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Biosynthesized Silver Nanoparticles from Brown Seaweeds *Padina tetrastromatica* and *Sargassum wightii* as Potential Antimicrobial Agents

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Abstract

In this study, silver nanoparticle (AgNPs) was synthesized using *Padina tetrastromatica* and *Sargassum wightii* and evaluated for antibacterial, antimycobacterial, anti-oxidant and cytotoxicity properties. Characterization was performed using UV-visible spectrometry, FTIR, HR-TEM, and STEM-EDX. UV spectra showed absorption peaks at 450nm (*P. tetrastromatica*) 430 nm (*S. wightii)*, While FT-IR indicated the role of algal metabolites as reducing and capping agents. TEM and SEM analyses revealed predominantly spherical nanoparticles ranging from 60-70nm. The AgNPs exhibited significance antibacterial activity against *Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcous aureus*. Anti-mycobacterial testing against *Mycobacterium* smegmatis showed IC50 values 31.25 µg/ml (*S. wightii*) and 6.25 µg/ml (*P. tetrastromatica*) indicating potent activity. Brine shrimp lethality assays confirmed low toxicity suggested therapeutic safety. These finding highlight the significant of marine algae-derived AgNPs as a potential therapeutic compound to combating the pathogenic bacterial and mycobacteria.

Keywords: Marine seaweeds, Nanoparticles, Antibacterial activity, Green synthesis

1. Introduction

World health organization revealed a report stating, infection caused by drug resistant microorganisms causing the higher mortality rates during the pandemics and epidemics. The development and resurgence of bacteria resistance to anti-microbial compounds have a become a critical problem. In response current research efforts have been develop the enhancement of antibacterial compound with increased the effectiveness (Das et al., 2020). Metal nanoparticles can be synthesised through various chemical and physical methods. However, these processes are often costly and include the use of high toxic compounds as reducing and capping agents posing harm to environment (Philip et al., 2021).

Silver nanoparticle possessing the electrical, thermal, magnetic and catalytic properties are well-known for their antimicrobial properties. This characteristic renders them most active against microorganisms, underscoring their possible for numerous biotechnology application (Aragao et al. 2021). Silver nanoparticle find application in both industrial and electrical sector, serving as conductors in transistors, catalysts and equipment for cancer detection (Hashmi et al. 2008). Recently, nanoparticles have been employed in diverse trans-disciplinary processes, encompassing separation procedures, resonance imaging, tumor detection, cancer treatment, and drug administration (Guo et al., 2018). The biological activities of nanoparticles can be attributed to their diminished size, magnetic properties, exceptional biocompatibility and facile surface modification (Wells et al., 2017). Recent advance in biosynthetic techniques involve the use of microbes to solve these restrictions. Biosynthetic techniques that use microorganisms and plant extract have gained popularity because to their ease of use, low cost and environmental friendliness. These methods have evolved as simple and feasible alternative to traditional physical and chemical processes in sensing, imaging and biomedicine (Philip et al., 2021).

Biological approaches offer cost-effectiveness, energy efficiency and environmental benefits (Thakkar et al., 2010). Biomolecules have been shown to play a significant role in lowering metal salts and converting them to their nano-forms inside biological processes (Chellapandian et al., 2019). Furthermore, these biomolecules act as covering agents, providing stability to the nanoparticles (Das et al., 2017). The Silver nanoparticles have gained great importance in recent times due to their biological and pharmacological versatility (Elahi et al., 2018). The surface of these nanoparticles can be readily decorated with diverse ligands, making it possible to form multifunctional nanoparticles which can be used for diagnostic and therapeutic

purposes (Jeong et al., 2019). Marine algae are an important source of bioactive compounds and metabolites. Algal metabolic compounds are famous for their anti-microbial activity (Selvaraj et al., 2020).

Nanoparticles have been successfully synthesized using eco-friendly techniques through the use of noble metals like ZnO, Sm_2O_3, and CdO, among others, without causing any chemicals harm, with smaller dimensions (Diallo et al., 2015). The utilization of biological components in the preparation of eco-friendly nanoparticles will assist in minimizing various disadvantages connected to physical and chemical procedures. This involves producing nanoparticles naturally at low pH, pressure and temperature without usage of harmful compounds (Usman et al., 2019). The potential application in biomedicine silver nanoparticle is garnering increasing attention. With their unique physicochemical characteristics silver nanoparticle stand out as one of the most fascinating nanomaterials in the realm of biomedicine This discussion delves into the fabrication processes, therapeutic applications and biosafety consideration associated with silver nanoparticles (Xu et al., 2020). Nanoparticle are regarded as the cornerstone of nanotechnology, with is distinguishing and critical attribute being a greater surface to volume ratio. Many researchers have favoured biological green synthesis because simplicity cost effectiveness and eco-friendly.

Seaweeds nanoparticles were potential source for bioactive compounds because they contain a varied types of secondary metabolites compounds such as flavonoids, saponins, alkaloids, phenol, polysaccharides (Sanaeimehr et al., 2018, Abdel et al., 2017). Seaweed like *Tubinaria conoides, Turbinaria ornata, Galaxaura elongata, Sargssum ilcifolium,* and *Padina tetrastromatica have* previously been found as effective source for generating nanoparticles with antibacterial, antiviral, anti-cancer and mosquito-larvicidal activities. Bio-nanotechnology eco-friendly synthesis of nanoparticle through the live cell stands out the as a potential and promising methods. Green synthesis eliminated the high pressure and temperature to avoid the toxic material making it an eco-friendly option that does not need the external reducing, capping agents (Salem et al., 2021).

Sargassum wightii (Macro algae) belonging to the family Sargassaceae is extensively distributed in temperate and tropical region of ocean. Sargassum exhibits a broad spectrum of bioactive compounds. Its widespread distribution in India and various parts of Asia. This makes it an ideal candidate for exploring biomolecules with potential medical and industrial application (Marimuthu et al., 2012). *Padina tetrastromatica* classified under Phaeohyceae and

distinctive fan shaped algae with a yellowish- brown hue that turns olive green upon drying. Abundantly growing in the Gulf of Mannar Southeast coast of India (Schnetter et al., 1976).

Silver nanoparticle (AgNPs) has demonstrated extraordinary efficacy at low concentration against different bacteria, viruses, fungus and other eukaryotic microorganisms in the context of nanoparticle manufacturing. While terrestrial plants have been extensively used for nanoparticles biogenesis, the promise of renewable marine organisms such as marine seaweeds has largely gone untapped. Notably, *Sargassum wightii and Padina tetrastromatica* two notable species found on the southern coasts of Tamil Naud, India and other parts of Asia. In this present research work, we synthesis and test silver nanoparticles made from marine algae *Sargassum wightii and Padina tetrastromatica concentrating on their antibacterial and antimycobacterial properties*

2. Material and Methods

2.1. Chemical

All the chemical and reagent employed in this study were of analytical grade and Procured from Himedia and Sigma Aldrich India

2.2. Sample collection and preparation for silver nanoparticle synthesis

Marine Seaweeds *Padina tetrastromatica* and *Sargassum wightii* were washed thoroughly water and then it crushed into powder. All the seaweeds were collected from costal region. We take seaweeds sample from Mandapam Beach, Rameshwaram, Tamil Nadu. The seaweeds are identified by the renowned seaweed research scientist and Seaweed Identification Expert Dr. P. Anantharaman, Dean and Professor, Centre for advance studies in marine biology, Faculty of marine science, Annamalai University, Parangipettai-608502, Cuddalore district, Tamil Nadu, India.

