

BIOSYNTHESIS OF SELENIUM NANOPARTICLES USING MICROALGAE FOR ANTI-TB ACTIVITY

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

by

DEEPIKA M (40770008)



**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 **DEEPIKA M (40770008)** who carried out the project entitled "**BIOSYNTHESIS OF SELENIUM NANOPARTICLES USING MICROALGAE FOR ANTI-TB ACTIVITY**" under our supervision from October 2022 to April 2023.

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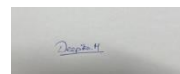
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DECLARATION

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SIGNATURE OF THE CANDIDATE

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TABLE OF CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
	ABSTRACT	i
	LIST OF FIGURES	ii
	LIST OF TABLES	iii
	LIST OF SYMBOL AND ABBREVIATION	iv
1	INTRODUCTION	1
	1.1 TUBERCULOSIS	1
	1.1.1 Multidrug resistant TB	1
	1.1.2 HIV with TB	2
	1.2 MICROALGAE	2
	1.2.1 Bioactive compounds from the microalgae	3
	1.3 NANOPARTICLES	4
	1.3.1 Selenium nanoparticles	4
	1.3.2 Mechanism of selenium nanoparticles	5
	1.4 ANTI- TB ACTIVITY (MABA ASSAY)	6
2	LITERATURE SURVEY	7
3	AIM AND SCOPE	14
4	MATERIALS AND METHODS	15
	4.1 CULTURE OF MICROALGAE	15
	4.2 ANALYSIS OF CULTURED MICROALGAE	15
	4.2.1 SEM	15
	4.3 EXTRACTION OF MICROALGAE	15
	4.4 BIOSYNTHESIS OF SELENIUM NANOPARTICLE	16
	4.5 ANALYSIS OF BIOSYNTHESISED SELENIUM NANOPARTICLES	16
	4.5.1 UV- Spectrophotometer	16
	4.5.2 Zeta potential	16
	4.5.3 FTIR	16
	4.5.4 XRD	17
	4.6 ANTI-TB ASSAY	17
	4.6.1 Inoculum preparation	17

	4.6.2 In vito Anti-Tubercular Assay	18
5	RESULTS AND DISCUSSION	20
	5.1 CULTURE OF MICROALGAE	20
	5.2 ANALYSIS OF CULTURED MICROALGAE	21
	5.2.1 Scanning Electron Microscopy	21
	5.3 BIOSYNTHESIS OF SELENIUM NANOPARTICLE	22
	5.4 ANALYSIS OF BIOSYNTHESISED SELENIUM NANOPARTICLE	22
	5.4.1 UV Spectrophotometer	22
	5.4.2 Zeta Potential	23
	5.4.3 FTIR	24
	5.4.4 XRD	27
5.5	ANTI-TB ACTIVITY MABAASSAY	27
6	SUMMARY AND CONCLUSION	29
7	REFERENCES	30

Abstract

Mycobacterium tuberculosis, the organism that causes tuberculosis, is a disease that mostly affects the lungs. Since MDR-TB is a strain of tuberculosis that is resistant to conventional first-line drugs, it is difficult and expensive to treat. One of the nutrients that humans require in their diets is selenium. It has been demonstrated that selenium enhances the activity of the selenium-enzyme glutathione peroxidase and guards against in vivo cell and tissue damage from free radicals. As a result, selenium has been employed in food and medicine. Numerous secondary metabolites produced by microalgae have various biological impacts. They are recognised for being abundant in bioactive compounds that may be used in pharmaceutical compositions. In this study the microalgae are grown and biosynthesized using sodium selenite to create selenium nanoparticles. When the stability of the nanoparticles was checked using the Zeta potential method, the nanoparticles produced a value of -14.5 mV. The nanoparticles were also investigated using a UV-Spectrophotometer, which produced a peak value of 200–300 nm. Then, the FTIR (Fourier Transform Infrared) analysis of several 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 comes to the conclusion that the sodium selenite, which is in crystal form, is reduced to the nanoparticle form during the biosynthesis. With this compound the anti-TB activity has been given where there has been an activity at 6.2 mg/ml. So, it is concluded that the selenium nanoparticle can be used for further studies in the future to find more novel compounds.

Keywords: Microalgae, Tuberculosis, Selenium Nanoparticle, Multi drug resistant, MABA Assay

LIST OF FIGURES

FIGURE NUMBER	FIGURE TITLE	PAGE NUMBER
5.1	Microalgae <i>Picochlorum. sp</i>	20
5.2	Culture of <i>Picochlorum. sp</i> in 25 ml of media	20
5.3	Subculture of <i>Picochlorum. sp</i>	20
5.4	Culture condition of microalgae	20
5.5	SEM image of <i>Picochlorum. sp</i> at the scale of 1µm	21
5.6	SEM image of <i>Picochlorum. sp</i> at the scale of 4µm	21
5.7	SEM image of <i>Picochlorum. sp</i> at the scale of 1µm	21
5.8	SEM image of <i>Picochlorum. sp</i> at the scale of 400nm	21
5.9	Before biosynthesis of Selenium nanoparticle	22
5.10	After biosynthesis of Selenium nanoparticle	22
5.11	UV Spectrophotometer attained OD values of 240nm and 200nm	23
5.12	Zeta potential of the Biosynthesised Selenium Nanoparticle	23
5.13	FTIR results of the Biosynthesised Selenium Nanoparticle	24
5.14	XRD Results of the biosynthesised Selenium Nanoparticle	27
5.15	MABA Assay for Anti-TB Activity of Biosynthesized selenium nanoparticle	27

LIST OF TABLES

TABLE NUMBER	TABLE TITLE	PAGE NUMBER
5.1	FTIR peak values	25
5.2	MABA Assay activity with respective concentrations	28

LIST OF ABBREVIATION

ACRONYM	DESCRIPTION
TB	Tuberculosis
MTB	Mycobacterium tuberculosis
MDR	Multi-drug resistant
XDR	Extensively drug resistant
MDR- TB	Multi-drug resistant – Tuberculosis
HIV	Human Immunodeficiency Virus
NP	Nanoparticles
Se np	Selenium nanoparticle
mg	Milligram
ml	Millilitre
nm	Nanometre
rpm	Rotation per minute
°C	Celsius
cm	Centimetre
CFU	Colony forming unit
MABA	Microplate Almar Blue Assay
MIC	Minimum Inhibitory Concentration
hr	hour
mV	megavolt
kV	kilovolt
Mm	Millimolar
ASN media	Artificial Sea water Nutrient media
FTIR	Fourier Transform Infrared
XRD	X- Ray Diffraction
SEM	Scanning Electron Microscope

CHAPTER 1

INTRODUCTION

1.1 TUBERCULOSIS

Tuberculosis (TB) is a disease caused by bacteria (*Mycobacterium tuberculosis*), primarily affecting the lungs. Tuberculosis spreads through the air from one individual to another. *Mycobacterium tuberculosis* (MTB) is a causative agent for TB which affects the lungs and other body parts except for hair and nail. TB is transmitted to other individuals through the aerosols expelled by infected people when they cough and the symptoms are cough, fever, night sweats and weight loss. Only a few of these germs must be inhaled for a person to get infected. A quarter of the world's population is thought to have been infected with tuberculosis bacteria, but the majority will not develop TB disease and some will clear the infection. Individuals who are infected but not unwell with the disease cannot spread it (WHO Global Report 2022).

