

***Biosynthesis of silver nanoparticles from the seaweed for
antimicrobial activity***

Submitted in partial fulfilment of the requirements for the award of

Bachelor of Technology degree in Biotechnology

By

B. NETHRA (38230023)



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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APRIL 2022



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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **B. Nethra (38230023)** who carried out the project entitled **“Biosynthesis of silver nanoparticle from the seaweed for the antimicrobial activity”** under our supervision from January 2022 to April 2022.

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Submitted for Viva voice Examination held on _____ 2022

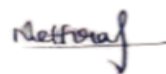
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DECLARATION

I, **B. Nethra (38230023)** hereby declare that the Project Report entitled **“Biosynthesis of silver nanoparticle from the seaweed for the antimicrobial activity”** done by us under the guidance of **Dr. Ramesh Kumar. V** is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

DATE: 21st April 2022
PLACE: Chennai



SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to Board of Management of SATHYABAMA for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to Dr. Ramesh Kumar. V, Head of the Department, Dept. of Biotechnology for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide Dr Rajasekar T for his valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology and Centre for the Drug Discovery and Development who were helpful in many ways for the completion of the project.

ABSTRACT

In this present study we synthesis the silver nanoparticle and evaluation the antibacterial activity of the silver nanoparticle by using *Padina tetrastromatica* and *sargassum wightii* marine algae. *Padina.sp* and *S.wightii* was collected from the Rameswaram, Southeast coast of India. The seaweed extract was used for the synthesis of AgNO₃ at room temperature. UV-visible spectrometry study revealed surface plasmon resonance at 413 nm. The characterization of silver nanoparticle was carried out using Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD) and transmission electron microscope (TEM). FT-IR measures revealed the possible functional groups responsible for reduction and stabilization of the nanoparticles. X-ray diffraction analysis showed that the particles were crystalline in nature. TEM micrograph has shown the formation of silver nanoparticles with the size in the range 50-100nm. The synthesized AgNPs have shown the best antibacterial activity against human pathogens such as *Staphylococcus aureus* and *Escherichia coli*. The above eco-friendly synthesis procedure of AgNPs could be easily gauged up in future for the industrial and remedial requirements.

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LIST OF SYMBOLS AND ABBREVIATIONS

i.	SEM	:	Scanning electron microscope
ii.	TEM	:	Transmission electron microscope
iii.	FTIR	:	Fourier transform infrared spectroscopy
iv.	UV	:	Uv-visible spectroscopy
v.	AgNO ₃	:	Silver nitrate
vi.	AgNPS	:	Silver nanoparticle
vii.	PADINA SP	:	Padina tetratomatica
viii.	<i>S.wightii</i>	:	Sarggasum wightii

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1. INTRODUCTION

Pathogenic bacteria are playing an important part in the creation of unknown conditions and the development of antibiotic resistance which are the major problems in the current script. The operations of nanoparticles are gaining an important function in the current script as they retain well- defined chemical, visual and mechanical attributes. Nanoparticles of essence are the most implicit agents as they show excellent antibacterial conditioning due to their large face area-to- volume rate, which is getting up. As the current interest in the experimenters due to the growing microbial resistance against essence ions, antibiotics and the growth of resistant strains. Antimicrobial nanoparticles offer colourful distinctive advantages in reducing acute toxin, prostrating resistance, and lowering cost, when compared to conventional antibiotics (Prakash et al., 2020).

Antibiotics in the NPs form may sustain for long run than in bitsy moles. Physical and chemical conflation styles, aimed at controlling the physical parcels of the patches are substantially employed for the product of essence nanoparticles. Utmost of the styles are yet in the experimental phase and colourful troubles are frequently endured with the Troop of the Nano particles medications, control of the charger's growth and aggregation of the patches. Accordingly, experimenters working in the field of nanoparticles medication turned their attention towards natural systems. In the biosynthesis of nanoparticles, natural organisms like bacteria, fungi, actinomycetes, incentive, algae and shops were employed as reducing agent or defensive agents. Biosynthetic system of nanoparticles has surfaced as a simple and feasible volition to more complex chemical synthetic procedures to gain nanomaterial. rate of reduction of essence ions using natural agents is observed to be important quicker with an ambient temperature and pressure conditions of all the different types of essence nanoparticles, the tableware nanoparticles are playing a major part in the field of nano-technology and nanomedicine (Rajeshkumar et al., 2012). A number of living organisms are formerly well known to unfold tableware nanostructured emulsion similar as cyanobacteria, bacteria, fungi, actinomycetes and shops similar as *Cinnamomumcamphora*, *Medicago sativa*; *Retchkiman-Schabesy*, *Pelargonium graveolens*, *Avena sativa*, *Azardirachtaindica*, *Tamarindusindica*, *Emblicaooffcinalis*, *Aloe vera*, *Coriandrum sativum*, *Carica papaya*, *Parthenium hystrophorus*, *Tritium vulgare*, *Acanthella elongata* and *Sesuvivmpotulacastrum*. Biosynthesis of tableware NPs using the marine seaweed *Sargassum wightii* was carried out by Shanmugam

etal., and they've shown that the sizes of the patches are in the range of 20 nm. In our present study, we report the conflation of AgNPs with sizes in the range of 10 nm using (Green algae) *Caulerpa racemosa* excerpt and also assessed their negative effect against gram-positive and gram-negative bacteria(Kathiraven et al.,2015)

Over the once many decades, nanoparticles in their nanometric scale (1-100 nm) have inspired important interest besides structurally parade considerably bettered physical, chemical, and natural parcels, features, and functionality. Nanomaterials parade unique, superior, and essential parcels and have attracted important attention for their distinct characteristics that aren't displayed in conventional macroscopic accoutrements. Their oneness arises specifically due to their advanced face to volume rate (RajeshkumarS, Rajivgandhi et.al- 2021). They represent the involvement of controlling and manipulating matter at their infinitesimal scale in the development of new bias that can be used in colourful physical, natural, biomedical, and pharmaceutical operations Over the once centuries, tableware has been used as a safe inorganic antibacterial agent which is non-toxic and well known in able of killing about 650 types of conditions causing microorganisms. Tableware has been described as being oligodynamic because of its capability to play a significant eventuality for a wide range of natural operations similar as antifungal agent and antibacterial agents for antibiotic-resistant bacteria, precluding infections, healing injuries, and anti-inflammatory indeed at nanosecond attention. Gray ions (Ag), are engaged in the expression of dental resin mixes, bone cement, ion exchange filaments and coatings for medical bias since its being an antibacterial element. Lately, studies conducted have shown that tableware nanoparticles parade strong inhibition and serve as a defensive hedge for utmost of the microorganism, for illustration, gram positive bacteria, gram-negative bacteria, medicine-resistant bacteria, contagions, fungus, and indeed some of the pathogen. The rubric *Caulerpa* is nearly comprised of 60 species which are astronomically distributed in tropical and tropical waters. Some species of *Caulerpa* (*Caulerpaceae*) are comestible and used as salad, still it has been shown that consummation of *Caulerpa* species especially during the stormy season could beget poisoning which ascribes to caulerpicin.