2.3 Hot water extract methods

Preparation for silver nanoparticle synthesis using hot water extract methods of seaweeds (*Padina tetrastromatica* and *Sargassum wightii*). The seaweeds Crude sample were extracted by using distilled water by mixing 5g of the sample in 100ml of double distilled water and kept in water bath at 60°C for 30 minutes. After that the samples were filter through muslin cloth. The filtered extract was passed into whatman filler no 1 and kept the sample into 4° for further analysis

2.4. Synthesis of silver nanoparticles

The 1 mM of silver nitrate ($AgNO_3$) was dissolved into 50 ml of double distilled water. After that 1ml of hot water extract of seaweeds were dissolved in 49 ml of silver nitrate (1mM). all the process was kept in dark room to avoid unnecessary photochemical reaction. The colourless solution ($AgNO_3$) changes to dark brown colour were obtained by visible and followed by confirmed by UV-Spectroscopy. Aqueous seaweeds can be used to negative control and all the test are done in triplicated. The synthetic nanoparticle was separated by centrifugation at 15000 rpm (8250 g) for 25 minutes. Afte that carefully remove the supernatant and pellet was dissolved in Milli-Q-water and kept at 4°C for further characterization

2.5 Phytochemical analysis of seaweeds

The analysis of phytochemical (Aminoacids, Protein, carbohydrate, saponins, tannins, diterpense, phytosterols, and phenol) from aqueous extract of *P. tetrastromatica and S. wightii* seaweeds (Farnsworth et al., 1966).

2.6. Characterization of synthesized silver nanoparticles

2.6.1. UV-Visible spectroscopy

One ml of silver nanoparticle was subjected to the UV spectral analysis. The absorbance spectrum was investigated using UV spectrophotometer (EPOCH 2 Microplate Spectrophotometer) (Li et al., 2020).

2.6.2. Fourier Transform Infrared Spectroscopy

The synthesis of the AgNPs was studied in the presence of biomolecules, which cause bio reduction using FTIR analysis (FTIR-SHIMADZU instrument), and to find possible functional groups in the biomolecules available in the AgNPs synthesized through the seaweed extract. The spectral data obtained by analysing the dried sample mixture is recorded using an FTIR spectrum (Li et al., 2020).

2.6.3 Scanning Electron and Transmission Electron Microscopic

The SEM technique was applied to the study of silver nanoparticles (AgNPs) for their morphology, shape and surface texture (Zeiss SEM). HR-SEM was performed to measure the size, shape and dispersion of the AgNPs with the aid of TEM JEOL3010. The instrument works at an accelerating voltage of 300 kV. The samples were sonicated for 5 minutes and dried under

a copper grid and then images captured at various magnifications. X-ray diffraction pattern was employed to assess the crystalline nature of the synthesizes silver nanoparticles (Li et al., 2020).

2.6.4 X-ray Diffractometric analysis and Dynamic light scattering

Scanning electron microscope (JSM-5910, jeol), which has been equipped with EDS detector INCA energy 200, was employed for elemental analysis of the AgNPs obtained from the extraction of seaweed extract. The particle size analysis was done through dynamic light scattering approach using the colloidal suspension. The density or the size of the nanoparticles was determined via Brookhaven Instrument (model 90 plus) particle size analyser (Li et al., 2020).

2.7. Biological activity of synthesized silver nanoparticles

2.7.1 *In-vitro* Antibacterial activity

The antibacterial efficacy of the synthesized silver nanoparticles was evaluated against a panel of human pathogens (Escherichia coli MTCC 443, Pseudomonas aeruginosa MTCC 424, Staphylococcus aureus MTCC 96, Enterococcus faecalis MTCC 439 and Klesbsiella pneumoniae MTCC 530) using a modified agar well diffusion assay. Bacterial suspensions, standardized to a 0.5 McFarland turbidity, were uniformly swabbed onto sterile nutrient agar plates. Using a sterile puncher, wells were created and loaded with 100 µL of the silver nanoparticles (5 mg/mL). Following overnight incubation at 37°C, the resulting zones of inhibition were measured in millimetres to quantify antibacterial activity (Valgas et al., 2007).

2.7.2 In-vitro antimycobacterial assay

The anti-mycobacterial efficacy of the silver nanoparticles was evaluated against Mycobacterium smegmatis using the agar well diffusion method on Middle brook 7H10 agar (pH 7.4). Agar plates were uniformly inoculated with a 4-day old broth culture using sterile cotton swabs. Wells (5 mm diameter) were created using a sterile cork borer and loaded with 100 µL of the nanoparticle solution. Following a 45 minutes pre-diffusion period at room temperature, the plates were incubated at 37°C for 72 hours. The resulting zones of inhibition (ZOI) were measured in millimeters to quantify the anti-mycobacterial activity (Syanthi et al., 2025)

2.7.3 In-vivo Anti-oxidant activity

DPPH free radical scavenging activity

The anti-oxidant property test involves reacting one mL of each sample and standard with one mL of 0.2 mM methanolic DPPH solution. The solutions were then mixed well and incubated

in the dark for 30 minutes. After this period, the absorbance was measured at 517 nm on the spectrophotometer. This experiment was repeated three times for reliability. Radical scavenging activity was calculated using the formula below:

$$\textbf{Percentage of Radical Scavenging} = \frac{\textbf{Ao} - \textbf{As}}{\textbf{Ao}} \; \textbf{x 100}$$

Where, A_o represents the absorbance of the blank solution while A_s represents the absorbance of the sample. IC_{50} is the concentration required for 50% inhibition of DPPH free radicals, which was determined by the linear regression equation from the graph of the inhibition percentage (Gulcin et al., 2023).

2.8 *In-vitro* Cytotoxicity Assay

Artemia salina cysts (50 mg) were hatched in a divided plastic vessel containing artificial seawater (38 g/L salinity), prepared by dissolving a commercial salt mixture in double-distilled water. One compartment was kept dark while others were exposed to a constant light source at 37° C. After 48 hours, the phototactic nauplii were collected from the illuminated partition using a Pasteur pipette for the bioassay. A stock solution of the nanoparticles (1mg/mL) was prepared in dimethylformamide (DMF). From this stock, calculated volumes were transferred into vials to achieve final concentrations of 10, 100 and 1000 µg/mL. The Solvent was allowed to evaporate, to completely prevent carrier-mediated toxicity. Following this, a volume of 5 mL of artificial solution is typically used. To each vial, seawater was added, and then ten active nauplii were introduced. Etopside was utilized as a positive control, while vials treated with 2 mL of DMF served. The samples were left to sit at room temperature for a whole day, about 24 hours, as a control to compare with the other results. Following the exposure period, the number of surviving nauplii was counted using a magnifying lens. The percentage of mortality was calculated for each concentration. The median lethal concentration (LD_{50}) was determined through probit analysis using Microsoft excel. According to established toxicity scales, samples exhibiting an LD_{50} levels below 1.0 mg/mL are considered to have an active or hazardous effect on the body (Meyer et al. 1982).

3. Result and Discussion

Green nanotechnology is a way to make tiny materials, between 1 and 100 nano-meters in size, using natural processes. This approach is good for the environment, doesn't cost too much, and is relatively simple. It uses nature's own abilities to reduce the risks to the environment and human health that can come with making these tiny materials. By doing so, it also lowers the costs associated with producing them. This method is a more sustainable way to create

nanomaterials, and it's an area that's getting more attention because of its potential to make a positive impact (Saravanan et al., 2021).

Nanoparticle have garnered significant attention from researchers due to their remarkable surface-to-volume ratio and effective interaction capabilities with other particles. The production of silver nanoparticles (AgNPs) can be achieved through various methods, encompassing chemical, physical, and biological approached. Among these, the biological method stands out as the cleanest and safest, as it avoids the use of hazardous chemicals throughout the synthesis process. Biological synthesis relies on bacteria, fungi, algae, and plant extracts, with algae demonstrating a particular appeal due to its remarkable capacity for metal absorption and reduction of metal ions. Algae, being abundant and easily cultivable in laboratory settings, adds an extra advantage to the biological synthesis of AgNPs (Chugh et al., 2021).