1.1.1 Multidrug resistant TB

Most forms of active TB can be treated with six months of medication. Unfortunately, the very success of the drug treatment of tuberculosis has been the catalyst for the emergence of a new wave of drug resistance (Prakash *et al.*, 2010). The World Health Organization (WHO) regards antimicrobial resistance as a "threat to effective infection prevention and treatment" MDR-TB is a type of tuberculosis that is challenging and expensive to treat since it does not respond to traditional first-line medications. Numerous countries have devised plans to combat MDR-TB, but the global response is still insufficient (Rajeev A *et al.*, 2006).

Alternative medications can be used to treat some infectious illnesses caused by drug-resistant microorganisms. This method, however, cannot be used for tuberculosis since the medications used to treat drug-resistant *Mycobacterium tuberculosis* strains are more expensive, less effective, and more hazardous (Ruiz-Güereca DA *et al.*, 2019).

1.1.2 HIV (*Human immunodeficiency virus*) with Tb

According to NIH (National Institute of Health) Tuberculosis is an opportunistic infection (OI). OIs are infections that occur more frequently or are more severe in people with weaker immune systems than in healthy people. HIV impairs the immune system, increasing the risk of tuberculosis in HIV patients. Infection with both HIV and tuberculosis is referred to as HIV/TB coinfection. In people with HIV, untreated latent TB infection is more likely to progress to TB disease than in people without HIV. Someone who has both HIV and TB is at least ten times more likely to acquire active TB, especially if their CD4 count is less than 200. Both infections, regardless of CD4 count, suggest you have AIDS, the advanced stage of HIV.

1.2 MICROALGAE

Due to their enormous biodiversity and quick development rate, microalgae have emerged as a significant source of antibacterial chemicals and provide several benefits for antimicrobial studies. There have been reports of bioactive compounds with anticancer, antileukemia, antibacterial, and antiviral effects worldwide. Antibacterial treatments that are both powerful and safe are in greater demand (Teja *et al.*, 2022).

The ocean occupies approximately 70% of the earth's surface and 80% of animal species resides in this continuous territory due to its immense source of food, mineral and energy. The ocean remains one of the natural resources to be utilized fully by man. So far, more than 10,000 chemicals have been extracted from marine creatures, with hundreds (or) more identified each year. Microalgae are one of the most varied species of microorganisms in both freshwater and marine environments. Eukaryotic organisms such as microalgae contribute 40% of worldwide production. They are distinguished by a large number of species that thrive in a wide range of settings and under severe circumstances such as high and low temperatures, light intensity, pH, and salinity (Saide *et al.*, 2021).

Antimicrobial agents from the marine environment have been studied with a particular focus to combat current antimicrobial resistance issues. Moreover, introducing enzymes from other organisms via biotechnological means has

succeeded in obtaining novel compounds. Owing to their potential as a source of industrial, economic and medical pharmaceutical interest microalgae have captured the scientific community's attention. Microalgae range from 0.2 to 2 μm (*picoplankton*) up to filamentous forms with 100 μm or higher sizes. These organisms have the potential for the co-production of other molecules such as pigments, proteins, polyunsaturated fatty acids and antioxidants are gaining attention as therapeutic agents for numerous health disorders and other applications in food, cosmetic, energy and pharmaceutical industries. Some microalgae are being studied and screened for the detection of active agents like anticancer, anti-inflammatory, antifungal, antibiotics and other pharmaceuticals. The secondary metabolites from microalgae are referred to as "High-Value Molecules" (Bule *et al.*, 2018)

Decades of study reveal that marine species provide enormous prospects for gathering antimicrobial compounds and offering insights for their laboratory production. Microalgae have recently gained popularity because of their rich hypometabolic content, including a wide range of biological activity. Algal biomass and its derivatives offer a wide range of potential applications, including animal feed and aquaculture, as well as human nutrition and health products.

1.2.1 Bioactive compounds from the microalgae

Microalgae-derived bioactive chemicals have the potential to be useful in the pharmaceutical industry. Terpenoids, steroids, phenolic compounds, alkenes, and Phlorotannins are among the bioactive substances found in algae that are effective as antibacterial agents (Bhuyar P *et al.*, 2020).

Microalgae have emerged as a significant source of antibacterial chemicals, and their vast variety and high growth rate provide various benefits for antimicrobial research. Marine algae produce many interesting bioactive molecules both lipid and water soluble which may turn out to be useful for the development of antimicrobial drugs. The pharmaceutical importance of marine algae is very well known all over to bring out bioactive substances. Marine planktonic algae have been recognized as a potential source of antibacterial substances. Marine microalgae have attracted increasing interest due to the possibility of cultivating them in large quantities in an eco-friendly and eco-sustainable way, thus overcoming the problem of supply for

chemical and bioactivity characterization and avoiding disruptive collection practices required for microorganisms (Saide A *et al.*, 2021)

3. NANOPARTICLES

Metallic nanoparticles (NPs) have a significant potential for dealing with problems and have been widely used in various biomedical applications such as antibacterial, antioxidant, and anticoagulant agents (Dinc *et al.*, 2022). Nanoparticles are often produced through chemical or physical processes. Physical approaches have several drawbacks, including a high energy requirement, a low yield of nanomaterials, and a high cost. Similarly, chemical procedures are environmentally detrimental due to the usage of dangerous substances. As a result, biosynthesis is an alternative eco-friendly, simple, and cost-effective technique for producing NPs that uses living things such as actinomycetes, algae, bacteria, viruses, fungi, yeast, and animal metabolites. biological synthesis is more important than the physical and chemical synthesis of nanoparticles paving the way for a new era of bio-nanotechnology (Saied *et al.*, 2020)

Surprisingly, repeated chemical and physical synthesis processes can result in high radiation, and radioactive reductants can have an impact on both people and other living things. The environmentally sustainable path to low-cost nanoparticle manufacturing involves just one step. Additionally, it is thought that biomolecule reductants are more significant than chemical reductants. Indeed, bio-nano research aims to develop new nanomaterials that are environmentally friendly and human-biocompatible (Rajagopal *et al.*, 2021).

1. *Selenium nanoparticles*

A vital micronutrient with outstanding antimicrobial, anti-cancer, anti-diabetic, and anti-inflammatory properties is selenium. Yet, it has a limited absorption rate and substantial toxicity levels in its conventional form. This issue has been solved by the nanoparticle system; selenium that is nanosized has high biocompatibility and increased biological effects. Due to its low toxicity, ability to deliver nanomedicine to specific areas, and stability, the biological method of creating selenium

nanoparticles has a wide range of applications in the field of biomedicine (Vikneshan *et al.*, 2020).

One of the nanoparticles that have been most thoroughly studied is selenium due to its growing importance in medicine. The trace element selenium (Se) has a pleiotropic impact and a high therapeutic potential for the treatment of numerous ailments; as a result, it has received a lot of interest in biotherapy and nanomedicine. Selenium is a crucial trace element for human health and is an important component of various enzymes, including glutathione peroxidase (GPx), thioredoxin reductase (TRx), and deiodinases. These enzymes play a crucial role in a variety of processes, including antioxidative and pro-oxidative processes that enhance muscular performance and cancer prevention. At least 25 selenoproteins, which serve as antioxidant, anti-inflammatory, antiviral, and antitumor agents, are combined with selenium in the human body (Ganeshkar *et al.*, 2022). Selenium plays a crucial part in cellular metabolism, is a necessary component of enzymes that shield the body from free radical species and is crucial for thyroid metabolism, human fertility, and many other crucial processes (Rajagopal *et al.*, 2021)

Comparing selenium nanoparticles (SeNP) to other substances containing selenium, there are several advantages. First of all, SeNP is not harmful. Second, SeNP exhibits selective cytotoxicity, killing only cancer cells while sparing healthy cells from damage. In recent decades, there has been intense research into SeNP's medicinal potential (Varlamova EG *et al.*, 2021)

1.3.2 Mechanism of selenium nanoparticles

Selenium nanoparticles have been shown to enhance the effectiveness of anticancer, anti-infection, and anti-inflammatory treatments by directly interacting with innate immune cells, such as macrophages, dendritic cells, and natural killer cells. Compared to conventional micron particles, nanoparticles larger surface areas offer greater interactions with biological agents like bacteria, giving them a better chance of penetrating bacterial cells. As a result, the nanoparticle system appears to be a reliable means of delivering pharmaceuticals as bioactive materials. (Vikneshan *et al.*, 2020).

