

BIOSYNTHESIS OF SELENIUM NANOPARTICLES USING MICROALGAE FOR ANTIBACTERIAL ACTIVITY

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

by

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

SATHYABAMA

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

Accredited with Grade “A” by NAAC

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This is to certify that this Project Report is the bonafide work of **KAVYA J (40770022)** who carried out the project entitled "**BIOSYNTHESIS OF SELENIUM NANOPARTICLES USING MICROALGAE FOR ANTIBACTERIAL ACTIVITY**" under our supervision from October 2022 to April 2023.

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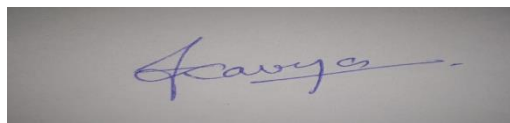
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DECLARATION

I, **KAVYA J (40770022)** hereby declare that the entitled **“BIOSYNTHESIS OF SELENIUM NANOPARTICLES USING MICROALGAE FOR ANTIBACTERIAL ACTIVITY”** done by us under the guidance of **Dr. R. THYAGARAJAN** (Internal) and **Dr. T. RAJASEKAR** (External) at **COL.DR. JEPPIAAR RESEARCH PARK, CENTRE FOR DRUG DISCOVERY AND DEVELOPMENT** is submitted in partial fulfillment of the requirements for the award of Bachelor of Science Degree in Biotechnology, School of Bio and Chemical Engineering.

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A rectangular box containing a handwritten signature in blue ink. The signature appears to be 'Kavya J.' with a long horizontal stroke extending to the right.

SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENT

We are pleased to acknowledge our sincere thanks to Board of Management of SATHYABAMA for their kind encouragement in doing this project work and for completing it successfully. We are grateful to them.

We convey our thanks to Dr. V. Ramesh Kumar, School of Bio and Chemical Engineering, Head of the Department, Department of Biotechnology for providing us with necessary support and details at the right time during the progressive reviews.

We would like to express our sincere and deep sense of gratitude to our Project Guide Dr. R. Thyagarajan (Internal Guide) and Dr. T. Rajasekar (External Guide) for their valuable guidance, suggestions and constant encouragement paved way for the successful completion of our project work.

I would like to thank Dr. S. Kumaran for being supportive of my project and contributing to my progress with the project at the Centre for Drug Discovery.

We wish to express our thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology who were helpful in many ways for the completion of the project.

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ABSTRACT

Selenium nanoparticles offer a wide range of possible uses. As a result, they have received greater attention recently, and several synthesis techniques have been used. Microalgae are abundant in antimicrobial compounds and offer a less risky, more affordable method of treating bacterial illnesses. It is known that the photoautotrophic microorganisms known as micro-algae create a variety of secondary metabolites with different biological effects. They are renowned for being rich in bioactive substances that have potential uses in medicinal formulations. To produce selenium nanoparticles, the microalgae are cultivated and biosynthesized using sodium selenite. Additionally, the nanoparticles were examined using a UV-Spectrophotometer, which produced a peak value of 200-300 nm; when tested for stability using the Zeta potential method, the nanoparticles produced a value of -14.5 mV that has a high stability. Then, the FTIR (Fourier Transform Infrared) analysis of a number of bio-organic compounds revealed with the peak values that these compounds aid in the synthesis and stabilisation of the nanoparticle, and the XRD (X-Ray Diffraction) that has been compared with the graphs of sodium selenite concludes that the sodium selenite, which is in crystal form, is reduced to the nanoparticle form during the biosynthesis. After the antibacterial activity is done by the agar well diffusion method and shows the positive activity against the five pathogens *Pseudomonas. sp*, *Klebsila. sp*, *Bacillus. sp*, *Staphylococcus. sp* and *E.coli. sp*.

Keywords: Microalgae, Antibacterial, Multidrug resistance, Selenium Nanoparticles, Agar-well diffusion.

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LIST OF SYMBOL AND ABBREVIATION

ACRONYM	DESCRIPTION
MDR	Multidrug Resistance
mm	Millimetres
μL	Microlitre
PUFAs	Polyunsaturated fatty acids
nm	Nanometer
Na ₂ SeO ₃	Sodium Selenite
PVA	Polyvinyl alcohol
NaSe	Sodium selenite
SCF	Stem cell factor
DI	Deionized water
SeNPs	Selenium Nanoparticles
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
FDA	Food and Drug Administration
PLGA	Poly lactide-co-glycolide
°	Degree
R	Resistance
UV	Ultra-violet
SEM	Scanning Electron Microscope
EDX	Energy Dispersive X-Ray
XRD	X-Ray Diffraction
FT-IR	Fourier Transform Infrared spectroscopy
Se	Selenium
PL	Post larvae
DLS	Dynamic Light Scattering
UV-Vis	Ultraviolet-Visible spectroscopy
AFM	Atomic Force Microscopy

mV	Millivolt
TEM	Transmission Electron Microscope
GC-MS	Gas Chromatography–mass spectrometry
<i>K.pneumonia</i>	<i>Klebsiella pneumoniae</i>
<i>P.aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
<i>A.baumannii</i>	<i>Acinetobacter baumannii</i>
CS	<i>Chitosan</i>
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
DPPH	2,2-diphenyl-1-picrylhydrazyl
ppm	Parts per million
AsAc	Ascorbic acid
%	Percent
ml	Millilitres
Mins	Minutes
Hrs	Hours
rpm	Rotation per minute
cm	Centimeter
kV	Kilovolt
mA	Milliampere
°C	Celcius
<i>He-Ne laser</i>	Helium-neon laser
<i>HR-TEM</i>	High-Resolution Transmission Electron Microscope
g	Gram
PI	Polydispersity
ROS	Reactive oxygen species
DNA	Deoxyribonucleic acid

CHAPTER 1

INTRODUCTION

1. Bacteria

1. *Bacterial infections*

The invasion and expansion of germs within the human body results in bacterial illnesses. They can induce a wide range of symptoms, from minor to severe, in various areas of the body. Antibiotics can be used to treat bacterial infections, but occasionally these bacteria develop antibiotic resistance and represent a major health danger. The bacteria *Staphylococcus aureus* is frequently responsible for skin diseases such as boils, abscesses, and cellulitis. More severe infections including endocarditis, bloodstream infections, and pneumonia can also be brought on by it. *Escherichia coli* is a bacteria that typically inhabits the human gut and can inflict diseases including diarrhea, bloodstream infections, and urinary tract infections (Bacterial infection, Medline Plus, 2021).

2. *Multidrug resistance bacteria*

- I. A public health issue is the rise of bacterial strains that are resistant to antibiotics. Compared to infections caused by sensitive strains, infections caused by antibiotic-resistant strains require a longer hospital stay, have a worse prognosis, and cost more money. As opposed to single resistances, multidrug resistance (MDR) may be associated with an even higher risk of improper therapy and a longer time to a suitable course of treatment. Despite the clinical significance of MDR, there are far less models looking into the variables that encourage MDR evolution than those that favour the evolution of single resistance. It is important to make a distinction between the elements that contribute to MDR's development and those that contribute to its spread. MDR strains can arise from a single biological mechanism that confers resistance to multiple medications, from multiple genes linked together genetically on a

chromosome or plasmid that confer multiple antibiotic resistances, or from numerous mutations that have evolved over the course of treatment in a host. Three theories have been put forth about the spread of MDR strains. When resistance has a fitness cost, aggregating the costs of individual resistances may not provide the whole cost of MDR, which would benefit the evolution of MDR over single resistances.

- II. MDR strains may be overrepresented at the population level if the host population is organised into distinct classes that receive antibiotics at varying rates because MDR strains are preferred in the classes that get high rates of antibiotic treatments.
- III. Multiple antibiotic prescriptions may encourage MDR in the host population. Bystander selection refers to the phenomena wherein many bacterial species are largely commensal and come into touch with antibiotics not because they themselves produced an illness but rather because their host is being treated for diseases brought on by other species. The whole range of antibiotics provided to the host population might occasionally select for MDR in these species, which is possible (Jacopin *et al.*, 2020).

