

**ANTIBACTERIAL & ANTI-BIOFILM ASSESSMENTS OF
BIOSYNTHESIZED SILVER NANOPARTICLES USING
ACANTHOPORA SPECIFERA**

PROJECT WORK

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science degree in Biochemistry

By

K.BHAVADHARANI (41070002)



SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

CATEGORY-1 UNIVERSITY BY UGC

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JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI – 600119

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of BHAVADHARANI. K (41070002), Who carried out the project entitled "ANTIBACTERIAL & ANTI-BIOFILM ASSESSMENTS OF BIOSYNTHESIZED SILVER NANOPARTICLES USING ACANTHOPORA SPECIFERA" under my supervision from January 2023 to April 2024.

Internal Guide

Dr. PRABHAKAR SINGH

External Guide

Dr.T.RAJASEKAR

Head of the Department

Dr. V. RAMESH KUMAR

Submitted for Viva-Voce examination held on.....

Internal Examiner

(Dr.S.JAYASHREE)

External Examiner

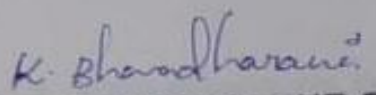
Dr. Prakash

DECLARATION

I, Bhavadharani. K (41070002), hereby declare that the Project Report entitled "ANTIBACTERIAL & ANTI-BIOFILM ASSESSMENTS OF BIOSYNTHESIZED SILVER NANOPARTICLES USING ACANTHOPORA SPECIFERA" done by me under the guidance of Dr. Prabhakar Singh Assistant professor Internal guide, Department of biotechnology and Dr.T.Rajasekar Assistant professor (Research park) External guide at Centre for drug discovery and development, is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Biochemistry.

DATE: 27/04/2024

PLACE: CHENNAI


SIGNATURE OF THE CANDIDATE

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ABSTRACT

Since ancient times, silver has been known to have antibacterial properties. Synthesising it via an eco-friendly process is beneficial because it is less expensive, more stable, and ecologically friendly. In this study, silver nanoparticles (AgNPs) were synthesized using a green technique. To convert silver nitrate to AgNPs, *Acanthophora specifera* was extracted with water. X-Ray diffraction (XRD) was used to analyze the structure of the produced AgNPs, which were seen to have a reddish brown color. The metallic silver of silver nanoparticles was face-centered cubic, with a size range of 33 nm to 81 nm. Additionally, infrared spectroscopy (FT-IR) was identified. Furthermore, the antimicrobial activity of AgNPs was determined utilizing well diffusion techniques. AgNPs greatly inhibited the development of Gram-positive *Staphylococcus aureus* 23.

Keywords: Marine macro algae; *Acanthophora specifera*; Silver nanoparticles; XRD; Antimicrobial activity

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LIST OF ABBREVIATIONS

AgNO₃	Silver nitrate
AgNPs	Silver nanoparticles
Ag⁺	Silver ions
ATCC	American Type Culture Collection
BDs	Biomedical Devices
CFU	Colony - forming unit
CG	Cytosine- guanine
EPS	Extracellular Polymeric Substances
FTIR	Fourier transform infrared spectroscopy
MTCC	Microbial Type Culture Collection and Gene Bank
NPs	Nanoparticles
PBS	Phosphate buffer saline
SEM	Scanning Electron Microscopy
UV	UV-visible spectrophotometer
XRD	X-ray diffraction
ZnO-NPs	Zinc oxide nanoparticles

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CHAPTER 1

INTRODUCTION

1.1 BIOFLIMS

Bacterial biofilm is common in nature, creating complex colonies that are adhered to biotic or abiotic surfaces. Scientists discovered that over 99% of the world's microorganisms reside as biofilms. (Abdel- Raouf *et al.*, 2017). Biofilm development occurs when microbial cells aggregate and build a matrix of polymeric chemicals known as Extracellular Polymeric Substances (EPS). The content of EPS depends on the type of bacteria and the environment. (Al- Amoudi OA. *et al.*, 2009). Biofilm is made up of millions of bacteria. Root cause of many serious infections such as cystic fibrosis, periodontal disease etc. Microbial biofilms are a severe hazard to the health of people implanted with synthetic Biomedical Devices (BDs).

Biofilms can form on a variety of medical equipment, including contact lenses, central venous catheters, artificial heart valves, hip prosthesis, and intrauterine devices. (Albrecht MA. *et al.*, 2006). were resistant to medicines and caused serious immune system problems. BDs are fairly significant. Support in A tool for effective clinical management that improves patients' lives. BD's subject Antibiotics frequently fail to prove their effectiveness against bacterial infections. (Feng QL. *et al.*, 2000). Antibiotic medication frequently fails to prove itself against bacterial illnesses. It has been shown that approximately 70% of bacteria are resistant to antibiotic therapy and frequently fail to prove themselves from bacterial illnesses with commercially available medicines.(Geoprincy G. *et al.*, 2013). Some well-known dangerous microorganisms. Associated with antibiotic therapy. Biofilm production on BDs includes *Escherichia*, *Legionella*, *Staphylococcus*, *Streptococcus*, *Salmonella*, *Shigella*, and *Vibrio*. The fact that antibiotic therapy frequently fails to cure bacterial infections contributes significantly to the development of chronic diseases. (Gnanadesigan M. *et al.*, 2012). The infection is induced by the surface alteration of biofilm, which provides an excellent environment for the exchange of extra-

chromosomal DNA, which is responsible for antibiotic resistance, virulence, and endurance, resulting in drug-resistant bacteria.

(Guzman G. *et al.*, 2009),

1.2 NANOTECHNOGY

Nanotechnology is a multidisciplinary field that includes catalysis, microelectronics, photonics, optics, and optoelectronics, sensors, treatments, DNA sequencing, pharmaceuticals, and nanocomposites. (Abdel-Raouf N. *et al.*, 2013). Nanoparticles can be classified as physical, chemical, physicochemical, or biological based on their synthesis processes. (Baruwat S. *et al.*, 2009) Each strategy has its advantages and downsides. The biological method is commonly employed among these manufacturing processes since incorporating living creatures in the synthesis pathway is safer, less expensive, and more ecologically friendly than other methods. Biologically manufactured nanoparticles primarily entail the employment of different biological entities, such as leaven, enzymes, mild, algae, bacteria, etc. seaweed extracts. The emphasis on the use of marine macro-algae is increasing, as they are a rich resource

of peptides, proteins, pigments, and secondary metabolites acting as nanobiofactories. They are also cost-effective, easy to harvest, and grow quickly, making them a good candidate for nanoparticle biosynthesis. They can also accumulate metals and reduce them to nanoparticles, making them the best choice for biosynthesis. Generally, several metal nanoparticles are biofabricated from all four types of algae: green (Chlorophyceae), blue-green (Cyanophyceae), red (Rhodophyceae), and brown (Phaeophyceae) (Mansour *et al.*, 2022).

Recent breakthroughs in nanotechnology have introduced produced nano-sized silver particles into biomedical and industrial applications for their inhibitory action against microorganisms. (Ibraheem I. *et al.*, 2016). Silver has long been known for its antibacterial qualities, but its significance in antimicrobial medical devices is still being contested. Silver compounds (silver nitrate, silver chloride, and silver oxide) are the preferred choice for infection-resistant coatings. AgNPs can be

utilized to treat human skin infections. (Ibraheem, Ibraheem BM. et al., 2017). used as arthroplasty and as dental materials Furthermore, AgNPs exhibit strong cytoprotective action against HIV-infected cells. Physical and chemical approaches are more expensive and more harmful to the environment than biological methods. Alternate biological sources for nanoparticle production include microbes, plant extracts, and plant biomass, which are environmentally acceptable.