1.4 ANTI-TB ACTIVITY (MABAASSAY)

A new innovative molecule can be discovered with the aid of natural compounds that are already accessible, and the nanoparticle is utilised as a therapeutic agent in the anti-TB activity due to the toxicity of the drugs that have been used up until now for many diseases that cause harm to organs, including the liver. The 96-well Microplate Alamar Blue Assay (MABA) enables the quantitative evaluation of drug susceptibility against any reproducing *Mycobacterium tuberculosis* strain within a week at a cheap cost. The 4-week incubation in microplates is employed in the microplate Alamar Blue assay (MABA) followed by the addition of the redox dye resazurin, which when oxidised is dark blue and nonfluorescent but when reduced to resorufin by cellular metabolism, becomes pink and fluorescent (Cho S *et al.*, 2015).

CHAPTER 2

LITERATURE SURVEY

Teja *et al.*, (2022) experimented the antibacterial activity of five marine microalgae. With an inhibition zone between 11.5 and 20mm, the ethanolic extract of *T.suecica*, *C. calcitrans*, and *N. oculata* showed inhibitory efficacy against *V. harveyi* and *S. aureus*. Only with an inhibition zone of 15 mm did the ethanolic extract of *I. galbana* exhibit inhibitory action against *S. aureus*. With a 20mm inhibition zone, *T. suecica* outperformed the other three extracts in terms of its ability to inhibit *V. harveyi*. With inhibition zones of 11.5 and 11mm, respectively, the methanolic extract of *T. suecica* moderately reduced the growth of *V. harveyi* and *S. aureus*, but the extracts of *I. Galbana* and *C. Calcitrans* only inhibited *V. harveyi* with inhibition zones of 10 and 12mm, respectively. But there was no evidence of any action against *S. aureus*, *A. hydrophila*, or *E. coli*.

Rajasekar *et al.*, (2022) reported a unique green synthetic method for producing - defined shaped seaweed silver nanoparticles, *Sargassum polycystin* algal extract, is the subject of the current studies, which examine its antimicrobial properties. Absorption spectrophotometers (UV–VIS), Fourier transforms infrared spectroscopy (FTIR), scanning electron microscopes (SEM), transmission electron microscopes (TEM), and X-ray diffraction (XRD) are some of the characterization methods used to determine their structure and size. Utilize silver nanoparticles to evaluate the antibacterial and antimycobacterial properties. Using zebrafish larvae, this silver nanoparticle's toxicity has been investigated. The biological nanoparticle has strong antimicrobial activity against *Staphylococcus aureus*, *Micrococcus gluteus*, *Pseudomonas fuorescens*, and *Candida albicans*. It also has strong antimicrobial activity against MTB H37Rv, SHRE-sensitive MTB, and Rifampicin-resistant MTB, which kills approximately 98% of the bacteria.

Jusidin *et al.*, (2022) reported that Antibacterial substances may be produced by marine microalgae. Four marine microalgae species were examined for their capacity to produce secondary metabolites that inhibit *Vibrio harveyi*'s growth during the exponential phase of growth: *Isochrysis sp.*, *Chaetoceros gracilis*, and

Nannochloropsis oceanica together with *Thalassiosira weissflogii*. The pathogenic bacteria *V. harveyi* is capable of causing severe aquaculture mortality and loss. Antibacterial activity was assessed through the co-culture assay and disc diffusion assay, respectively. The disc infused with microalgae and extracted with ethanol, methanol, saline water, and dimethyl sulfoxide (DMSO) was tested for *V. harveyi* at concentrations of 1.0×10^5 , 10^6 , and 10^7 CFU mL⁻¹ on TSA % NaCl media. The disc diffusion assay revealed that the largest inhibitory zone against *V. harveyi* was found in the ethanol-extracted *N. oceanica*. In the meantime, only *Isochrysis sp.* and *N. oceanica*, In the co-culture assay, *T. weissflogii* inhibited *V. harveyi*'s growth by 105 CFU mL⁻¹ (p 0.05)

Alipour S *et al.*, (2022) Bioactive compounds like selenium nanoparticles (SeNPs) are well-known. SeNP synthesis has utilized a variety of chemical and biological approaches. Blue-green microalgae *Spirulina platensis* are frequently utilized in a variety of industries. The SeNP synthesis was carried out under a variety of cultivation conditions, including variations in pH and the illumination schedule. FT-IR, XRD, size, and zeta potential measurements were used to identify the SeNPs, and DPPH and FRAP assays were used to assess the antioxidant properties of a few of the SeNPs. SeNP production was revealed by FT-IR analysis. At pH 5, continuous light exposure and 12h dark/12h light cycles resulted in the production of stable SeNPs with sizes of 145 ± 6 and 171 ± 13 nm, respectively. Some SeNPs had a higher antioxidant activity than sodium selenite. Green synthesis appears to be a convenient and risk-free strategy for scaling up the production of SeNPs.

Dinc *et al.*, (2022) report the use of *Citricoccus sp.* in the manufacture of selenium nanoparticles (SeNPs). The effects of a few factors, including time, pH, temperature, and stirring rate, were investigated for the formation of SeNPs. pH 8, 24 hours of reaction time, 37 degrees, and 150rpm were discovered to be the ideal synthesis conditions. The created particles had a zeta potential of -20.43 ± 0.41 mV and were spherical, measuring 104.46 ± 50.82 nm. Following UV-C irradiation of *Chlorella vulgaris*, the effects of the nanoparticles on oxidative stress biomarkers, hydrogen peroxide (H₂O₂), malondialdehyde (MDA), chlorophyll (a + b), and growth rate were examined. As compared to the UV-applied group, the amount of H₂O₂ and MDA reduced on the first, third, and seventh days after UV exposure in the culture that

contains nanoparticles, while optical density and cell density increased where the amount of chlorophyll a and b changed were same.

Ganeshkar *et al.*, (2022) Due to its lower toxicity towards the environment, which involves living organisms like plants, microalgae, and other microbes, the biological technique of NP synthesis is chosen over the traditional chemical and physical approaches. SeNPs have garnered a lot of interest in niche fields such as antibacterial, antioxidant, and anticancer. SeNPs provide a desirable platform for medication delivery to the site of action. The biosynthesis of SeNPs carried out with *Averrhoa carambola* leaf extract is the main topic of this investigation. With the help of zeta sizing, potential, TGA-DSC, FTIR, SEM-EDS, and UV-Visible spectroscopy, the SeNPs were further characterised. Using the MTT assay, therapeutic activity such as cell viability was assessed against several cancer cell lines.