With an estimated 100000 tonnes of antibiotics generated annually on a worldwide basis, antibiotic use has significantly impacted the existence of bacteria on earth. Antibiotic resistance in pathogen strains is increasing, and some have evolved tolerance to many drugs. Antibiotics and chemotherapeutic medications are both affected by the syndrome known as multidrug resistance (Nikaido *et al.*, 2014).

1.2.1 Microalgae

Marine microalgae are viewed as a potentially innovative and significant pharmaceutical, nutraceutical and cosmetic sectors they may be easily grown and are short drug discovery generation times may be sped up and an ecologically sustainable approach to drug research made possible by addressing difficulties with the overuse of marine resources and the use of risky collecting techniques. Microalgae are photosynthesis-

producing eukaryotes those are critical elements of aquatic and ocean microalgae and act major basic producers and nutrition sources. Several aquatic life are also exceptional producers and suppliers for lipids, carotene, the omega-3 fatty acids and some other great compounds (Lauritano *et al.*, 2016). A diverse family of plants known as microalgae produces unique bioactive compounds carbohydrates make up the bioactive chemicals that algae developing cells create lipids and proteins enzymes free amino acids from proteins fats lipids circumstances of growth pigments threatening substances only a few examples are antibiotics phenol flavonoids and vitamins (Hamayeli *et al.*, 2019).

While not being required for fundamental cellular function, the broad collection of chemicals known as microalgal secondary metabolites are acknowledged to contain major biological characteristics, such as antibacterial properties. From among microalgae-based compounds having antibacterial properties includes fats, glycolipids, polyphenolic compounds, terpene, -diketone, and indo alkaloid. Researchers have been able to study the challenging subject of natural product screening as technology to detect, filter, isolate, and identify chemicals found in complex extracts has become more readily available (Little *et al.*, 2021).

Prokaryotic and eukaryotic cellular creatures, microalgae range in size from a few millimetres to about 200 millimetres, and they can generate around 50% of the oxygen that exists on Earth. Photosynthesis which produces a variety of bio products like as polysaccharides, lipids, pigments, proteins, vitamins, bioactive substances, and antioxidants. Only 104 species, which are divided into the *Chlorophyceae* (green algae), *Cyanophyceae* (blue-green algae), *Chrysophyceae* (golden algae), *Rhodophyceae* (red algae), *Phaeophyceae* (brown algae), and *Bacillariophyceae* families, have been described in the literature, according to estimates of the number of species that are currently available. Microalgae have sparked an increasing amount of attention in both academic and professional circles because of their wide range of

applications in sectors including nutraceuticals medicines and renewable energy they certainly do in some aspects they differ from bioprocesses that use feed stocks such as lingo cellulosic bio masses and need transportation to the bio refinery facilities since they may be farmed nearby established industrial areas with pre-existing infrastructure. Many elements including climate sunlight as well as the nutrient availability have an impact on the development of microalgae as well as operational factors like fluid and hydrodynamic stress mixing culture depth dilution rate and harvest frequency elements including gas exchange saline cell density and pH should also be examined (Zuccaro *et al.*, 2020).

1.2.2 Bioactive compounds from microalgae

Aquatic habitats are home to a variety of single-celled, photosynthetic organisms known as microalgae. They have attracted interest lately because of their potential as a source of bioactive substances. Bioactive chemicals are naturally occurring substances with the capacity to influence bodily physiological processes. Among the bioactive substances discovered in microalgae are:

- I. Carotenoids are the pigments responsible for the distinctive hues of microalgae. They possess antioxidant qualities and are linked to a lower chance of developing chronic illnesses including cancer and cardiovascular disease.
- II. Microalgae are abundant in polyunsaturated fatty acids (PUFAs), particularly omega-3 and omega-6 fatty acids. These important fatty acids help keep people healthy and are linked to a lower risk of heart disease, stroke, and other chronic illnesses.
- III. Phycobiliproteins: Some species of microalgae have these water-soluble pigments. They have been investigated for their potential as a natural food colourant and for use in medical imaging because they contain anti-inflammatory and antioxidant qualities.
- IV. Polysaccharides: Complex carbohydrates found in microalgae have a variety of biological benefits, including as anti-inflammatory, immune-modulatory, and antioxidant properties. It has been demonstrated that certain microalgal polysaccharides

have the ability to treat a number of disorders.

All things considered; microalgae are a promising source of bioactive substances with a variety of potential health advantages. Identification, characterization, and exploration of these molecules possible uses in the food, pharmaceutical, and other sectors are the main goals of ongoing study (Guedes *et al.*, 2011).

1.3.1 Nanoparticles

Nanoparticles are defined as particles with a size of 1–100 nanometers (nm). Numerous processes, including as chemical synthesis, mechanical abrasion, and laser ablation, can be used to create them. Nanoparticles differ from their bulk counterparts in a variety of physical, chemical, and biological ways because of their tiny size and high surface area to volume ratio. They may be applied in a variety of fields, including as electronics, imaging, catalysis, and medication delivery. Metals, semiconductors, ceramics, and polymers, among other materials, can be used to create nanoparticles. The nanoparticles gold, iron oxide, quantum dots, and carbon nanotubes are a few examples of those that are often employed.

Drug delivery is one application for nanoparticles in science. Drug delivery may be made more effective and efficient by using nanoparticles, which can be created to target certain cells or tissues in the body. They can also make medications more soluble and bioavailable while preventing them from degrading. The potential toxicity of nanoparticles, especially when they are discharged into the environment, is a matter of concern. Researchers are developing techniques to reduce any bad effects and striving to understand the potential hazards. Overall, the usage of nanoparticles shows tremendous promise for a variety of applications; nevertheless, before using them, it is crucial to carefully weigh their potential advantages and hazards (Makadia and Siegel, 2011)

2. Selenium nanoparticles

The trace selenium element is an inorganic kind with a possible anti-inflammatory action, whereas sodium selenite (Na_2SeO_3) seems to be a water-soluble and white crystalline solid. The raw materials were sodium selenite, which came in the form of white, crystalline solid powder with a molecular weight of 172.94 g/mol. Without additional purification, both research items were obtained. Using ultrasonic waves and the solution casting process, a combination of PVA doped with $\text{Na}_2\text{O}_3\text{Se}$ was effectively synthesised to produce PVA: NaSe SCF samples with various Selenite fillers. To create the clear PVA solution, 45 g of PVA powder was sprayed into 1 Liter of deionized water (DI) using a combination of stirring magnetic equipment for 48 hours at a temperature of 80°C (Bouzidi *et al.*, 2022).

Selenium nanoparticles are extremely small selenium atom-based particles with distinct physical and chemical characteristics. These nanoparticles have drawn a lot of interest recently because of the potential uses for them in a variety of

industries, including electronics, agriculture, and medicine. Selenium nanoparticles have demonstrated remarkable anti-inflammatory, anticancer, and antioxidant properties in the medical area. They have been utilised as imaging agents for the detection and treatment of cancer as well as in medication delivery systems. The use of selenium nanoparticles in agriculture has improved plant growth and resilience to a variety of environmental stresses. Overall, selenium nanoparticles show great promise for a variety of applications, and scientists are still learning more about how to use those (Wang *et al.*, 2019).

3. Antibacterial activity with the help of biosynthesized selenium nanoparticles

Due to their distinctive characteristics, including their high surface area-to-volume ratio, strong reactivity, and capacity to permeate cell

membranes, selenium nanoparticles (SeNPs) have demonstrated potential as antibacterial agents. An economical and environmentally beneficial technology, biosynthesis of SeNPs utilising microbes or plant extracts has attracted growing attention in recent years. Biosynthesized SeNPs have been shown in several investigations to exhibit antibacterial action against both Gram-positive and Gram-negative pathogenic bacteria. SeNPs have been found to suppress bacterial growth by causing oxidative stress, bacterial cell membrane damage, and cell metabolic disruption.