It has been mentioned that carnivorous fish and pets avoid consuming of the *Caulerpaceae* and *Codiaceae* shops. Algae of *Caulerpaceae* family produce several secondary metabolites including sesquiterpenoids and diterpenoids which protects shops from beasties. It seems that caulerpenyne, a sesquiterpen, plays a major part

in chemical defense . In addition, triterpenes; squalene, squalene epoxides, sterols, di-indolocolors; caulerpin and its analogues, caulersin, an admixture of ceramides derivations; caulerpecin are the other secondary metabolites that insulated from different *Caulerpa* species. Before studies had shown anti HSV-1, anti-bacterial exertion, antitumor exertion and factory nonsupervisory goods for caulerpin.

Grounded on the factors mentioned over, seaweeds have attained great attention in the medicinal and biomedical fields. Still, different rubrics and species of seaweed have a implicit variation in bioactive emulsion types and functions. A many former study reported that, fucosterol and hexadec-4-enoic acid uprooted from *Sargassum longifolium*, have inhibitory goods on *Vibrio parahaemolyticus*, *Pseudomonas fluorescens*, and *V. vulnificus*, while focusterol in *Eklonia stolonifera* showed anti-fat exertion in 3T3-L1 adipocytes. Thus, species specific identification is essential before studying the exertion of bioactive composites of seaweeds.

Thus, it's necessary to exhaustively characterize seaweeds, which might help in understanding their eventuality in the product of value- added composites. In this study, seaweeds species from brown, red, and green algae were named grounded on physiology, geographical position and edibility. The named seaweeds were linked grounded on morphological characterization and molecular comment. Also, the characterizations including bio-components analysis (carbohydrates, proteins, and lipids), proximate and ultimate analyses were performed along with FTIR and TGA/DTG analyses to estimate their implicit operations. In this present study we synthesis and evaluation antibacterial activities of the silver nanoparticle from *Padina tetrastromatica* and *sargassum wightii* marine algae.

2. REVIEW OF LITERATURE

Multidrug-resistant bacteria (MDR) are becoming a worldwide unhealthiness, and scientists are continuously investigating new strategies to fight against MDR. Seaweeds are a crucial source of biological compounds and should function as natural sources for bacterial infection control. This study evaluated the antimicrobial and anti-quorum sensing (QS) activities of phlorotannins from *Hizikia fusiforme*. The phlorotannins exhibited antimicrobial activity against selected bacterial pathogens and inhibited QS activity of the reporter strain *Chromobacterium violaceum* 12472 by inhibiting purple pigment production. Phlorotannins can decrease the bacterial motility, reduce the assembly of extracellular protease, hemolysin, and pyocyanin and inhibit biofilm formation of *Pseudomonas aeruginosa*. In vivo studies showed that phlorotannins can reduce *Paeruginosa* inflicted mortality in *Caenorhabditis elegans*. Seaweeds are non-vascular, photosynthetic plants that inhabit near-coastal regions and are one of the richest and most promising sources of primary and secondary bioactive metabolites with antimicrobial properties. Phlorotannins are polyphenolic compounds of seaweeds and have many biological activities including antioxidant, antidiabetic, anti-inflammatory, and antimicrobial. In this study, they investigated the results of phlorotannins from brown seaweed on QS activity in *Chromobacterium violaceum* and virulence factors and biofilm formation in *Pseudomonas aeruginosa*. Furthermore, we determined the survival rate of *Caenorhabditis elegans* against *Paeruginosa* infect (Jiali Tang et al.,2020)

Opportunistic pathogen is also known as rapidly growing mycobacterium. In antibacterial treatment the mycobacteria are highly resistant. In this study we using Antimicrobials Amikacin, Ciprofloxacin for testing it were evaluated for susceptibility of pathogen and in this study, we investigate the anti-biofilm activity of antimicrobial it's against mycobacterium. Non-tuberculous is caused by Mycobacterium. It was in air liquid surfaces and mycobacterium has Hydrophobicity and it allows subsequent formation and adhesion of cell on the surfaces (Vanessa Da Costa Flores et al.2016).

Dental diseases are caused by various dental plaque biofilm. To prevent the dental disease and to inhibit the growth of dental plaque bacteria because it produces the biofilm. Marine algae had certain compound sulphate polysaccharides which exert antimicrobial activities and it against human bacterial pathogen and its physiological benefits. It has fucoidan which from *Fucus vesiculosus*. It was able to notify the antimicrobial activities against dental plaque. And it has inhibitory concentration which

is above $250 \mu\text{g ml}^{-1}$ it was able to suppress biofilm completely. In this study we evaluated the observation of antimicrobial and antibiofilm activities of sulfate polysaccharides from different marine algae which against dental plaque bacteria. The heat treatment was changing the molecular weight of fucoidan which affect the activity of antimicrobial. (Joon-young Jun et al.,2018)

Marine algae and seaweeds are widely used for the human's purpose, and they used marine algae for the various purpose food and drug development in some seaweed have the abundant source that namely phytochemical property it was used to develop for the new insecticides and antimicrobial compounds. In this study we investigate the two species of the brown *sargassum wightii* and the green *halimeda gracilis* this extract was used to obtained five solvents for varying polarity and then the bioactive compound of the seaweed was tested by the uses of FTIR spectroscopy. The biofilm formation was detected by the compound of antimicrobial activity, the light microscopy was detected the morphological change that exspore in the seaweed extract and the result of this study reveals that the relevant bioactivity of the phytochemical compound occur in two seaweed it proves the possibility of that seaweed can use to develop the biodegradable pesticide and to inhibit the development of microbial biofilm formation by the help of antimicrobial drug.(S,suganya et al.,2015

Antibiotics in the NPs form may sustain for long run than in bitsy motes. Physical and chemical conflation styles, aimed at controlling the physical parcels of the patches are substantially employed for the product of essence nanoparticles. Utmost of the styles are yet in the experimental phase and colourful troubles are frequently endured with the Troop of the Nano particles medications, control of the charger's growth and aggregation of the patches. Biosynthetic system of nanoparticles has surfaced as a simple and feasible volition to more complex chemical synthetic procedures to gain nanomaterial. rate of reduction of essence ions using natural agents is observed to be important quicker with an ambient temperature and pressure conditions. .(rajeshkumar,2012)Of all the different types of essence nanoparticles, the tableware nanoparticles are playing a major part in the field of nano-technology and nanomedicine. A number of living organisms are formerly well known to unfold tableware nanostructured emulsion similar as cyanobacteria, bacteria, fungi, actinomycetes and shops similar as Cinnamomumcamphora, Medicago sativa; Retchkiman-Schabesy, Pelargonium graveolens, Avena sativa, Azardirachta indica,

Tamarindus indica, Emblica officinalis, Aloe vera, Coriandrum sativum, Carica papaya, Parthenium hysterophorus, Tritium vulgare, Acanthella elongata and Sesuvium portulacastrum.