3.1 Phytochemical analysis

The tiny details in the image showed some clumps, probably caused by things like polysaccharides, flavonoids, and terpenoids in the plant extracts, which helped create the silver nanoparticles (Menon et al., 2017). Studies have shown that the things that reduce and stabilize the metal ions, and the conditions they're kept in, have a big impact on the nanoparticles that are made. When using biopolymers to make nanoparticles, the size of the particles is similar to what's been found in other studies on making silver nanoparticles in a green way (Abdellatif et al., 2022). The phytochemical constituents are detailed in Table 1. The characteristic absorption of metallic silver nanoparticles was observed to peak at approximately 3 KeV. Eluvakkal et al. (2010) emphasized the abundance of secondary metabolites in marine seaweeds such as alkaloids, glycosides, flavonoids, saponins, tannins, and steroids, demonstrating substantial therapeutic potential and widespread use in the pharmaceutical and medical sectors.

Table 1. Phytochemical analysis

Phytochemical	***Padina tetrastromatica***	***Sargassum wightii***
Alkaloid	+	+
Carbohydrates	+	+
Flavonoid	+	+
Phenolics	++	++
Saponin	-	+

Steroids	-	-
Tannins	++	+

Nanotechnology is gaining traction as a possible approach to solving biological issues, notably in cancer therapies. With the growing demand for nanostructures, it is critical to ensure both their efficacy and sustainability. Given its capacity to reduce dangers to both human health and the environment, green synthesis has emerged as a fascinating field of research. Microbes are a key source of nanoparticle (NP) synthesis due to their plentiful availability and simplicity of culture (Truong et al., 2022).

The algal extract is abundant in a wide variety of nutrients, which include carbohydrates, proteins, minerals, oils, fat and polyunsaturated fatty acids, among other, together with different classes of bioactive compounds like pigments, anti-oxidants (polyphenols, tocopherols), and carotenoids (carotene, xanthophylls) (Michalak et al., 2018). It has been noted that such bioactive compounds act as stabilizers and reducers in the synthesis pf nanoparticles. In the synthesis of NPs using algae, three main steps include the production of algal extract, metal precursor solution, and the mixing of the algal extract and metal precursor solution (Sharma et al., 2015). Reaction starts through the mixing of the liquid algal extract with metal precursor solution. Observable changes in the color of the reaction mixture act as a visible indicator, signifying nucleation. Subsequently, NPs undergo growth resulting from this nucleation process, yielding thermodynamically stable NPs of diverse sizes and shapes (Prasad et al., 2017, Fawcett et al., 2017). The pH, temperature, concentration, and time emerge as pivotal regulating variables in the cascade of nanoparticle synthesis, influenced by the bioactive components of the extract. In this synthesis process, two methods, extracellular and intracellular, coexist, each subject to distinct controlling factors.

3.2 Synthesis and Characterization of silver nanoparticles

3.2.1 UV-vis spectra analysis

Characterizing metallic nanoparticles is a common practice, and it usually involves evaluating their stability and production. When these tiny particles are made from their respective salts, they often produce a strong peak with significant absorption in the visible spectrum. Many studies have found that the absorption band between 200 and 800 nanometers is useful for identifying particles with diameters between 2 and 100 nanometers. For instance, researchers have used this approach to study the properties of various nanoparticles. In one

study, Honary et al. used mathematical methods to optimize the biosynthesis of iron oxide nanoparticles using a fungus called Penicillium waksmanii, which was isolated from soil. This fungus was used to produce the nanoparticles, and the resulting particles were then characterized using various techniques. The absorption spectrum of these particles was likely evaluated to determine their size and stability.

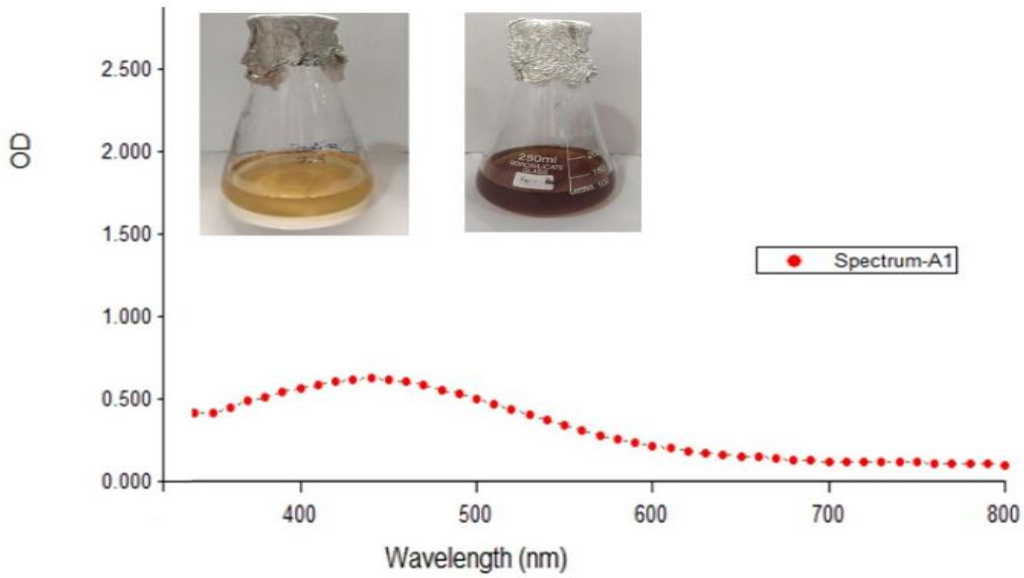


Figure 1: UV-vis spectra of the synthesis of silver nanoparticles of *Padina tetrastromatica*.

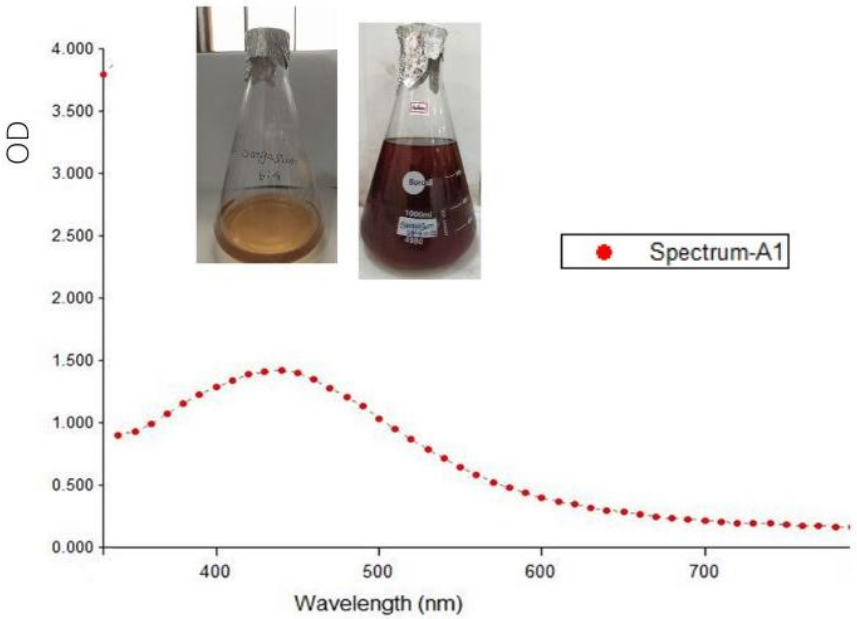


Figure 2: UV-vis spectra of the synthesis of silver nanoparticles of *Sargassum wightii*

For the silver nanoparticles, the valance band and conduction band are relatively near to each other, making it possible to create a surface plasmon resonance absorption band due to the ease of movement of electrons in these bands. There are many factors that affect the absorption of silver nanoparticles such as the chemical nature of the surrounding atmosphere, the type of dielectric medium, and the size of the particles. Analysis of the surface plasmon peak is fairly well-known and studied for many types of metal nanoparticles that lie in the 2 – 200 nm range of size. The surface plasmon resonance peak was observed at the same wavelength through spectrophotometric techniques after 12 months of stability testing of the silver nanoparticles (Zhang et al., 2016).