Saide *et al.*, (2021) reported that Numerous compounds derived from primary and secondary metabolism are known to be produced by microalgae. These compounds may have allelopathic, antimicrobial, and antipredator properties, according to several studies. In addition, in vitro and in vivo screenings have demonstrated that several compounds possess intriguing bioactivities (such as antioxidant, anti-inflammatory, anticancer, and antimicrobial) that may be useful in the treatment and prevention of human diseases. For a few species, the targets and mechanisms of action of these compounds, as well as the enzymatic pathways that are responsible for their synthesis, have also been investigated. However, their full exploitation and potential pharmaceutical and industrial applications necessitate additional research.

ElSaied B *et al.*, (2021) reported that the Several characterisation methods, including UV-Visible, transmission electron microscopy, Fourier transform infrared spectroscopy, X-ray diffraction, and energy dispersive X-ray, were used to evaluate the photosynthesized *S. platensis* SeNPs. They were successfully produced utilising various concentrations of sodium selenite (Na_2SeO_3) (1, 5, and 10 mM), resulting in size averages of 12.64, 8.61, and 5.93 nm, respectively, with spherical forms and strongly negative zeta potentialities. According to infrared studies, numerous phytochemicals were shown to be involved in forming SeNPs. *Salmonella typhimurium* and *Staphylococcus aureus* were used as foodborne pathogens for the

qualitative and quantitative confirmation of SpSeNPs antibacterial capabilities. The antibacterial activity was connected with and grew with SeNPs' size reduction.

Alipour S *et al.*, (2021) reports that it has been created to produce SeNPs by the biosynthesis of *Spirulina platens* and sodium selenite. Different growing conditions, such as pH and illumination schedule change, were used to produce SeNP. The FT-IR, XRD, size, and zeta potential measurements were used to characterise the SeNPs, and the DPPH and FRAP assays were used to determine the antioxidant activity of certain SeNPs. An FT-IR investigation revealed SeNP synthesis. Stable SeNPs with diameters of 145.6 and 171.13 nm were produced as a result of the 12 h dark/12 h light cycles and continuous light exposure at pH 5. Selected SeNPs had more antioxidant activity than sodium selenite.

Rajagopal *et al.*, (2021) discussed Ultraviolet-Visible analysis, X-Ray Diffraction analysis, Scanning Electron Microscopy-Energy Dispersive X-ray analysis, X-ray Photoelectron Spectroscopy, and Fourier Transform Infrared studies were used to characterise the synthesised nanoparticle. Analysis using the UV-Vis spectrum has verified the absorption value at 306nm. The *A. pinnata* stabilised nanoparticle's crystallite size was determined to be 36.45nm. The selenium nanoparticle that was created was spherical in shape. The XPS examination verified that the 3d core level region contains significant amounts of elements like selenium. The yield from the conversion of p-tolualdehyde to p-toluic acid was determined to be 78.3%. According to the antioxidant analysis, at a concentration of 500g/mL, free radical scavenging potential was determined to be high. From the antimicrobial activity, the synthesized Ap-Se NPs showed the maximum zone of inhibition in *E. coli* 17 ± 0.67 mm followed by *E. faecium* 15 ± 0.13 mm, *C. albicans* 15 ± 0.32 mm and *S. aureus* 14 ± 0.93 mm

Bhuyar P *et al.*, (2020) proposed to investigate the antibacterial properties of Cyanobacteria-derived extracts. The *Cyanobacteria* were gathered, and an isolated colony of *Oscillatoria sp.* was discovered. and it was grown for mass production in BG 11 medium. The biomass that had been centrifuged was then weighed and used to extract bioactive compounds. *Oscillatoria sp.* GCMS analysis determines the fatty acid, as well as the derivatives of triazine, pyridine, and acridine. The conclusion was that the species *Oscillatoria* because the methanolic extract inhibits the growth

of *S. aureus*, a gram positive bacteria, and *P. aeruginosa*, a gram negative bacteria, can play crucial roles as antibacterial agents. With a concentration of 100mg/ml and a zone area of $14.10 \pm 0.8\text{mm}$, the crude extract was effective against *S. aureus*. At 75 and 100 mg/ml, the crude extract effectively inhibited *P. aeruginosa*, with average inhibition areas of 13.23 ± 0.1 and $15.43 \pm 0.2\text{mm}$. Lastly, the antimicrobial activity was tested using the disc diffusion method with different concentrations of active compounds

Vikneshan *et al.*, (2020) report that Transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction were used to characterise the nanoparticles after their creation. (XRD). The nanoparticle's average size and shape, as revealed by TEM, were spherical and 85 nm in size. Additionally, the MTT assay 3T3-L1 cell line was used to test the cell viability of nanoparticle conjugates, and the results showed 34% cell viability at 30 ml/ml. Oral pathogens such as *Streptococcus mutans*, *Staphylococcus aureus*, *Lactobacillus*, and *Candida albicans* were used as test subjects for SeNPs mouthwash's antibacterial efficacy. At a concentration of 100mg/ml, all of the microorganisms were successfully eradicated.

Ruiz-Güereca *et al.*, (2019) gave an account of One of the most significant epidemiological diseases in the world tuberculosis. A combination of antituberculosis medications for at least nine months is the current treatment for tuberculosis. The search for new pharmacological agents to treat tuberculosis is a pressing need right now. Microalgae produce a wide range of molecules with potential pharmaceutical-useful biological functions.

Kashyap M *et al.*, (2019) experimented with an algal extract used in this straight forward and novel biosynthesis method to convert silver metal ions into Ag/AgCl nanoparticles. Using an alcoholic extract made from the biomass of *Chlorella sp.*, various concentrations of metallic precursors of silver nitrate (0.1mM, 0.2mM, 0.5mM, and 1mM) were tested. for the screening of four species for nanoparticle biosynthesis, *Chlorella sp.*, *Oocystis sp.*, *Lyngbya putealis* and *Vacuolatus Scenedesmus*. By reducing the metallic salt to metal ions and acting as capping agents to stabilize the particles, the biomolecules in the alcoholic extract helped the synthesis of nanoparticles. The physicochemical properties of the synthesized

particles were examined. *Chlorella sp.* prepared particle DLS analysis shows the particles that are 90.6nm in size.

Lauritano C *et al.*, (2017) Marine microalgae are thought to be a promising new and useful source of biologically active compounds that could be used in a variety of biotechnology fields. By overcoming issues associated with the excessive use of marine resources and destructive collection methods, they are able to be cultured easily, have short generation times, and enable an environmentally friendly approach to drug discovery. This study screened 46 microalgae for potential antibacterial and antifungal activities in light of the rising rate of antibiotic-resistant bacteria and fungi infections. In order to increase the likelihood of finding positive hits, two distinct extraction methods have been used. Specifically examined microalgae under both nutrient stress and control conditions. In addition, tested various strains for seven species to investigate the possibility of distinct bioactivities due to strain diversity. *Skeletonema costatum* and *Chaetoceros pseudocurvisetus*, two diatoms, had anti-tuberculosis activity when cultured in the control and phosphate starvation conditions, while the nitrogen starvation condition had no activity, according to the findings. They have also tried the water and organic extracts, and only the organic extracts for the two diatoms were active. These two diatom species' organic extracts did not harm human cell lines.

de Aragaõ *et al.*, (2016) employed a straight forward green synthesis of silver nanoparticles (AgNPs) using a polysaccharide extracted from the red algae *Gracilaria birdiae* on the Piau coast as a reducing and stabilizing agent is presented in this work. The AgNPS was made by stirring for 30 minutes at 90°C with three different polysaccharide concentrations (0.02, 0.03, and 0.05% v/v) and two pHs (10 and 11). Measurements of UV–vis and FTIR were used to track the formation of silver nanoparticles, and DLS and TEM were used to measure size and zeta potential. The silver nanoparticle-specific surface plasmon peak was observed at 410nm in the UV–vis absorption spectrum. The Fourier transform infrared spectroscopy (FTIR) analysis revealed the polysaccharide's functional biomolecules and the nanoparticles' interaction. Over the course of four months, the stability of the synthesized silver nanoparticles was examined, and no significant agglomeration was found. The AgNPs' hydrodynamic diameter ranged from 20.2nm to 94.9nm. *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-

positive) were used to test the AgNPs' antimicrobial activity, and all of the samples showed activity against *E. coli*. The environmentally friendly AgNPs were synthesized in a straightforward, quick, and one-step process from naturally occurring red algae with advantageous properties like spherical shape, small size, and negative zeta potential.