Pathogenic bacteria are playing an important part in the creation of unknown conditions and the development of antibiotic resistance which are the major problems in the current script. The operations of nanoparticles are gaining an important function in the current script as they retain well-defined chemical, visual and mechanical attributes. Nanoparticles of essence are the most implicit agents as they show excellent antibacterial conditioning due to their large face area-to-volume rate, which is getting up. As the current interest in the experimenters due to the growing microbial resistance against essence ions, antibiotics and the growth of resistant strains. Antimicrobial nanoparticles offer colourful distinctive advantages in reducing acute toxin, prostrating resistance, and lowering cost, when compared to conventional antibiotics. (Prakash et al, 2020)

The marine brown algae have the higher levels of the carbohydrates, minerals etc. they also considered as the food source, and they widely used for food and food additives. The *cystoseria hakodatensis* is a brown alga it has been unutilised it belonging to the family of *sargassaceae*. In this study we showed the crude methanol extract from the algae, and it has inhibitory effect for the growth of *bacillus cereus* and *bacillus licheniformis*. The process were applied in these studies is liquid-liquid column chromatograph it has marked to the antimicrobial agent was isolated to cause hydrophobic fraction to determine the phenolic form without the phytochemical. The conclusion of the studies refers to prove the *cystoseria hakodatensis* have the potential source of antimicrobial agent with the capable of preventing the two bacterial growth. (Joon-young jun et al., 2015)

The silver nanoparticle it has the red algae named *gelidium corneum* extract that used as the reducing agent. The silver nanoparticle were obtained by the characterization of uv-vis, xrd and FTIR. It indicated the measurement of reduction of nanoparticle. xrd analysis it showed the crystalline structure in the centric cubic geometry form. The result obtained by the broth microdilution test, and it showed the agnp has higher antimicrobial activity and lower mic. The antibiofilm efficacy were examined in two types of biofilm namely prebiofilm and postbiofilm. The result of this

study showed that the silver nanoparticle have the friendly feature that used for the process of industrial and therapeutic (Betulyilmazozturk et al.,2019)

To reduce the Ferric chloride and stabilizing the formed a Fe_3O_4 -Nps they were three samples of iron oxide nanoparticles. It was successfully synthesized by using *Sargassum vulgare*, *Ulva Fasciata* and *Jania Ruben* Aqueous extract. It was characterized by the uses of standard methods in these studies they have evaluated the antibiofilm activity of the selected a seaweed and their biosynthesized a Fe_3O_4 -Nps against the marine bacterial biofilm where examine in vitro. Furthermore, it was showing a good result of tested the seaweed. Green synthesis of nanoparticles by the use of a seaweed, and it reduces the toxic substance, and it maintains the environment.(Dalia M.S.A.Salem et al.,2020)

In the global many have health challenges and the antimicrobial resistance were rapidly spreading by the various microorganisms namely bacteria, fungi, viruses etc. They were resistance to antibiotic many of parts in the world they were affected by the antimicrobial resistance .the resistance were divided into selective favors of pan-resistance, MDR stains etc. The result the shown the increase in mortality, morbidity there range were increasing to treat the bacterial infectious diseases. They initiated to change the genetically modified which they include the genetic transfer of resistance gene through the form of plasmid and mutation of target genes, biofilm mechanisms means which the bacteria able o survive in the presence of an antibiotic because they have the ability to communities for themselves which they are ebbed with exopolysaccharide matrix .in this biofilm state bacteria can either stay biotic or abiotic surface because they are association of different microorganisms and the pathogen, but they do not attach to the surface which embed in self producing matrix, and they could form the filamentous bacteria strains in the form of tightly held together by the help of EPS which is known as extracellular polymeric substance. In this study there aim is to evaluate the antibiotic is study against n.mucosa, ecoil by the method of antibacterial potential of cr-sps it evaluated by agar cup diffusion, mi, CFU and bacterial concentration assays.(Jyoti vishwakara and sirisha L vavilala,)

Seaweed is the most important source in marine kingdom its has bioactive compound. In this research we evaluated the anti-biofilm active compound in the ethanol extract. They were isolated, and their structure were identified. The term ethanol where extract

by the compound called *Gracilaria fisheri* marine seaweed, and it were effectively decreased the formation of biofilm in *Vibrio Harveyi*. Minimum inhibitory concentration it where have biofilm assay, and it produced two fractions which produced by maximal inhibitory toward the biofilm formation of *V.harveyi* strains 1114and BAA116 it has the method called chromatographic separation for the bioactive formation they were isolated and their structure where explain by the use of FTIR, NMR etc. in the invitro activity were demonstrated by the both compound to inhibit the biofilm formation and anti quorum sensing. This study were observed by the property of N- benzy cinnamamide which extract by the ethanol it leads by the antimicrobial drug against by the *V.harveyi*. (Kulwadee Karnjana et al.2020)

Gram negative bacteria it uses the molecule named n-acyl homoserine lactone (AHL) for quorum sensing signal.which acts as a signals for regulate the various biological functions.the term quorum sensing inhibitor it stimulates the bacteria and inactive the qs signals for producing the molecule in other bacteria. The aim of this study is to screen and identified the epibiotic bacteria that associated with the producing the qs inhibitory to isolate the serratia rubidaea they use the jcm 14263 as an indicator organism and this indicator controls by the production of the red pigment. It also shows that the epibiotic bacteria play the role in defensive mechanism by the methods of producing qsi like compounds and its suppress the other competitive bacteria.(M.kanaga sabhapathy et al.2009)

In the marine environment algae were ever present, and they were interacting with many of the microorganisms such as bacteria these interaction leads to an impact for the physiology of the both. In this study we evaluated the interaction between and studied the information regarding the chemical -ecological role to the quorum sensing. Which stimulate the response to correlated to the population density, in this article they summarize how to modulate the behavior of microbial and how they affect the symbiotic relationship which regulates the phytoplankton and its chemical process .it modulated the evolution relationship between the algae and the bacteria plankton, and it impacts that the structure and the function of the population in the symbionts.(Jin zhou et al.,2016)