1.3 SEAWEED

Seaweeds are a rich source of numerous natural byproducts and are used to synthesis nanoparticles to battle various biological disorders, which is now an increasing subject of interest in scientific research. (Mohanpuria *et al.*, 2008) (Murugesan *et al.*, 2017). *Acanthophora spicifera*, a non-native macroalga, interacts with native macroalgae (*Sargassum spp.*) and sponge assemblages in a subtropical Mexican Pacific embayment. The level of *A. spicifera* epiphytism on the native seaweed *Sargassum* fluctuated dramatically over time and was inversely proportional to *Sargassum* density and size. The highest intensity (up to 28 individuals per host plant) occurred when *Sargassum* was smaller and less dense (senescence period).

The lower intensity was recorded during *Sargassum* growth period, and the subsequent increase in intensity was attributed to *A.spicifera* high fragmentation period, as evidenced by a decrease in its average size and biomass, as well as the presence of larger free-floating accumulations in the subtidal zone. The facultative interaction between *A.spicifera* and *Sargassum* appears to be neutral, as no detrimental or positive impacts were observed on epiphytic or basibiont seaweeds. This invasive seaweed dominates hard substrates and poses a threat to other algae and benthic animals, warranting further research. Furthermore, *A. spicifera* was frequently found epizoic on epilithic sponges. Rhizome-like structures anchor this invasive seaweed to sponge tissue. Additionally, free-floating (Okafor *et al.*, 2013), (Parveen *et al.*, 2016) Antimicrobial activities and discovery of polyenoic fatty acids.

The current investigation reveals the anti-biofilm and anti-microbial effectiveness of synthetic AgNPs derived from *A. spicifera* against five well-known pathogenic strains, whose biofilm formation Plays a vital function in antibiotic resistance and pathogenesis. As far as we know, this work has Not previously described. in *A. spicifera*. This strategy is thus a viable method for green production of AgNPs with increased competency against biofilm-forming bacteria.

CHAPTER 2

REVIEW OF LITERATURE

Priyanka Singh proposed that the antibacterial and antibiofilm activity of AgNPs against *P. aeruginosa* strains is due to their tiny size and wide surface area, which allow them to interact with bacterial cells and rupture their membranes. AgNPs also prevent biofilm formation by altering the biofilm structure and causing oxidative stress Using naturally existing biological systems, including plants, bacteria, and yeast. (Osman A.I. *et al.*, 2023).

Green synthesis methods provide a clean, safe, and cost-effective process by using naturally occurring starting materials and reducing the use of toxic chemicals and huge energy inputs that are common in conventional approaches. Huston M. *et al.*, 2021).

Because of their unique properties, green-synthesised nanoparticles can be employed in a range of industries, including biomedical, agricultural, environmental remediation, culinary, sensors, electronics, and more. (Singh J.*et al.*, 2018).

This approach not only reduces pollution but also has promise antibacterial qualities, making green nanoparticles valuable in biomedicine for treating cancer and other infections, as well as enhancing medication delivery methods. (Farghali M. *et al.*, 2024).

Green-synthesized nanoparticles' small size, unusual morphology, and specific physical and chemical properties have made them appealing for usage in a range of industries, including biomedical, agricultural, environmental remediation, food, sensors, and electronics.(Vijayaram.S. *et al.*, 2024).

In materials science, "green" synthesis has received a lot of attention as a reliable, ecologically friendly, and sustainable methodology for synthesizing a variety of materials/nanoparticles, such as metal/metal oxide nanomaterials, hybrid materials, and bioinspired materials. (Dutta. T. *et al.*, 2018).

Green synthesis is a great alternative to traditional methods for producing metal and metal oxide nanoparticles. This process operates under ambient conditions and is not only safe and benign but also affordable, scalable, and simple. Metals and metal oxide nanoparticles, including gold, silver, and iron oxides, by employing a variety of bio-reductants derived from plant extracts. (Abuzeid HM. *et al.*, 2023).

Natural extracts, such as *M. oleifera* leaf extract, are utilized as reducing agents in the green manufacturing of silver nanoparticles to convert silver ions into metallic silver atoms. To make silver nanoparticles, the natural extract is often mixed with a solution of silver nitrate and heated to facilitate the reduction of silver ions. (Kim, KH *et al.*, 2018).

Using seaweed extracts in the environmentally friendly and sustainable synthesis of nanoparticles offers various advantages. Zinc oxide nanoparticles (ZnO-NPs) can be manufactured in an environmentally friendly manner utilizing seaweed extracts. This offers various benefits, including the capacity to manufacture nanoparticles of a variety of sizes and shapes, the utilization of non-toxic and economical bioactive chemicals, and applications in biomedicine, wastewater treatment, and food packaging. (Alprol AE. *et al.*, 2023)

The environmentally friendly and non-toxic technique known as "green synthesis" of silver nanoparticles (AgNPs) uses biological, plant, or microbial reducing and capping agents. Because of concerns about the use of hazardous solvents and precursor chemicals, as well as the production of toxic byproducts in traditional synthesis procedures, this method is becoming increasingly popular. (Simin Wei, *et al.*, 2022).

Because of the differences in bulk material behavior at the nanoscale, this technology has recently piqued the curiosity of a wide range of application fields. One example is the use of nanostructured materials as catalysts or adsorbents to extract toxic and harmful chemicals from air and water. Neem leaf extract was added to an aqueous solution of silver nitrate (AgNO₃), and the formation of crystalline structure was seen using a UV spectrometer. (Namratha N. *et al.*, 2013).

Environmentally friendly synthetic AgNPs have proved their potential as antimicrobial agents, with applications including clothing, medical equipment, medicines, water purification, and metal and pesticide adsorption. (Rautela .M. *et al.*, 2019).

AgNPs' antimicrobial activity is affected by their size, shape, and concentration; smaller, more anisotropic particles have higher antibacterial properties. (Varthini T. *et al.*, 2018).

Using plant extracts to create metal nanoparticles is one of the most simple, practical, cost-effective, and environmentally beneficial strategies to eliminate the usage of dangerous chemicals. (Ariana A. *et al.*, 2024).

Algae, particularly marine algae, are numerous and widely distributed, making them ideal for low-cost, mass production. (Chugh D, *et al.*, 2018).

Numerous studies have demonstrated the generation of silver nanoparticles using various algae species, including *Sargassum polycystum*. (Thiurunavukkarau R. *et al.*, 2022).

The scientific community is interested in green silver nanoparticle synthesis utilizing seaweed macroalgae due to its potential applications in a range of industries, including electronics, biotechnology, medicines, cosmetics, and antimicrobial

chemicals. The biological synthesis of nanoparticles is regarded to be the safest and cleanest approach because it does not require any toxic chemicals. (Das B. *et al.*, 2021).

According to these findings, the generated silver nanoparticles show high antibacterial and antimycobacterial capabilities. (Shanmugam S. *et al.*, 2022).

Silver particles can be manufactured in a more cost-effective and ecologically friendly manner by changing their size and stability using capping agents and algae-mediated synthesis. (Viswamalya VS. *et al.*, 2022).

We show how silver nanoparticles are produced and antibacterial active using the sea algae *Caulerparacemosa*. Fresh *C. racemosa* was collected in India's Southeast coast, in the Gulf of Mannar. (Kathiraven T. *et al.*, 2014).

Red macroalgae seaweed is employed in the green manufacture of silver nanoparticles via a process in which the seaweed acts as a natural reducing agent. This approach avoids the harmful effects of chemical manufacture and is environmentally friendly. (Bhuyar P. *et al.*, 2020).

Silver nanoparticles produced from marine seaweed have been shown in studies to have antibacterial activity against *Salmonella* and *Staphylococcus aureus*. These nanoparticles are characterized using a range of approaches, including as X-ray diffraction, Fourier transform infrared spectroscopy, scanning electron microscopy, transmission electron microscopy, and dynamic light scattering. (Shanmugam N. *et al.*, 2014).