Sivakumar *et al.*, (2011) In developing nations like India, tuberculosis (TB) has been a major health issue. Finding newer anti-mycobacterial agents to combat *Mycobacterium tuberculosis*'s rise in multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains is crucial. There are a lot of interesting secondary metabolites with anti-mycobacterial activity from natural sources, which suggests that natural products could be a lucrative area for finding new anti-TB leads. *Allium sativum*, *Allium cepa*, *Syzygium aromaticum*, and *Cinnamomum verum* were found to have anti-TB activity against *M. tuberculosis* H37Ra in the current study. On the other hand, *Zingiber officinale*, *Camellia sinensis*, *Curcuma longa*, and *Elettaria cardamomum* had poor or no activity against *M. tuberculosis* H37Ra. By using the microplate Alamar blue assay (MABA) method, they were able to determine the growth and minimum inhibitory concentration (MIC) (g/ml) at which the ethanolic and aqueous extracts of medicinal plants inhibited *M. tuberculosis* H37Ra after seven days of incubation at 37°C.

CHAPTER 3

AIM AND SCOPE

1. AIM

Biosynthesis of Selenium nanoparticles using microalgae for evaluating the anti-tuberculosis activity.

2. SCOPE

1. Biosynthesize selenium nanoparticles using microalgae
2. Characterize the biosynthesised selenium nanoparticles
3. Evaluate the anti-tuberculosis activity of the biosynthesized selenium nanoparticles

CHAPTER 4

MATERIALS AND METHODS

4.1 CULTURE OF MICROALGAE

Microalgae strain *picochlorum. sp BDUG100241* is bought from Bharathisadan University, National Repository for Microalgae and Cyanobacteria (NRMCC), Trichy. The microalgae were cultured in 25ml of ASN III (Artificial Seawater Nutrient Medium) with 1ml of *picochlorum. sp* and incubated for 15 days with occasional shaking. The growth of microalgae is checked with a microscope.

2. ANALYSIS OF CULTURED MICROALGAE

1. SEM (Scanning Electron Microscope)

The topography, morphology, composition, and crystallographic information of the specimen can all be obtained through the use of SEM. The best tool for producing a finely detailed visual image of a particle with a spatial resolution of 1 nm is the scanning electron microscope (SEM). Using this kind of equipment, you can get up to 300,000 times more magnification (Akhtar K *et al.*, 2018).

The cultured microalgae morphology and size are analysed with the help of a Scanning electron Microscope. The microalgal cells were analysed at 2 kV at room temperature in a high-resolution scanning electron microscope (Salim *et al.*, 2014)

4.3 EXTRACTION OF MICROALGAE

The cultured microalgae have been centrifuged at 10,000 rpm for 15 minutes the pellet is then collected and the supernatant is discarded, the pellet is dried using a hot air oven for 20 minutes. After drying, the pellet is mixed with methanol and left for incubation for 24 hours (Prakash *et al.*, 2020).

4.4 BIOSYNTHESIS OF SELENIUM NANOPARTICLES

After the culture of microalgae with the suitable media, Sodium selenite of 5 Mm is mixed with the extracted microalgae sample with a ratio of 0.5:5, 1:5, 5:5, 7.5:5, 10:5 and incubated for 2 days. While adding Sodium selenite to the microalgae sample; it was constantly stirred with a magnetic stirrer and sodium selenite is added in small amounts continuously (Alipour *et al.*, 2021).

5. ANALYSIS OF BIOSYNTHESISED SELENIUM NANOPARTICLES

1. *UV-Spectrophotometer*

UV spectroscopy is a type of absorption spectroscopy in which molecules absorb light in the UV region (200–400 nm). When UV rays are absorbed, the electrons are excited from their ground state into a higher energy state. (Chirayil CJ *et al.*, 2017).

The biosynthesised nanoparticle is scanned with the UV- Spectrophotometer with spectral scanning ranging from 250nm – 500nm (Ganeshkar *et al.*, 2022)

2. *Zeta potential*

The scientific name for the electrokinetic potential in colloidal dispersions is zeta potential. The stability of colloidal dispersions is largely determined by the z potential. The size of the z potential represents how much electrostatic attraction there is in the dispersion between nearby particles with identical charges. A high z potential will give stability, meaning that the solution or dispersion will resist aggregation, on molecules and particles of sufficient size. (Chirayil CJ *et al.*, 2017)

The surface zeta potential of synthesized SeNPs was measured in ultrapure water at 25°C using the Malvern Nano ZSP (Malvern Instruments, UK). The data obtained by analysing the electrophoretic mobility of 1 mg/mL nanoparticle solution was converted to the zeta potential value by using the Helmholtz-Smoluchowski equation. (Dinc *et al.*, 2022)

3. *FTIR (Fourier Transform Infrared)*

Fourier transform spectrophotometers provide the infrared spectrum much more quickly than standard spectrophotometers. An interferogram, also known as

constructive and destructive interference, is produced by the recombination of beams with various path lengths in the interferometer. The beam enters the sample compartment, where it is absorbed by the sample at precise frequencies that are specific to the sample from the interferogram. The detector then simultaneously measures the energy against time for all frequencies of the particular interferogram signal. Different frequencies give different compounds present (Mohamed MA *et al.*, 2017).

Using Bruker Optics TENSOR 27 FTIR spectrometer (Ettlingen, Germany), the IR spectra of the lyophilized powders were noted at a wave number range of 1000–4000 cm^{-1} (Kora, 2018)

4.5.4 XRD (X-Ray Diffraction)

In order to learn more about the structure of crystalline materials, X-ray diffraction (XRD) uses the X-rays' dual wave/particle nature. The technique is primarily used for the characterization and identification of substances based on their diffraction pattern. According to Bragg's law ($n\lambda = 2d\sin\theta$), crystals can diffract X-rays. This method calculates the typical distance between atom layers or rows.

For X-Ray Diffraction Analysis, the Selenium nanoparticles were dried, powdered, and then analysed using Powder X-Ray Diffractometer. The 2θ peaks were noted to confirm the presence of the nanoparticles. The instrument used for the analysis was Model-D8 Advanced, BRUKER, Germany (Whitfield P *et al.*, 2014)

6. ANTI-TB ASSAY

The Selenium nanoparticle sample is centrifuged at 15000 rpm for 15 minutes and the pellet is collected and dried with the help of a hot air oven for 30 minutes and the dried material is scrapped to a powder. The powdered sample is mixed with the water and subjected to MABA assay (Microplate Almar Blue Assay)

1. Inoculum Preparation

Mycobacterium tuberculosis, Reference strain H37Rv was sub cultured and incubated at 37°C using Middle brook 7H9 broth which is supplemented with 0.2% glycerol and 10% OADC (to prepare 500ml 7H9 broth 50ml OADC was added) enrichment for 21 days (until logarithmic phase). The standard inoculum was

prepared in sterile 7H9-medium adjusted to a McFarland standard No.1 (Equivalent to a standard suspension of 10^7 CFU/ml). This concentration was further diluted to 1: 25 and then used as an inoculum, to make that the bacteria were at the start of the log phase when the test commenced and 100 μ l was added to the inoculums to make the final volume of 200 μ l.