Marine organism variety of drug it has chemical structures it provided major source for pharmaceutical. The pathogenic bacteria synch their expression of gene to cell to cell communication it can be defined as quorum sensing it enables bacteria to monitor their cell density. This system classified into two proteins AHL synthase (LuxI-homologue) it responsible for AHL regeneration. LuxR it used for dual function receptor, and it recognizes and binding the molecules, and it allows interaction with the QS controlled promoter sequence. This concept is providing from the source of *halogenation furanes* it was derived from marine macro algae namely *delisea pulchra*. This experiment held in both invivo and invitro which inhibit the QS. The overview of this study showed that the discovery and reports on QS compound from marine organisms and potential application. The virulence and pathogenic bacteria.(Fohad Rabood husain and Iqbal Ahmad 2015)

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Extracellular substances have been released from the source of macro algal it serves as a feed for the microorganisms, and they have two type of bacteria epiphytic and endophytic, and it were associated with green and red macroalgae *ulva gracilaria* it were identified by different seasons .in this research we evaluated the effect of quorum sensing molecules on *curpospores* liberation from *gracilaria dura*. Seven type of gram negative bacteria were found, and they produced the different type of n-acylhamoserin lactones (ALS) and the *shewanella* algae it produced five type these were isolated screened the carpospore liberation from *g.dura*. They were compared the

control and confirming their indirect effect on the *carpospore* liberation.(Ravindra pal singh et al.,2015)

The red seaweed acts as bioactive compounds rich in the source, and it was the secondary metabolite to improve the human and animal health. In this study we evaluate the antimicrobial property of the red seaweed extract it against the *Salmonella enteritidis* by the use of the *Caenorhabditis elegans* infection model. The research has proved that the SWE also impacts beneficial effects on the animal and human health.(Garima Kulshresh et al.,2016)

DESCRIPTION OF SELECTED PLANTS

SARGASSUM WIGHTII

- Class phaeophyceal
- Order fucales
- Family sargassaceae
- Genus sarragassum
- Phylum ochrobhyta
- Clade SAR

Sargassum is a tropical and subtropical brown seaweed and its as other called gulf-weed or sea holly belonging to order fucales,sub-class cyclosporeae and class phaeophyceae. Sargassum is most diverse genus among phaeophyta its having 15 species .its is established that the species of sargassum act as biofilters accumulating toxic heavy metals thereby preventing the coast from the land based pollution .seasonal changes influence various abiotic factors such as temperature ,ph, salinity etc.the biotic factors like morphology and epifaunal diversity ultimately reasonable for the biosynthesis of secondary metabolites

Sargassum wightii is one of the most marine algal species rich in sulated polysaccharides Its as free radical scavenging and antioxidant effects and its as good source of carbohydrate,minerals,essential amino acids .they are generally inhabit shallow water and coral reefs and the genus is widely knowns as the planktonic speciesand they are mostly cold water organisms



Figure 1: Marine Seaweeds *Sargassum wightii*

Padina tretrastromatica

- Class phaeophyceae
- Order dictyotales
- Family dictyotaceae
- Genus padina
- Phylum ochrophyta

Padina tretrastromatica is the family of dictyotaceae one of the most pertinent marine brown sea weed and it will contain the major component of fucopyranosyl residues containing sulphated fucans called fucoidans, alginic acid and glucans were isolated from the seaweed and minor amount of glucuronic acid and uronic acid are also found and they play major role in the field of antiviral activities

The plant are flat, thin and 4 cells thick, soft and slightly leather-like surface and reach about 15-20 cm in height and the blades are divided into numerous flabellate pieces thallus flabellate are divided into several narrow overlapping blades and in the base



thallus split into small blades with in rolled edges 7-15 cm high 15-29 cm broad and it were in yellowish brown colour.

**Figure 2: Marine Seaweeds *Padina*
*tretrastromatica***



3. AIM

Biosynthesis silver nano particle from the Marine seaweed for the Evaluation of antibacterial activities

OBJECTIVES

- ❖ Evaluation of marine brown algae for the biological activities
- ❖ Synthesis of silver nanoparticles using marine seaweeds
- ❖ The antibacterial and Antimycobacterial tested
- ❖ Characterized of silver nanoparticles using UV-vis Spectrophotometer, FTIR, XRD, and SEM.

4. MATERILAS AND METHODS

4.1.SAMPLE COLLECTION

Marine Seaweeds like *Padina tetrastromatica* and *sargassum wightii* were collected from the Rameshram Tamil Nadu India and washed thoroughly water and then it crushed into powder.

4.2. HOT WATER EXTRACT PREPARATION

The compound is extracted by using distilled water by mixing 5g of the sample in 100ml of distilled water and kept in water bath at 60°C for 30 mins and then filter the extract by using Whatman filter.

4.3. SYNTHESIS OF SILVER NANOPARTICLE

1mm of silver nitrate were dissolve into 1000ml of distilled water and then sample and silver nitrate were diluted 9ml of AgNO₃ and 1ml of sample 8ml of AgNO₃ and 2ml of sample 7ml of AgNO₃ and 3 ml of sample 6ml of agno3 and 4 ml of sample 5ml of agno3 and 5ml of sample kept in incubation for 72 hrs and the colour change were observed

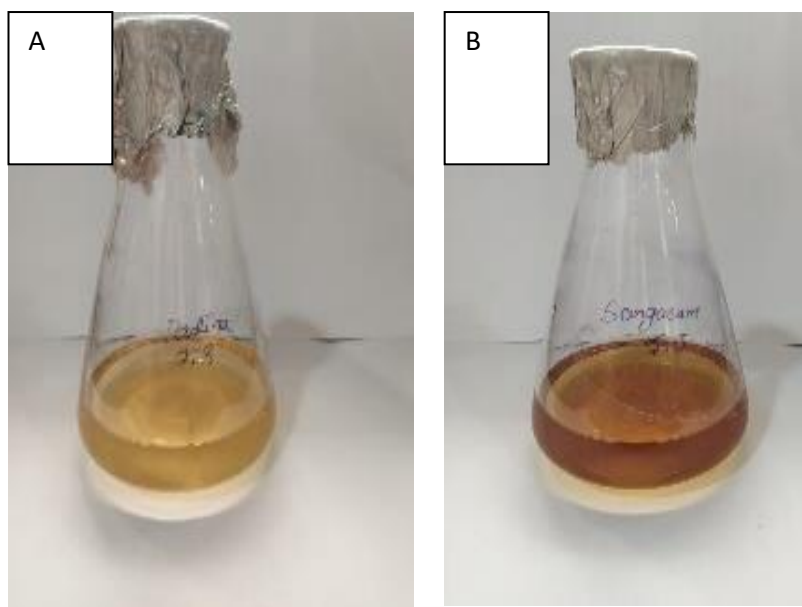


Figure 3: Synthesis of Silver nanoparticles using *Padina tetrastromatica* and *Sargassum wightii*