Escherichia coli, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were all effectively inhibited by SeNPs created utilising the fungus *Aspergillus terreus*. According to the study, SeNPs damaged the bacterial cell membrane and allowed internal components to flow out, which finally caused the bacteria to die. Strong antibacterial efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA) was demonstrated by biosynthesized SeNPs. According to the study, SeNPs prevented MRSA from spreading by producing oxidative stress and rupturing the bacterial cell membrane. Overall, biosynthesized SeNPs have demonstrated significant promise as antibacterial agents, and additional study in this field may result in the creation of fresh, strong antibacterial treatments. Prior to its application in clinical settings, SeNPs must first be completely understood in terms of their mode of action and potential toxicity through more research (Kumar *et al.*, 2019).

CHAPTER 2

LITERATURE SURVEY

Nikaido *et al.*, (2009) reported that high doses of antibiotics used to humans, farmanimals, and even fish in aquaculture led to the selection of pathogenic bacteria that were resistant to a number of different medications. Bacteria may develop multidrug resistance by one of two ways. Secondly, each gene that codes for drug resistance can accumulate many times within a single cell of these bacteria. This build-up often takes place on resistance (R) plasmids. Second, increased gene expression for multidrug efflux pumps, which extrude a variety of medicines, may result in multidrug resistance.

Guedes *et al.*, (2011) stated that Astaxanthin, a well-known antioxidant generated by *Haematococcus pluvialis*, is an instructive example with significant anti- inflammatory and antitumoral effects. A few chemicals synthesised by microalgae have in fact demonstrated to exhibit anti-inflammatory, antiviral, antibacterial, and antitumoral properties. Many of these substances are polysaccharides or long- chain fatty acids, which can be saturated or unsaturated. Furthermore, they frequently have unusual chemical structures, and their concentrations can be higher than those in many other natural sources. The mode of culture, make-up of the growing medium, and nutrient profile all have a significant impact on the productivity and biochemical makeup of the microalgae.

Makadia and Siegel, (2011) experimented that in addition to having a wide variety of erosion times, customizable mechanical characteristics, and most significantly, being an FDA-approved polymer, PLGA is also biocompatible and biodegradable. In particular, PLGA has received a great deal of attention in the creation of tools for the controlled administration of small molecule medications, proteins, and other macromolecules in both research and commercial applications. The

numerous manufacturing methods for these devices are covered in this book, along with the elements influencing their deterioration and drug release.

Sarkar *et al.*, (2011) experimented that the atomic force microscopy, scanning and transmission electron microscopy, as well as dynamic light scattering investigations, all contributed to the understanding of how monodisperse spherical α -selenium nanoparticles in the size range of 30 to 150 nm came into being. The nano-Se's X-ray diffraction spectrum showed a large peak at 2θ angles of $15-35.8^\circ$, indicating its amorphous nature. Research using energy-dispersive X-rays identified selenium in the nanoparticles. The existence of a protein shell around the nanoparticles was verified by Fourier transform infrared spectroscopy, which in turn supports their stabilisation. Here, a unique technique for producing nano-selenium has been described. A recent literature review revealed that this is the first account of mycosynthesis of selenium nanoparticles.

Von *et al.*, (2013) proposed that the best prospects for waste-gas and water treatment are microalgae. Salinity, however, frequently changes across several places. The ideal microalga for uniform culture across multiple remediation sites would have a broad tolerance for salinity and consistent biochemical profiles. This study sought to understand how salinity affected *Picochlorum atomus* growth, biomass production, nutrient absorption, and metabolic profiles. The results of 4-day nutrition restriction were also studied to see if target end-products might be altered.

Lauritano *et al.*, (2016) experimented that the food business, as well as the pharmaceutical, nutraceutical, and cosmetic industries, are thought to benefit from the use of marine microalgae as a potentially new and important source of biologically active chemicals. By resolving issues related to the overuse of marine resources and the use of hazardous collecting methods, they can be readily cultivated, have quick generation periods, and provide an environmentally responsible approach to drug development. In this work, 21 diatoms, 7 dino-flagellates, and 4 species

of flagellates were cultured under three distinct conditions, and extracts from these cultures were examined for potential antioxidant, anti-inflammatory, anti-cancer, anti-diabetes, antibacterial, and anti-biofilm properties. To show the variability in clone bioactivity, we evaluated two more clones for three diatoms.

Satgurunathan *et al.*, (2017) stated that sodium selenite was used to create selenium nanoparticles (SeNPs), which were then characterised by UV, SEM, EDX, XRD, and FT-IR. These tests indicated that the particles were uniform, monodispersive, and very stable, and ranged in size from 48 to 87 nm. *Macrobrachium rosenbergii* post-larvae (PL) were fed the enriched *Artemia* nauplii for 30 days as live feed. The results showed that the 12 h enriched *Artemia* nauplii significantly ($P < 0.05$) increased the survival rate, growth rate, nutritional indices, and concentrations of tissue biochemical constituents of *M. rosenbergii* PL, followed by the 1 h enriched *Artemia* nauplii.

Fritea *et al.*, (2017) experimented that a vital trace mineral with a variety of biological impacts is selenium (Se). Numerous physicochemical and biological techniques for the manufacture of selenium nanoparticles (SeNPs) have been documented in the literature. The purpose of this work was to develop an environmentally friendly approach to produce SeNPs using an extract from parsley (*Petroselinum crispum*) leaves. Using analytical methods including dynamic light scattering (DLS), UV-vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and Atomic Force Microscopy (AFM), SeNPs were characterised from a structural and morphological point of view. Particles with a maximum distribution measured by the DLS technique were 400 nm in size and had an apparent zeta potential of - 14.2 mV.

Hussein *et al.*, (2019) stated that the effectiveness of certain negative and positive gramme pathogenic bacteria against crude bioactive compounds derived from *Chlorella vulgaris*. *Chlorella vulgaris*' antibacterial activities have been examined in vitro utilising agar well diffusion techniques against certain gram-positive and gram-negative

harmful bacteria. The results obtained have demonstrated that the basic bioactive components of Wide-ranging antibacterial activities of *Chlorella vulgaris* are present against gram-negative and gram-positive bacteria isolated from tooth decay. This study demonstrates that it is reasonable to assume that large concentrations of the crude bioactive chemicals in *Chlorella vulgaris* have a specific impact on a variety of pathogenic life forms.

Hamayeli *et al.*, (2019) reported that the sea anemone's crude extract (tentacle, disc, and whole body) was produced using methanol as the solvent. Using the disc diffusion approach, antibacterial experiments were conducted. The microtiter plate technique was used to assess how well the sea anemone extracts prevented oxidation and decreased metabolic activity. GC-MS analysis was used to pinpoint the bioactive substances. According to data, the "whole body" section extracts have the strongest antibacterial action (as it relates to *P. aeruginosa*). Maximum antibacterial activity occurs at 10–20 mg/ml concentrations, according to data on minimum inhibitory and bactericidal concentrations. In particular, inhibition of biofilm was shown by the tentacle, disc, and complete body of the sea anemone against *P. aeruginosa*, *K. pneumonia*, and *A. baumannii*, respectively.

Boroumand *et al.*, (2019) proposed that this study focuses on the environmentally friendly synthesis of selenium nanoparticles (Se-NPs) by chemical reduction, employing polyvinyl alcohol (PVA) or chitosan (CS) as stabilisers and ascorbic acid (Vitamin C) as a reagent. The primary characterisation procedures were transmission electron microscopy (TEM), dynamic light scattering (DLS), fourier transform infrared spectroscopy (FT-IR), and ultraviolet-visible (UV-vis) absorption spectroscopy. The MTT test was used to evaluate the cytotoxicity of Se-NPs on fibroblast L929 cells. The examined cell line exhibited little toxicity towards the nanoparticles, remaining more than 50% alive at 170 ppm for 24 hours. By using a radical scavenging (DPPH) test, the in-vitro

antioxidant potential of the synthesised Se nanoparticles was assessed. Jacopin *et al.*, (2020) reported that the major public health problem is the emergence of multidrug antibiotic resistance in commensal microorganisms. As opportunistic pathogens, commensal bacteria like *Escherichia coli*, *Streptococcus pneumonia*, or *Staphylococcus aureus* are responsible for a significant portion of community- and hospital- acquired bacterial illnesses. Antibiotic treatment of these illnesses becomes more difficult due to multidrug resistance (MDR), which may result in significant extra morbidity and death.