4.6.2 *In vitro* Anti-Tubercular Assay

Mycobacterium tuberculosis reference strain H37Rv was used to evaluate the preliminary screening of given extracts at concentrations of 250mg/mL, 500mg/mL and 1000mg/ml. Microplate Alamar Blue Assay (MABA) method was used for the study. Prior to the bioassay, stock solutions of Rifampicin with a concentration of 32 μ g/mL were prepared and stored to be used for the positive control. Control wells without the tested extracts and sterility controls were assayed simultaneously. Rifampicin was prepared from the stock solution just prior to inoculation time to the concentration of 5 μ g/mL in the total volume of 200 μ l. The growth inhibition result was explained by Microplate Alamar Blue Assay (MABA) using 1% resazurin. The reagent allows the detection of microbial growth in microtiter plates without the use of a spectrophotometer.

The susceptibility test conducted by the Microplate Alamar Blue Assay was using 96 well microtiter plate to evaluate the susceptibility of H37Rv MTB reference strain to the extract. The inhibitory concentration of all extracts was evaluated with concentrations of 250mg/ml, 500mg/ml and 1000mg/ml in the total volume of 200 μ l. Prior to inoculation, the crude extracts of 50mg, 100mg and 200mg were measured to make the proposed concentration in 200 μ l. The measured extracts were mixed with the bacterial suspension and the diluent media (7H9) in the well. One hundred microlitres of Middle Brook 7H9 broth and the test inoculum were added to all testing wells, as well as to the drug/extract-free control wells.

Then, one hundred micro litres working extract solutions were poured into the first well of each row from which two-fold dilution series were made through the micro plate column. Each extract concentration was assayed in triplicate. Each micro plate was then sealed with parafilm and incubated for 5–7 days at 37°C in normal atmosphere. After the incubation period, 25 μ L of resazurin 0.02% w/v was added to each wells and re-incubated at 37°C for 24 hr for colour development. The Alamar Blue oxidation-reduction dye is a general indicator of cellular growth and/or viability; the blue, non-fluorescent, oxidized form becomes pink and fluorescent upon

reduction. Growth was therefore determined by a visual colour change. Minimum inhibitory concentration (MIC) was determined for those extracts showing inhibitory effect. The MIC was conducted at various concentrations of 1, 6, 12.5, 25, 50, 100 mg/ml of the extracts. The visual MIC was defined as the lowest drug/extract concentration that prevented the colour change of resazurin reagent from blue to pink. Blue colour in the well was interpreted as there is no mycobacterial growth and pink colour was scored as growth occurrence (Arokiarajan *et al.*, 2023)

CHAPTER 5

RESULTS AND DISCUSSION

5.1 CULTURE OF MICROALGAE



Figure 5.1 Microalgae *picochlorum. sp*

The stock *picochlorum. sp* culture was inoculated into ASN III (Artificial Seawater Nutrient medium), and after 15 days of incubation with occasional shaking under light 12hr:12hr (light: dark), the medium's colour changed to a green, suggesting that the culture had grown. SEM was used to conduct further study on the culture.



Figure 5.2 Culture of *Picochlorum. sp* in 25ml of media



Figure 5.3 Subculture of *Picochlorum.sp*



Figure 5.4 Culture conditions of microalgae

2. ANALYSIS OF CULTURED MICROALGAE

For a further culture study with scanning electron microscopy, the cultivated microalgae are centrifuged at 8000rpm for 15 minutes to separate the medium from the microalgae. The green pellet is collected, dried, and powdered.

1. Scanning Electron Microscopy

The SEM images show the structure of the microalgae *picochlorum. sp*

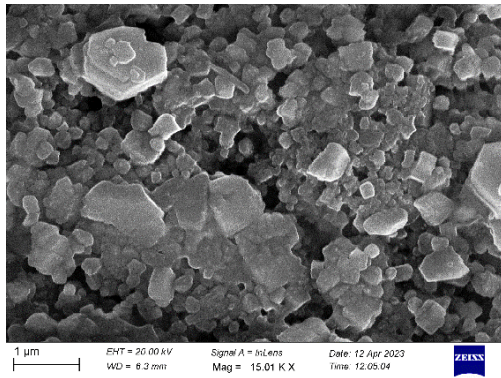


Figure 5.5 SEM images of microalgae *Picochlorum. sp* at the scale of 1μm

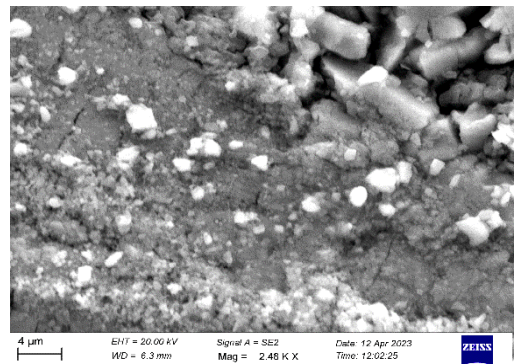


Figure 5.6 SEM images of microalgae *Picochlorum. sp* at the scale of 4μm

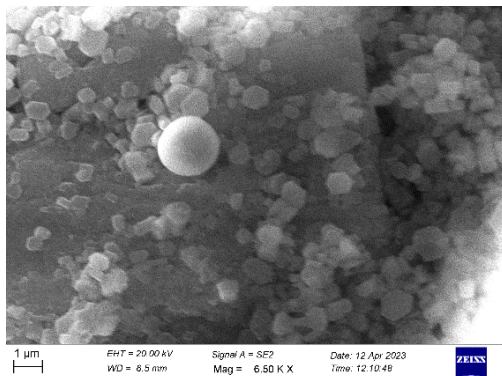


Figure 5.7 SEM images of microalgae *Picochlorum. sp* at the scale of 1μm

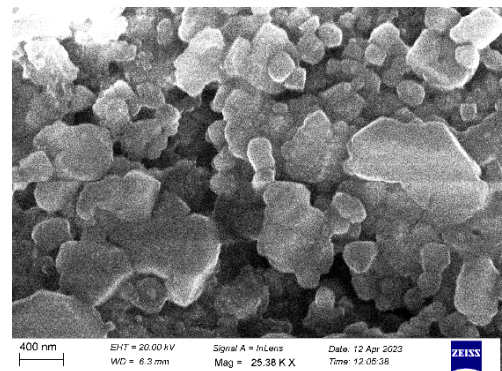


Figure 5.8 SEM images of microalgae *Picochlorum. sp* at the scale of 400nm

5.3 BIOSYNTHESIS OF SELENIUM NANOPARTICLE

When compared to physical or chemical approaches, the creation of nanoparticles using green sources like phytochemicals of microorganisms, plants, proteins, or enzymes as reducing agents in the case of microbes is one of the extensively used reaction strategies. The lack of harmful chemical use, low-cost machinery, manufacture of biodegradable products, enhanced selectivity, and high yield are benefits. Characterising the capping agent and the nanoparticle production using imaging tools is a vital step since it plays a significant role in stabilising the nanoparticles and aids in improving their biological applications. (Menon *et al.*, 2019) Visual colouring is the first step in the synthesis of nanoparticles. It came to light that the colour of the green solution (Figure 5.9) turned red after the incubation period (Figure 5.10).