4.4. ANTIBACTERIAL ACTIVITY

Antibacterial activity was assayed by using the agar diffusion technique. Nutrient agent medium was prepared the pH was maintained and then it was sterilized by autoclaving at 121°C and 15 IBS pressure for 15 min. 20ml of the sterilized media was poured into sterilized petri dishes and allowed to solidify at room temperature. A sterile cotton swab is used for spreading each microorganism from the 24 h inoculation broth evenly on the nutrient agar plates. Left for a few minutes to allow complete absorption of the inoculums. In each of these plates 5-mm diameter wells were made at the centre using a sterilized cork bores different concentration of each algal extract were added to the respective wells on the nutrient agar plates. after incubation a clear inhibition zone around the well's incubation the presence of antimicrobial activity.

4.6. CHARACTERIZATION OF SILVER NANOPARTICLES

4.6.1. UV(VISIBLE SPECTROSCOPY)

The characterization of silver nanoparticle was observed by the colour changes. A quantity of silver nitrate was diluted 9ml of agno3 and 1ml of sample 8ml of agno3 and 2ml of sample 7ml of agno3 and 3 ml of sample 6ml of agno3 and 4 ml of sample 5ml of agno3 and 5ml of sample kept in incubation for 72 hours and the colour change were observed and scanned at wavelength of 420nm to 580nm in uv spectroscopy to determine absorption of the sample

4.6.2. FTIR (Fourier Transform Infrared Spectroscopy)

To identify the potential biomolecules responsible for the conversion of AgNO₃ into AgNO₂, FT-IR spectra were collected for sargassum. we extract and manufactured silver nanoparticles., The FTIR spectrum reported from the freeze-dried powder of silver nanoparticle, formed after 72hr of incubation with seaweed aqueous extract. The characteristic bands are related to the chemical and physical changes of the OH stretching vibration

4.6.3. SEM(SCANNING ELECTRON MICROSCOPE)

The morphology and shape of these silver nanoparticles were carried out using SEM After 2 hours of exposure to 10⁻³ M aqueous silver nitrate solution, nanoparticles biosynthesized from *sarggasum.w* biomass displayed the colloidal form of the particles in solution, which microprecipitated. on the surface of the experimental

seaweed's biomass During the. The SEM investigation of silver nanoparticles in this study, in addition to being interesting, was also useful. It was also microprecipitated when it was present in colloidal form in solution. on the biomass's surface, which were clearly showing that The reduction of Ag⁺ ions produces nanoparticles that are bonded together. To the cell's outer surface Biosynthesis of silver nanoparticles produced from *Sargassum.w* were dominated by cubical and spherical formations with homogeneous shape The more luminous cubical and spherical region of the electrons scattering in the back

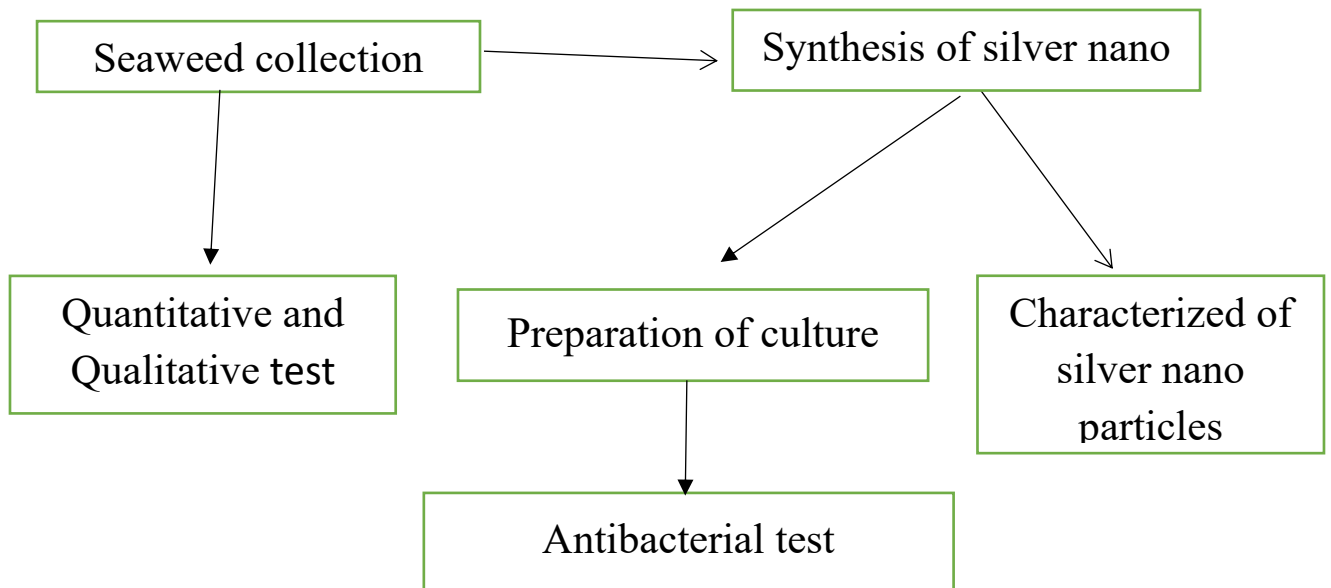
4.6.5. TEM (TRANSMISSION ELECTRON MICROSCOPE)

HR-TEM is a technique for determining the size and particle dispersion of silver nanoparticles. The magnitude and distribution of the group Observations of biosynthesized silver nanoparticles were made using the micrograph of silver complex solution drop-coated sheets with *sargassum.w*, an experimental seaweed extract The TEM is a term that refers to a. The production of spherical shaped particles was visible in images. Silver nanoparticles are found in aggregates with diameters ranging from 5 to 50 nanometers as depicted in The size of silver nanoparticles on average *sargassum.w* has a diameter of 32 used edulis extract as a reducing and antioxidant agent of capping The form of metal nanoparticles has changed dramatically. they've altered their optical.

4.6.4 XRD (X-RAY DIFFRACTION)

The X-ray diffraction (XRD) method was used to confirm the creation of a single-phase compound. The XRD pattern of synthetic AgNPs was examined and compared to that of natural AgNPs.