Absorption spectroscopy in the UV-vis range, spanning from 300 to 800 nm, is commonly referred to as UV-vis spectroscopy. Noble metallic silver nanoparticles typically exhibit a maximum absorption wavelength (max) falling between 400 and 450 nm. Consequently, the confirmation of the formation of corresponding nanoparticles can be established by examining the max value within these wavelength regions (Soliman et al., 2023).

For the initial characterization of the synthesized nanoparticles and to monitor the synthesis and stability of Silver Nanoparticles, UV-vis spectroscopy stands out as a highly valuable and reliable approach. After a 24-hour incubation period, a noticeable change in color was observed in the silver nanoparticle sample, manifesting as a shift from its original brown hue. This change in color was considered a prominent indication of the creation of silver nanoparticles. The UV-visible spectrum of the silver nanoparticles was recorded in the reaction solution using a UV-1100 Shimadzu spectrophotometer. Different ratios of silver nanoparticles were generated, with ratios of 9:1, 8:2, 7:3, 6:4, and 5:5 being explored. The UV spectrum exhibited peak values at 420 nm for *Padina tetrastromatica* with a ratio of 8:2 and at 450 nm for *Sargassum wightii* with a ratio of 9:1 (Figure 1).

UV-vis spectroscopy, is known for its speed, simplicity, sensitivity, and selectivity for various types of nanoparticles, proved to be a rapid and efficient method requiring minimal time for measurement (Figure 2) (Wypij et al., 2018).

3.2.2 Fourier Transform Infrared Spectroscopy

For the study of the presence of various functional groups that contribute to the reduction of $AgNO_3$ to AgNPs, the FTIR analysis was performed on samples from the *P. tetrasomatica* and

S. wightii species (Fig. a, b, c, d). The Fig. 3a shows the FTIR spectra of *P. tetrasomatica* seaweed extract with different major peaks positioned at 3399.89, 2558.11, 1633.41, 1427.07, 1139.72 and 608.431. The Wide presence of 3399.89 peak could be due to the vibrations of phenol groups with -O-H. The small peak observed at 2558.11 is due to CH-stretching of alkanes. The small yet sharp peak 1633.42 is due to -C=H bending vibrations. The peaks positioned 1427.07 due to the vibrations of alkenes – C-H bending. The peaks 1139.72 may be due to C-N stretching vibrations of aliphatic amines. The observed peaks of 608.431 is due to the N-H stretching vibrations of amines. The Fig 3b shows the FTIR spectra of silver nanoparticles synthesized from *P. tetrasomtica* with the peaks positioned at 3423.48, 2924.16, 1631.06, 1384.87, 1226.93, 1081.28. The respective position assignments may be O-H stretching band, short narrow CH2 stretching, C=C alkene group, phenolic groups, C-O stretching vibrations of carboxylic acids and a short band of aliphatic amines. It is predicted that the O-H, CH2, C=C and the C-O functional groups are responsible for the reduction of Ag from AgNO3 which serve as the reducing and capping agents in the nanoparticle synthesis. The above-mentioned assignments were confirmed in-accordance with Golla et al., 2013. The assignments for the specific peaks of both the seaweed extract and silver nanoparticles were represented in the table 2.

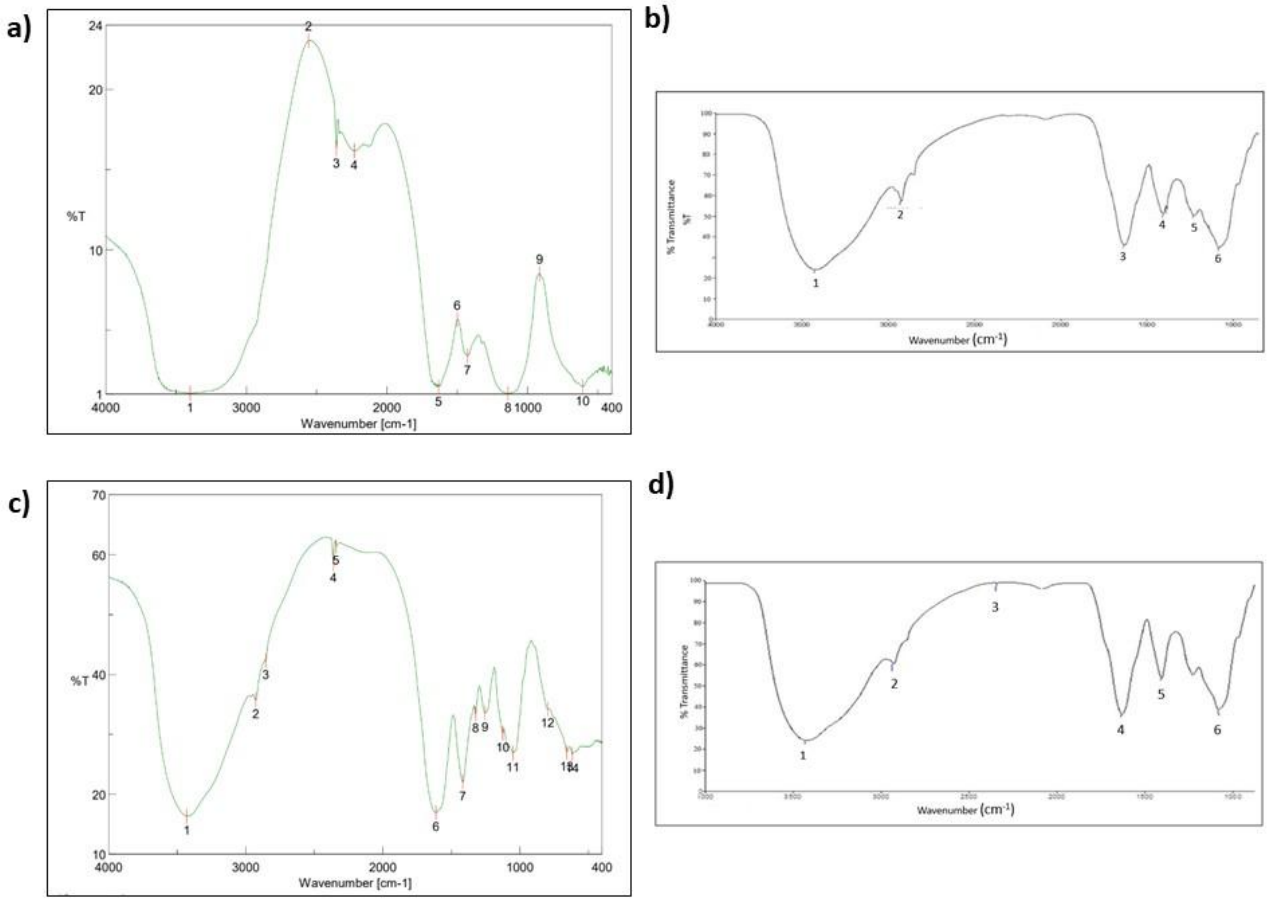


Figure 3: FTIR analysis of both the crude extracts and silver nanoparticles synthesized.

a) FTIR spectra of *Padina tetrastromatica* seaweed extract b) FTIR spectra of AgNP'S from *Padina tetrastromatica* c) FTIR spectra of *Sargassum wightii* seaweed extract d) FTIR spectra of AgNP'S from *Sargassum wightii*

Table 2. FTIR Spectra position and Assignments of *Padina tetrasomatica* seaweed extract and silver nanoparticles.

