

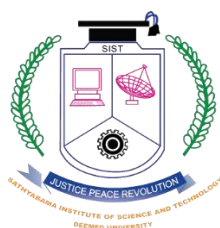
**PERENNIAL COGON GRASS EXTRACTS FOR GREEN-SYNTHESIS OF SILVER
NANOPARTICLES: PHYTOCHEMICAL ANALYSIS, COMPOUND
CHARACTERIZATION ANTIOXIDANT & ANTI-MICROBIAL STUDIES**

Submitted in Partial Fulfillment of the requirement for the award of a Bachelor 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 **SIVA SHANKARAN V (39230044), VINOTH KUMAR V (39230057)**, who carried out the project entitled **"PERENNIAL COGON GRASS (*IMPERATA CYLINDRICA*) EXTRACTS FOR GREEN-SYNTHESIS OF SILVER NANOPARTICLES: PHYTOCHEMICALS ANALYSIS, COMPOUND CHARACTERIZATION ANTIOXIDANT & ANTI-MICROBIAL STUDIES"** under our supervision from September 2022 to April 2023.

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To whomsoever it may concern

This is to certify that the dissertation entitled “Perennial Cogon Grass (*Imperata cylindrica*) extracts for green-synthesis of silver nanoparticles: Phytochemical analysis, compound characterization, antioxidant, & anti-microbial studies” submitted to ‘Sathyabama Institute of Science and Technology (Deemed to be University), Chennai’ for the partial fulfilment of *Bachelor of Technology in Biotechnology* is an authentic record of the work carried out by **Mr. Siva Shankaran. V. (39230044)** under my supervision and guidance at the Department of Biotechnology, Indian Institute of Technology Madras, Chennai during the period from December 2022 to May 2023.

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ABSTRACT

Traditional medicine has been recently used as a fallback option for treating human disorders, confirming the link between increased microbial resistance to modern medications and the need to verify the antibacterial effectiveness of therapeutic herbs. *Imperata cylindrica*, a type of rhizomatous grass endemic to tropical and subtropical Asia, Micronesia, Melanesia, Australia, Africa, and southern Europe, is one such plant popularly known as "Cogon grass." As a hepatoprotective agent, cogon grass is useful for nephritis, fever, hypertension, dyspnea, and epitaxy. It also lowers cholesterol and blood sugar levels. Due to this, the purpose of the current study was to examine the various assay solvents and the antibacterial, antioxidant, and phytochemical components of *Imperata cylindrica*.

Keywords: Antibacterial activity, Antioxidant activity, Phytochemicals, Solvents.

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LIST OF ABBREVIATIONS

I.cylindrica – *Imperata cylindrica*

AgNPs – *Silver Nanoparticles*

GC-MS – Gas Chromatography-Mass Spectrometry

FTIR -Fourier-Transform Infrared Spectroscopy

OD – Optical Density

UV – Ultra Violet

CHAPTER – 1

INTRODUCTION

1.1 MEDICINE PLANTS

India has a long history of learning about using plant-based medicines for both therapeutic and preventative purposes. India is ideally suited for the creation of pharmaceuticals from medicinal plants. The Indian subcontinent is ideal for growing a variety of medicinal and aromatic plants that may be utilized as novel raw materials for the pharmaceutical, fragrance, cosmetics, flavour, food, and agrochemical sectors due to its vast range of soil types and different climatic conditions (Saranraj & Sivasakthi, 2014). Medicinal plants are considered clinically effective and safer alternatives to synthetic antibiotics. The current scenario is to isolate the active constituents present in the plant material to develop medicinally drugs which are having rare chances of adverse effects ((Mahato & Sharma, 2018)). Natural products and their derivatives have historically been exploited as a valuable source of novel therapeutic agents ((*Extraction Techniques of Medicinal Plants*, n.d.)). WHO report depicts that plant-based products or their derivatives account for nearly 28% of drugs available in the market ((Mahato & Sharma, 2018)). Phytochemicals offer a unique platform for structural diversity and biological functionality which is indispensable for drug discovery. In particular, the emergence of resistance to antibiotics has hampered the pace by which newer antibiotics are being introduced into the public domain ((Deshmukh Krishi Vidyapeeth et al., 2017)). Phytochemicals play a critical role in diversity-oriented synthesis (DOS) of natural products like pharma compounds ((Soni & Sosa, 2013)).

1.2 NANOTECHNOLOGY

The National Nanotechnology Initiative has defined nanotechnology as “working at the atomic, molecular and supramolecular levels, in the length scale of approximately 1–100 nm range in order to understand and create materials, devices and systems with fundamentally new properties and functions because of their small structure”. It is also defined as an exciting and rapidly emerging technology to manipulate and create tools, materials and structures at the molecular level, often atom by atom into functional structures having nanometre dimensions. There are two different methods to synthesise nanoparticles they are top-down and bottom-up methods. The top-down

approach comes under the Physical process namely includes ball milling, thermal evaporation, spray pyrolysis, microwave-assisted, hydrothermal, electrochemical, laser ablation, and pulsed wire discharge. Bottom-up includes chemical and biological processes. The chemical process involves electrodeposition, electrodeposition, chemical solution deposition, chemical vapour deposition, sol-gel process, catalytic route, co preparation and wet chemical method. In the Biological process synthesis of metallic nanoparticles done through the bioreduction method, the reduction reaction is achieved through biological agents such as plants, Bacteria, fungi, Yeast, and Algae.

1.3 NEED FOR GREEN SYNTHESIS

The need for the biosynthesis of nanoparticles rose as the physical and chemical processes were costly and non-eco-friendly. Often, the chemical synthesis method leads to the presence of some of the toxic chemicals absorbed on the surface that may have adverse effects in medical applications ((Fairuzi et al., 2018)). This is not an issue when it comes to biosynthesized nanoparticles via the green synthesis route ((Fairuzi et al., 2018)). So, in the search of cheaper pathways for nanoparticle synthesis, scientists used microbial enzymes and plant extracts (phytochemicals). With their reducing properties they are usually responsible for the reduction of metal compounds into their respective nanoparticles. Green synthesis provides advancement over chemical and physical methods as it is cost-effective, environment-friendly, and easily scaled up for large-scale synthesis and in this method, there is no need to use high pressure, energy, temperature and toxic chemicals.

1.4 SILVER NANOPARTICLES

One of the substances used in nano formulation is silver (Silver nanoparticles). Due to its antimicrobial properties, silver has also been incorporated into filters to purify drinking water and clean swimming pool water. To generate nano silver, metallic silver has been engineered into ultrafine particles by several methods; including spark discharging, electrochemical reduction, solution irradiation and cryo chemical synthesis. In addition, nanostructures can be produced as tubes, wires, films etc. At the nano-scale, the silver particles exhibit deviating physicochemical properties (like Ph-dependent partitioning to solid and dissolved particulate matters) and biological activities compared with the regular metal. This is due to the higher surface area per

mass, allowing a larger number of atoms to interact with their surroundings. Due to the properties of silver at the nanoscale, nano silver is nowadays used in an increasing number of consumer and medical products. Because, silver is a soft white lustrous element, an important use of silver nanoparticles is to give a product a silver finish. Still, the remarkably strong antimicrobial activity is the major direction for the development of nano silver products. Examples are food packaging materials and food supplements, odour-resistant textiles, electronics, household appliances, cosmetics and medical advices, water disinfectants and room sprays.

1.5 IMPERATA CYLINDRICA

The perennial rhizomatous grass *Imperata cylindrica*, sometimes known as cogon grass, is a native of tropical and subtropical Asia, Micronesia, Melanesia, Australia, Africa, and Southern Europe. The flowers, roots and leaves have antibacterial, diuretic, skin softening (emollient), antipyretic (febrifuge), salivating (sialagogue), anticoagulant (styptic), urine (haematuria), blood in the vomiting (haematemesis), and nosebleed (epistaxis), Jaundice and dropsy (oedema) ((Rusdy, 2020)) antifebrile, antihelmintic, antidiabetic, and antidiarrheal properties, ((Brook, 1989)). The rhizome-root portion is used in traditional medicine across Southeast Asian civilizations for the management of many illnesses. Experimental evidence suggests that the leaves have vasodilatory and neuroprotective properties. It is employed as an anti-inflammatory and diuretic in Chinese medicine ((Abubakar & Haque, 2020a)). It is also a significant medication in folk medicine The medication from *Imperata cylindrica* is for the treatment of gout, diabetes, heart conditions, urinary stones, and urine retention. *Imperata cylindrica* is said to have an antimicrobial effect on various varieties of bacteria.

Table 1.1: SCIENTIFIC CLASSIFICATION

Taxonomic Hierarchy	
Kingdom	Plantae
Sub kingdom	Viridiplantae
Super division	Embryophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Super order	Liliana
Order	Poales
Family	Poaceae
Genus	Imperata
Species	cylindrica (L.) Raeusch
Botanical Name	<i>Imperata cylindrica</i>

CHAPTER – 2

LITERATURE SURVEY

2.1 INTRODUCTION

Phytochemistry, often known as plant chemistry, has emerged as a separate field of study in recent years and is near to both plant biology and natural product organic chemistry. It addresses the vast array of organic compounds that plants produce and collect, as well as their chemical compositions, biological synthesis, turnover, and metabolism, as well as their natural distribution and biological functions. The principal phytochemical elements reported in *I. cylindrica* include saponins, glycosides, coumarins, flavonoids, and phenols. A total of 72 compounds have been isolated and identified from the plants ((Soni & Sosa, 2013; Yadav & Agarwala, 2011)). Modern pharmacology research on *I. cylindrica* has revealed that numerous of its compounds demonstrate a variety of biological functions, including haemostasis, improved urination, anti-inflammatory, antibacterial, anticancer, and immune system stimulation (). In Methanol solvents, the phytochemicals saponins, flavonoids, phenol, coumarin and Tannins are present through the chemical analysis method. The maximum number of flavonoids were found in the chloroform extract, which had a value of 30.88 mg QE/g dry wt., followed by petroleum ether extract, which had a value of 22.05 mg QE/g dry wt., and methanol extract, which had the lowest value at 7.35 mg QE/g dry wt.((Deshmukh Krishi Vidyapeeth et al., 2017)).

2.2 SYNTHESIS OF SILVER NANOPARTICLES

Silver nanoparticles were synthesized from *Imperata cylindrica* by adding 10ml of the aqueous extract into 90ml of 5mM aqueous silver nitrate, the mixture was heated using a water bath at 60°C for 10min ((Fairuzi et al., 2018)). The silver nanoparticles of *I. cylindrica* leaves extract were synthesized by adding 10 ml of the aqueous leaf extract into 90 ml of 5 mM aqueous AgNO₃. The mixture was stirred for 5 min at 150 rpm before being heated using a water bath at 60°C for 10 min ((Fairuzi et al., 2018)).

Three different conditions were employed for the synthesis of silver nanoparticles using aqueous and methanolic extract of *Imperata cylindrica* with 1 mM silver nitrate (AgNO₃). In the first condition, 10 mL of aqueous extract was mixed with 90 mL of 1 mM aqueous AgNO₃ solution at room temperature. Similarly, 10 mL of methanol extract was treated with 90 mL of 1 mM AgNO₃ solution at room temperature. The second condition involved boiling of 10 mL of extract at 60°C and adding 90 mL of 1 mM AgNO₃ at room temperature. 10 mL of extract and 90mL of 1mM AgNO₃ were boiled at 60°C in the third condition.