Zuccaro *et al.*, (2020) states that specific environmental factors, such as temperature ranges, light intensities, mixing circumstances, nutritional content, and gas exchange, are needed for microalgae production. They may be raised utilising various metabolic and cultivation methods, which are categorised as open and closed systems, respectively. An overview of the fundamentals necessary to predict microalgae's potential contribution to bio refinery applications will be given in this chapter.

Cavalu *et al.*, (2021) experimented that by using dynamic light scattering, which reveals a gaussian size distribution with the largest percentage falling between 20 and 40 nm, the creation of nanoparticles was verified. In addition to UV-visible spectroscopy, which further confirms the stability of selenium colloidal sol, zeta potential measurements show a negative charge of -38.2 mV. The homogenous, spherical shape was shown by TEM and AFM, revealing that the nanoparticles were in the 20–40 nm size range. The amorphous structure of the synthesised selenium is highlighted by structural analyses of powdered selenium nanoparticles using FTIR spectroscopy and XRD patterns. These analyses highlight the existence of stretching and bending vibrations of Se-O bonds.

Little *et al.*, (2021) reported that the serious problem that has raised the demand for new antibacterial chemicals is the rise of germs that are resistant to antibiotics. Depending on the kind of algae, type of bacterial pathogen, and solvent employed to extract the metabolites, antibacterial activity in extracts from photosynthetic green microalgae have been

assessed. The potential for development varies. Extreme environmental circumstances have forced green microalgae to adapt to habitats that are inhospitable to a variety of other creatures. Green microalgae can survive in these harsh settings thanks in part to the synthesis of antimicrobial substances, which may also play other possible roles.

Alvi *et al.*, (2021) states that the creation of materials and particles at the nanoscale, which has dimensions between 1 and 100 nm, is the subject of nanotechnology. In terms of simplicity and dependability, biological production of nanoparticles utilising microorganisms and plants is the most effective way. Selenium metal salt from citrus fruit extracts was used to synthesise metallic nanoparticles, and their characterisation and assessment for antibacterial properties against pathogenic microorganisms were key goals of this work.

Hernandez *et al.*, (2021) proposed that for an alternative to the rising bacterial antibiotic resistance, selenium nanoparticles (SeNPs) have been used more often in the biomedical field. Ascorbic acid (AsAc) was used as a reducing agent and methanolic extract of *Calendula officinalis* L. flowers as a stabiliser in the green synthesis process used in this study to create SeNPs. Transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), and ultraviolet-visible spectrophotometry (UV-VIS) methods were used to characterise SeNPs. Spherical morphologies and SeNPs with sizes between 40 and 60 nm were produced. By using the disc diffusion technology, the bactericidal activity of marigold extracts and fractions was assessed.

Bouzidi *et al.*, (2022) stated that we looked at the microstructural, optical, conductivity, and AC dielectric impedance characteristics of both pure PVA and PVA: NaSe SCF samples. The semi-crystalline structure of the PolVA:NaSe SCF samples was validated by the XRD research, which explained the semi-crystalline nature of the pure PolVA as a matrix. This has to do with PolVA chains making strong intermolecular contacts

across intermolecular hydrogen bonds. Band gap energies dropped when NaSe salt fillers in the PolVA matrix increased.

CHAPTER 3

AIM AND SCOPE

1. AIM

To find an antibacterial compounds from microalgae with the biosynthesized selenium nanoparticles.

2. SCOPE

- Culture and maintenance of microalgae.
- Biosynthesis of selenium nanoparticles using microalgae
- To determine the antibacterial activity of microalgae *Picochlorum.sp.*
- To determine the characterization of bioactive compound.

CHAPTER 4

MATERIALS AND METHODS

1. Collection and culture of Microalgae

The samples are bought from Bharathidasan University. The microalgae sample *Picochlorum. sp* (BDUG100241) was bought. The microalgae were cultured in 25 ml of Artificial Sea Water medium (ASN III media) with 5ml of *picochlorum. sp* in 100ml conical flask for free air circulation. The cultured microalgae are kept under light for a faster growth. The incubated condition of microalgae is 12h: 12h cycle (light: dark). The microalgae were maintained for 15 days. After the growth of the microalgae, the cultured microalgae structure was checked under a microscope. Then from the 25ml of grown microalgae 10ml of microalgae is sub-cultured to a 250ml of ASN III media in 500ml conical flask. And the 25 ml microalgae culture were stored as a mother culture (Von *et al.*, 2013).

2. Screening of cultured Microalgae

Scanning Electron Microscope (SEM)- SEM analysis is commonly used to study microalgae due to their small size and intricate morphologies. Microalgae are a diverse group of unicellular or multicellular photosynthetic organisms that are often found in aquatic environments. SEM analysis of microalgae can provide detailed information on their morphological features, such as cell shape, size, surface structures, and arrangements. These features can be useful for taxonomy and identification of microalgae species.

4.2 Extraction of Microalgae

The cultured microalgae have been centrifuged for 20 minutes at 8,000 rpm. After centrifugation the supernatant were removed and the pellet were collected and kept for drying in hot air oven for 30 mins. Then the dried pellet was mixed with themethanol and left for incubation (Lauritano *et al.*, 2016).

4.2.1 Synthesis of selenium nanoparticles

After the extraction of the cultured microalgae, the extracted microalgae is added with a Selenium nanoparticle with a ratio of 0.5:4.5, 1:4, 1.5:3.5, 2:3, 2.5:2.5 and incubated in a dark for colour change. After 2 to 3 days the indication for the biosynthesis of selenium nanoparticles occurs by the change of a colour to a dark red (Satgurunathan *et al.*, 2017).

4.2 Analysis of the biosynthesized nanoparticles

Samples are dried and powdered and used for the analysis.

4.3.1 UV-Spectrometry

The synthesized nanoparticle is taken UV-Spectrometry readings for the OD value with a wavelength range from a 220nm-700nm (Alvi *et al.*, 2021). The basic principle of spectroscopy is the absorption of ultraviolet or visible light by chemical substances, which generates distinctive spectra. The basis of spectroscopy is the interaction of light and matter. A spectrum is created when the substance absorbs the light through excitation and de-excitation processes.

The electrons existing in matter experience excitation when it absorbs UV energy. They go quickly from a relaxed state to an ecstatic one as a result. It is essential to remember that the quantity of visible or ultraviolet light absorbed by an electron is always equal to the energy difference between its ground state and excited state.

4.2.2 FT-IR (Fourier Transform Infrared Spectroscopy)

In order to do Fourier transform infrared spectroscopy (FTIR) analysis, vacuum- dried selenium nanoparticles were combined with potassium bromide at a ratio of 1:100. Under neutral pH settings, a Shimadzu 8400S FTIR was used to record the spectra using a diffuse reflectance accessory (PH 7.2). The scanning data came from an average of 50 scans between 4000 and 400 cm⁻¹ (Sarkar *et al.*, 2011).

3. XRD (X-Ray Diffraction)

The liquid reaction mixture was dried at 458°C in a vacuum drying oven following bio-reduction. A powder X-ray diffraction (XRD) study was performed using the vacuum-dried combination. A PW 3040/60 Analytical X-ray diffractometer (Cu Ka radiation, 1.54443) operating at 45 kV and 30 mA was used to record the spectra. From 2 to 90° 2θ angles, diffracted intensities were observed (Sarkar *et al.*, 2011).

4. Zeta-Potential

Using a Beckman Coulter Delsa™ Nano Particle analyzer, the charge distribution (zeta-potential) of a selenium particle solution was illuminated with a He-Ne laser (658nm) in a sample cell (Sarkar *et al.*, 2011). Zeta potential is a physical characteristic that every suspended particle, macromolecule, or material surface demonstrates. It may be used to anticipate interactions with surfaces, improve the production of films and coatings, and optimise the formulations of protein solutions, suspensions, and emulsions. The time required to create experimental formulations can be decreased with knowledge of the zeta potential. It can also be helpful in making long-term stability predictions. When charged colloidal dispersions are introduced into a zeta cell for a zeta potential study, the particles move towards the electrode that has the opposite charge to the particle when an external electric field is applied.