Sargassum latifolium, a brown seaweed, produces sufficient alginate. Alginate silver nanoparticles outperformed alginate in terms of antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia*,

Escherichia coli, *Bacillus subtilis*, and *Bacillus cereus*. Alginate, on the other hand, is not effective against *E. coli*. (El-Sheekh M.M. *et al.*, 2022).

The process of producing nanoparticles (NPs) by microalgae-mediated synthesis is a relatively new topic of nanobiotechnology that uses microalgae's biomolecular corona as capping and reducing agents. To establish if a specific microalgal strain can create silver nanoparticles (Ag-NPs), this study first assessed the NPs' biological activities. The chemical composition of the algal extract was evaluated by GC-MS analysis. (Hamida RS. *et al.*, 2002).

Around the world, rocky shorelines are home to marine algae, which are red, green, or brown in color and serve as a food source for marine life. *Padina sp.*, a marine alga, synthesized and characterized silver nanoparticles using a UV-visible spectrophotometer, Fourier transform infrared spectroscopy, scanning electron microscopy, field emission scanning electron microscopy, and energy-dispersive X-ray spectroscopy. (Rahim M.H.A. *et al.*, 2020).

Specifically, reports of the green manufacture of silver nanoparticles have been reported using various algae strains, including *Ulvalactuca*. (Senthilkumar P. *et al.*, 2019).

We report a green method for producing pharmaceutically active gold nanoparticles by reducing chloroauric acid in *Acanthophora spicifera*, a marine red algae. When compared to *A. spicifera* extract, As-AuNPs have much stronger antioxidant activity. At 100 µg/ml, As-AuNPs had higher antibacterial activity against *Vibrio harveyi* than *Staphylococcus aureus*. As-AuNPs showed the maximum cytotoxicity against HT-29 colon cancer cells, with an IC₅₀ of 21.86 µg/ml. These findings confirmed that the synthesized (Babu B. *et al.*, 2020).

Although algae have long been used in agriculture and industry to produce food, feed, fertilizer, additives, cosmetics, and pharmaceuticals, the current trend is

toward green nanoparticle (NP) synthesis using algae. Algae are a rich source of secondary metabolites, easy to produce, develop quickly, and are scalable, therefore this trend is rising by the day. (Chaudhary R. *et al.*, 2020).

Algal biosynthesised nanoparticles have also been studied for their medicinal applications, which include antibacterial, antifungal, anti-cancerous, anti-fouling, bioremediation, and biosensing properties. These uses are made possible by the NPs' unique physicochemical properties, safety, and biocompatibility. This study discusses the thinking behind the biosynthesis of metallic, metallic oxide, and bimetallic nanoparticles (NPs) from various algae using algae as a mediator. In addition, a thorough examination of the process of NP biosynthesis from algae and its prospective applications in biomedicine was carried out. (Khan AK. *et al.*, 2020).

It has been reported that the production of AgNPs with the marine red algae *Acanthophora spicifera* possesses antibacterial properties. (Abbasi BH. *et al.*, 2020).

The environmentally benign, trustworthy, and financially viable approach of manufacturing nanoparticles (NP) is gaining traction. Biosynthesis uses biological agents such as yeast, fungi, bacteria, plants, and algae to produce desired compounds or materials. This chapter focuses on algae, a prolific and widely available source of sustainable resources for the manufacture of silver nanoparticles (AgNPs) and their distinctive properties. Furthermore, the distinguished AgNPs work toward cutting-edge applications in various areas of research and technology, including medicine. As a result, as compared to other NPs, the applications of AgNPs in biomedicine have been thoroughly investigated. (Khandegar V. *et al.*, 2022).

The antibacterial effectiveness of *Acanthophora spicifera* extract against human pathogens was investigated in the green production of silver nanoparticles. (Kaur P.J. *et al.*, 2022).

Using biological agents such as algal extracts, the production process converts silver ions (Ag⁺) to silver nanoparticles (Ag⁰). Enzymes like naphthoquinones found in algae and functional groups on bacteria's cell walls can convert Ag⁺ to Ag⁰. (Anjum S. *et al.*, 2020).

It has also been demonstrated that polysaccharides such as starch and chitosan may be employed to produce AgNPs. (Roy A. *et al.*, 2019).

The silver particle coated with green macroalgae synthesis demonstrated notable antibacterial and anti-mycobacterial activities. *Sargassum polycystum*-coated synthetic silver nanoparticles had the highest anti-pathogen activity of the three seaweed varieties tested. The silver nanoparticles behaved differently under several spectroscopic measurements. There was no or minimal toxicity observed against zebrafish. It may serve as a replacement agent with antibacterial and anti-mycobacterial activities. (Muralitharan G. *et al.*, 2022).

Silver nanoparticle (AgNP) biosynthesis is becoming a popular and ecologically friendly alternative to conventional chemical synthesis methods. AgNPs are becoming increasingly used as sensors, antibacterial and anticancer agents, imaging contrast agents, and nanoelectronic devices. Extracts of various marine algae species, such as *Ulvarigida* (green alga), *Cystoseiramyrica* (brown alga), and *Gracilaria foliifera* (red alga), were utilized as reducing and capping agents to synthesis biogenic AgNPs.(Gab-Alla A. *et al.*, 2022).

We report a green method for producing pharmaceutically active gold nanoparticles by reducing chloroauric acid in *Acanthophora spicifera*, a marine red algae. (Palanisamy S *et al.*, 2020).

Bacterial biofilm can be found almost wherever in nature, forming complicated colonies that adhere to both biotic and abiotic surfaces. Researchers have revealed that nearly all microbes on Earth live in biofilms. Microbial cells combine to create extracellular polymeric substances (EPS), which is responsible for biofilm formation. The content of extracellular polymeric substances (EPS) is influenced by the kind of

microorganism and the environment. Millions of bacteria encased in biofilm are the root cause of many serious disorders, including periodontal disease and cystic fibrosis. (Kumar P. *et al.*, 2012).

CHAPTER 3

AIM AND OBJECTIVES

1. AIM

To determine the antimicrobial properties of silver nanoparticles from seaweeds.

2. OBJECTIVES

- ❖ Collection and preparation of the seaweed extracts
- ❖ Green synthesis and Characterization of Silver nanoparticles using *Acanthopora specifera*.
- ❖ *Invitro* Antimicrobial activity of silver nanoparticles
- ❖ Anti-biofilm activity of silver nanoparticles

CHAPTER 4

MATERIALS AND METHODOLOGY

1. PREPARATION OF THE SEAWEED EXTRACT

1. Collection of the sample

Acanthophora specifera. Seaweed extract was used to create silver nanoparticles because it is affordable, widely available, and has medicinal effects. Fresh seaweed was collected from the Gulf of Mannar. They were surface cleaned with running tap water to remove dirt and other organic contaminants, then rinsed with double distilled water and air dried at room temperature. About 10 g of finely cut leaves were placed in a beaker with 100 mL of double distilled water and boiled for 30 minutes. The extract was cooled, filtered using Whatman No.1 filter paper, and stored at 4°C for future use.

2. Extraction of the sample

Extraction was carried out using hot water. The powdered seaweed was sterilized at 120°C for 15 minutes. Then, 20g of powder was combined with 100ml of distilled water and boiled in a boiling water bath at 60°C. The extract was filtered using Whatman No.1 filter paper and stored at 4°C for further research.

2. BIO-SYNTHESIS OF SILVER NANOPARTICLES

1. Synthesis of silver nanoparticles

To create AgNPs, 10 ml of seaweed extract was combined with 90 ml of 1 mM silver nitrate (AgNO_3) in a 250ml conical flask. The reaction mixture was then kept at room temperature and agitated magnetically. Color changes were noticed, and the formation of nanoparticles was studied with a UV-vis spectrophotometer, FTIR, XRD, particle size analyzer, Zeta potential, and transmission electron microscopy.