2.2.1 ANTIBACTERIAL ACTIVITY

Imperata cylindrica solvent extracts were tested for antibacterial activity in vitro using Kirby-Bauer disc diffusion methods ((Mahato & Sharma, 2018)). For microorganisms that grow quickly, the disc diffusion method is quite successful, and the activities of the test complexes are expressed by measuring the diameter of the zone of inhibition. The extracts were dissolved in 1% dimethyl sulfoxide (DMSO) and kept dry at room temperature. In nutrient agar medium, both Gram-positive (*S. aureus*, *B. cereus*, and *B. subtilis*) and Gram-negative (*P. aeruginosa* and *P. vulgaris*) bacteria were cultivated before being incubated at 37°C for 48 hours. Using a sterilized glass spreader, bacterial culture was evenly distributed over fresh culture discs after being infected with a loop-full of the culture. Each sterile disc received a different concentration of solvent extract, such as 2g/l, 4g/l, 6g/l, 8g/l, and 10g/l. A reference standard, Norflaxin (20g/l), was also applied using a sterile micropipette. The plates were then left to grow bacteria for 24-48 hours at 35°C. After the duration of incubation, the inhibition was measured by determining the diameter of the inhibitory zone ((Nascimento et al., 2000)). The findings showed that, when compared to extracts from other solvents, including water, chloroform, ethanol, and petroleum ether, methanol alone shown potential action. Only *B. cereus* was the target of the ethanol extract's action, and the concentration that caused the greatest inhibition was 10 g/l (7.2 mm). While *S. aureus*, *B. cereus*, *P. aeruginosa*, and *P. vulgaris* were all susceptible to the methanol extract. The concentration of 10 g/l showed the maximum zone of inhibition. Aerial part of the aqueous and ethanol extract of *Imperata cylindrica* has antibacterial activity against *Staphylococcus aureus* and *E.coli*. In their study, *E. coli* and *S. aureus* were used as test microorganisms. Based from their study the antibacterial substances were observed to be more soluble in water than in alcohol.

2.2.2 ANTIOXIDANT ACTIVITY:

Antioxidants effectively treat a variety of human diseases, including as cancer, cardiovascular disease, and inflammatory disorders, by reducing the oxidative stress in cells. Antioxidants capacity to alleviate oxidative stress by neutralizing or scavenging reactive species by hydrogen donation is the cause of this action ((Veeru et al., 2009)). The primary cause of phenolics' antioxidant activity is their redox characteristics, which enable them to function as reducing agents and hydrogen donors. Particularly abundant in leaves, floral tissues, and woody portions like stems and bark are phenolic compounds. In the nutraceutical, pharmaceutical, and food sectors, the oxygen radical absorbance capacity (ORAC) test has gained widespread acceptance as a standard method for assessing antioxidant activity ((Sultana et al., 2009)). ORAC test was used to assess the antioxidant capacity of *I. cylindrica* methanol extract. In a 96-well plate, 25 L of the Trolox (Standard) and methanol extract of *I. cylindrica* were placed in separate wells at varied concentrations (20, 40, 60, 80, and 100 g/l), and then 150 L of the fluorescein solution was added to each well. After carefully combining the contents of each well, the plate was incubated for 30 min. at 37°C. Then, 25 L of the AAPH solution was poured into each well to start the production of free radicals. Pipetting was used to properly combine the reaction mixture and achieve homogeneity ((Moon & Shibamoto, 2009)). The fluorescent microplate reader was used to read the standard and sample wells right away at 37 °C with an excitation computed using an emission wavelength of 520 nm. and a wavelength of 480 nm. According to the findings, methanol extract has more antioxidant capacity than regular (Trolox). The standard's IC₅₀ value was 69.369 whereas the methanol extracts were 72.754. In research on natural antioxidants, the DPPH test has gained considerable popularity. One of the causes is that this technique is straightforward and extremely sensitive. The idea behind this test is that an antioxidant is a hydrogen donor. The reduction of DPPH in test samples reflects the antioxidant action. There have been reported many techniques for measuring the concentration of DPPH in the antioxidant test system: Plant powders and electron spin resonance spectroscopy (ESR). The antioxidant activity of this plant, as is demonstrated by our current analysis, may account for its therapeutic effects. However, further research is required to determine its value in the prevention, diagnosis, and treatment of a variety of disorders.

CHAPTER – 3

AIM AND OBJECTIVES

3.1 AIM

The aim of this study was to carry out “Perennial Cogon Grass (*Imperata cylindrica*) Extracts for green-synthesis of silver nanoparticles: Phytochemical analysis, compound characterization & anti-microbial studies”.

3.2 OBJECTIVES

- ❖ To Leaves and stem of *Imperata cylindrica*.
- ❖ To Extract the *Imperata cylindrica* Plant Extract using the Maceration method.
- ❖ Qualitative analysis of Phytochemical Screening by the Harborne method.
- ❖ To Characterize the Phytochemical Using UV- Vis Spectroscopy & GCMS.
- ❖ To Evaluate the Antibacterial Activity Using the Disc Diffusion method.
- ❖ To Synthesis the silver Nanoparticles in Aqueous Extract and 1Mm AgNO₃ at 70C.
- ❖ To Characterize the Silver Nanoparticles Using UV-Visible Spectrophotometer, Fourier Transform Infrared Spectroscopy, X-ray Powder Diffraction, and Scanning Electron Microscope.
- ❖ To Evaluate the Antioxidant Activity Using Hydroxy Radical Scavenging Activity and Reducing power methods.
- ❖ To Evaluate the Antibacterial Activity Using Disc Diffusion method and Minimum Inhibitory concentration.

CHAPTER-4

MATERIALS AND METHODS

4.1 COLLECTION OF PLANT MATERIALS

Perennial Cogon *Imperata cylindrica* was collected from Navalur, Chennai in the month of September. The leaves and stems were washed thoroughly 2-3 times with running water and the final wash with drinking water. Fresh plant material was shade-dried for 10 days and then powdered using a mortar and pestle. The powder was kept in an airtight container at ambient temperature for future use.

4.2 EXTRACT USING THE MACERATION METHOD

25 g of plant material was mixed with 200 ml of distilled water, Methanol and hexane individually in each flask and kept in the shaking incubator at 24 °C at 120 rpm for 24hrs then the flask was kept at 12 hours at room temperature for the sedimentation process and then filtered through three layers, primarily Muslin cloth, secondary Filter paper and tertiary using Whatman 1 under aseptic conditions and the filtrate was collected and concentrated using a rotary evaporator. The resultant Extract was stored at 4 °C for future use (Abubakar & Haque, 2020b).

4.3 PHYTOCHEMICAL ANALYSIS

Qualitative analysis of phytochemicals using the Harbone method

4.3.1 TANNINS:

1 ml of the sample taken to that few drops of 0.1% ferric chloride was added a blue/black colourization or brownish green colour indicates the presence of tannins.

4.3.2 SAPONINS:

1ml of a sample taken to that 2ml of water was added (shaken vigorously) observed for foaming appearance.

4.3.3 FLAVONOIDS:

1 ml of a sample taken to that 1 ml of conc. Hydrochloric acid was added and one or two salts of magnesium chloride were added a pink-red tomato color indicating the presence of flavonoids (Deshmukh Krishi Vidyapeeth et al., 2017).

4.3.4 ALKALOIDS:

1ml of a sample taken to that few drops of Dragandoff reagent (gram's iodine) was added an orange colour indicates the presence of alkaloids.

4.3.5 CARBOHYDRATES

A few drops of alpha naphthol add 1ml of extract and a few drops of con H₂SO₄. A violet ring indicates the presence of carbohydrates

4.3.6 PROTEINS:

1ml of a sample taken to that few drops of Bradford reagent were added blue colour development indicates the presence of proteins.

4.3.7 STEROIDS:

1ml of a sample taken to that few drops of 10% sulphuric acid were added observed green colour indicates the presence of steroids.

4.3.8 TERPENOIDS:

1ml of a sample taken to that few drops of Chloroform and few drops of conc. Sulphuric acid was added. A reddish-brown colour indicates the presence of terpenoids.

4.3.9 CARDIO GLYCOSIDES:

1 ml of a sample taken to that 0.4 ml of glacial acetic acid and few drops of ferric chloride and a few drops of conc. Sulphuric acid was added. A brown ring indicates the presence of cardio glycosides.

4.3.10 PHENOL:

1ml of a sample taken to a few drops of ethanol and a few drops of ferric chloride was added a blueish green colour indicating the presence of phenol(Yadav & Agarwala, 2011).

4.4 CHARACTERIZATION OF PHYTOCHEMICALS

4.4.1 UV - SPECTRA ANALYSIS

The analytical method of UV-vis spectroscopy is affordable, straightforward, adaptable, non-destructive, and suited for a wide range of organic chemicals and certain inorganic compounds. UV-vis spectrophotometers calculate the absorption or transmission of light through a material as a function of wavelength (Lin, n.d.). Wavelength was carried out between 200 – 700 nm.



Fig 4.1: UV- Vis spectrophotometer

4.4.2 GAS CHROMATOGRAPHY-MASS SPECTROSCOPY (GCMS)

Gas Chromatography-Mass Spectroscopy (GCMS) Analysis: Gas Chromatography-Mass Spectrometry (GC-MS) plays a key role in the analysis of unknown components of plant origin. The chemical composition of ethanolic extracts of the leaf *Imperata cylindrica* was subjected to GC-MS analysis. GC-MS analysis of these extracts was carried out using the equipment GC Clarus 500 Perkin-Elmer system comprising an AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following condition: Equipped with a column Elite-1, fused silica capillary column (30 m × 0.25 mm ID × 1 μm df, composed of 100% dimethyl polysiloxane), operating in electron impact mode at 70 eV; helium gas (99.999%) was used as carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 μl was employed (split ratio of 10:1). The injector temperature is set at 250 °C, and the ion-source temperature is 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min, to

200°C/ min, then 5°C/ min to 280 °C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds, and fragments from 45 to 450 Da. Total GC running time was 36 min.



Fig 4.2: GC Clarus 500 Perkin-Elmer system comprising an AOC-20i

4.5 PREPARATION OF PLANT EXTRACT

5g of fresh aerial parts of *I.cylindrica* was dissolved in 100ml of double distilled water and kept in the shaking incubator at 24C at 120 rpm for 24hrs then the flask was kept at 12 hours of room temperature for the sedimentation process and then filtered through three layers, primarily Muslin cloth, secondary Filter paper and tertiary using Whatman 1 under aseptic conditions and the filtrate was collected and used for nanoparticle synthesis.

4.6 BIOSYNTHESIS OF SILVER NANOPARTICLES

12 ml of 5% aqueous extract of *I.cylindrica* (pH 7.4) was added to 400 ml of 1mM aqueous extract of silver nitrate solution kept at 70 C. The time of addition of aqueous extract into 1mM silver nitrate solution was considered as the start of the reaction, at regular intervals, samples are collected for Uv spectra Analysis, and sometimes the

colour of the solution changes gradually to brownish colour indicating the formation of silver nanoparticles and absorbance was read from 300 - 600 nm.