4.2.4 HR TEM (High-Resolution Transmission Electron Microscope)

The TEM method was employed to confirm the size and form of the synthesised SeNPs. At 80kV and 120kx magnification, TEM pictures were captured. A spherical shape and an average frequency size distribution of 40 to 60 nm in diameter were seen in the SeNPs micrographs produced by AsAc-mediated green synthesis and Cof-Met extract (Hernandez *et al.*, 2021).

4.5.1 Antibacterial activity assay

The biosynthesized selenium nanoparticle is used as a sample for

antibacterial activity. The agar well diffusion method is used for the antibacterial activity. The Synthesized nanoparticle were centrifuged for 15 minutes at 8,000 rpm and the supernatant were removed and pellet were collected and kept for drying in hot air oven for 45 minutes. After drying, 1g of pellet were mixed with a 1ml of distilled water (Alvi *et al.*, 2021).

4.5.2 Agar well diffusion method

Nutrient agar and nutrient broth were used for the activity. At first the nutrient broth was prepared for 5 bacterial pathogens. The pathogens are *Klebsilla. sp*, *Pseudomonas. sp*, *Staphylococcus. sp*, *E-coli. sp*, *Bacillus. sp*. In 25ml of distilled water 0.65g nutrient broth were added and separated to 5 test tubes each 5 ml and autoclave it for 20mins at 121°C and the pathogen were inoculated in each test tube with an inoculation loop in laminar flow and left for incubation for 24 hours. Then the nutrient agar was prepared, in 100ml of distilled water 3.8g of nutrient agar and 0.1g of agar agar were mixed and kept for autoclave at 121°C for 20 minutes. And 20ml of agar were added to 5 petri plates and left to solidify for 15 to 20 minutes. After solidify the inoculated pathogen were swab in a plate with a help of a swab for all plate with a different pathogen. And then the petri plate was marked consisting of three well which is cut using the well cutter, first well consist of bio-synthesized selenium nanoparticle sample, second well consist of a sodium selenite and the other is a control, the control used Gentamycin. In the well the 50µl of the samples were added and left for the incubation for 24 hours. The formation of the zone around the well shows the antibacterial activity against the sample. And the radius of the zone was measured and tabulated (Hussein *et al.*, 2019).

CHAPTER 5

RESULT AND DISCUSSION

5.1.1 Collection of microalgae

The *Picochlorum.sp* was brought from Bharathidasan repository. The colour of the *Picochlorum.sp* culture was green. The bought sample was shown in figure 5.1

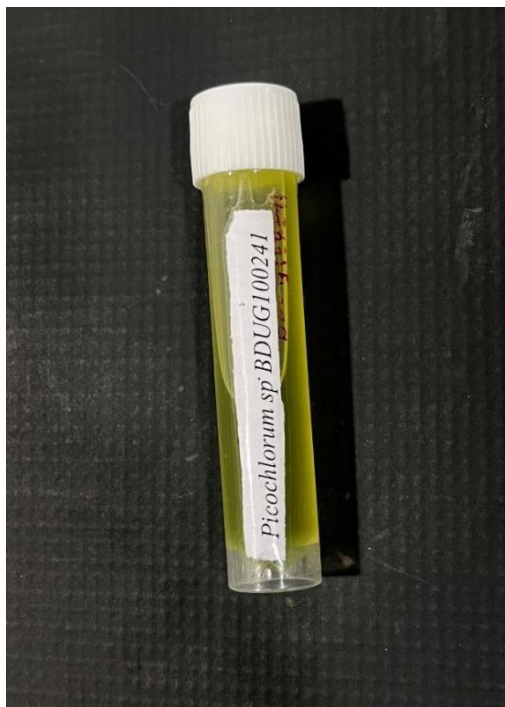


Figure 5.1 *Picochlorum.sp*

5.1.2 Culture and growth of microalgae

After fifteen days of incubation with periodic shaking under light 12h:12h (light:dark), the stock *Picochlorum.sp* cultures was inoculated into ASN III (Artificial Seawater Nutrient medium), and the medium's colour changed to green, confirming that the culture had developed. SEM was employed to carry out additional studies on the culture. The inoculated microalgae was shown in figure

5.2 , the growth of inoculated microalgae was shown in figure 5.3 .



Figure 5.2 Inoculated microalgae sample in ASN III medium



Figure 5.3 Growth of microalgae

5.2 Analysis of cultured microalgae

Centrifuging the cultured microalgae at 8000 rpm for 15 minutes separates the media from the microalgae in preparation for a subsequent culture analysis with scanning electron microscopy. The emerald green pellet has been collected, dried, and crushed. Before centrifugation of algae was shown in figure 5.4, the pellet collection was shown in figure 5.5, the dried pellet was shown in figure 5.6 and the

extraction of the dried sample with methanol for the biosynthesis of seleniumnanoparticles was shown in figure 5.7 .



Figure 5.4 Centrifugation of cultured microalgae



Figure 5.5 Collection of pellet.

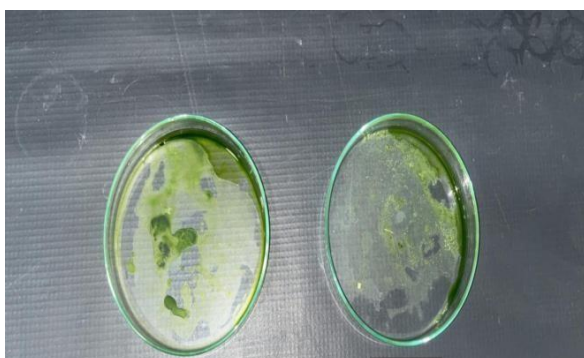


Figure 5.6 Dried sample of microalgae



Figure 5.7 Dried sample extracted with methanol.

5.2.1 SEM (Scanning Electron Microscope)

The microalgae *picochlorum*'s structure is seen in the SEM pictures shown in figure 5.8, 5.9, 5.10 and 5.11.

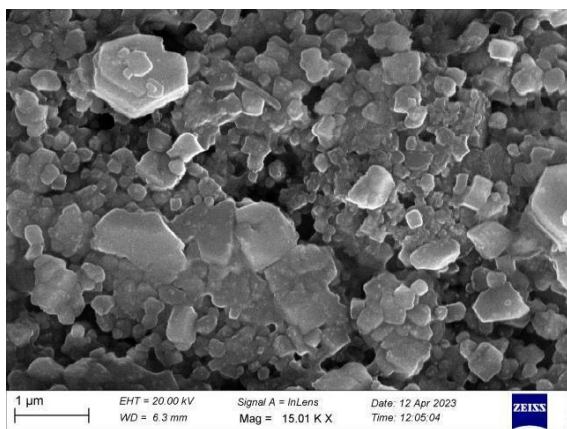


Figure 5.8

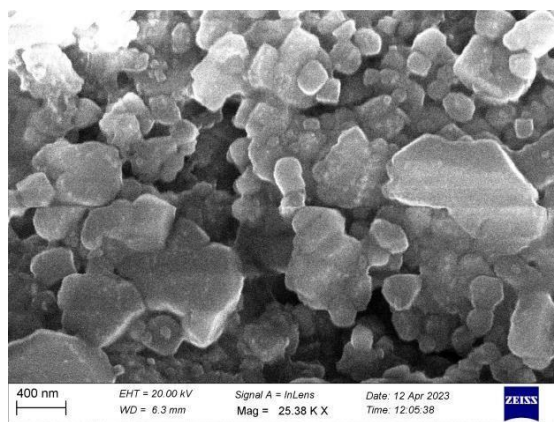


Figure 5.9

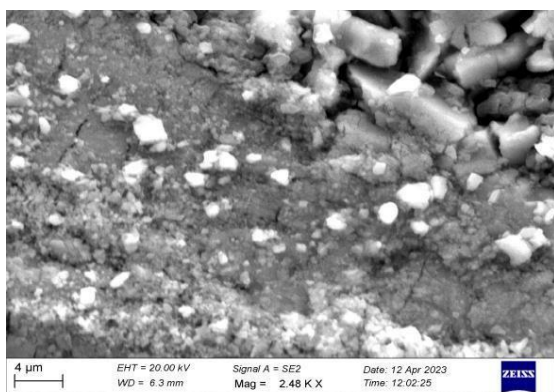


Figure 5.10

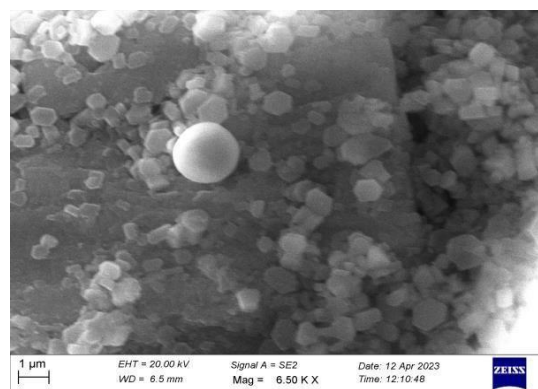


Figure 5.11

Picochlorum microalgae SEM pictures are shown in Figure 5.8, 5.9, 5.10, 5.11 at the 1µm, 4µm, and 400 nm scales.