3. CHARACTERIZATION OF SILVER NANOPARTICLES

1. *UV-vis spectroscopy*

To study the optical characteristics of biosynthesized Ag NPs, samples were submitted to UV-vis spectroscopic examinations (Shimadzu UV/vis 1800 spectrophotometer) at room temperature with a resolution of 1 nm between 190 and 1100 nm. (Dash *et al.*, 2014).

2. *Fourier transform infrared spectroscopy*

Fourier transform IR spectroscopy was carried out on Ag NPs using a Perkin Elmer Spectrum RX I Fourier transform IR system with a frequency range of 500 to 4000 cm⁻¹ and a resolution of 4 cm⁻¹. The samples were prepared with the KBr pellet technique. (Chattopadhyay *et al.*, 2013).

3. *Particle size analysis and Zeta potential*

The zeta potential of the Ag NPs was evaluated using a Zetasizer-Nano ZS (Malvern, Malvern Hills, United Kingdom). Milli-Q water was used to generate a 1 mg/mL Ag NPs solution. Then this suspension was employed for the experiment. (Dash *et al.*, 2014).

4. *Scanning electron microscopy*

The particle size and microstructure were studied using high resolution scanning electron microscopy (SEM; Nikon, Japan) (Chattopadhyay *et al.*, 2013a). In summary, 1 mg/mL of Ag NPs were suspended in deionized water and sonicated in a sonicator bath until the sample formed a homogenous suspension. For size assessment, a sonicated stock solution of Ag NPs (1 mg/mL) was diluted 20 times with one drop of sonicated aqueous solution.

5. *X-ray diffraction study*

The solid state dispersions of Ag NPs were studied using X-ray powder diffraction. Diffraction patterns were obtained with a 240 mm-radius XPERT-PRO diffractometer (PANalytical Ltd., the Netherlands). The Cu K α radiation (K α 1.54060 Å) was Ni-filtered. A system with diverging and receiving slots measuring 1° and 0.1

mm was used. The pattern was acquired with 40 kV of tube voltage and 30 mA of tube current and scanned across a 2-hour range of 10-90°.

4. INVITRO ASSESSMENT OF SILVER NANOPARTICLES

1. **Antibacterial and antifungal activity**

1. *Test organism and Inoculum preparation*

Bacterial cultures such as *A.baumannii* (MTCC 9829), *E.coli* (MTCC 1302), and *S. aureus* (MTCC 740) were received from MTCC and kept at 4°C in the refrigerator until subcultured. The preserved bacterial strains were subcultured on nutrient agar and broth. The cultures were swabbed onto solidified nutrient agar plates using cotton swabs and incubated at room temperature for 24 hours.

2. *Agar well diffusion method*

The antibacterial and antifungal properties of *A.Specifera* was assessed using the agar well diffusion method. Nutrient agar was employed to create the bacterial medium. The agar medium was autoclaved at 121°C for 15 minutes, then put into sanitized plates and allowed to solidify. After the hardened plates had been swabbed with cultures, four wells were cut into the sterile cork borer and the samples were placed within. The plates were then incubated at room temperature for 24 hours, and the zone of inhibition was determined.

4.4.2 **Inhibition of biofilm formation Assay**

The reduction of biofilm development was investigated in *Pseudomonas aeruginosa*, a mucoid bacterium that may form biofilms. The cultures were grown overnight in MHB and diluted to 1×10^6 CFUs/mL. Bacterial suspensions (50 µL) in MHB were added to sample solutions (50 µL) in 96-well plates and incubated at 37°C for 18 hours. The samples were analyzed within the same concentration range as reported in the previous section. After incubation, remove the medium and fix the bacteria for 5 minutes in 200 µL of methanol anhydrous 99.8%. After removing the

methanol solution, cells were stained with 200 μ L of 0.2% crystal violet for 5 minutes, then washed .

CHAPTER 5

RESULTS AND DISCUSSION

1. PREPARATION OF THE SEAWEED EXTRACT

1. *Collection of the sample*

The seaweeds were processed according to the above mentioned protocol.



***Fig 5.1.1 Depicts the A.specifera
Seaweed***



***Fig 5.1.2 Depicts the grinded
seaweed sample***

5.2 Extraction of the Sample

Extraction was carried out using hot water. The powdered seaweed was sterilized at 120°C for 20 minutes. Then, 10g of powder was mixed with 100ml of distilled water and heated in a water bath at 60°C. The extract was filtered using Whatman No.1 filter paper and stored at 4°C for further research.



Fig 5.2.1 shows (A) Extraction & filtration Of the sample



Fig 5.2.2 shows (B) Hot water extraction

5.3 SYNTHESIS OF SILVER NANOPARTICLES

The silver nitrate solution was added with pure algae extract. The brown tint that formed within a few minutes indicates the formation of silver nanoparticles. The color changed to dark brown after one hour of incubation (Fig. b), indicating that free electrons in the reaction mixture had been activated.

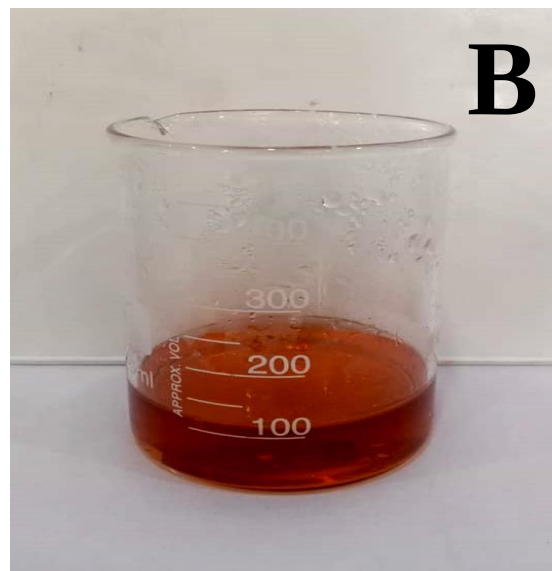
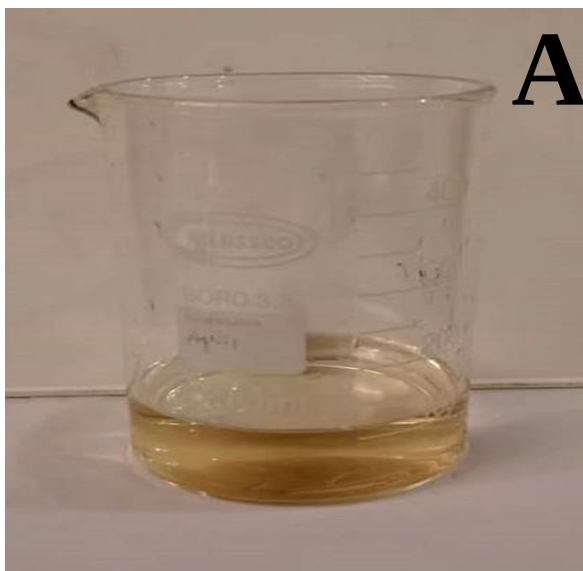


Fig 5.3.1 (A) *A.Specifera* extract diluted with distilled water

Fig 5.3.2 (B) Colour change during the reduction of AgNO_3 into AgNps by the *A.Specifera*.

4. CHARACTERIZATION OF SILVER NANOPARTICLES

1. ***UV-vis spectroscopy***

Using a UV-vis spectrophotometer, the reduction of silver ions into silver nanoparticles with *Acanthopora specifera* seaweed was studied. The UV absorption spectra of silver nanoparticles produced from brown seaweed extract are plotted as a function of reaction time. The absorption spectra show a peak at 400-500 nm, which suggests the creation of silver nanoparticles.