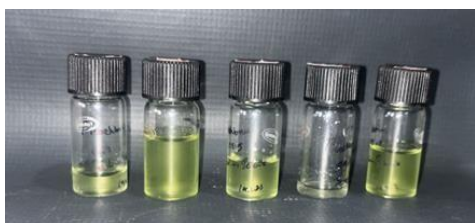


Figure 5.9 Before biosynthesis of selenium nanoparticle



Figure 5.10 After biosynthesis of selenium nanoparticle

4. ANALYSIS OF BIOSYNTHESISED SELENIUM NANOPARTICLE

1. UV Spectrophotometer

The peak was noticed at about 240nm in Spectrum A3 (1:5 Sample) as shown in figure 5.11 whereas the sample 5:5 has a characteristic peak value at 200nm during the spectrum scanning of the biosynthesized selenium nanoparticle from 250 to 500nm. According to a UV-Vis examination, the maximum absorption was between 200 and 300. *Pseudomonas aeruginosa* ATCC 27853 (Kora and Rastogi 2016), *Z. ramigera* (Srivastava & Mukhopadhyay, 2013), and *Vitis vinifera* (Raisin) Extract (Sharma *et al.*, 2014) all saw a similar colour shift, which denotes the synthesis of the elemental form of selenium from sodium selenite. According to Rajeshkumar *et al.*, (2013) and Zhang *et al.*, (2004), the process known as SPR (surface Plasmon resonance) is what causes the particular colour.

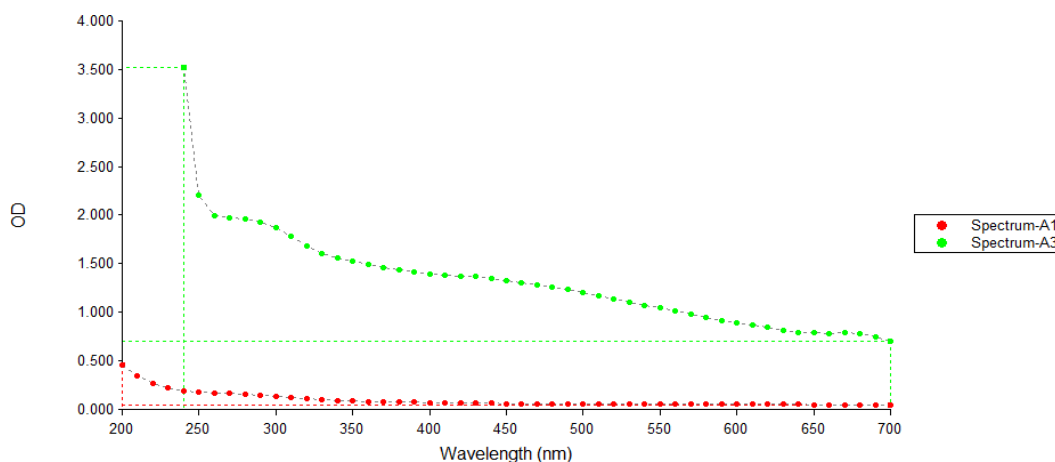


Figure 5.11 UV Spectrophotometer attained OD values of 240nm and 200nm

5.4.2 Zeta Potential

The Zeta Potential of the biosynthesised selenium nanoparticle is -14.5 mV, and the negative charge indicates that the nanoparticle is stable. 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°

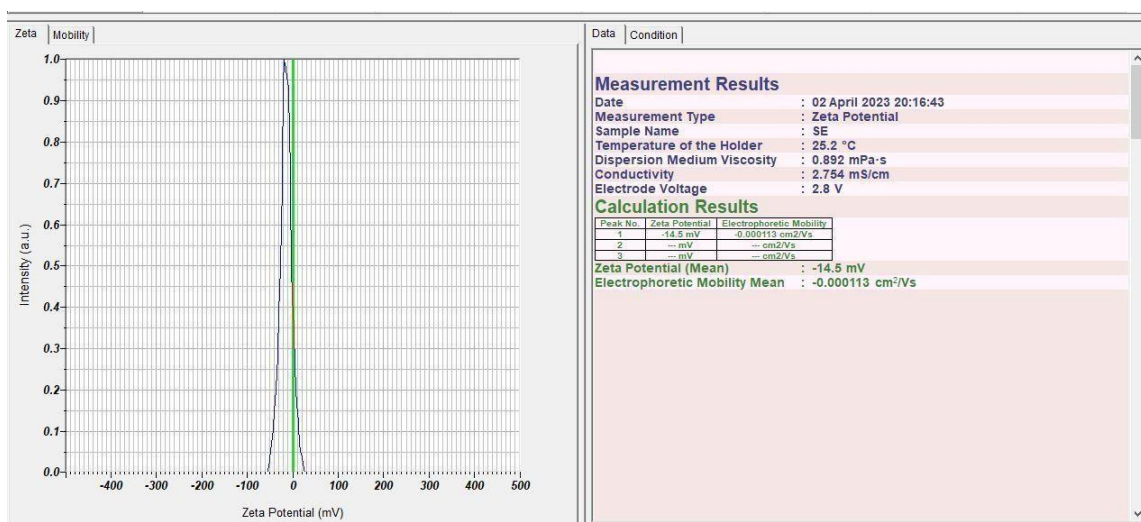


Figure 5.12 Zeta Potential of the Biosynthesised Selenium Nanoparticle

5.4.3 FTIR (Fourier Transform Infrared)

The FTIR spectrum (Figure 5.13) was taken for biosynthesized and compared with the cow urine extract. These functional groups gave information on stabilising or reducing the precursor to elemental selenium.

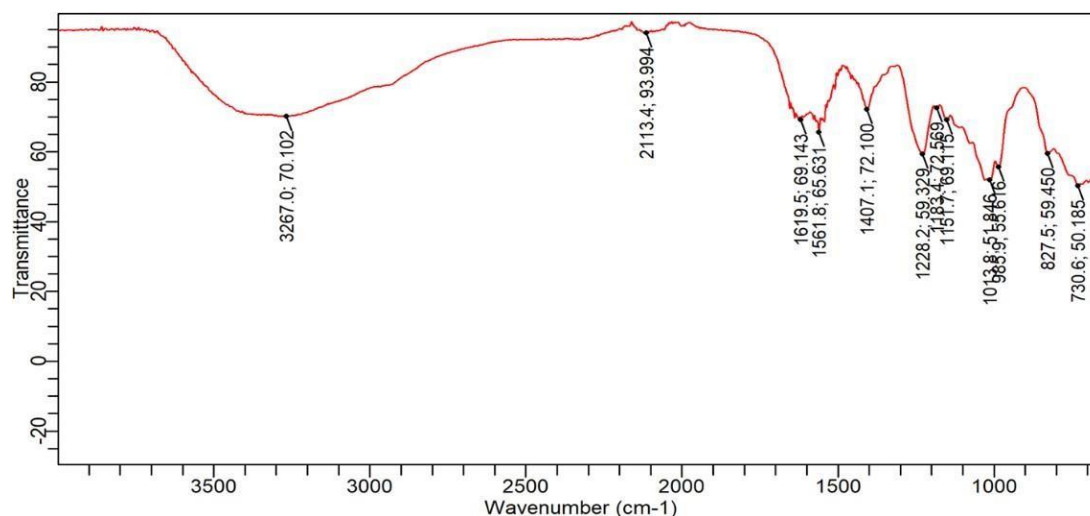


Figure 5.13 FTIR results of the Biosynthesised Selenium Nanoparticle

Absorption bands around 1619.52829 and 1561.75455 are attributed to amide I and amide II linkage, and absorption bands around 3267.01161 are known to result from O-H and N-H stretching vibration from polysaccharides and proteins. concluded that the proteins in the sample play a role in the synthesis and stabilization of selenium nanoparticles. From the FTIR, it can be shown that the bio-organics from *S. platensis* extract served as potent capping and reducing agents on Selenium Nanoparticles including proteins, esters, amino acids, and carbonyl-unsaturated ketone amides (Basant ElSaied *et al.*, 2020). The absorption bands from the graph is tabulated in Table 5.1