Padina tetrastromatica					
Seaweed extract			**Silver Nanoparticles**		
Position Number	**Position (cm^{-1})**	**Assignments**	**Position Number**	**Position**	**Assignments**
1	3399.89	phenol groups - O-H stretching vibrations	1	3423.48	O-H Stretching Band
2	2558.11	CH-stretching of alkanes	2	2924.16	CH2 stretching -short narrow
5	1633.41	-C=C- stretching vibrations	3	1631.06	C=C- Alkene- short band
7	1427.07	alkenes - C-H bending vibrations	4	1384.87	The phenolic groups
8	1139.72	C-N stretching vibrations, Aliphatic amines	5	1226.93	C-O stretching vibration of carboxylic acids - short band
10	608.431	OH group, N-H stretching Vibrations 1º & 2º amines	6	1081.28	Aliphatic amines - short band

Table 3. FTIR Spectra position and Assignments of *Sargassum wightii* seaweed extract and silver nanoparticles.

Sargassum wightii					
Seaweed extract			**Silver Nanoparticles**		
Position Number	**Position**	**Assignments**	**Position Number**	**Position**	**Assignments**
1	3429.78	O–H group in polyphenols	1	3423.69	–OH, stretching vibrations
3	2926.45	CH-stretching of alkanes	2	2927.54	C–H of alkanes
6	1612.2	Stretching vibration of the (NH)=O group	3	2338.06	amide 1 band of proteins
10	1125.26	C–N stretching vibration of aliphatic amines	4	1630.59	C–N stretching vibration of amines

11	1051.01		5	1405.83	C–N stretching vibration of aliphatic amines
13 14	657.607 618.074	α-glucopyranose rings deformation of carbohydrates	6	1081.11	α-glucopyranose rings deformation of carbohydrates

The Fig. 3c shows the FTIR spectra of seaweed extract of *Sargassum wightii* with the peaks assignments at 3429.78 may be due to the presence of 0-H group in polyphenols. The peak at 2926.45 represents the presence of CH- stretching of alkanes. Peaks positioned at 1612.2 is due to the stretching vibrations of (NH)=O groups which play a vital role in stabilizing the silver nanoparticles. The peptide peak at 2338.06 due to the band of proteins responsible for the reduction of the silver nanoparticles. The peaks at 1125.26 and 1051.01 are due to the vibrations of C-N aliphatic amines. The α-glucopyranose rings deformation of carbohydrates are represented by the peaks at 657.607 and 618.074. On the other hand, the FTIR spectra of *S. wightii's* silver nanoparticle is represented in the fig. 3d, which shows the peaks 3423.69, 2927.54, 2338.06, 1630.59, 1405.83 and 1081.11. The assignments of the peaks were -OH stretching vibrations, C-H of alkenes, amide 1 of proteins, C-N stretching vibration of amines and aliphatic amines and α-glucopyranose rings deformation of carbohydrates. The assignments for the specific peaks of both the seaweed extract and silver nanoparticles were represented in the table 3.

The FTIR analysis of silver nanoparticles revealed the presence of functional groups and demonstrated the seaweed extract's dual action as a reducing and capping agent. The FT-IR spectrum of silver nanoparticles synthesized from *Padina tetrastromatica* and *Sargassum wightii* biomass showed variations in band intensities at various positions (Figure 3). Tables 2 and 3 show the numerous wide bands observed in *Padina tetrastromatica* and *Sargassum wightii*, respectively.

The simplicity and low cost of FTIR make it a great way to study how biological molecules help turn silver nitrate into silver. Researchers like Xu et al (2020) have used this technique to learn more about this process. They found that certain organic compounds, such as flavonoids and terpenoids, play a role in this transformation, Additionally, proteins in leaf extract seem to help stabilize the silver nanoparticles that form around parts of the plant. The FTIR spectra showed some interesting changes, like a shift in the peak that suggests silver ions are being reduced to nanoparticles. There was also a small change in the (NH)C=0 band, which indicated

that a member of this group is involved in stabilizing the nanoparticles. The UV-visible spectra showed a surface resonance peak at 415 nm, which further confirms that biological molecules are involved in the synthesis of silver nanoparticles. This is similar to what Bykkam et al (2015) found in their research. Overall, FTIR is a useful tool for understanding how biological molecules contribute to the formation of silver nanoparticles (Al-Rajhi et al., 2022).

3.2.3 Scanning electron microscope (SEM) and Transmission electron microscopy (TEM)

The SEM images of lyophilized silver nanoparticles from *Padina tetrastromatica* revealed predominantly irregular spherical particles less than 100 nm in size (Figure 4). Similarly, SEM pictures of lyophilized silver nanoparticles from *Sargassum wightii* revealed particles with a spherical shape and diameters ranging from 44 to 200 nm (Figure 5) (Al-Zahrani et al., 2022). While the majority of the silver nanoparticles were equally distributed, aggregation developed about 14 weeks. The extract's organic components or reducing agents may impact the form and size of the nanoparticles by interlinking with them, resulting in their decrease Menon et al. (2017).

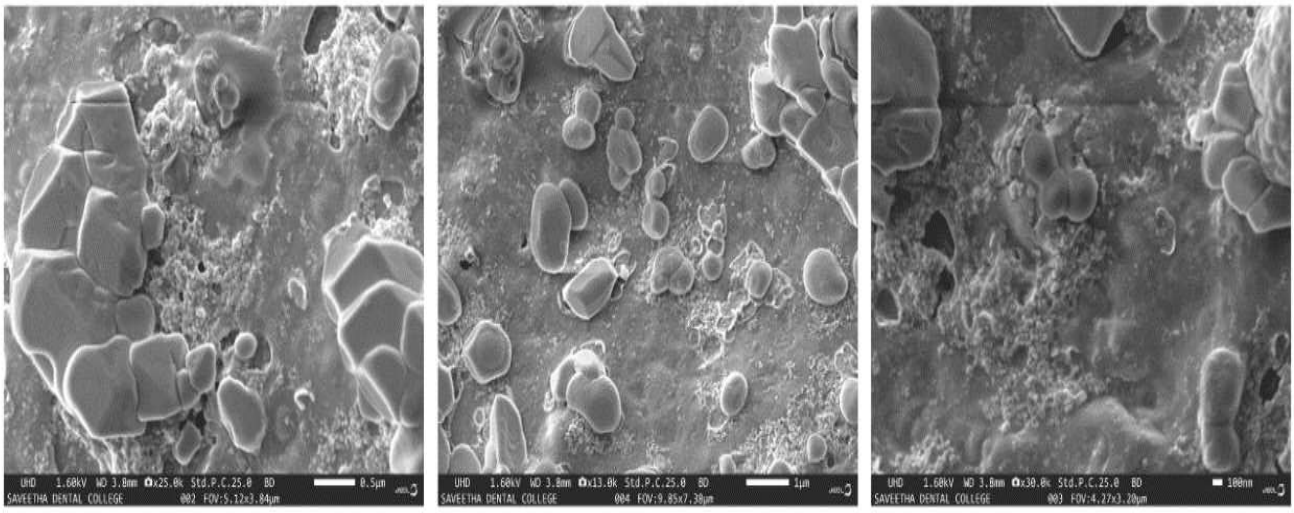


Figure 4. Scanning electron microscope image of silver nanoparticles synthesized using *Padina tetrastromatica* Aqueous extracts at different magnifications