FLOW CHART



5.RESULTS

The synthesis of silver nanoparticle was characterized by UV visible spectroscopy at wavelength between 420nm to 550nm.the chemical composition of the synthesised agno3 was determined using FTIR spectroscopy at a wavelength 4000and 500cm. XRD analysis of 20 ranged from 10 to 80.the morphological structure and element composition of AgNPs were determined using SEM and the arrangement of AgNPs were determined by TEM.

Antibacterial activities

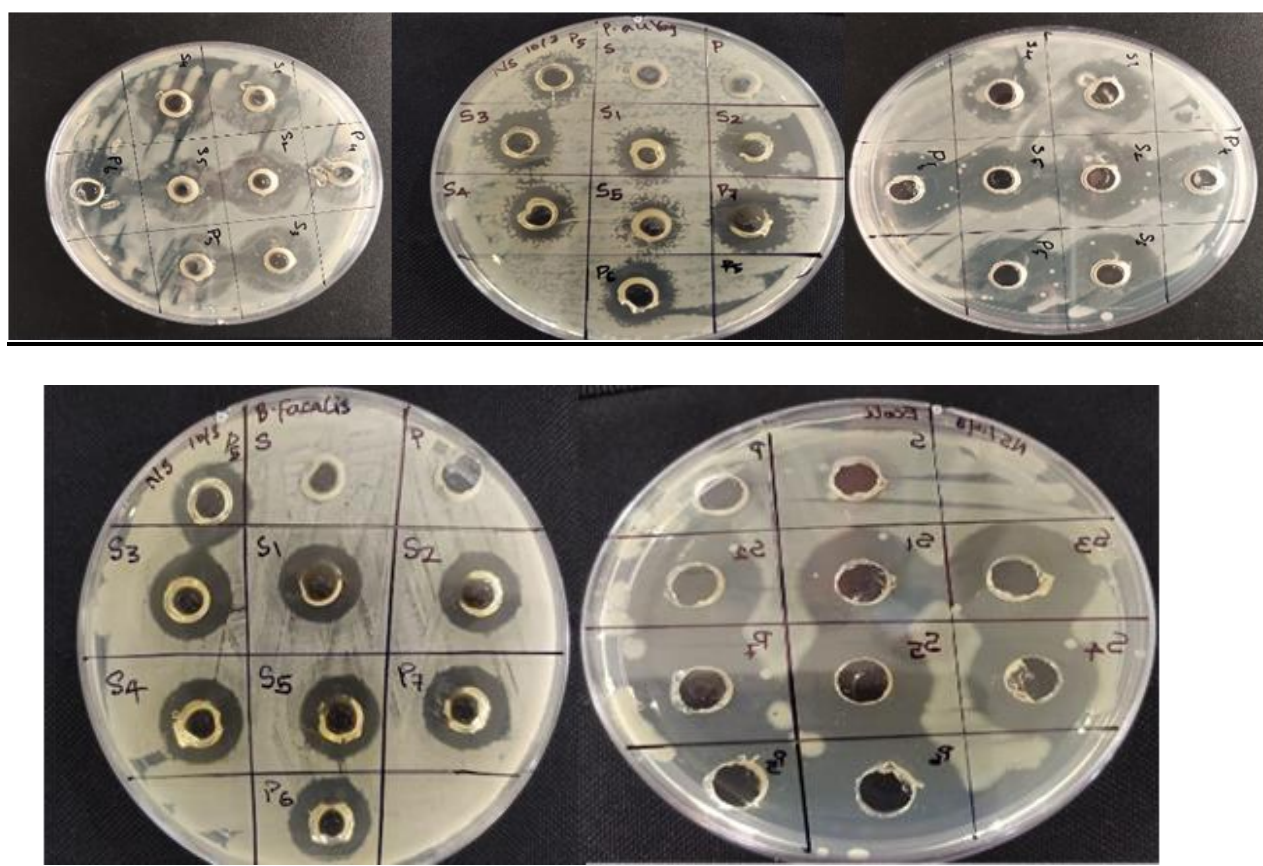


Figure 4: Zone of Inhibition

Table – 1: Antibacterial activities of silver nanoparticles

BACTERIA NAME	S1	S2	S3	S4	S5	P7	P5	P6
<i>E.coli</i>	25mm	20mm	16mm	-	-	-	-	-
<i>P.aeruginosa</i>	18mm	22mm	-	-	-	-	-	-
<i>S.aureus</i>	-	-	-	-	-	14mm	-	-
<i>E.faecalis</i>	23mm	24mm	15mm	17mm	18mm	20mm	15mm	20mm
<i>K.pneumoniae</i>	20mm	22mm	20mm	19mm	20mm	21mm	19mm	22mm

UV-VISIBLE SPECTROSCOPY

The characterization of silver nanoparticles based on surface plasmon resonance vibration observed 445nm conformed the synthesis of silver nanoparticles using marine algal extract. The silver surface plasmon resonance band occurred at 420nm to 450nm showed the study increase in absorbance until 48 hours. The broadening of the signified that the particle is polydispersed. The peak intensity increased as the sample are treated for a longer time.

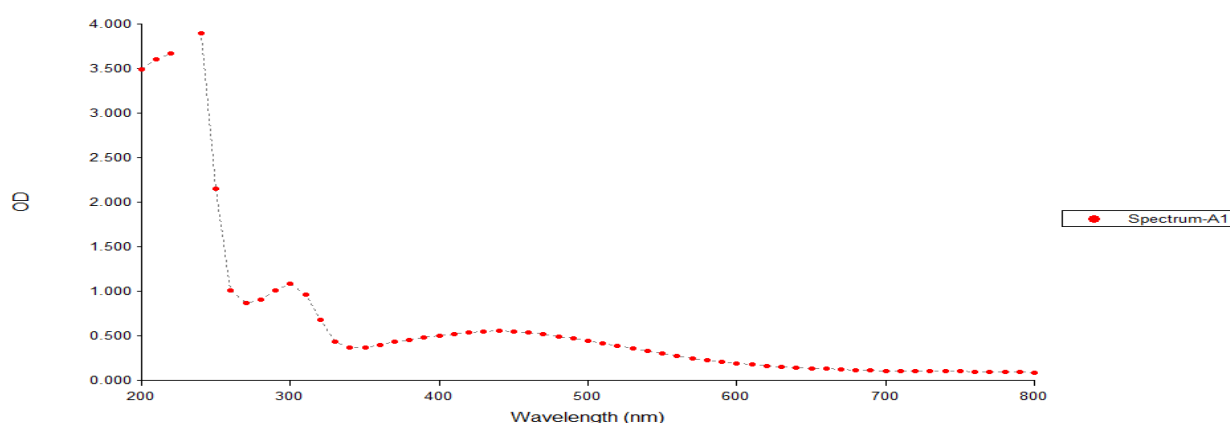


Figure No: 5. -UV spectroscopy biosynthesis of silver nanoparticle of *Sargassum wightii* (450nm)

