azithromycin, except for Staphylococcus sp. and Bacillus sp. where the base antibiotic performed better. The results suggest that silver nanoparticles may have potential as an antibacterial agent against the tested bacteria, as they showed a zone of inhibition of 7 mm against Staphylococcus sp. and 9 mm against Pseudomonas sp. and Bacillus sp., which is comparable to that of azithromycin. However, azithromycin and silver nanoparticle did not show any activity against Escherichia coli, which is a gram-negative bacteria. Silver nanoparticles have a 7 mm zone of inhibition against Staphylococcus sp., and therefore have potential as antibacterial agents against the tested bacteria. 9 mm for Pseudomonas species and Bacillus species comparable to azithromycin. However, azithromycin and silver nanoparticles showed no activity against the Gram-negative bacterium Escherichia coli.

CHAPTER - 4
CONCLUSIONS

Using Biophytum Sensitivum leaf extract and microwave assisted synthesis, silver nanoparticles were effectively bio synthesized from silver nitrate. The presence of silver nanoparticles was confirmed by the UV-visible absorption spectrum. The absorption peak at 418 nm proved the presence of SPR in the sample. The existence of biofunctional groups such as carboxyl, phenolic, and alcoholic groups was found by FTIR spectroscopic analyses, which aid in the reduction of silver nitrate solution into silver nanoparticles and the stabilization of the nanoparticles. We learned from the particle size analyzer that the average particle size is around 715.6 nm, which is the size of the solvated nanoprticles. The SEM investigation yielded the rod-like microstructure. The antibacterial activity of silver nanoparticles against Staphylococcus sp., Pseudomonas sp. and Bacillus sp. is comparable to that of azithromycin.

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