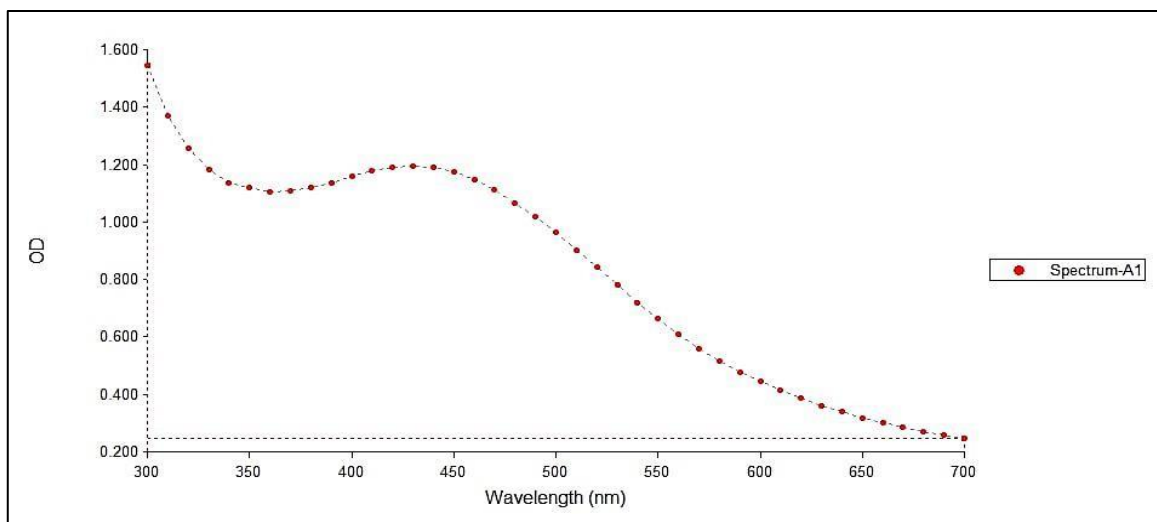
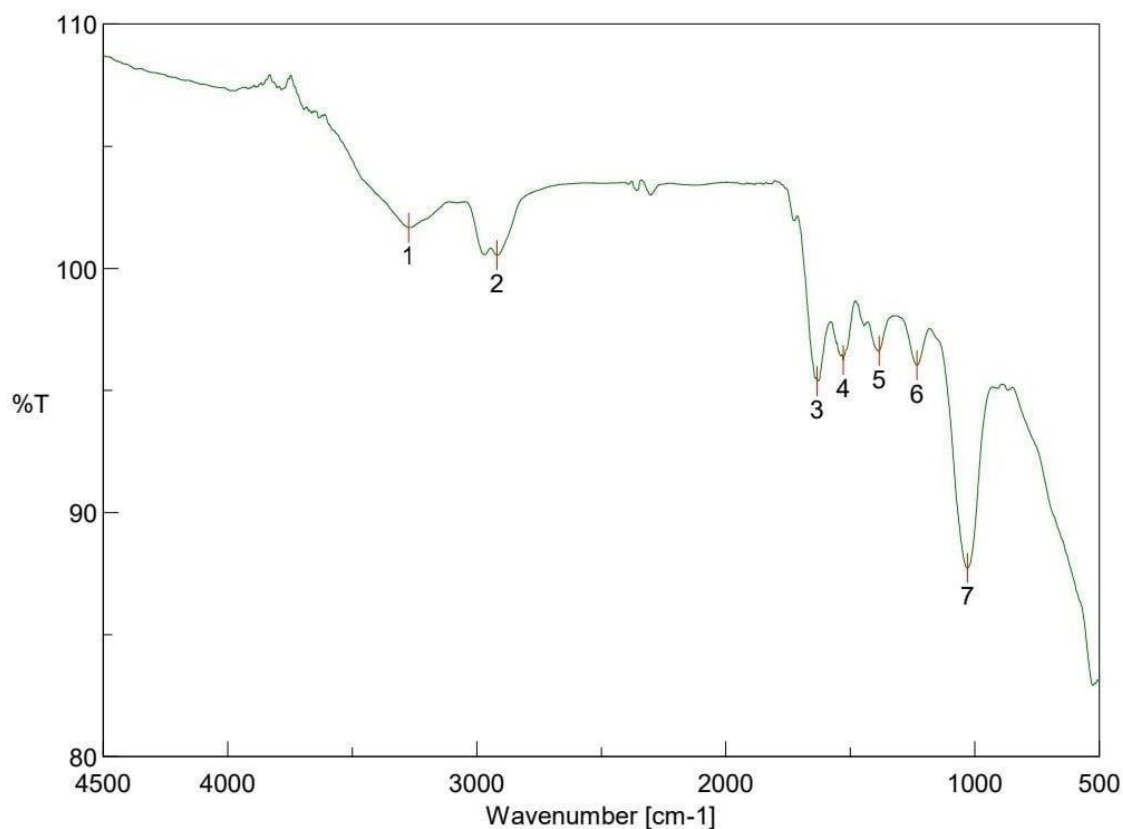


Fig 5.4.1 UV-vis spectroscopy representation of silver nanoparticle

5.5 Fourier transform infrared spectroscopy

FTIR spectra for nano composite films. Pure CG film spectra show a strong band at 3320 cm^{-1} due to stretching vibrations of pendant groups such as NH_2 and OH in CH and GE, respectively. The asymmetric stretching vibrations of CH_2 groups in the CH chain are assigned to their band at 2874 cm^{-1} . The IR bands at 1640 cm^{-1} represent $\text{C}=\text{O}$ stretching (amide I), 1551 cm^{-1} for N-H bending (amide II), and 1345 cm^{-1} for C-N stretching. The IR band at 1058 cm^{-1} represents the C-O-C stretching of the polysaccharide chain's link. The chemical structure of CH was unaffected by additions. The inclusion of chemicals does not ensure the emergence of a novel.



Result of Peak Picking:

1 - 3273.57; 2- 2918.73; 3 - 1633.41; 4 - 1528.73; 5 - 1383.68; 6 - 1232.29; 7 - 1029.8

Fig 5.5.1 Represents the FTIR analysis of the Silver nanoparticles

5.6 Particle size analysis and Zeta potential

The zeta potential of the Ag NPs was evaluated using a Zetasizer-Nano ZS (Malvern, Malvern Hills, United Kingdom). Milli-Q water was used to generate a 1 mg/mL Ag NPs solution. Then this suspension was employed for the experiment. (Dash *et al.*, 2014).