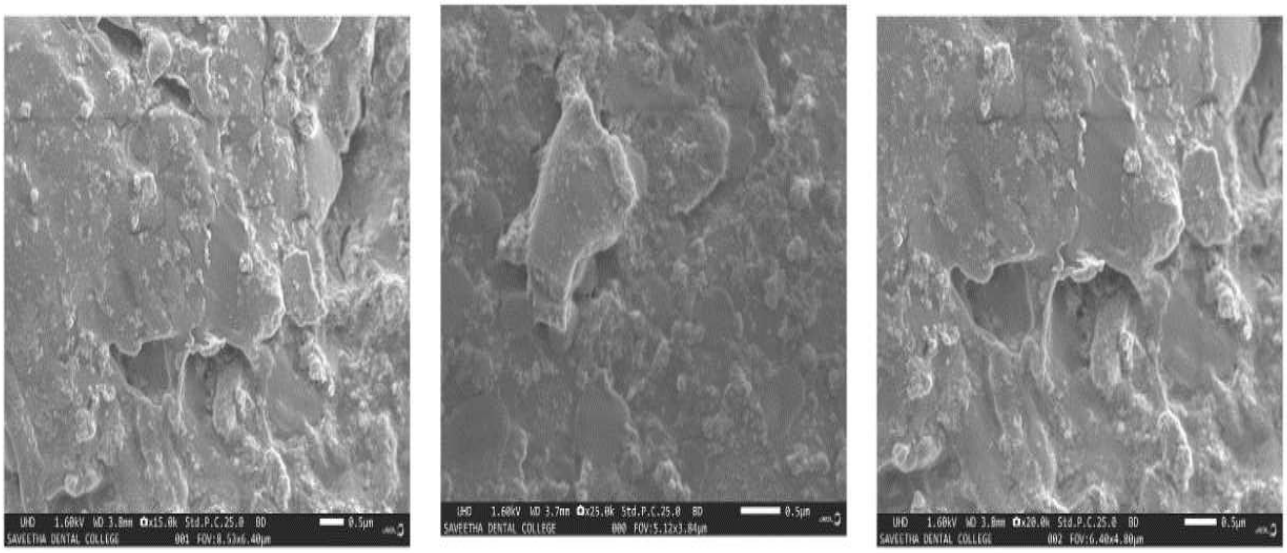


Figure 5. scanning electron microscope image of silver nanoparticles synthesized using *Sargassum wightii* Aqueous extracts at different magnifications

Transmission electron microscopy (TEM) revealed AgNPs with particle sizes ranging from 2.4 to 53.5 nm (Salem et al., 2022). Further high-resolution analysis was conducted using field emission scanning electron microscopy (FESEM) on the cyclic peptides of the nanoparticles. The shape and size characteristics of the synthesized Silver Nanoparticles were elucidated using TEM, which displayed the spherical and cubic nature of the nanoparticles, with an average diameter of 40–80 nm (Figure 6).

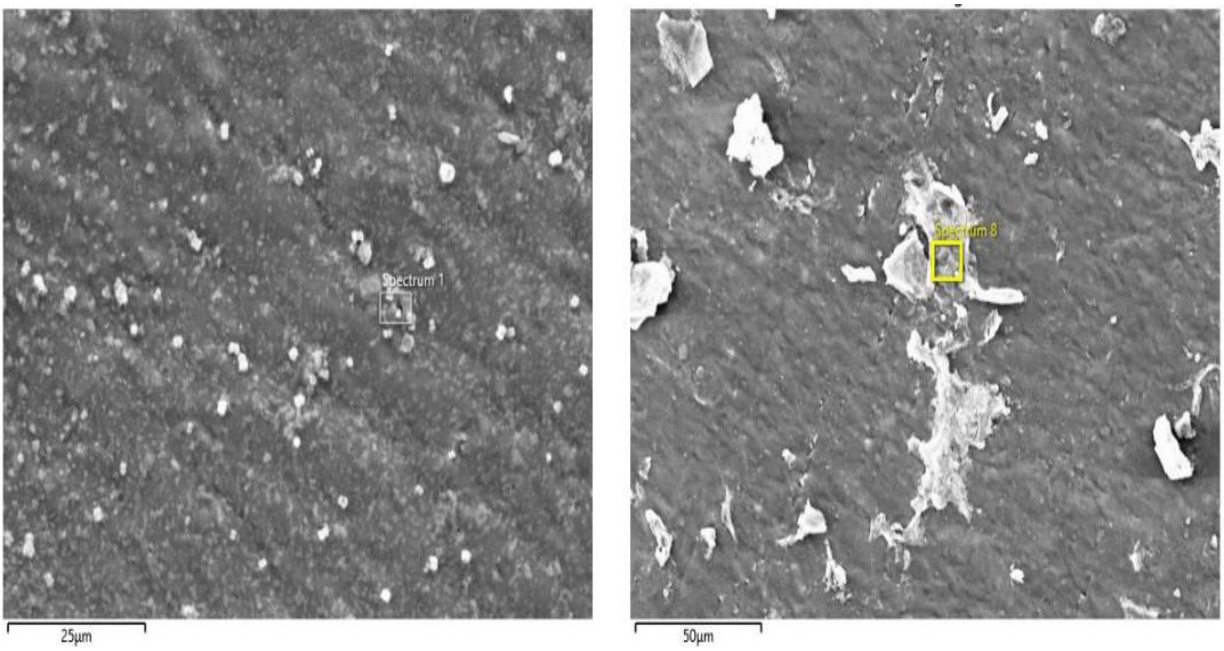


Figure 6. Transmission electron microscopy image of silver nanoparticles synthesized using Seaweeds extract. a. *Padina tetrastromatica* aqueous extracts and b. *Sargassum wightii* Aqueous extracts

3.2.4 Energy dispersive X-ray and Dynamic light scattering

The EDS analysis provides both quantitative and qualitative information about elements involved in nanoparticle formation. Confirmation of silver nanoparticle formation is obtained through the elemental profile analysis of synthesized nanoparticles using the Seaweed extract of *Sargassum wightii* aqueous extract, revealing higher counts at 3 keV attributed to silver. The energy dispersive spectrum demonstrated the presence of elemental silver, with a sharp peak at 3 keV indicative of metallic silver. Additional peaks (Ca, Si, and Na) were likely associated with the underlying glass substrate that supported the sample (Zhang et al., 2011).

3.3 In-vitro Assays

3.3.1 In vitro Antibacterial and antimycobacterial Activity

Different concentrations of silver nanoparticles were synthesized using the extracts of two seaweeds. The S2 (9:1) sample exhibited significant activity against pathogens, with a maximum activity of 24 mm observed against *E. faecalis* and the lowest activity against *E. coli*. However, S2 did not show activity against *S. aureus*. *P. tetramatica* P4 (8:3) demonstrated notable activity against various pathogens, with the highest activity observed against *K. pneumoniae* and the lowest against *S. aureus*. It was ineffective in treating E. coli and P. aeruginosa infections (Fig.9). The antimicrobial activity of the biosynthesized nanoparticles specific to the pathogen was investigated. The MIC of AgNPs for *S. aureus* and *C. albicans* was 12.5 µg/mL, whereas for *E. coli, P. aeruginosa* and *B. subtilis*, it was 25 µg/mL. AgNPs exhibited significant anti-microbial properties against several pathogens such as *E. coli, P. aeruginosa, B. subtilis, S. aureus and C. albicans* (Salem et al., 2020).