4.7 CHARACTERIZATION OF SILVER NANOPARTICLES

4.7.1 UV SPECTROSCOPY

UV-Visible Spectroscopy of the silver nanoparticles was recorded using Jasco V- 750 UV-Visible Spectrophotometer. It was carried out at room temperature at a resolution of 1nm and the spectra scanning ranges from 300 – 600 nm. The absorption spectra of AgNO₃ solution, aqueous extract of *I.cylindrica* and silver nanoparticles at different time intervals were recorded.



Fig 4.3: UV-Vis spectrophotometry

4.7.2 FOURIER TRANSFORM INFARED (FTIR)

The AgNPs (1 mg) powder was taken in an Eppendorf tube and the suspension was prepared in 1 ml of distilled water. This suspension was sonicated. In a clean, dry watch glass a potassium bromide (KBr) powder was taken and 3 to 4 drops of suspension were added to it. The mixture was ground using a mortar and pestle to make it a fine powder. FTIR measurement was carried out to identify the group of biomolecules responsible for the surface capping and reducing the Ag salt. Fourier Transform Infrared Spectroscopy (FTIR) identifies chemical bonds in a molecule by producing an infrared absorption spectrum. FTIR is an effective analytical instrument

for detecting functional groups and characterizing covalent bonding information. An FTIR spectrum was recorded using the Perkin Elmer FTIR Spectrum-100 model.

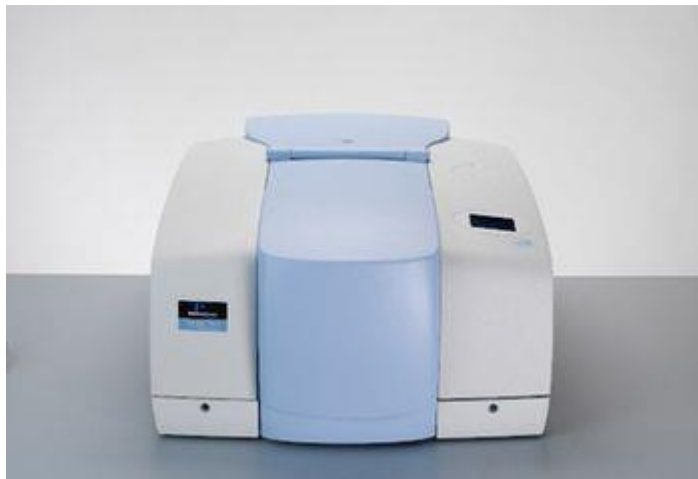


Fig 4.4: Perkin Elmer FTIR Spectrum-100 model

4.7.3 X-RAY DIFFRACTION (XRD)

1 mg of nanoparticles powder was dissolved in 1 ml of distilled water in the Eppendorf tube to obtain a thick film. The dry powder of the silver nanoparticles was used for XRD analysis. The diffraction pattern was recorded from a diffraction angle range of 20° to 80° . The XRD method is suitable to determine the crystal structures by analyzing the positions and intensities of diffraction peaks typically observed for well-crystallized material. The diffraction pattern corresponds to the pure silver metal powder. The XRD studies were conducted using a Rigaku mini flex benchtop X-ray spectrophotometer. The X-rays are generated using a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. X-ray diffraction (XRD analysis) is a unique method in the determination of the crystallinity of a compound. XRD analysis is based on constructive interference of monochromatic X-rays and a crystalline sample (Fairuzi et al., 2018).



Figure 4.5: Rigaku mini flex benchtop X-ray spectrophotometer

4.7.4 SCANNING ELECTRON MICROSCOPE (SEM)

SEM analysis of the silver nanoparticles provides information regarding the dimensions including the surface, shape, and size. The sample was prepared by sonicating the sample solution for 15 minutes at room temperature. A small drop of the sonicated sample was dried on a glass slide, and it was coated with gold and observed under VEGA3 TESCAN.



Fig 4.6: VEGA3 TESCAN

4.8 ANTIOXIDANT ACTIVITY

4.8.1 HYDROXYL RADICAL SCAVENGING ACTIVITY

The Hydroxyl radical scavenging activity of the extract was measured according to the method of Halliwell(Veeru et al., 2009).

4.8.2 REAGENTS

1. Phosphate buffer (pH 7.4)
- 2.1 mM Ferric Chloride
3. 1 mM Ethylene diamine tetra acetic acid
4. 1 mM Ascorbic acid
5. 30 mM Deoxyribose
6. 20 mM H₂O₂
7. Standard Butylated hydroxytoluene (BHT)

4.8.3 PROCEDURE

1 ml of the final reaction solution consisted of aliquots (500 µl) of various concentrations of the extract, 1 mM FeCl₃, 1 mM EDTA, 20 mM H₂O₂, 1 mM Ascorbic acid and 30 mM de-oxy ribose in potassium phosphate buffer (pH 7.4). The reaction mixture was incubated for 1hr at 37°C and further heated in a boiling water bath for 15 minutes, after the addition of 1 ml of 28% (w/v) TCA and 1ml of 1% (w/w) 2-Thiobarbituric acid(Razafindrakoto et al., 2021). The colour development was measured of 532nm against a blank containing phosphate buffer. Total Hydroxyl radical scavenging activity was expressed in %(Huang et al., 2002).

Hydroxyl radical scavenging activity (%) = [Abs (control) – Abs (sample) / Abs (control)] × 100.

4.8.4 REDUCING POWER ACTIVITY

The reducing power of the extract was determined by the method of Yen and Duh 2.

4.8.5 REAGENTS REQUIRED

- 1)1% Potassium Ferric cyanide

2)10% TCA

3)0.1% Ferric chloride

4.8.6 PROCEDURE

Different concentrations of extracts were mixed with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of 1% Potassium Ferricyanide. The mixtures were incubated for 20 min at 50°C. After incubation, 2.5 mL of 10% Trichloroacetic acid was added to the mixtures, followed by centrifugation at 650×g for 10 min. The upper layer (5 mL) was mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride and the absorbance of the resultant solution was measured at 700 nm(Moon & Shibamoto, 2009)

4.8.7 RESULT

The reducing power of the extracts was expressed as Vitamin E equivalence in mg/g.

4.9 ANTIBACTERIAL ACTIVITY

4.9.1 ANTIBACTERIAL ACTIVITY BY DISC DIFFUSION METHOD

The antibacterial activity of the Plant & Nanoparticles sample was determined by the disc diffusion method on Muller Hinton agar (MHA) medium. The Muller Hinton Agar medium was weighed as 3.8gms and dissolved in 100ml of distilled water and added 1gm of agar. Then the medium is kept for sterilization. After sterilization the media was poured into sterile petri plates, these petri plates were allowed to solidify for twenty minutes(Nascimento et al., 2000). After the medium was solidified, the inoculums were spread on the solid plates with sterile swabs moistened with the bacterial suspension. The well was placed in an MHA plate and add 25µl of the sample was [concentration: 100µg, 75µg, 50µg, 25µg]. The plates were incubated for 24 hrs, at 37°C. Then the microbial growth was determined by measuring the diameter of the zone of inhibition(Parkavi et al., 2012).

4.9.2 MINIMUM INHIBITORY CONCENTRATION

The antimicrobial activity of the silver nanoparticles was determined by the resazurin microtiter assay method (Fairuzi et al., 2018). The inoculum, equivalent to a 0.5 McFarland turbidity standard, was prepared from each bacterial isolate in MH broth. Sterile PBS (50 µL) was dispensed in each well of a sterile 96-well flat bottom plate. The root extract of various concentrations from 5000 µg/mL to 2 µg/mL was added by twofold serial dilutions. Tetracycline was used as the positive control in separate wells. Separate wells with negative control (culture with no plant extract) were also performed. To each well, 10 µL of resazurin indicator solution was added, followed by the addition of 30 µL of threefold strength MH broth. The plates were incubated at 37 °C in an incubator for 12–24 h for further growth if any was detected. The change in colour of the dye added to the wells from blue to pink indicated the growth of bacteria (Sundaram et al., 2021). The MIC was defined as the lowest concentration of each test compound or drug that prevented any colour change observed visually, i.e., the concentration that inhibited microbial growth.

CHAPTER-5

RESULTS

5.1 PLANT POWDER OF *IMPERATA CYLINDRICA*



5.2 THREE LAYERS OF FILTRATION

Aqueous Extract Filtration



Methanol Extract Filtration



Hexane Extract Filtration



1. Muslin cloth filtration
2. Filter paper
3. Whattmann filter

Figure 5.1: a) Plant powder of *Imperata cylindrica*, **b)** Three layers of Filtration

The highest percentage of yield was gained in Aqueous at 27.98% followed by hexane at 16.19, and then methanol at about 15.22%.

Table 5.1: Yield calculation

S.NO	Extract	Empty Weight	Extract Weight	Percentage
1	Aqueous	106.2 g	107.2 g	27.98
2	Methanol	195.7 g	197.1 g	15.22
3	Hexane	184.4 g	185.2 g	16.19

5.3 QUALITATIVE PHYTOCHEMICAL ANALYSIS OF THE VARIOUS EXTRACTS OF *IMPERATA CYLINDRICA*

Table 5.2: Phytochemical analysis

S.NO	Phytochemicals	Test Name	Aqueous	Methanol	Hexane
1	Alkaloids	Iodine test	Presence	Presence	Presence
2	Carbohydrates	Molish's test	Presence	Presence	Absence
3	Tannin	Lead acetate test	Presence	Presence	Absence
4	Saponin	Foam test	Absence	Absence	Absence
5	Flavonoids	Alkaline reagent test	Presence	Presence	Presence
6	Glycosides	Glycosides test	Absence	Presence	Absence
7	Terpenoids	Terpenoids test	Presence	Presence	Absence

8	Protein	Bradford reagent	Presence	Presence	Presence
9	Phenol	Phenol Test	Absence	Absence	Absence
10	Cholesterol	Cholesterol	Absence	Presence	Absence

Phytochemical analysis of *Imperata cylindrica* showed the presence of alkaloids, flavonoids, and protein in Methanol aqueous and hexane extract. Carbohydrates, tannin, flavonoids, and terpenoids present in Aqueous and methanol extract compared to all three-extract showed methanol has good phytochemicals.