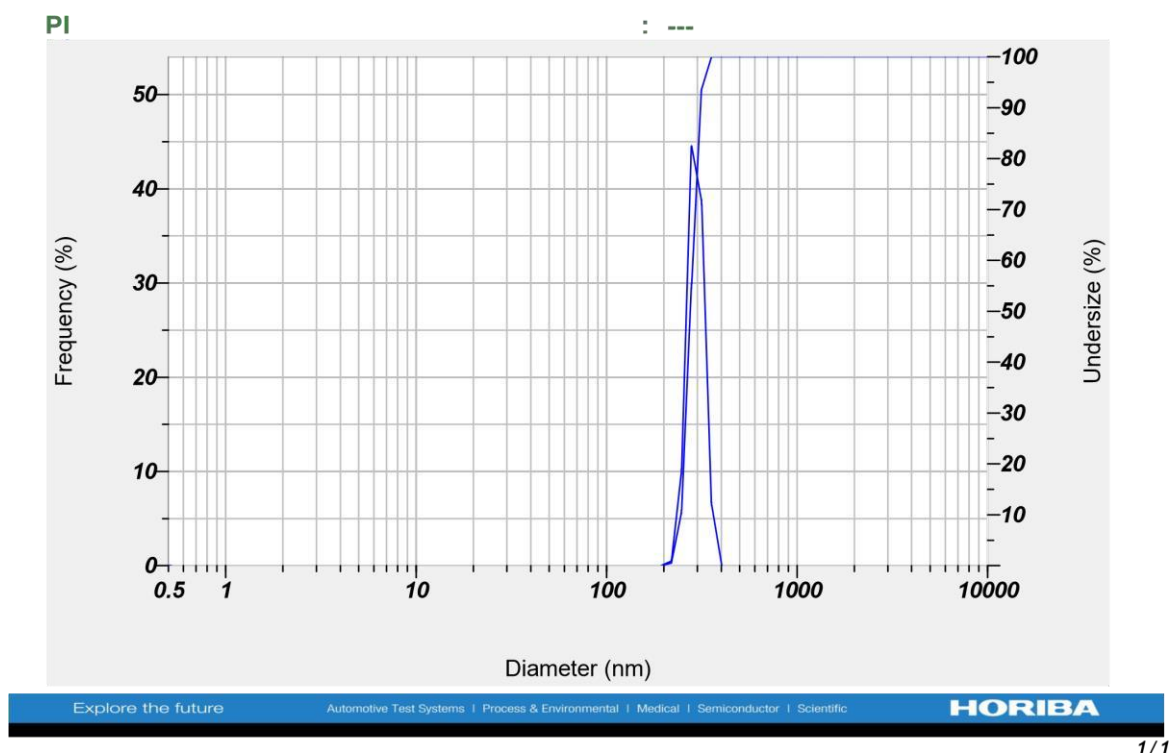


Fig 5.6.1 Represents the particle size analysis of the Silver nanoparticles

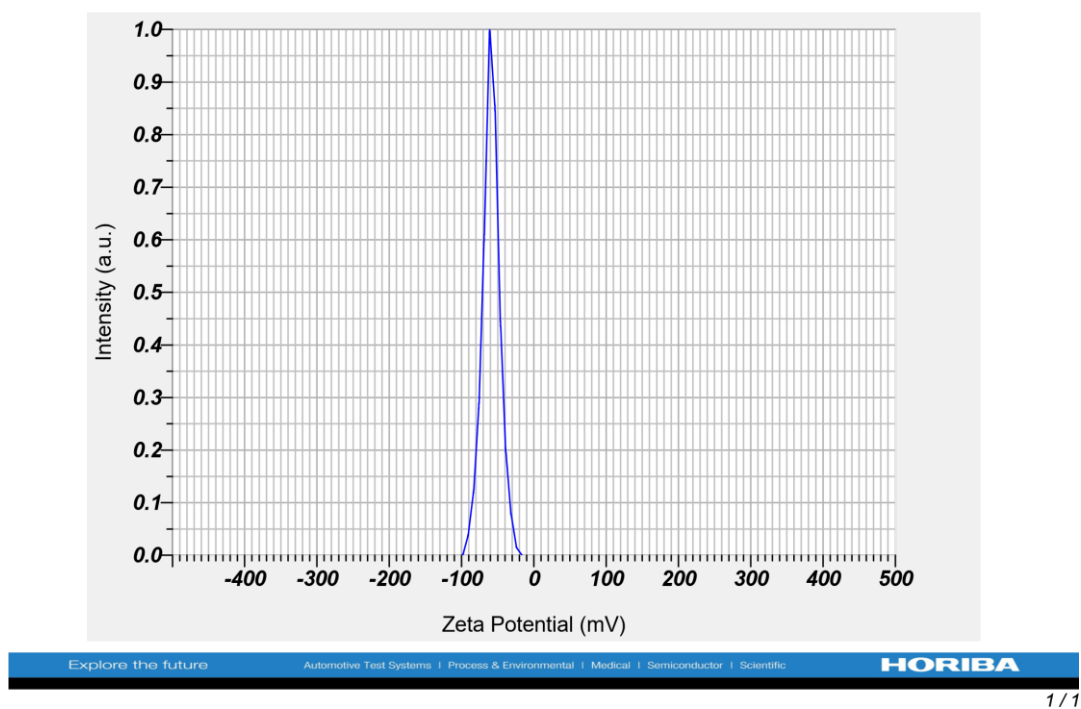


Fig 5.6.2 Represents the zeta potential analysis of the Silver nanoparticles

5.7 Scanning electron microscopy

The particle size and microstructure were studied with high resolution scanning electron microscopy (SEM; Nikon, Japan) (Chattopadhyay et al., 2013). In brief, 1 mg/mL of AgNPs was suspended in deionized water and sonicated in a sonicator bath until a homogenous suspension formed. To calculate size, a sonicated stock solution of Ag NPs (1 mg/mL) was diluted 20 times. A single drop of sonicated aqueous solution was then placed on a glass plate and allowed to dry. The sample was then coated in gold and photographed. The diameters of 27.24 nm, 43.58 nm, and 50.12 nm demonstrate the presence of silver nanoparticles. SEM was used to analyze the size and form of Ag nanoparticles.

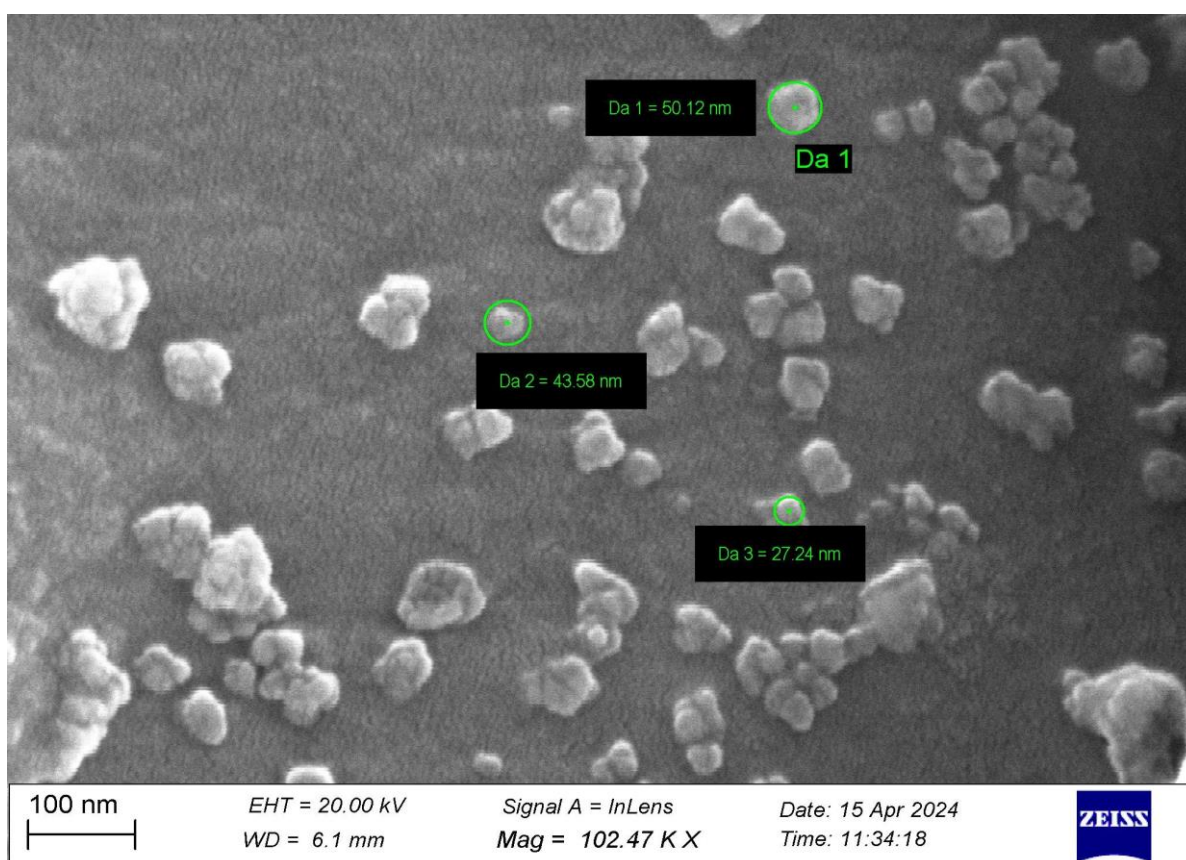


Fig 5.7.1 Represents the SEM characterization of Silver Nanoparticles

5.8 X-ray diffraction study

In XRD investigation, the peaks of photosynthesized AgNPs are assigned to the face-centered cubic (fcc) forms of metallic silver (JCPDS 04-0873).