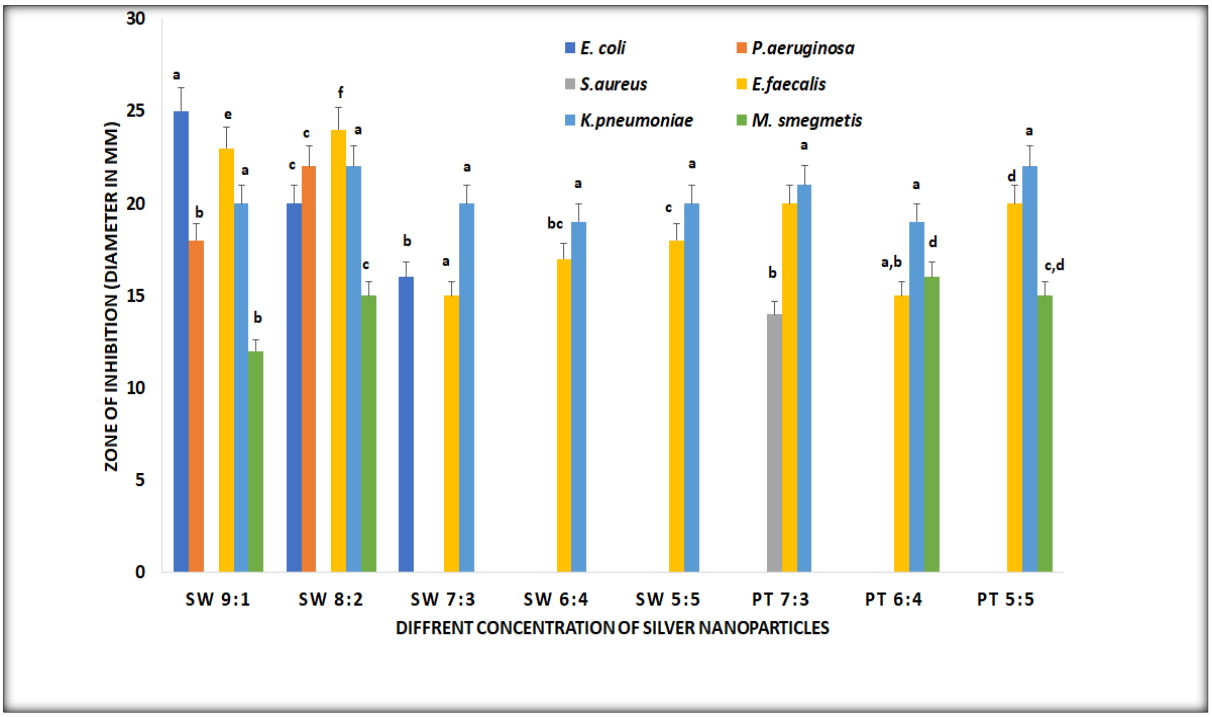


Figure 8. Antibacterial activity of the silver nanoparticles synthesized using a. *Padina tetrastromatica* b. *Sargassum wightii* Aqueous extracts

The agar well diffusion technique was used in this work to evaluate the antimycobacterial activity of silver nanoparticles synthesized from *Sargassum wightii* and *P. tetrasomatica*. The results showed that *M. smegmatis* was very active. Streptomycin was used as a positive control, while molecular grade water was used as a negative control, indicating that the silver nanoparticles were antibacterial. Silver, renowned for its antibacterial qualities, has been used in medicine to inhibit HIV attachment to host cells (Abilash et al., 2015). Differences in membrane structure may explain why antibacterial activity against Gram-positive and Gram-negative bacteria differ. Gram-positive bacteria have a thick peptidoglycan layer that is externally surrounded by a lipid layer, whereas Gram-negative bacteria have a thinner peptidoglycan layer that is externally surrounded by a lipid layer (Bekele et al., 2016).

Silver nanoparticles possess great anti-microbial effects since they are small in size and large in the ratio of surface area to volume. Various micro-organisms, such as E. coli, S. aureus, K. pneumoniae, Candida albicans, Aspergillus niger, HIV and HBV have been successfully eradicated by low concentrations of silver nanoparticles (Pei et al., 2019). Several cancers, including ling cancer, cervical cancer, hepatocellular carcinoma, breast cancer, nasopharyngeal carcinoma, prostate carcinoma, colorectal adenocarcinoma, and glioblastoma, have proven to be effective for the treatment by silver nanoparticles. Moreover, silver nanoparticles showed

strong broad-spectrum anti-bacterial effects against bacteria like s. aureus, b. subtilis, E. coli and P. aeruginosa with inhibition zones being 19.6 ± 0.8 mm, 19.1 ± 0.7 mm, and 15.1 ± 0.4 mm, respectively (Aref et al., 2020, Eid et al., 2020).

The antimicrobial activity of biosynthesized Ag/Fe_2O_3 against bacterial pathogens was tested, and it was found to have broad-spectrum activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa* (Al-Zahrani et al., 2022).

Antimicrobial activity of biosynthesized AgNPs against diverse pathogens such as E. coli, P. aeruginosa, B. subtilis, S. aureus and C. albicans was tested. It was observed that MIC of AgNPs against S. aureus and C. albicans is 12.5 µg/mL, while for E. coli, P. aeruginosa and B. subtilis, it is 25 µg/mL (Salem et a., 2022).

To understand the process of action by AuNPs against the pathogens, microbes were exposed simultaneously to AuNPs and observed under the scanning electron microscope, where it was observed that nanoparticles get accumulated on the cell membrane followed by cell wall breakage after exposure to silver nanoparticles (Zamakhaev et al., 2020).

The current study demonstrated the potential activity against *M. smegmatis* using agar well diffusion techniques for antimycobacterial activity, employing *Padina tetrastromatica* and *Sargassum wightii* silver nanoparticles. Similarly, these silver nanoparticles can be targeted against pathogenic drug-sensitive, MDR, and XDR-MTB strains for more focused applications. The introduction of CFM to the Ag-Se nanoparticles showed inhibition to S. aureus (96.09%), E. coli (98.32%) and C. albicans (95.93%) biofilm growth. Based on these results, there is a possibility that the developed nanomaterials have great anti-microbial activity at lower concentrations, implying future usage in the medical industry. (Elakraa et al., 2022).

3.3.2 In vitro anti-oxidant activity

The antioxidant activity of silver nanoparticles derived from seaweeds (*Padina tetrastromatica and Sargassum wightii*) was assessed through DPPH scavenging. Significant differences were observed among the respective values obtained, showing an increase in DPPH values in a quantity-dependent manner, as depicted in Figure 10. In the present study, *Padina tetrastromatica and Sargassum wightii* exhibited IC_{50} values of 6.25 and 31.25 µg/mL, respectively. The DPPH radical scavenging assay, a fundamental and straightforward method for evaluating the radical scavenging capability of various antioxidant molecules, demonstrated dose-dependent behavior for the AuNPs. These findings align with previous reports on radical scavenging activities.