Aqueous extract



Methanol extract

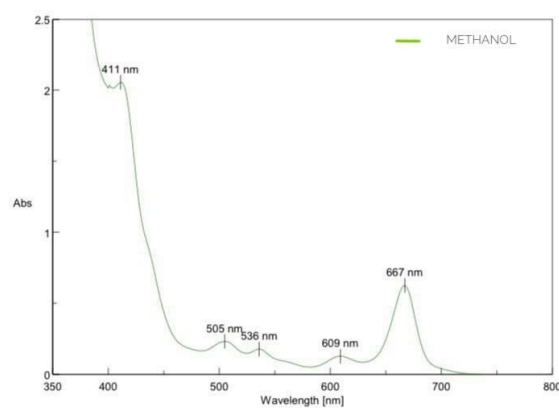
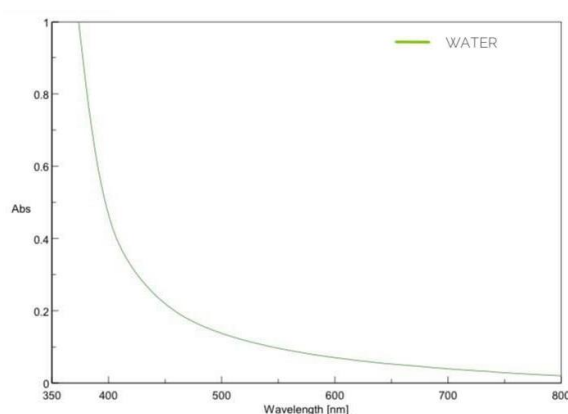


Hexane extract



Figure 5.2: Phytochemical analysis

5.3.1 UV SPECTRA ANALYSIS OF PHYTOCHEMICALS



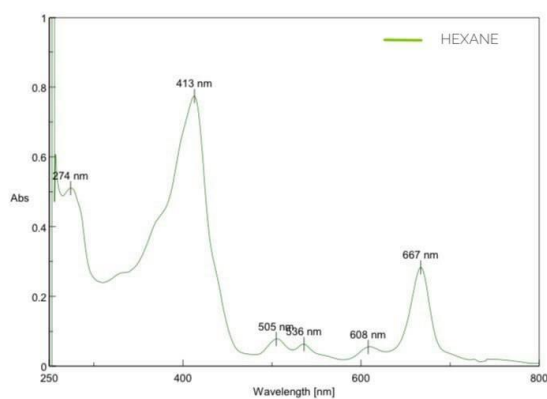
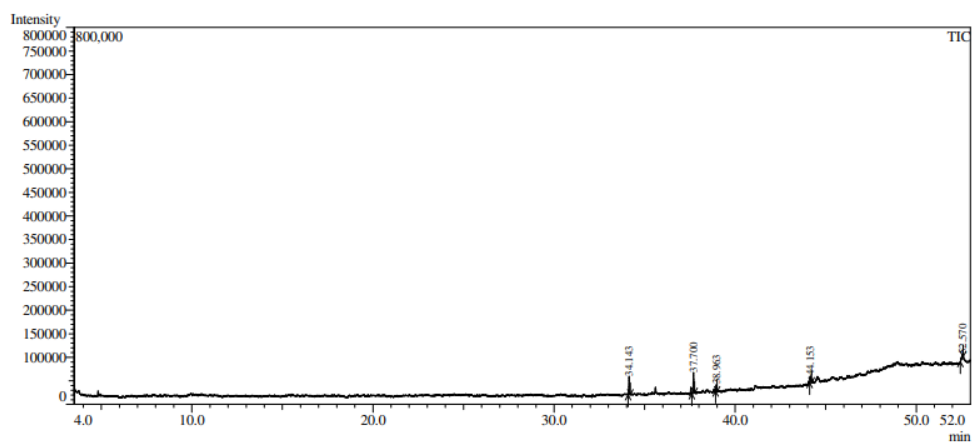


Figure 5.3: UV Spectra Analysis of Phytochemicals

Table 5.3: Presence of phytochemicals

S.NO	Extract	Nanometer	Phytochemicals
1	Methanol	411,505,536,609,667	Flavonoids, Alkaloids.
2	Hexane	274,413,505,536,608,667	Alkaloids, Flavonoids, Protein.
3	Aqueous	NIL	NIL

5.3.2 GAS CHROMATOGRAPHY-MASS SPECTROSCOPY



Peak Report TIC				
Peak#	R.Time	Area	Area%	Name
1	34.143	101495	29.27	HEXADECANOIC ACID, METHYL ESTE
2	37.700	117103	33.77	9-Octadecenoic acid, methyl ester, (E)-
3	38.963	36719	10.59	Ethyl Oleate
4	44.153	54262	15.65	9-Octadecenoic acid, 1,2,3-propanetriyl ester
5	52.570	37225	10.73	STIGMAST-7-EN-3-OL, (3.BETA.,5.ALPH
		346804	100.00	

Figure 5.4: GCMS analysis

GCMS analysis of *Imperata cylindrica* extracts the peaks are shown. The obtained results Hexadecanoic acid, 9-Octadecenoic acid, Ethyl Oleate, 9-Octadecenoic acid, 1,2,3-propanetriyl ester, STIGMAST-7-EN-3-OL were detected in whole *Imperata cylindrica* with R. time 34.143, 37.700, 38.963, 44.153 and 52.570.

5.4 BIOSYNTHESIS OF SILVER NANOPARTICLES FROM *IMPERATA CYLINDRICA*

On adding the plant extract to the AgNO₃ solution, the solution changed from yellowish orange to brown colour. The intensity of the absorbance was found to increase as the reaction proceeded further. AgNPs display intense yellowish-brown colour in dipole oscillation arising when an electromagnetic field in the visible range is coupled to the collective oscillations of conduction electrons.

5.4.1 UV SPECTRA IMAGES OF SILVER NANOPARTICLES

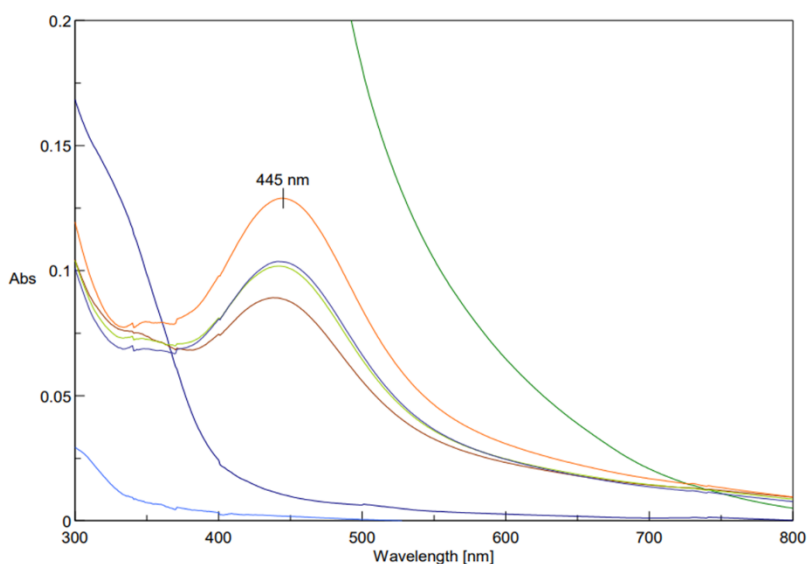


Figure 5.5: UV spectra of silver nanoparticles

The synthesis of AgNPs was performed using *Imperata cylindrica* extract. The Ag⁺ ion reduction was confirmed by the apparent transformation in the colour of the reaction mixture from light brown to dark brown. The formation of AgNPs was further confirmed by UV-Vis spectral study, which is an authentic technique to monitor the progress of the reaction during the reduction of Ag⁺ ions. The peak was measured at 445nm in 90mins of the formation of AgNPs.

5.4.2 FOURIER TRANSFORM INFRARED (FTIR)

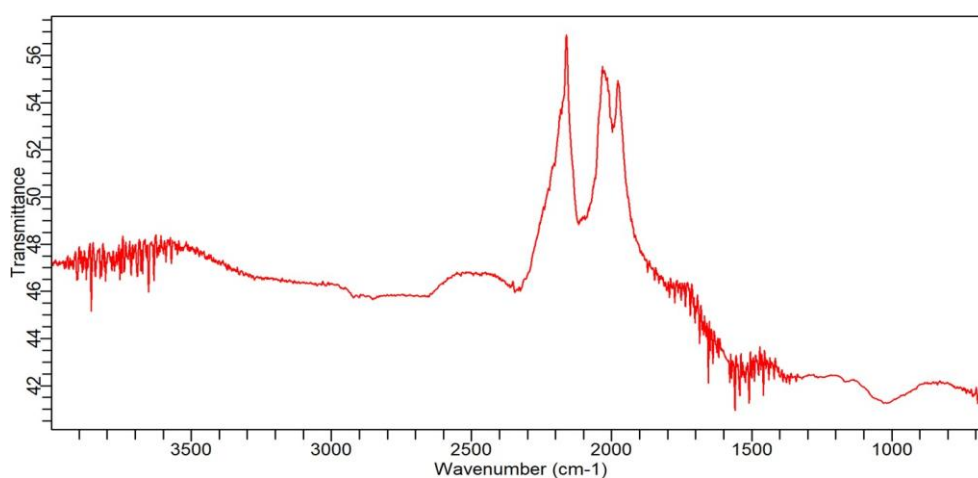


Figure 5.6: FTIR Analysis of silver nanoparticles

Table 5.4: FT-IR Functional group

S.no	Wavenumber	Functional group
1	2939.006	O-H group
2	2163.72	S-C=N
3	2029.536	N=C=S
4	1979.216	N=C=S
5	717.5125	C-H group

The FT-IR spectrum of *Imperata cylindrica* extract stayed on the surface of the biosynthesis of AgNPs. The FT-IR spectrum exhibits several absorption peaks at

different locations including at $\sim 2939\text{ cm}^{-1}$, $\sim 2164\text{ cm}^{-1}$, $\sim 2030\text{ cm}^{-1}$, $\sim 1979\text{ cm}^{-1}$, $\sim 717\text{ cm}^{-1}$. The corresponding functional groups are the O-H group, S-C=H, N=C=S, N=C=S, and C-H group.

5.4.3 X-RAY DIFFRACTION(XRD) SPECTRA OF *IMPERATA CYLINDRICA*

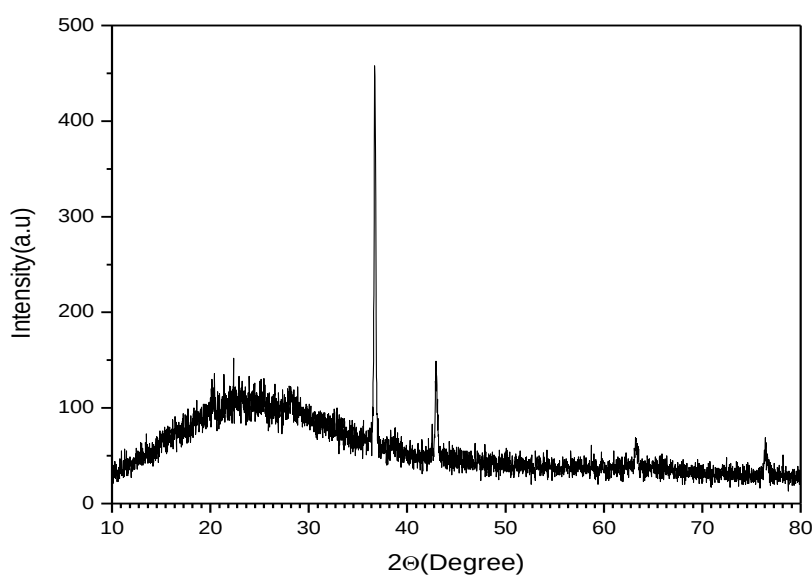


Figure 5.7: XRD spectra

The XRD pattern for the synthesized AgNP of *Imperata cylindrica* and it is observed that the particles are crystalline in nature. A strong peak from Bragg's reflection was obtained in the XRD pattern at $2\theta = 38.12^\circ$, 44.5° , 64.6° and 77.5° . The X-ray diffraction peak corresponding to 38.12° , 44.5° , and 64.6° is due to AgNP corresponding to the lattice plane (111), (200), (220), and (311) respectively. The peak match with the joint committee on powder diffraction standards proves the formation of crystal AgNP.