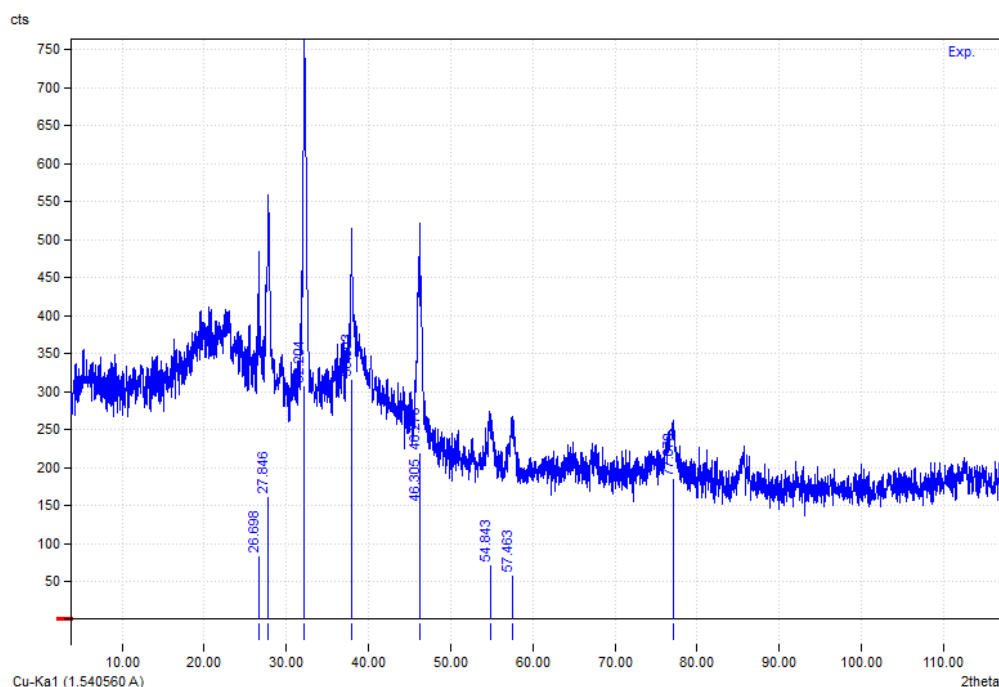


Fig 5.8.1 Represents the X-ray diffraction characterization of Silver Nanoparticles

9. INVITRO ASSESSMENT OF SILVER NANOPARTICLES

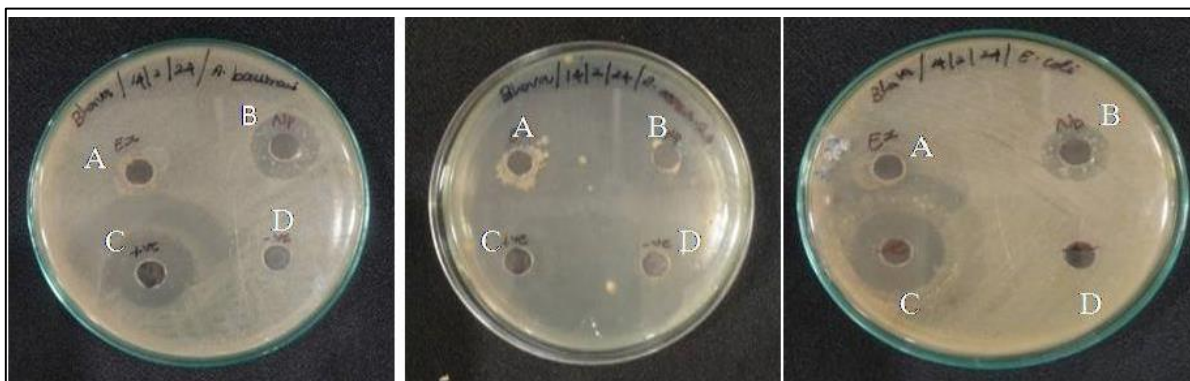
1. Antibacterial Activity of the Silver Nanoparticles

The created silver nanoparticles from *A. specifera* were tested against four distinct bacterial infections, as shown in the table. The activity of both the silver nanoparticle and the seaweed extract was tested to determine the nanoparticle's efficacy. Among the pathogens, the silver particles produced were more effective against *Staphylococcus aureus* 23, with a 6mm zone of inhibition.

Table 5.9.1 Test Organisms of Antibacterial Activity of the Silver Nanoparticles

Sl.No	Organism
1.	<i>Acinetobacter baumannii</i>
2.	<i>Staphylococcus aureus</i> 23
3.	<i>Escherichia coli</i>

5.9.1 Antibacterial Activity of Silver nanoparticles against the Test pathogens



(A- Aqueous extract of *Acanthophora specifera*; B- Silver Nanoparticle; C-Positive control; D- Negative Control).

Fig 5.9.1	Fig 5.9.2	Fig 5.9.3
Represents the Antibacterial activity of Ag Np's against <i>Acinetobacter baumannii</i>	Represents the Antibacterial activity of Ag Np's against <i>Staphylococcus aureus</i> 23	Represents the Antibacterial activity of Ag Np's against <i>Escherichia coli</i>

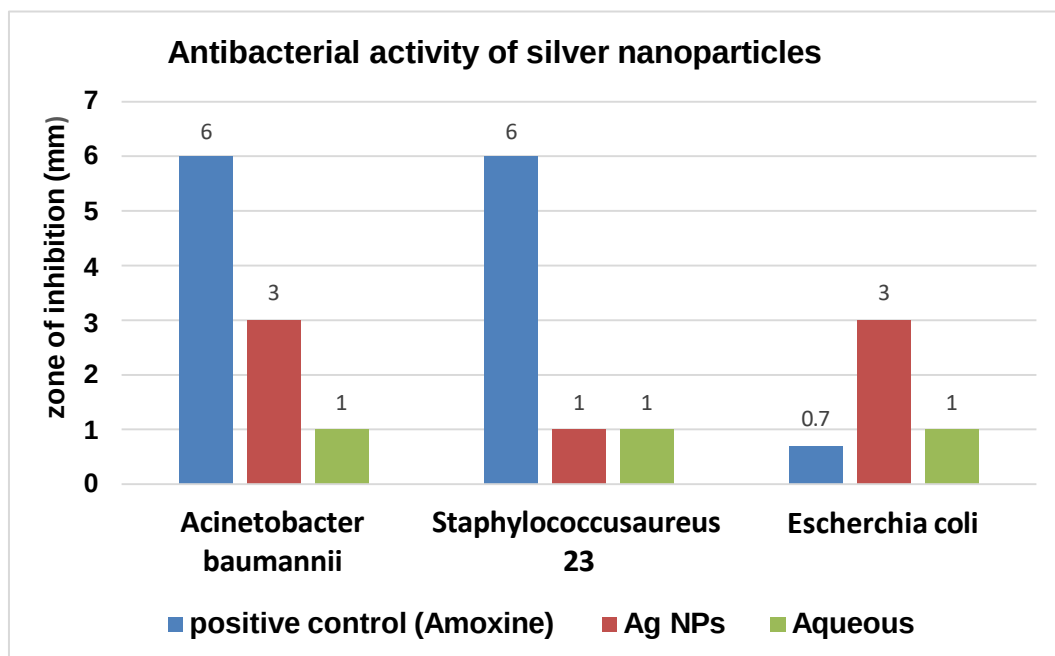


Fig 5.9.4 *Graphical representation of antibacterial activity of silver nanoparticles using agar well diffusion method*

Table 5.9.4 Zone of inhibition of the Silver nanoparticles antibacterial assessment.