Table 5.1 FTIR Peak Values

Peak Number	Wavenumber (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

5.4.4 XRD (X-Ray Diffraction)

Characteristic peak at 2θ value of 26.941 and 44.894 (Figure 5.14) indicates the presence of selenium nanoparticle which is reduced from sodium selenite. The crystalline nature and purity of nanoparticles were determined using the powder X-ray diffraction technique. The diffraction peaks were observed at 23.3 (100), 29.6 (101), 43.5 (012), and 50.05 (201) which are in good agreement. The unassigned peaks (*) present in the XRD data could be due to the biomolecules present in the Seaweed leaf extract (Khatoon *et al.*, 2015). Whereas Jayanthi Abraham *et al.*, suggested a characteristic peak at 2θ value of 23.680, 29.788 and 43.9 indicating that the presence of selenium nanoparticles reduced from selenite ions present in the medium.

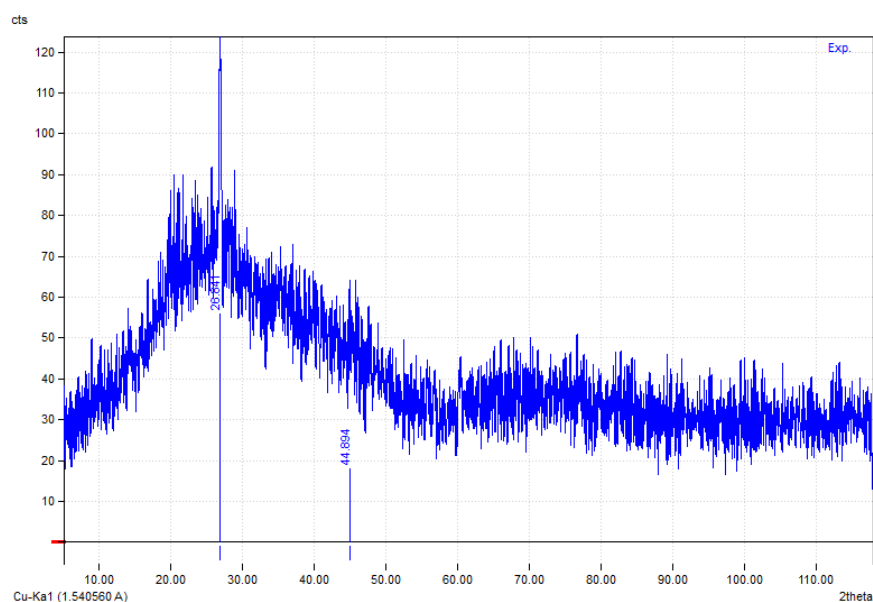


Figure 5.14 XRD Results of the biosynthesised Selenium nanoparticle

5.5 ANTI-TB ACTIVITY MABAASSAY

The biosynthesised sample is incubated in the MABA Assay, and sample 2 (1:5) was found to be the most effective compound (MIC: 1 / mL) among the compounds whose anti-tb activities were evaluated against *Mycobacterium smegmatis*. Sample 1 does not show activity. Sample 2 show activity at 6.21 mg/ml. Which is explained in Figure 5.15

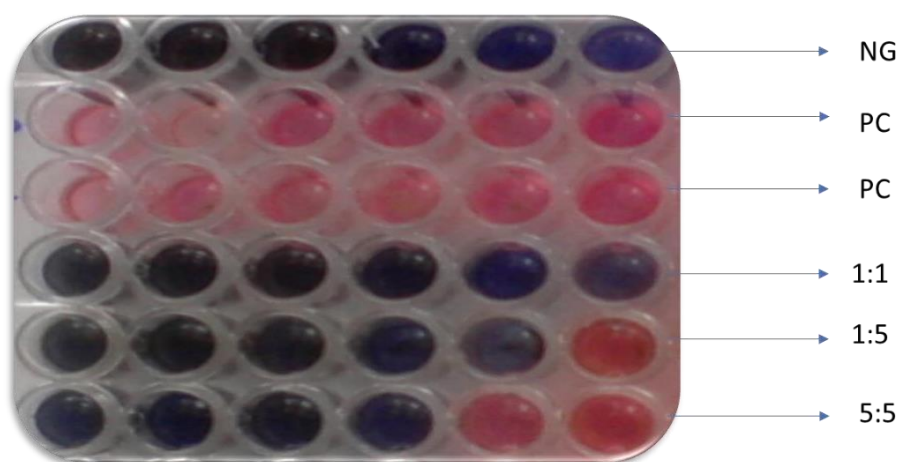


Figure 5.15 MABA assay for Anti-TB activity of Biosynthesized selenium nanoparticle

NG-Negative control; PC-Positive Control; 1:1-Selenium: microalgae extract; 1:5-Selenium: microalgae extract; 5:5-Selenium: microalgae extract.

Table 5.2 MABA assay activity with respective concentrations

Concentration (mg/ml)	NG (Negative Control)	1:1	1:5	5:5	PC (Positive Control)
200	-	-	-	-	+
100	-	-	-	-	+
50	-	-	-	-	+
25	-	-	-	-	+
12.5	-	-	-	+	+
6.2	-	-	+	+	+

CHAPTER 6

SUMMARY AND CONCLUSION

Major health issues in developing nations, particularly India, have included tuberculosis (TB). Finding novel anti-mycobacterial treatments is urgently needed to counteract the rise in MDR and XDR strains of *M. tuberculosis*. In this study, the microalgae *Picochlorum.sp* are cultured and biosynthesised with sodium selenite to obtain selenium nanoparticles further, the nanoparticles has been analysed with a UV- Spectrophotometer which gave a peak value of 200nm-300nm, with Zeta potential the nanoparticles obtained a value of -14.5mV indicating the stability of the nanoparticle, then the FTIR (Fourier Transform Infrared) analysis several bio-organic compounds shown with the peak values that these compounds help in the synthesising and stabilising of the nanoparticle, with 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, and after concluding that the compound is a nanoparticle, further the sample is now given for Anti-TB activity with MABA Assay (Microplate Almar Blue Assay) and the sample with ratio of 1:5 showed a positive activity by resazurin reduced to resorufin upon cellular metabolism.

By the above experiment, we conclude that the Selenium nanoparticle of microalgae shows anti-tubercular activity at 1mg/ml and it may be used for further investigations. The presence of flavonoids, alkaloids, tannins, triterpenoids, steroids and carbohydrates in microalgae extract alone or in combination might be responsible for the observed activity. Further studies need to be carried out to isolate the individual compounds from the crude extract, their purification, characterization and pharmacological screening will be an informative tool in revolutionizing marine organism-based medicine for the treatment of TB.

CHAPTER 7

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