5.3 Biosynthesis of selenium nanoparticles

The synthesised SeNPS were characterised using a variety of spectroscopic and microscopic techniques to determine their precise shape, elemental content, and other physicochemical characteristics. UV-Vis absorption spectra were taken in order to show the production of Se nanoparticles. The reaction mixture's transition from being green to reddish verifies the synthesis of SeNPs. A similar colour change was observed from the colour alteration of the aqueous parsley extract, which changed from its normal light brown to an orange-red peculiar to

SeNPs, served as confirmation (Fritea *et al.*, 2017).



Figure 5.12 Before incubation



Figure 5.13 After incubation.

4. Characterization of biosynthesized selenium nanoparticles

1. UV-Vis Analysis

UV-Vis analysis the maximum absorbance was in the range from 200-300. A similar one from PVA-SeNPs and Cs-SeNPs, respectively, significant absorption peaks were seen about 264 and 310 nm, which are connected to Se-NP production during the reduction process. Due to the varying sizes and coatings of the green synthesised Se-NPs, the spectra of the synthesised nanoparticles varied from one another (Boroumand *et al.*, 2019). The UV-visible absorption spectra of selenium nanoparticles is shown in figure 5.13.

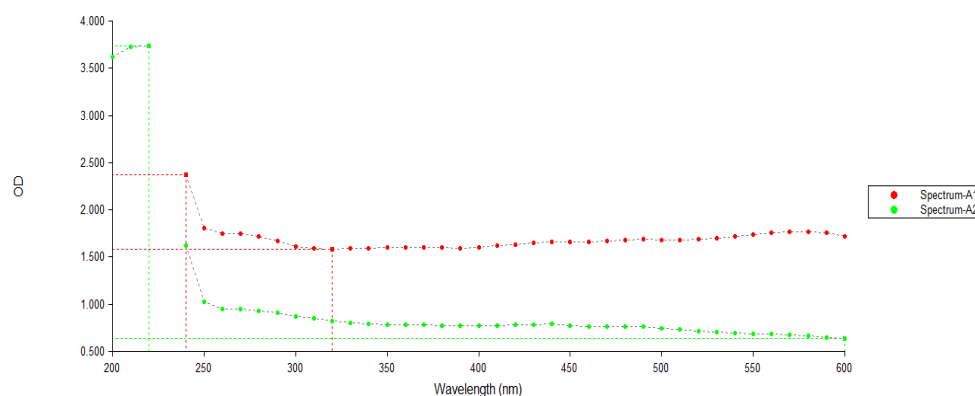


Figure 5.14 UV-Vis analysis of biosynthesized SeNPs using microalgae extract

5.4.2 FT-IR (Fourier-Transform Infrared) Analysis

FTIR measurements were carried out to identify the possible interactions between selenium with culture under normal pH condition. The absorption bands around 1619 and 1561 cm^{-1} are attributed to amide I and amide III linkage. The absorption bands around 3267 cm^{-1} are known to result from O-H and N-H stretching vibration from polysaccharides and proteins. The protein in the sample play a role in the synthesis and stabilization of selenium nanoparticles. The FTIR spectrometer of selenium nanoparticles is shown in figure 5.14. The similar value for SeNPs produced from Citrus limon (Lemon) peel extract, wavelength absorbance

measurements of infrared light between the range of 1000 and 3500 cm^{-1} were assessed. Fresh lemon peels create SeNPs with two absorbance peaks at 3274.74 cm^{-1} and 1637.05 cm^{-1} , respectively. Due to the presence of alcohol and phenol functional groups, the peak between 3200 and 3300 cm^{-1} confirmed the occurrence of O-H bonded stretching and considerable vibrations. N. H. Amid that displays C=C stretching, C-N stretching, and Amides functional groups of amides I and II with C-H bonds follows the same logic (Alvi *et al.*, 2021).

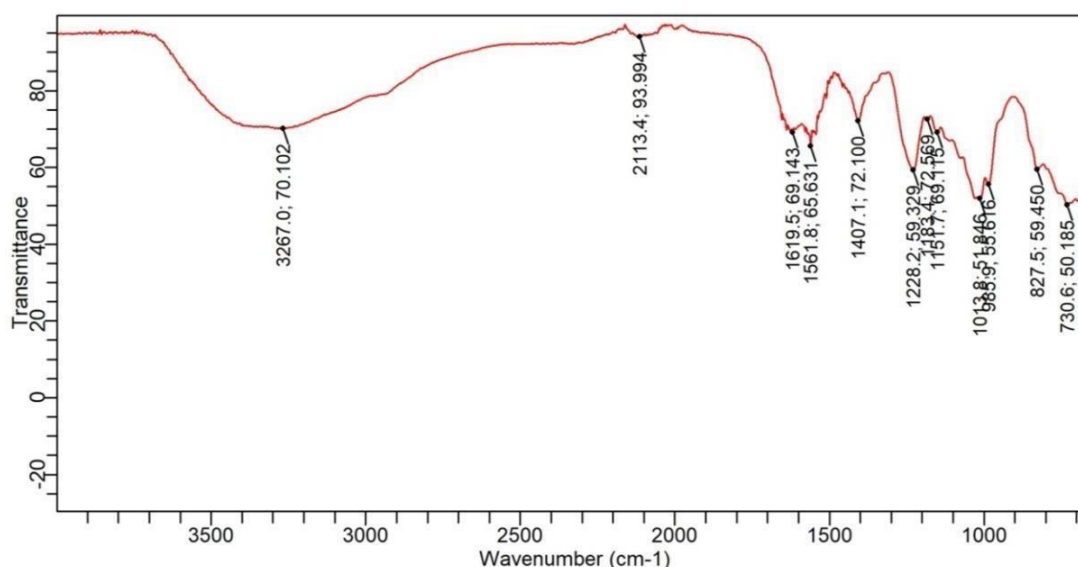


Figure 5.15 FTIR analysis of biosynthesized SeNPs using microalgae extract.

TABLE 5.4.2.1: FT-IR Analysis value

PEAK NUMBER	WAVELENGTH (cm ⁻¹)	INTENSITY	FUNCTIONAL GROUP
1	730.55822	50.18502	Halogen compound [chloro-compound] (C-Cl)
2	872.46900	59.45022	Bending -C=O inorganic carbonate
3	985.88086	55.61617	COO ⁻
4	1013.83589	51.84630	Polysaccharide
5	1151.74739	69.11460	C-O-C polysaccharide
6	1183.42976	72.56905	Ester carbonyl
7	1228.15782	59.32856	C=C unsaturated compounds
8	1407.07003	72.09970	Stretching -C=O inorganic carbonate
9	1561.75455	65.63132	C-N amide III bond
10	1619.52829	69.14254	C-N amide I bond
11	2113.40055	93.99448	C=C conjugated and C ⁻ =C
12	3267.01161	70.10192	NH ₂ aminoacidic group

5.4.3 XRD (X-Ray Diffraction) Analysis

The crystalline nature and purity of nanoparticles were determined using powder X-ray diffraction technique Figure 5.15. The diffraction peaks were observed at 2θ value of 23.3 (100), 29.6 (101), 43.5 (012), and 50.05 (201) some of previous study of selenium nanoparticles in the crystalline phase indicated that stable amorphous forms the Se nanoparticles XRD pattern has a large and strong peak at roughly $2^\circ=23^\circ$, indicating that they are not crystalline (Cavalu *et al.*, 2021).