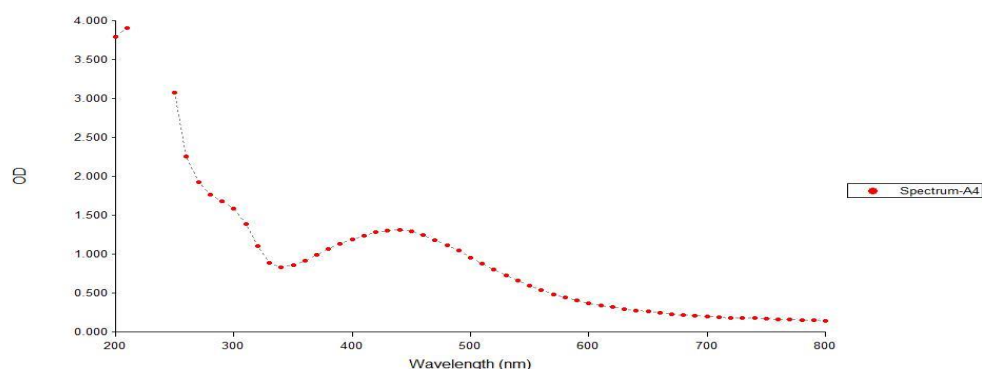


Figure No: 6 - UV spectroscopy biosynthesis of silver nanoparticle of *Padina tetrastromatica* (420nm)

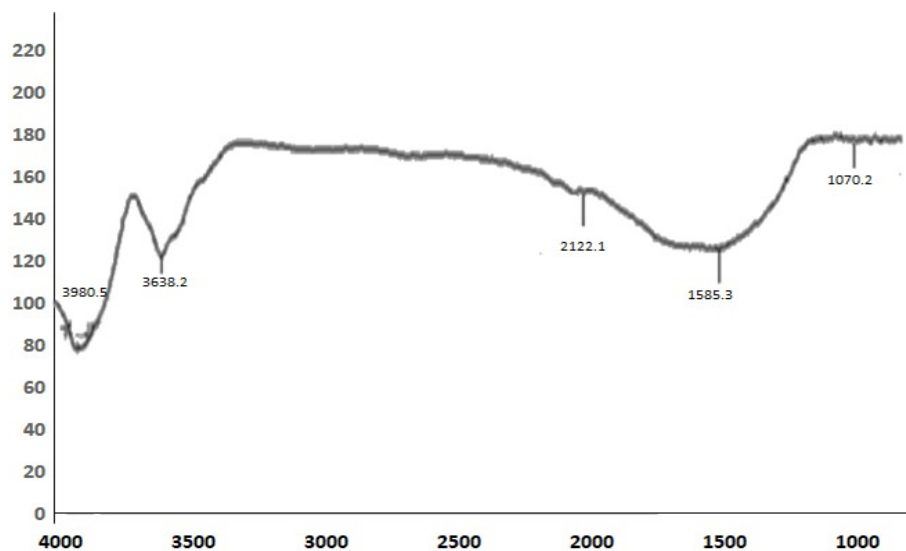


Figure: 7 FTIR Fourier Transform Infrared Spectroscopy of *Sargassum wightii*

The presence of functional groups in the aqueous extract of the marine alga *sargassum.w* was determined via FTIR analysis. According to Fig.7 prominent transmission peaks for *sargassum.w* extract were identified at 3980.5cm⁻¹, 3638.2cm⁻¹, and 2122.1 cm⁻¹ [20] for O–H and C=O stretching for hydroxyl group and carboxylic acid, and 1585.3 cm⁻¹, 1070.2cm⁻¹ for hydroxyl group and carboxylic acid, respectively.

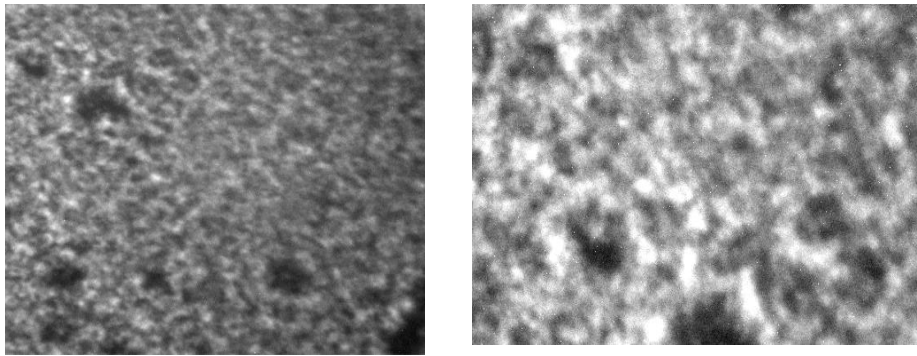


Figure: 8 Transmission Electron Microscope of *Sargassum wightii*

The silver nanoparticles made with sea weed extract TEM sample was prepared by dropping a drop of the reaction mixture onto carbon coated copper grids and allowing the acetone to evaporate. Micrographs of the sample were acquired using a high-resolution transmission electron microscope (HRTEM). The particle size of the prepared CuO-NPs ranged between 50-100nm.

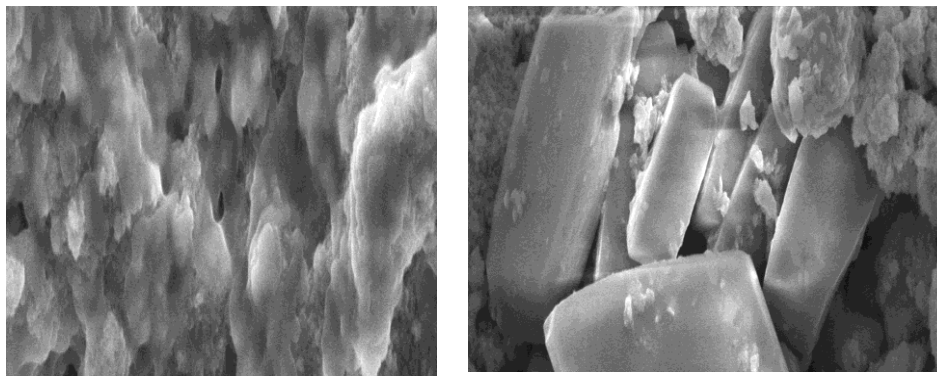


Figure:9 Scanning Electron Microscope of *Sargassum wightii*

The high-density silver nanoparticles generated by treating *sargassum.w.* extract in the SEM further demonstrated the growth of silver nanostructures. The silver nanoparticles appear to be evenly dispersed, with a mean size of 33.75 nanometres. For a higher-resolution examination and sharper pictures, the nanoparticles shape cubie and cuboctahedron.