5.4.4 SCANNING ELECTRON MICROSCOPE (SEM)

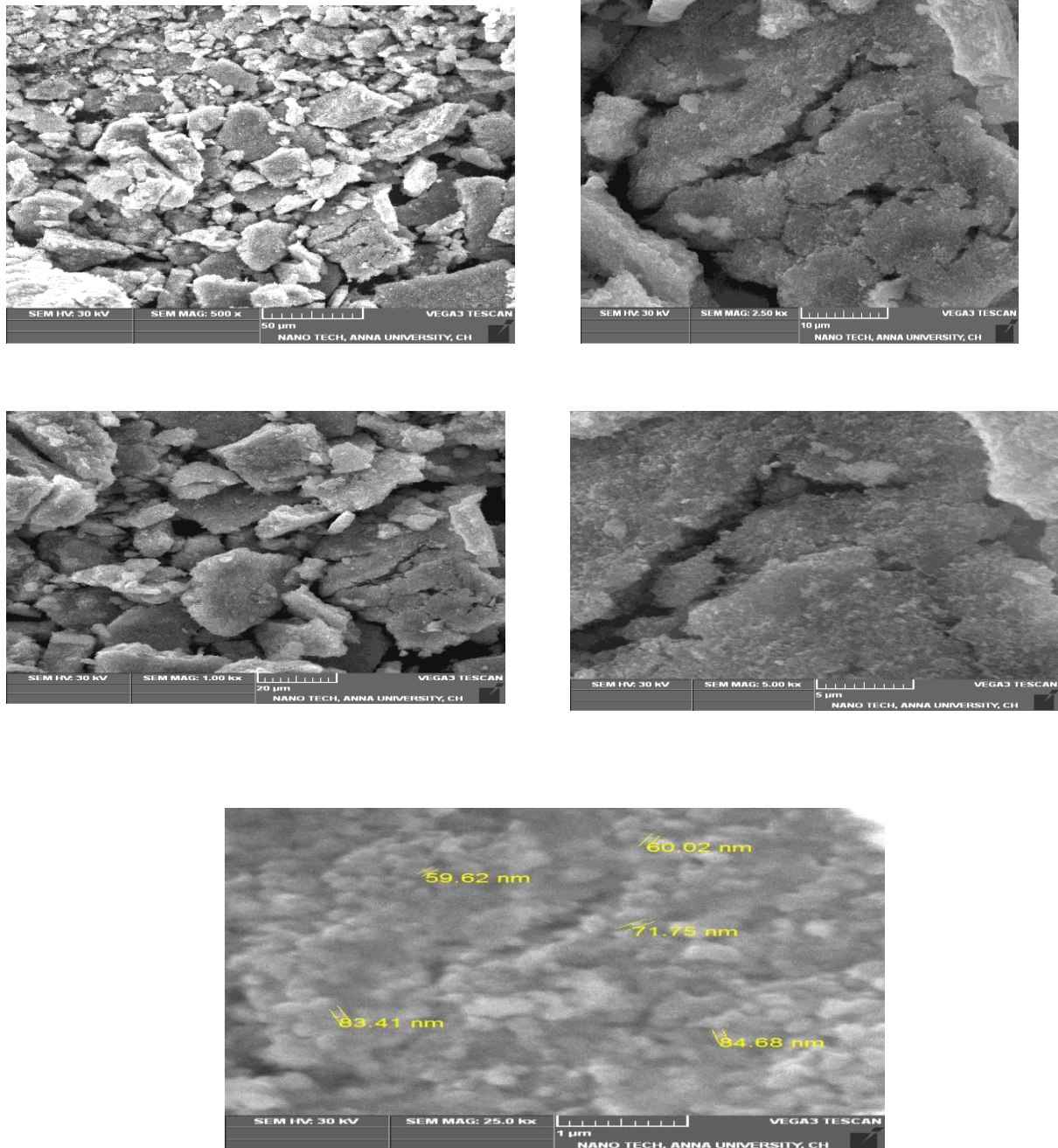


Figure 5.8: SEM Images

Scanning Electron Microscopic (SEM) analysis was carried out using the Vega3 TEScan SEM machine. The average diameter of the silver nanoparticles is 72 nanometers.

5.5 ANTIBACTERIAL ACTIVITY OF *IMPERATA CYLINDRICA* EXTRACT

5.5.1 CONTROL

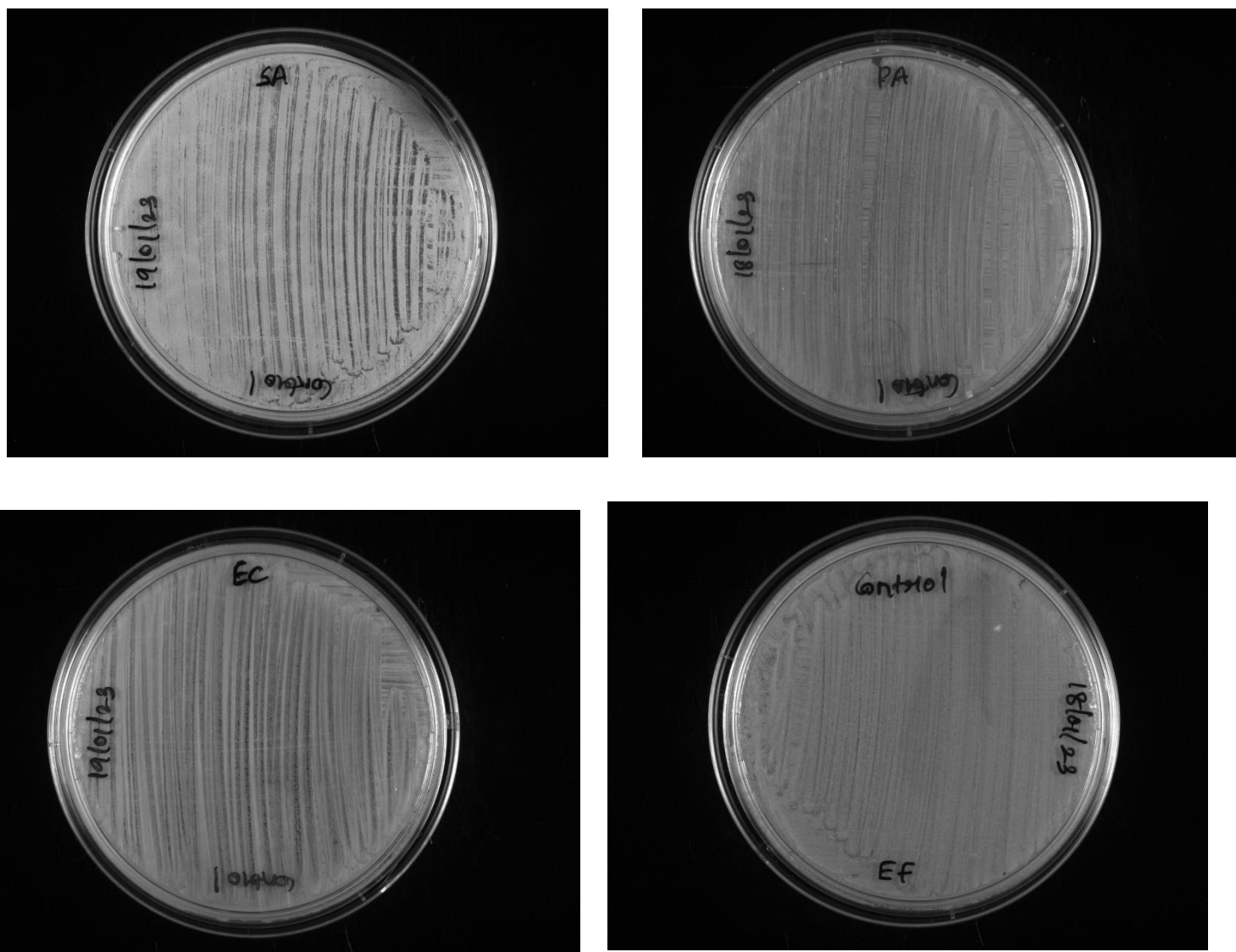


Figure 5.9: Antibacterial activity of *Imperata cylindrica* extract Control images