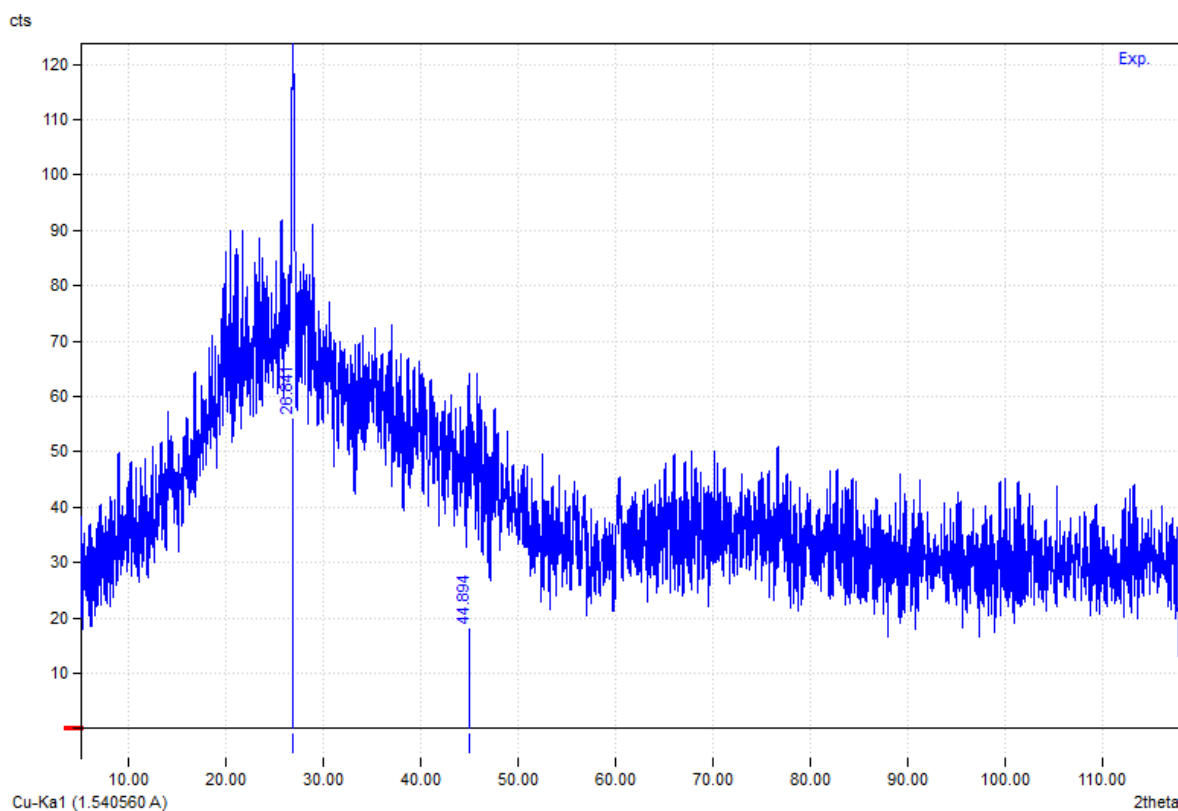


Figure 5.16 XRD pattern of biosynthesized SeNPs

5.4.4 Zeta-Potential or DLS Analysis

The particle size analyzer or DLS (dynamic light scattering) with zeta average value and PI (polydispersity) > 0.5, which proves to be either monodisperse or polydisperse in colloidal solution, helps in evaluating the hydrodynamic diameter in colloidal solution which was measured at 25 °C by means of a scattering angle of 90°. Most experts agree that the nanoparticles are sufficiently repulsed by zeta-potential. This was determined to be a crucial indicator of the stability of the dispersion and the strength of the particle interactions. The selenium nanoparticles negative charge denotes their extreme stability in figure 5.16.

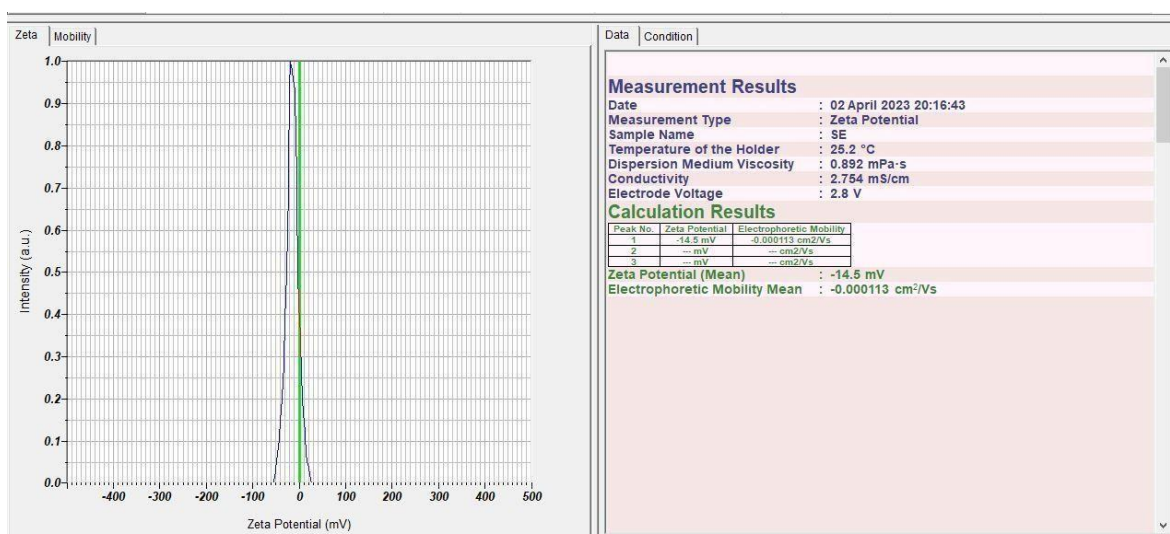


Figure 5.17 Zeta potential or DLS of biosynthesized SeNPs.

5.5 Antibacterial activity

The growth of bacterial cultures under normal conditions showed all the phases including the decline phase of the bacterial growth curve, but when the bacterial cultures were treated with the biosynthesized selenium nanoparticles, reduction of the log phase of the bacteria was showed signifying the bacteriostatic activity. It can be assumed that the selenium nanoparticles must have penetrated into the bacterial cells through the cell membrane and started exhibiting its bacteriostatic effect on it, as shown in figure 5.17, 5.18, 5.19, 5.20, 5.21. The mechanism explained can be due to the disruption of the cell wall indicating that the selenium nanoparticles were capable of entering, while the cell wall remained intact when the cultures were not treated with SeNPs. The antibacterial activity of these selenium nanoparticles can be identified by the morphological changes in the bacterial strain which can occur either intracellular or extracellular. The antimicrobial activity of selenium nanoparticles is mainly due to the production of ROS, which leads to disruption of the phospholipid bilayer where the SeNPs interact with the intracellular proteins and inactivate them or may react with the sulphhydryl and thiol groups present in the membrane proteins, eventually denaturing

them. Oxidative stress induced by the reactive oxygen species causes cell death by altering the protein synthesis cycle, interfering in the respiratory or food metabolism pathway or impairing the DNA replication (Vadivel *et al.*, 2012 and Shah *et al.*, 2013).

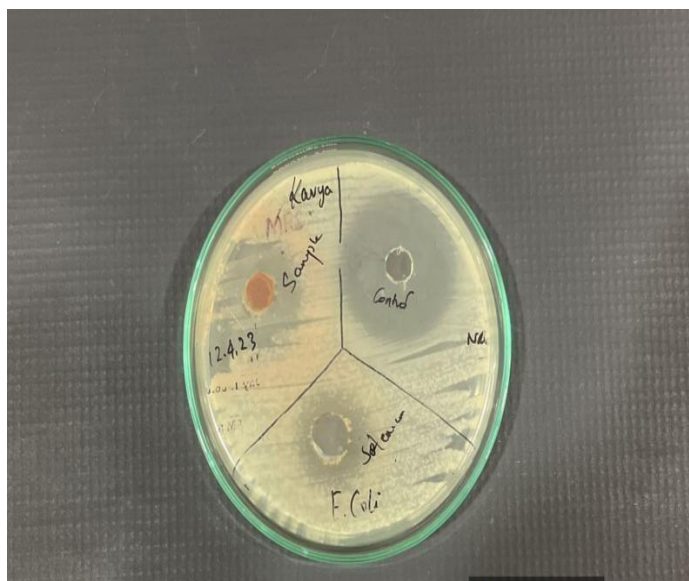


Figure 5.18 Antibacterial activity against E.coli. sp pathogen.