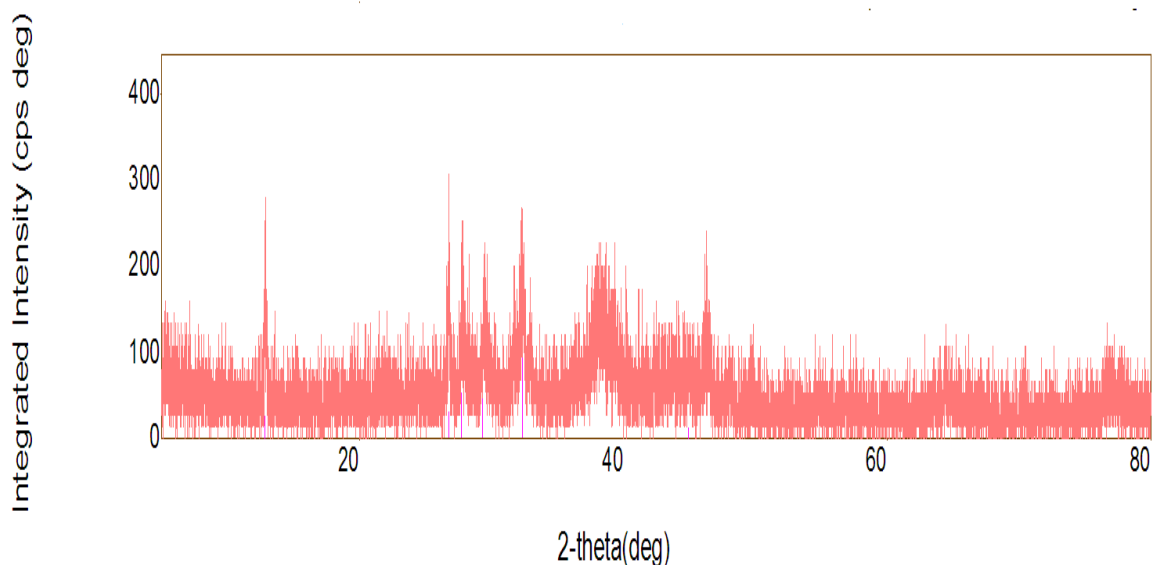


Figure :10 X-Ray Diffraction of *Sargassum wightii*

XRD is a widely used technique to assess the size of nanoparticles in the range of 1 to 100 nm due to the often-employed x-ray wavelength. An x-ray diffractogram of biosynthesize a nanosilver revealed Bragg reflection, which corresponded to face to the centre cubic (fcc) type bulk silver. The x-ray diffractogram of biosynthesize nanosilver revealed Bragg reflection due to (111), (290), (300), (299) and (260), similar to fcc type bulk silver. The bases of the diffraction peaks widened, indicating that the silver nanoparticle was nanosized.

6. Discussion and Summary

The present study show that biosynthesis of silver nanoparticle from the seaweed and its antibacterial studies and its characterization.in the toxic compound silver nitrate were reduced into silver nanoparticle and the addition silver nitrate solution in sargassum wightii colour changes absorb within 24hrs it were change light brown to dark brown . in *padina.sp* colour changes were taken longer duration.In this study sargassum only showed the uv visible spectroscopy peak value at 420nm the average size of nanoparticle was showed in TEM and the crystalline nature was clearly showed in XRD pattern and the biomolecules responsible for the formation of silver nanoparticle were proved. For (rajeshkumar,2012) have showed padina. sp uv visible spectroscopy at the peak 580 nm. The Padina tetrastrum, which contains many polysaccharide components, revealed that carbohydrates can be used to fabricate nanoparticles. *Sargassum.w* also has many polysaccharide compounds because it has shown the sharp occurred at 3980.5cm⁻¹, 3638.2cm⁻¹ was assigned to be strong nitro compounds. FT-IR measurements were used to identify the potential biomolecule responsible for the reduction of Ag⁺ ions and capping of the bio-reduced silver nanoparticles generated by the seaweed *S. wightii*. Silver nanoparticles have antibacterial activity that kills germs locally or slows their growth without being harmful to surrounding tissues. Antibacterial action of silver nanoparticles depends on the nanoparticle's physiochemical components and the bacteria's kind. Based on the structure, components, and functions of bacterial cell walls. *Pseudomonas aeruginosa* and *Escherichia coli* are Gram negative microorganisms used in this experiment. Gram-positive bacteria are considered *Staphylococcus aureus*. The structure of the cell wall where to play a vital role in bacteria in the presence of the silver nanoparticle. gram negative bacteria *E. coli* was the greatest zone of inhibition compare to the other bacteria and the gram-positive bacteria *Staphylococcus aureus* also shown similar result . These bacteria absorb nanoparticles on their cell surfaces, reducing the number of bacteria on the bacterial growth plate. As a result, silver nanoparticles derived from the seaweed *Sargassum.w* can be considered viable candidates for bacterial infection inhibition. For *Sargassum.w* result were not similar to (Shanmugam et al) they have shown maximum zone of inhibition in gram positive bacteria and minimum zone of inhibition in gram negative bacteria. In this study we collect the seaweed *Sargassum.w* and *padina. Sp* and then wash it in tap water and

pat dry it and crushed into fine powder to synthesis silver nanoparticle boils the extract and then filter it and add silver nitrate leave at room temperature for 24 hours after it will change light brown into dark brown and then will take uv-visible spectroscopy it reaches the peak at 480 nm. After it will go under antibacterial activity and calculate the zone of inhibition. silver nanoparticle characterized by TEM, SEM, FTIR, XDR. The x-ray diffractogram of biosynthesize nanosilver revealed Bragg reflection due to (111), (290), (300), (299) and (260), similar to fcc type bulk silver. The nanoparticles shape cubie and cuboctahedron. The particle size of the prepared CuO-NPs ranged between 50-100 nm. FTIR analysis prominent transmission peaks for sargassum.w extract were identified at 3980.5cm⁻¹, 3638.2cm⁻¹, and 2122.1 cm⁻¹.

7. Conclusion

The marine seaweed has eco-friendly and low cost. The biosynthesis of silver nanoparticle from the seaweed is great method. the biomolecule extracted of *sargassum wightii* and *padina tetrastrum* were get processed. The *sargassum wightii* have lots of application in the field if medical biochemistry. The brown seaweed silver nanoparticle characterized and synthesised the characterization of *Sargassum wightii* examined by UV visible spectroscopy it shows the peak value above 420nm.

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