5.5.2 METHANOL

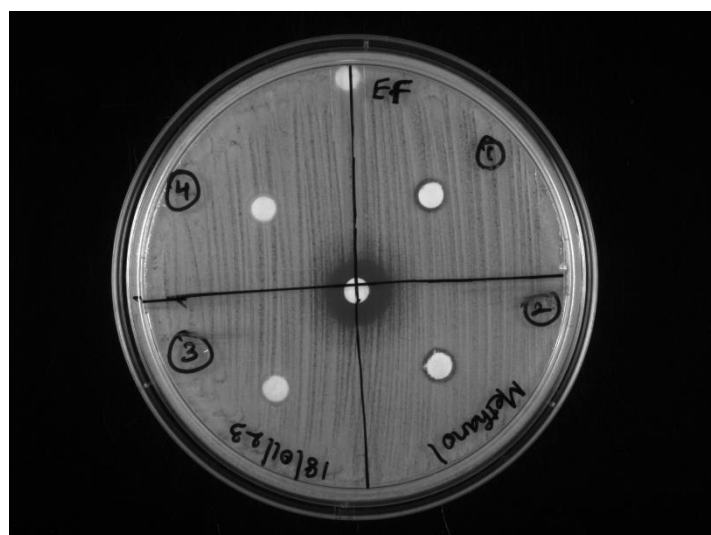
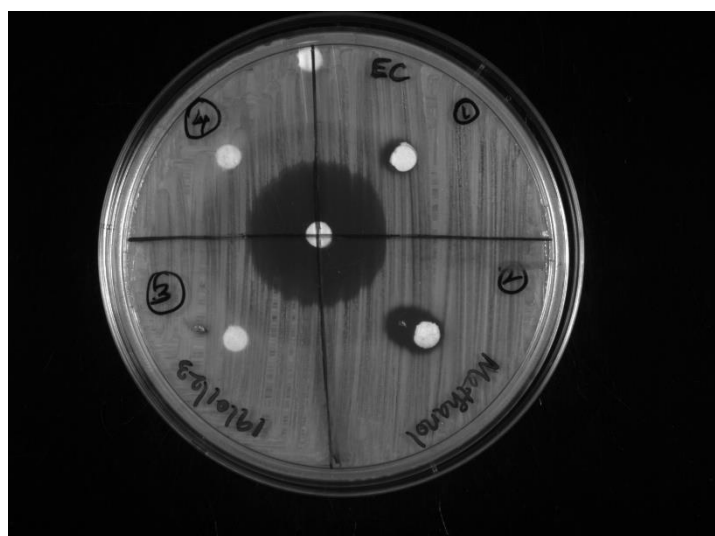
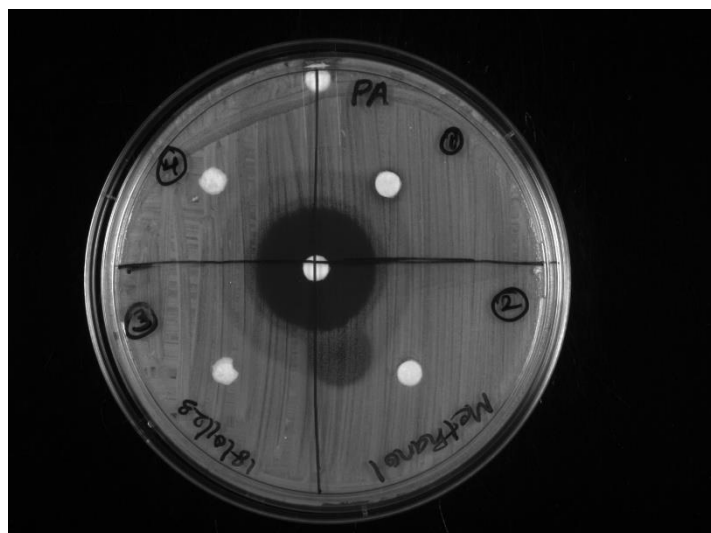
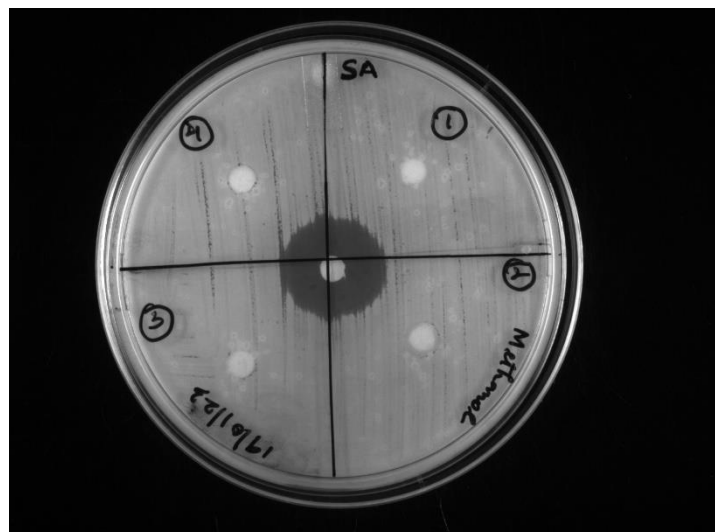


Figure 5.10: Methanol extract

5.5.3 HEXANE

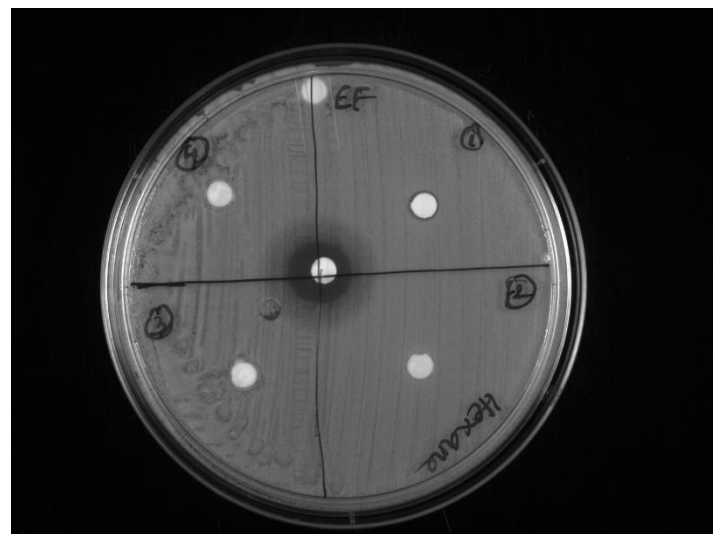
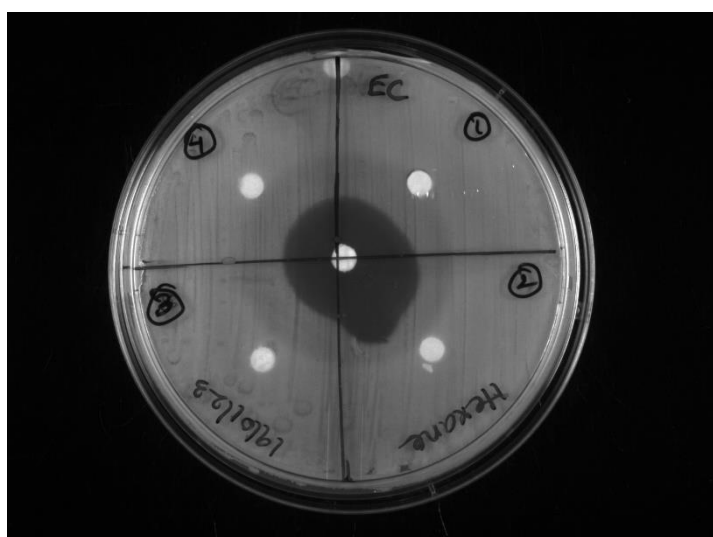
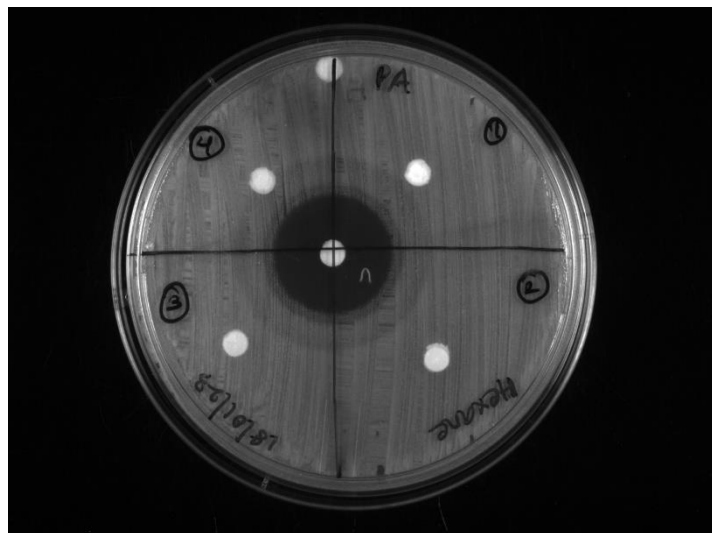
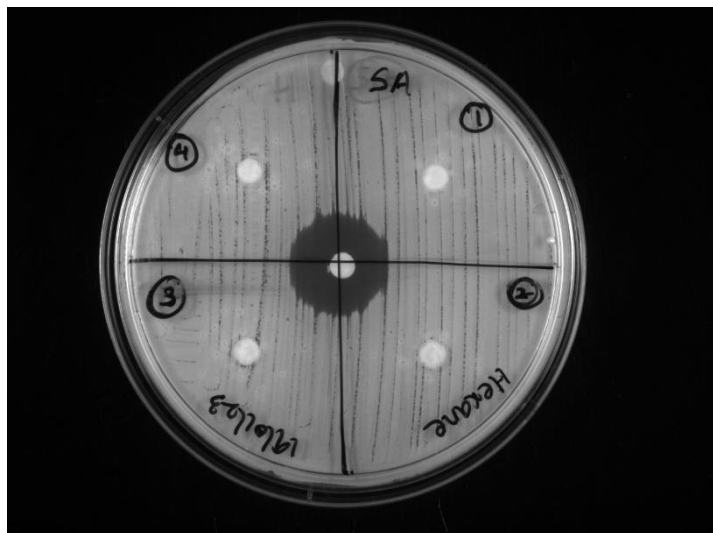


Figure 5.11: Hexane extract

5.5.4 AQUEOUS

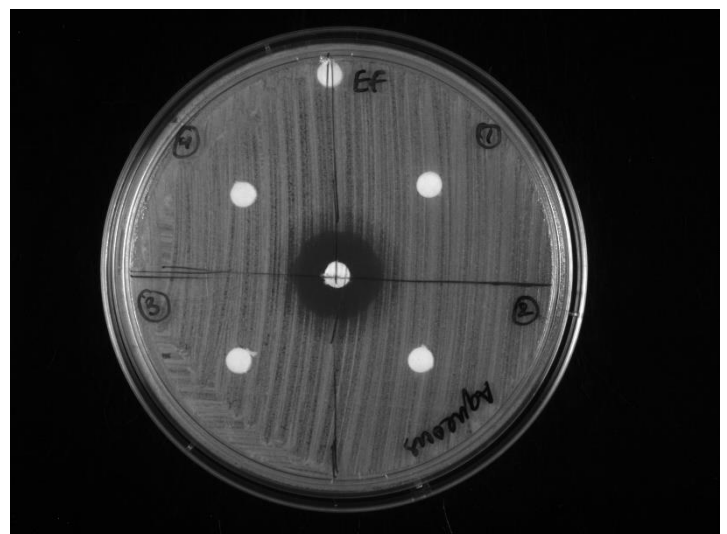
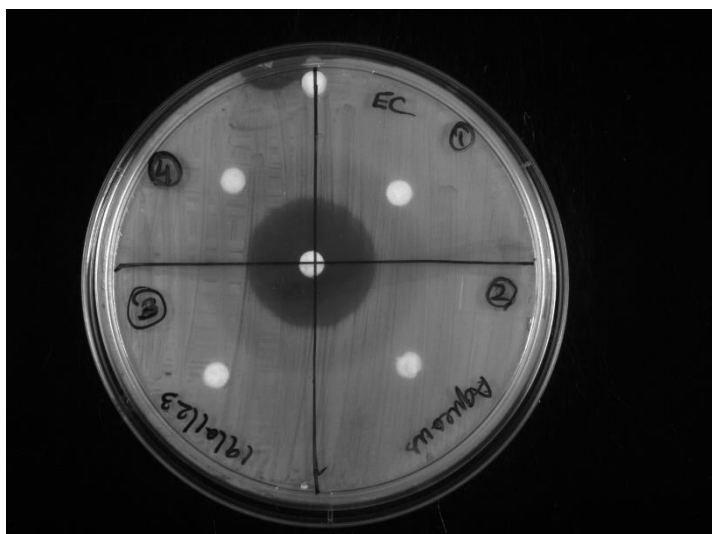
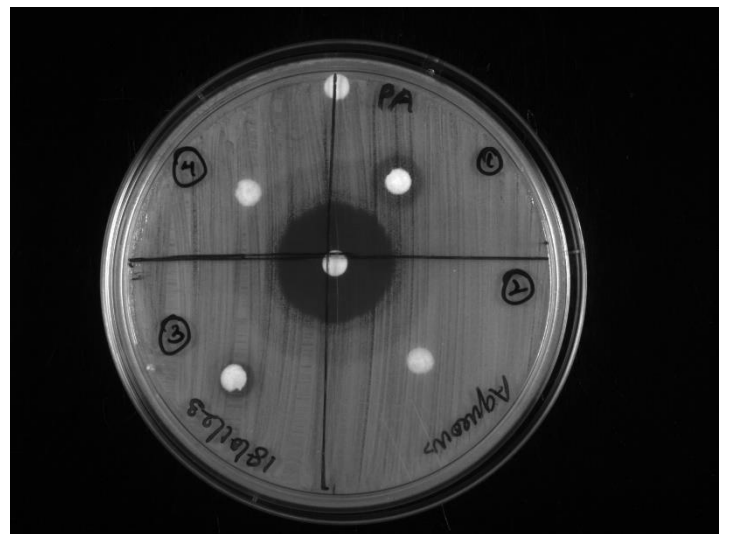
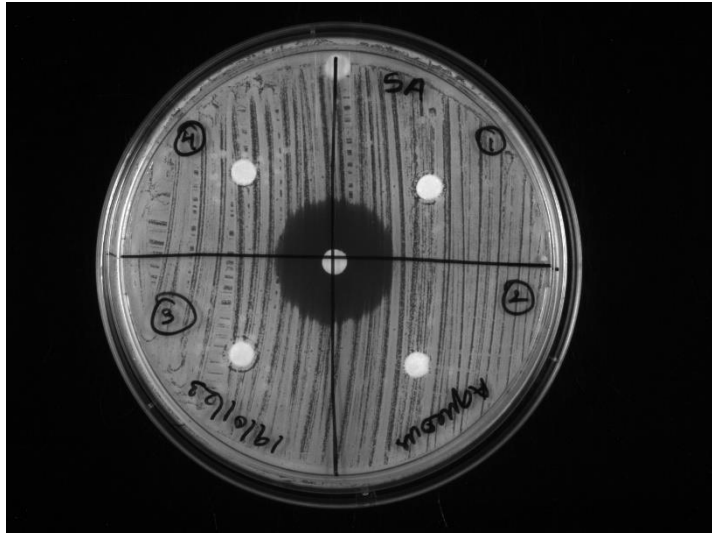