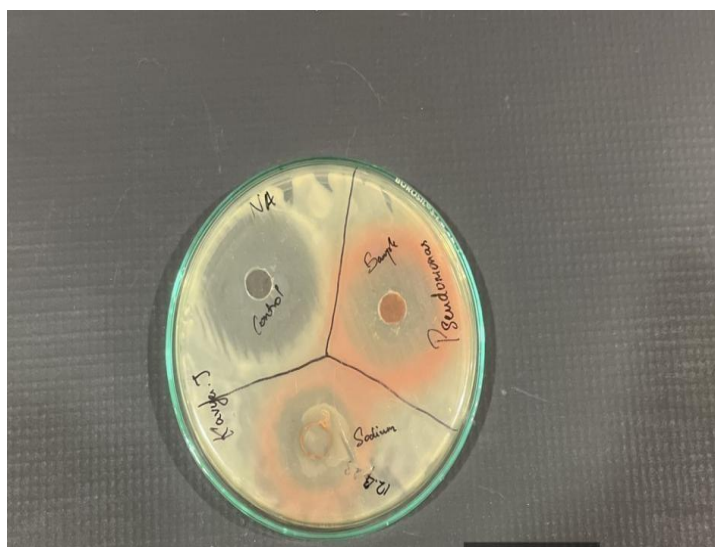


Figure 5.19 Antibacterial activity against Pseudomonas.sp pathogen.

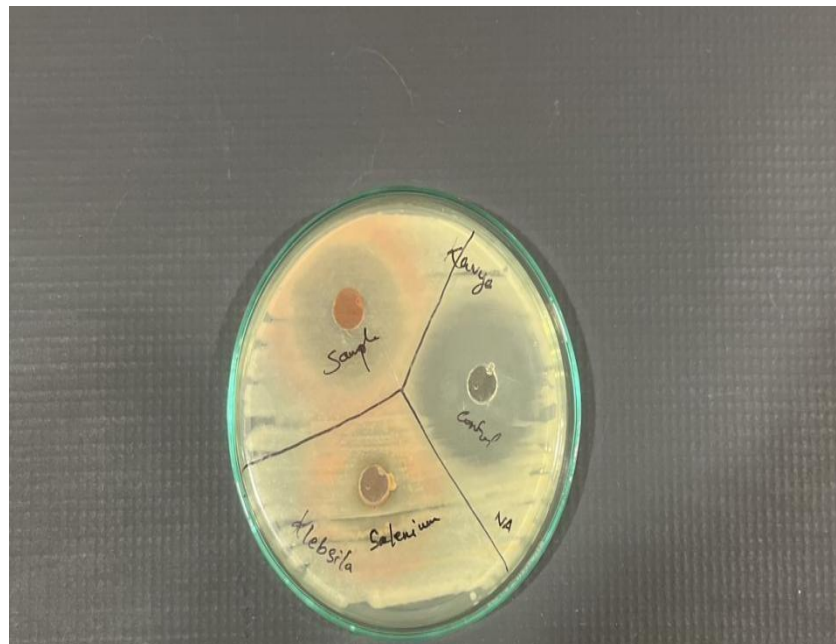


Figure 5.20 Antibacterial activity against *Klebsiella* sp. pathogen.



Figure 5.21 Antibacterial activity against *Staphylococcus* sp. pathogen.

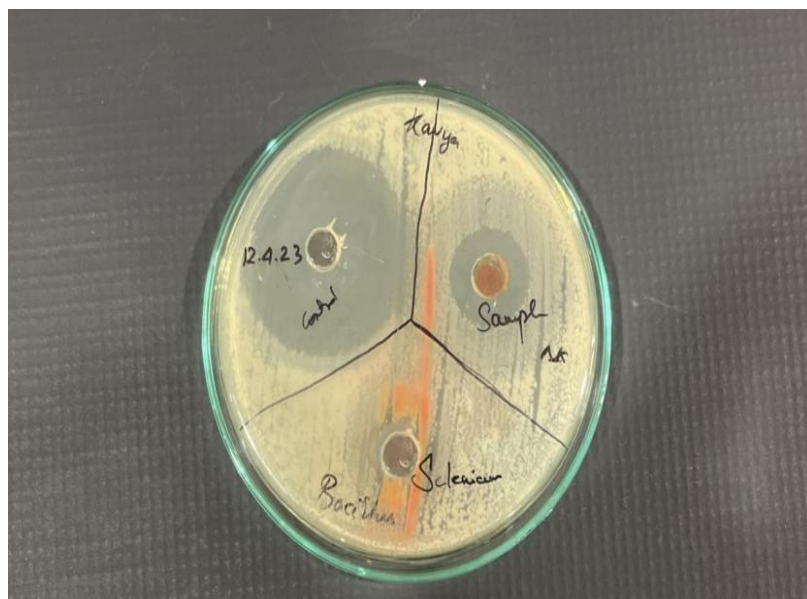


Figure 5.22 Antibacterial activity against *Bacillus. sp* pathogen.

TABLE 5.5.1: Zone of Inhibition

BACTERIAL STRAIN	SELENIUM NANOPARTICLES WITH ALGAE	CONTROL	SODIUM SELENITE
<i>Klebsilla. sp</i>	3.4cm	3.5cm	2.9cm
<i>Staphylococcus. sp</i>	3.2cm	4cm	2.8cm
<i>Pseudomonas. sp</i>	3cm	3.8cm	2cm
<i>Bacillus. sp</i>	3cm	4cm	2.2cm
<i>E.coli. sp</i>	3.5cm	3.7cm	2.2cm

CHAPTER 6

SUMMARY AND CONCLUSION

The capacity of bacteria to withstand the effects of antibiotics that were formerly successful in treating bacterial illnesses is an issue that is becoming more and more of a worry on a worldwide scale. When bacteria undergo genetic changes or gain resistance genes, they can withstand antibiotic exposure. Infections that are resistant to antibiotics can be challenging to treat, and they can result in serious disease or even death. It is crucial to take action to stop the emergence and spread of antibiotic-resistant bacteria. Some of these actions include avoiding the overuse of antibiotics, maintaining proper hand cleanliness, and receiving vaccinations against bacterial diseases. In further the microalgae are cultured and biosynthesized with selenium nanoparticles and then they had been analysed with the UV-Spectrometry which gave a peak value on 240nm and 270nm, with zeta potential obtain a value of -14.5mV that shows the high stability of the nanoparticles, then the diffraction peaks were observed at 2θ value of 23.3 (100), 29.6 (101), 43.5 (012), and 50.05 (201) and then to discover potential interactions between selenium and culture under neutral pH conditions, FTIR studies were performed. The amide I and amide III linkages are responsible for the absorption bands at 1619 and 1561 cm^{-1} . It is known that the O-H and N-H stretching vibrations from polysaccharides and proteins cause the absorption bands at 3267 cm^{-1} . After the agar well diffusion technique is used to test the antibacterial activity, results are favourable against the five pathogens *Pseudomonas sp.*, *Klebsiella sp.*, *Bacillus sp.*, *Staphylococcus sp.*, and *E. coli sp.*

By above experiment we concluded that the utilization of green chemistry has helped in the production of selenium nanoparticles from microalga extract, which is efficient, quick, and environment-friendly when compared with other techniques. The synthesized nanoparticles will be optimized using the statistical procedure Box-Behnken method, which requires

lower experimental runs. The optimization helps in enhancing the production and yield of the SeNPs and improvises the application of these nanoparticles in various biomedical fields.

CHAPTER 7

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