SI. NO	Test Organisms	Positive Control (Amoxylin)	Negative control (DW)	Zone of Inhibition (mm)	
				Ag NPs	Aqueous Extract
1	<i>Acinetobacter baumannii</i>	6 mm	-	3 mm	1 mm
2	<i>Staphylococcus aureus 23</i>	6 mm	-	1 mm	1 mm
3	<i>Escherchia coli</i>	0.7 mm	-	3 mm	1 mm

5.10 ANTIFUNGAL ACIVITY OF THE SILVER NANOPARTICLES

The generated silver nanoparticles from *A.specifera* were tested against the fungal diseases indicated in the table. The activity of both the silver nanoparticle and the seaweed extract was tested to determine the nanoparticle's efficacy. The generated silver particles showed a 1mm inhibitory zone against *Candida albicans*.

5.10.1 Antifungal Activity of Silver nanoparticles against the Test pathogens



(**A**-Aqueous extract of *Acanthophora specifera*; **B**-Silver Nanoparticles; **C**- Positive Control ; **D**- Negative Control).

Fig 5.10.1 shows the zone of inhibition by Ag nanoparticle against *Candida albicans*

Table 5.10.1 Test Organisms of Antifungal Activity of the Silver Nanoparticles

Sl.No	Organism
1	<i>Candida albicans</i>

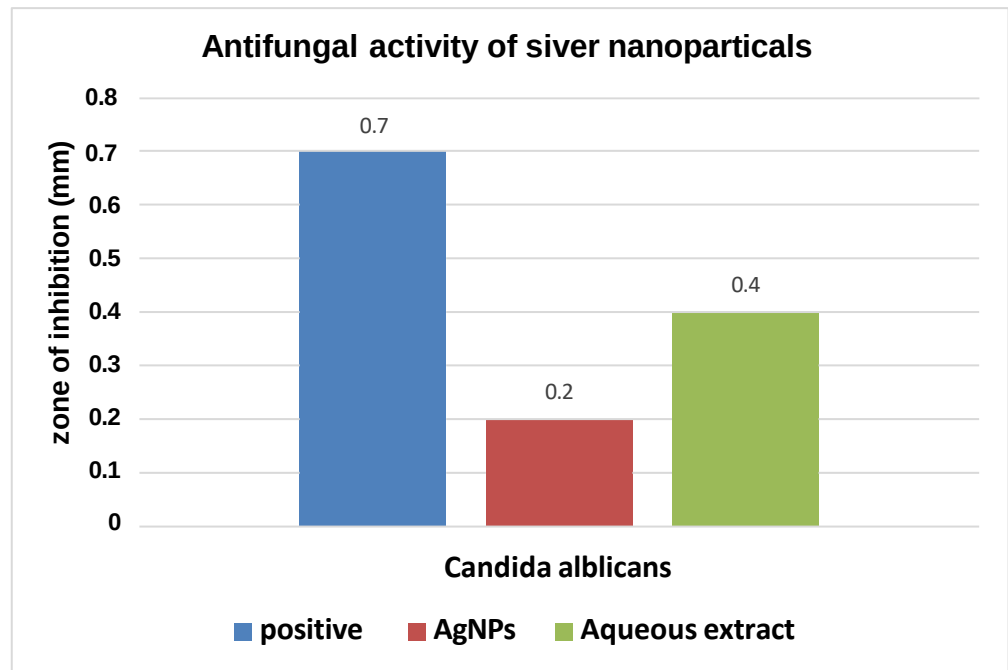


Fig 5.10.2 Graphical representation of antifungal activity of silver nanoparticles using agar well diffusion method

Table 5.10.2 Zone of inhibition of the Silver nanoparticles antifungal assessment.

SI.No	Test Organisms	Positive Control (Nalidixic Acid)	Negative control (DW)	Diameter of Zone of Inhibition (mm)	
				Ag NPs	Aqueous Extract
1	<i>Candida albicans</i>	0.7 mm	-	0.2mm	0.4mm

5.11 ANTIBIOFLIM ACTIVITY OF SILVER NANOPARTICLES

Our studies revealed that silver nanoparticles had active bio-film characteristics that may aid in biofilm resistance. At a dosage of 200 µg/mL, 70% of *Pseudomonas aeruginosa* biofilm was suppressed, compared to the untreated biofilm. AgNps treatment at IC50 resulted in a considerable reduction (65-70%) in biofilm growth. Overall, silver nanoparticles inhibited biofilm development in *Escherichia coli* in a dose-dependent way.

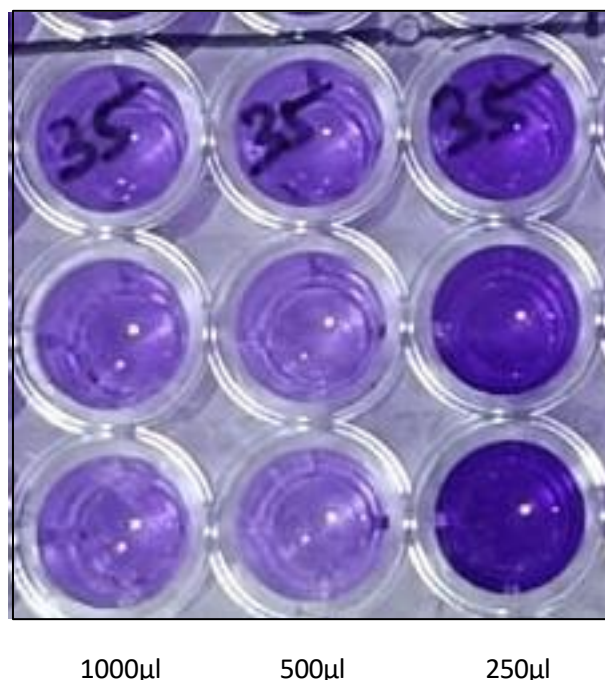


Fig 5.11.1 Represents the Biofilm inhibition assay against *Psueodomonas aeruginosa* using Silver nanoparticle by Mtp assay

The production of silver nanoparticles (AgNPs) from the marine seaweed *Acanthophora specifera* has gained significant attention due to its potential applications in medicine and biotechnology. *A.Specifera*, with its abundance of bioactive compounds, is an ideal source for green AgNP synthesis. This session will go into the manufacturing method and the antibacterial, antifungal, and antibiofilm properties of these nanoparticles against human illnesses. The green synthesis of AgNPs involves reducing silver ions with bioactive compounds present in *A.Specifera*. To obtain silver nanoparticles, we used a biological synthesis technique. This approach is safe for the environment, inexpensive, and offers a long-term alternative to standard chemical synthesis. The use of seaweed extracts, particularly those from marine sources, ensures the presence of several phytochemicals that contribute.

CHAPTER 6

SUMMARY AND CONCLUSION

In this study, we proposed using a low-cost marine red seaweed called *Acanthophora specifera* as a reducing mediator to generate silver nanoparticles in an environmentally acceptable way. We used a UV-Vis spectrophotometer to investigate the nanoparticle formation process. This yielded a peak value of 470nm. X-Ray Diffraction patterns were utilized to determine the nanoparticles' crystalline nature, and a SEM micrograph revealed that the nanoparticles had an average size of about 96 nm and a spherical shape. FT-IR is used to identify possible biomolecules such as amide and polyphenol groups that could be responsible for converting silver nitrate into silver nanoparticles. It was revealed that the produced metal nanoparticle had broader antibacterial activity against *Acinetobacter baumannii*, *Staphylococcus*, and *Escherchia coli*.

To summarize, this effort effectively produced silver nanoparticles from *Acanthophora specifera* and investigated their diverse bioactivities. The antibacterial, antifungal, and antibiofilm capabilities of these nanoparticles make them interesting candidates for pharmaceutical and medical applications, opening up new pathways in the fight against microbial illnesses. Furthermore, their antibiofilm activity research adds to the developing field of nanotechnology-based healthcare solutions and emphasizes the need of using natural resources, such as marine seaweed, to synthesize nanoparticles in a sustainable and effective manner.

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