Figure 5.12: Aqueous extract

5.6 ANTIBACTERIAL ACTIVITY OF AGNPS *IMPERATA CYLINDRICA* EXTRACT

5.6.1 CONTROL

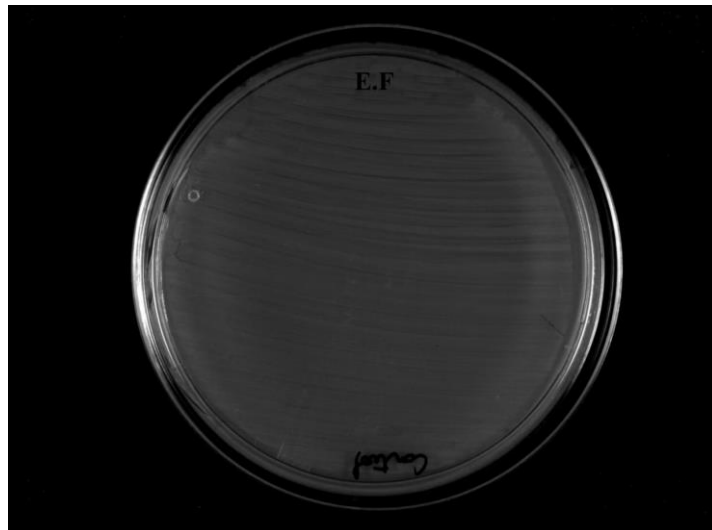
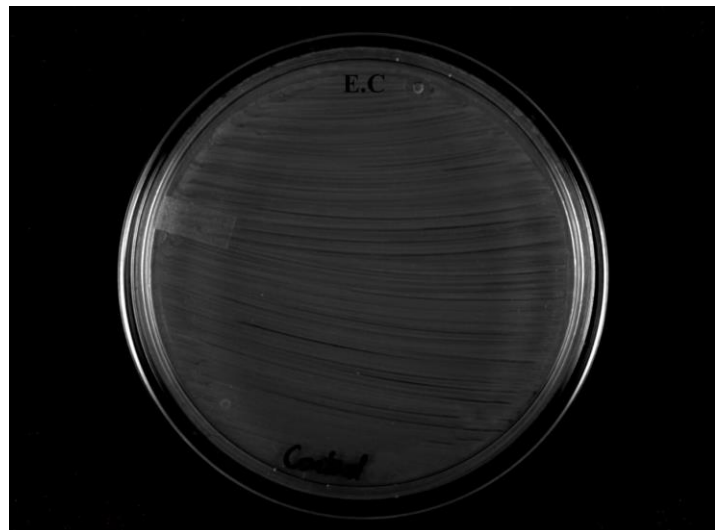
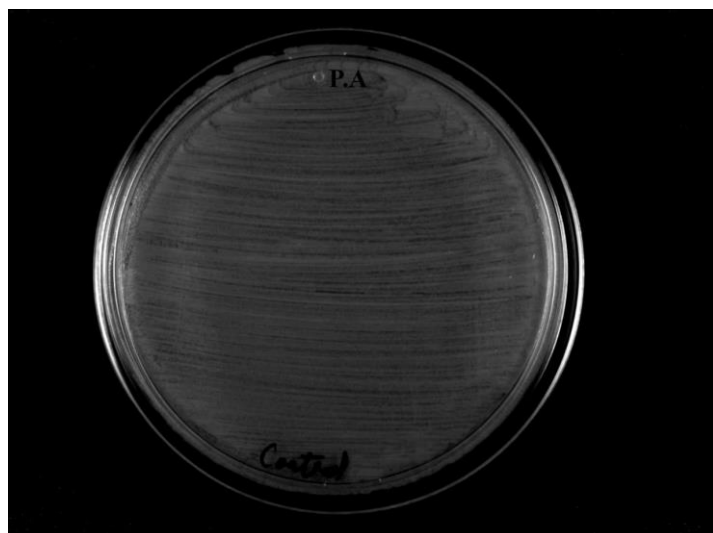
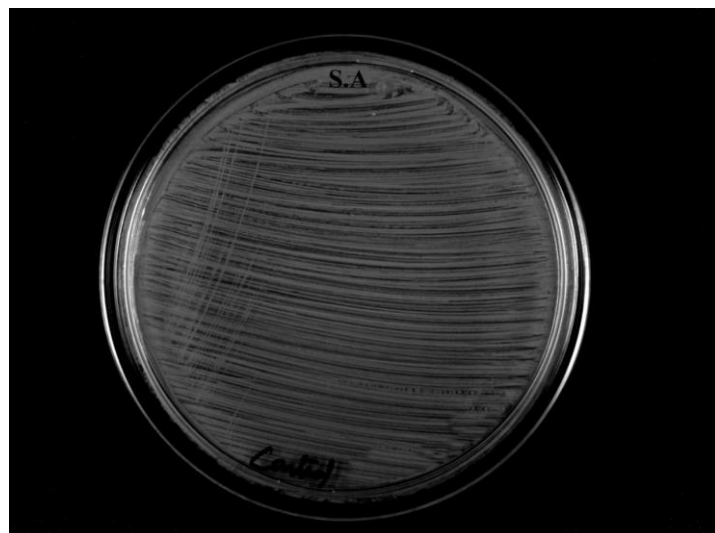


Figure 5.13: Antibacterial activity of AgNPs with *Imperata cylindrica* extract, Control Images

5.6.2 AQUEOUS

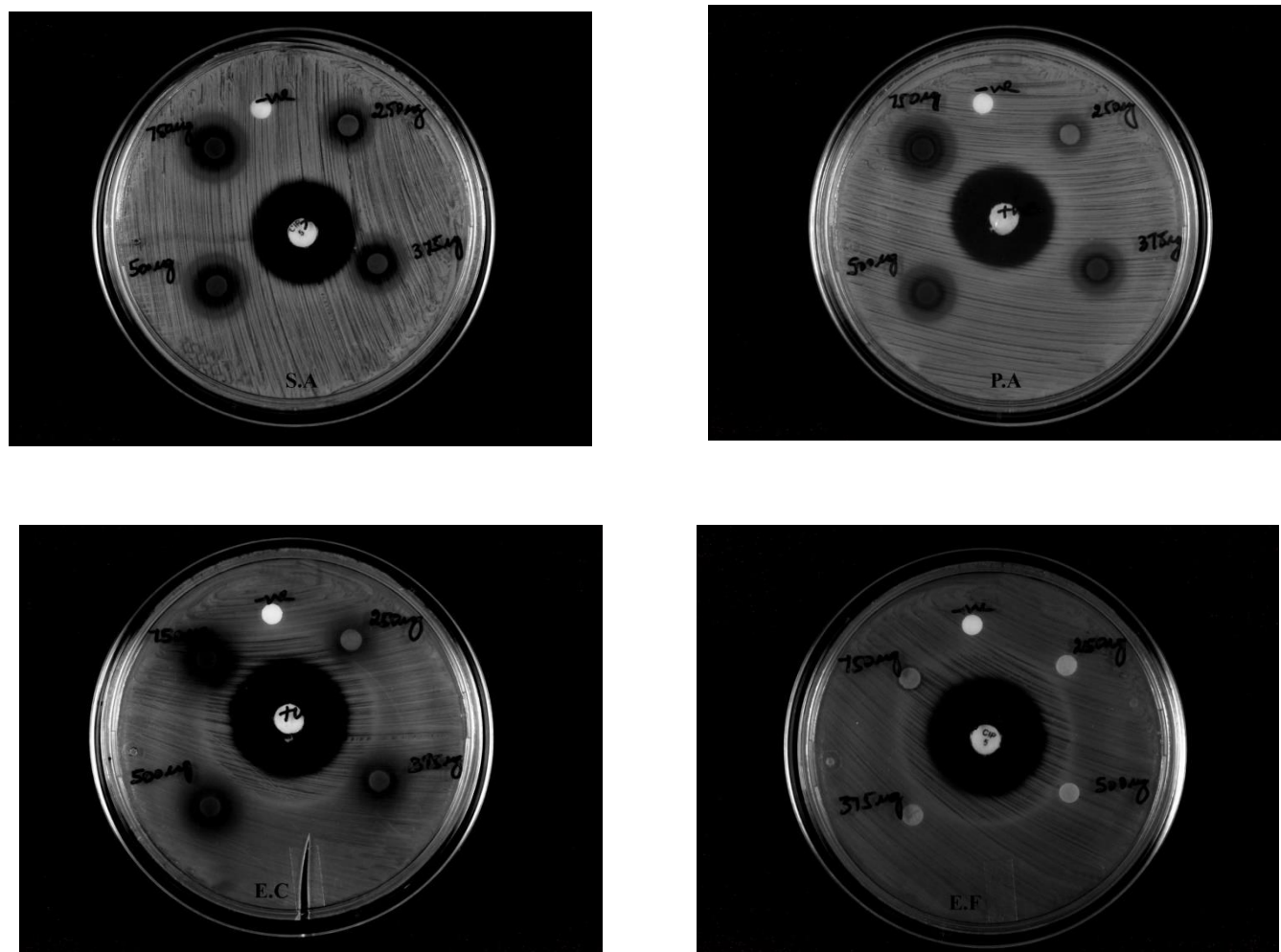


Figure 5.14: Aqueous extract

Table 5.5: Antibacterial activity Zone of inhibition

S.NO	ORGANISM	ZONE OF INHIBITION				ANTIBIOTIC
		25µg	50µg	75µg	100µg	
1	Staphylococcus aureus	6mm	8mm	10mm	12mm	Ciproflaxin
2	Pseudomonas aeruginosa	5mm	7mm	9.6mm	11mm	Ciproflaxin
3	Escherichia coli	4mm	6.2mm	8mm	11mm	Ciproflaxin
4	Enterococcus faecalis	8mm	9mm	11mm	12mm	Ciproflaxin

5.7 MINIMUM INHIBITORY CONCENTRATION

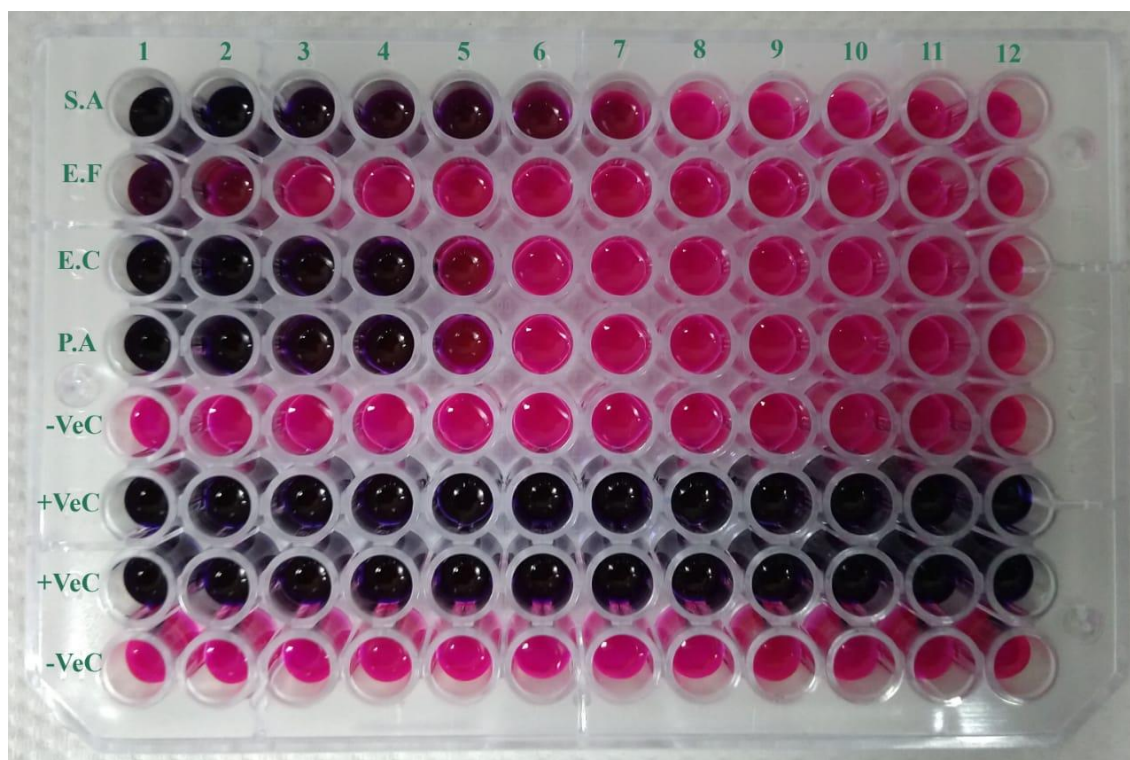


Figure 5.15: Minimum Inhibitory Concentration (MIC)

Staphylococcus aureus (S.A) showed no change in the colour of the resazurin indicator at a concentration of 0.31mg/mL. Similarly, *Escherichia coli* (E.C) and *Pseudomonas aeruginosa* (P.A) showed no change in the colour of the resazurin indicator at a concentration of 0.62mg/mL. The complete colour change was observed for the negative control, indicating the growth of the organism in all wells. The presence of blue colour for the positive control in all wells indicates complete inhibition of growth of the organism (Antibiotics).

5.8 ANTIOXIDANT ACTIVITY

The results of *in-vitro* antioxidant data showed a significant free radical scavenging 1mM of silver nanoparticle in a dose-dependent manner. (**Figure 5.16**). The maximum scavenging activity of hydroxy radical for Aqueous Extract of *Imperata cylindrica*(AEIC), AgNPs & std BHT was found to be 45, 34 & 30% at 1000 µg. Reducing Power activity was depicted in **Figure 5.17** and the silver nanoparticles of IC showed greater reducing ability than AEIC.

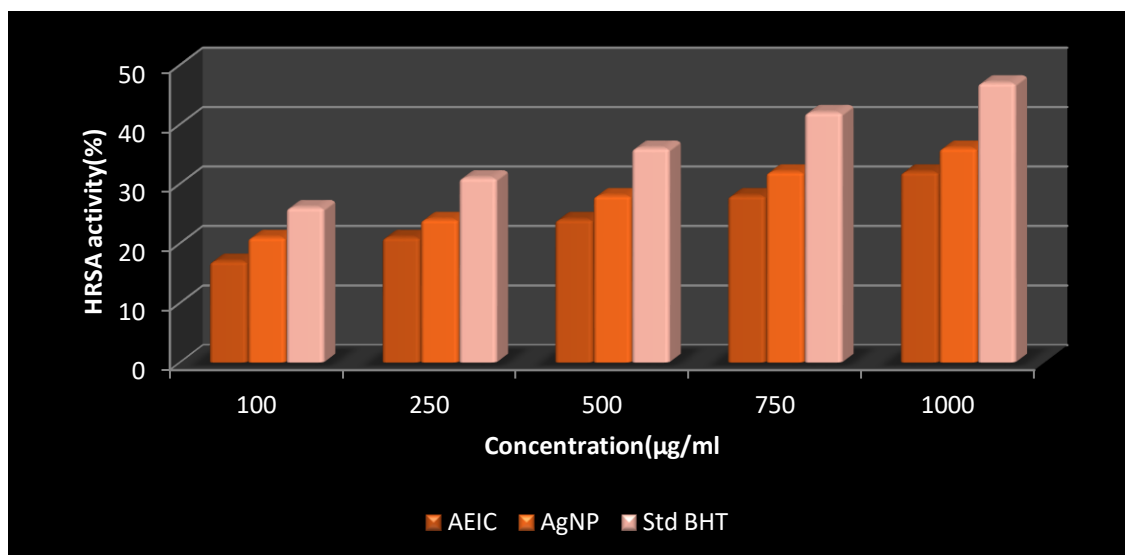


Figure 5.16: Hydroxy radical scavenging activity of AgNP's of *Imperata cylindrica*

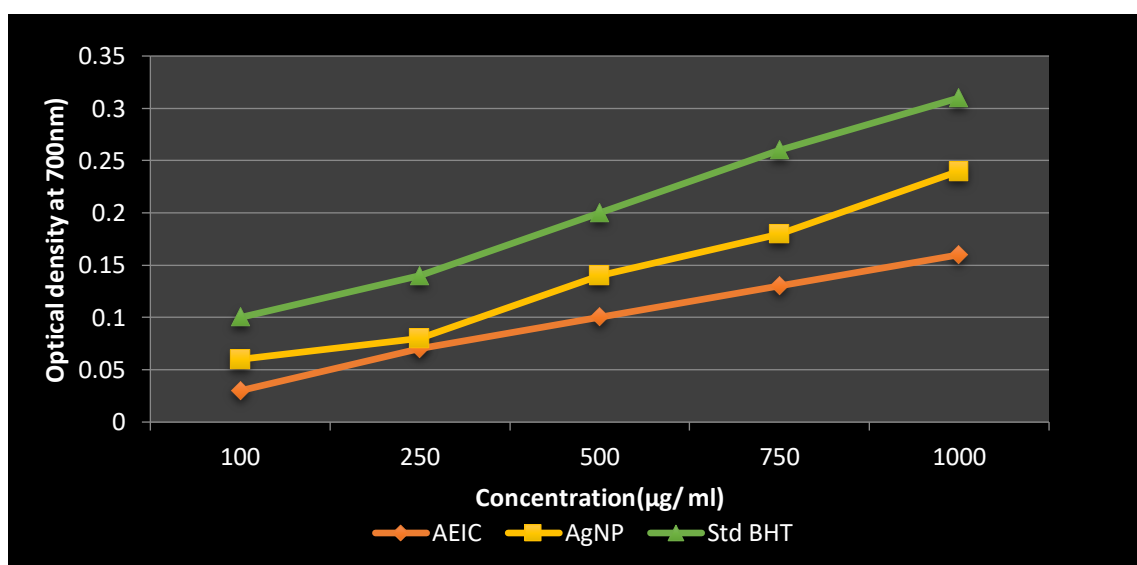


Figure 5.17: Reducing power activity of AgNP's of *Imperata cylindrica*

CHAPTER 6

SUMMARY

- ❖ The plant *Imperata cylindrica* was collected from Navalur, Chennai. Then the plant is extracted using Three solvents (Methanol, Hexane and Aqueous).
- ❖ Phytochemical analysis is a qualitative analysis using the harbour method.
- ❖ The analysis is done for Methanol extract, Hexane extract and Aqueous extract of the plant *Imperata cylindrica*. The result shows that Flavonoids, Alkaloids are present in the Methanol extract, and Then Flavonoids, Alkaloids and protein are present in the Hexane extract.
- ❖ The characterization done for plant extract is UV-Vis spectra analysis and GC-MS.
- ❖ The biosynthesis of silver nanoparticles with plant *Imperata cylindrica* extract was prepared and the characterization was also done.
- ❖ The characterization of silver nanoparticles is UV-Vis spectra analysis, FTIR, XRD and SEM analysis done.
- ❖ AgNP exhibited potent reducing power and hydroxyl radical scavenging activities than the plant extract. The maximum scavenging activity of hydroxyl radical for 1mM AgNP was 45% at concentration of 1mg/ml.
- ❖ The antibacterial activity for both plant extract and AgNPs was determined by the disc diffusion methods.
- ❖ The antimicrobial activity of the silver nanoparticles was determined by the resazurin microtiter assay method using a 96-well bottom plate, Then the Minimum inhibitory concentration (MIC) was also calculated.

CONCLUSION

Plant-based synthesis of metal nanoparticles is an emerging technique in nanoparticle synthesis because this type of technique is showed a more effective impact on antibacterial activity than antibacterial activity with plant extract. The characterization results obtained from FT-IR and XRD analyses revealed the presence of plant extract *Imperata cylindrica* on the surface of the nanoparticles. The Results of the phytochemical analysis of the plant extract showed that the methanol extract shows more compounds than Hexane and Aqueous Extract. The plant extract and Biosynthesized AgNPs obtained were subjected to biological evaluation and tested against Gram-positive and Gram-negative bacterial strains. It shows that the plant extract does not have good microbicidal, and then the synthesized AgNPs obtained higher microbicidal activity.

CHAPTER 7

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