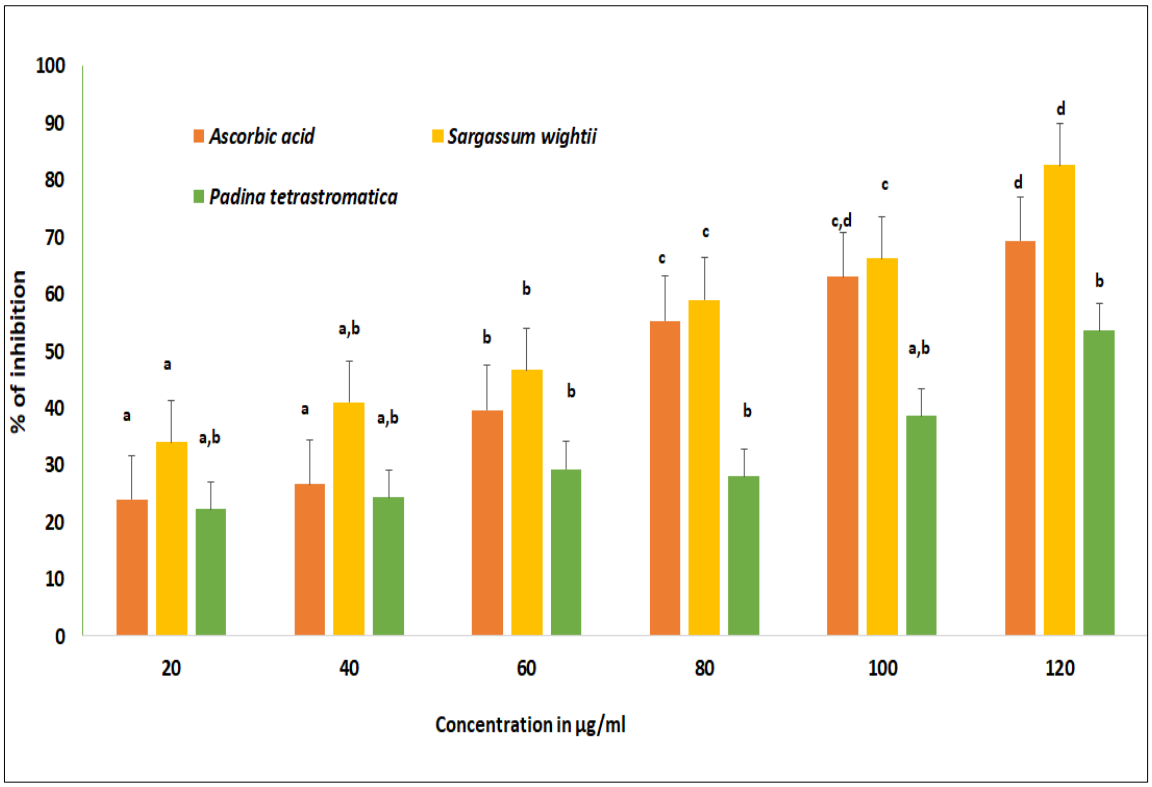


Figure 9. Anti-oxidant activity of biosynthesized silver nanoparticles

Screening a large number of chemicals or extracts on a high-throughput basis poses challenges when using a slow-growing mycobacterium like MTB. As a result of its non-pathogenic nature and higher growth rate than MTB, *M. smegmatis* has previously been chosen as the surrogate of choice for antimycobacterial screening. *M. smegmatis* is often used as a surrogate in anti-TB screening bioassays to identify candidates. The physicochemical characterization of produced nanomaterials is critical for investigating biological activities, and radiation scattering methods are frequently used for this (Lin et al., 2014).

3.4 In-vitro Cytotoxic activity

The cytotoxicity of silver nanoparticles synthesized using *Sargassum wightii* was assesses for their cytotoxic activity. Our findings revealed a dose-dependent increase in death rates, as illustrated in Figure 11. In our studies, the concentration of 100 mg/mL showed lower toxicity on *Artemia salina*. Lee et al. (2014) have described the Artemia cytotoxicity (Brine Shrimp Assay) as a reliable method for screening the cytotoxicity of products. In our present study, we observed mortality of Artemia based on the concentration of silver nanoparticles from *Sargassum wightii*. Vijyaan et al. (2014) had found that tiny silver particles from a type of algae called *Turbinaria conoides* can be harmful. More recently, Al-Zahrani et al (2022) and

their team discovered that these small silver particles have antioxidant properties, which means they can help prevent damage to cells. They used a test called DPPH to measure this, and found that it looks a certain amount of the silver particles to have an effect. Another group, led by Chugh et al. (2021) looked at how to make these silver particles using algae. They found that using different helpers, called capping agents, can affect how stable and big the particles are. This is important because it can make the process of making the particles more efficient and better for the environment. By using the right capping agents, we can make the process of making silver particles more cost-effective and friendly to the planet. This is a key part of making sure that the silver particles are made in a way that is good for both people and the environment.

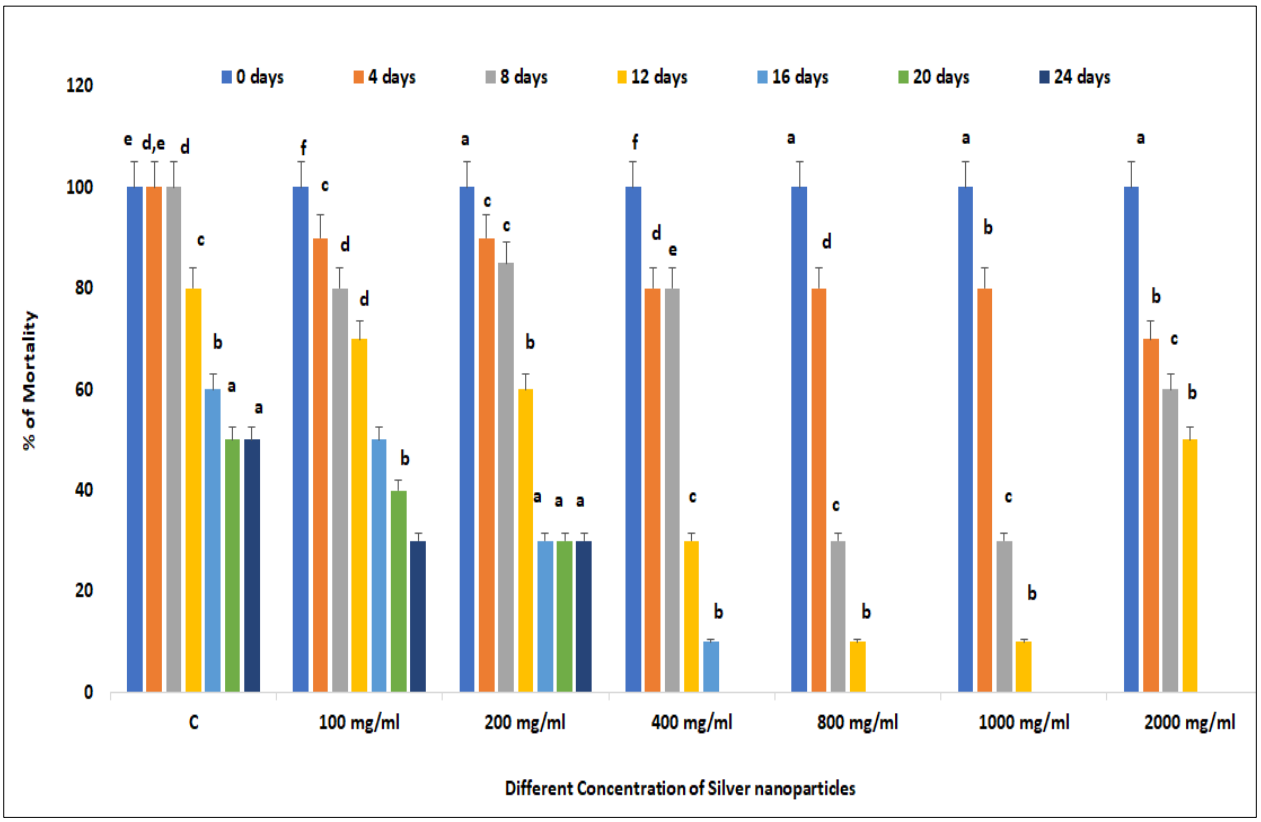


Figure 10. Analysis of Mortality rate using *Artemia salina* treated with the silver nanoparticle of *Sargassum wightii*

Researchers found that tiny particles called Ag-NPs, made using natural methods, can stop cancer celles from growing. They tested these particles on a type of cancer cell called $CaCO_2$

and found that it took a concentration of 5.7 ± 0.2 ppm to inhibit the cells by half. In another test, they saw that the particles could kill cancer cells in a dose-dependent way, with an IC_{50} value of about 200 µg/mL. Another study looked at how biogenic AuNPs affect cells and found that they can be toxic to cancer cells. However, more research is needed to see how these particles work in living things, which is something that's missing from current studies.

Due to their physiochemical properties, silver and gold nanoparticles have attracted significant interest for use in breast cancer therapy. These nano biomaterials, synthesized via a biological process, may be used as smart nanomedicine for both treatment and diagnosis.

Using tiny silver and gold particles, we can create systems that deliver drug straight to the target, which is really promising. These particles can be combined with medicines that are already approved by the FDA, as well as agents that help diagnose or target specific areas, forming tiny structures that fight cancer cells. The idea of green nanotechnology is to use natural systems to create, change, and control these tiny materials in a way that's good for the environment and doesn't cost too much or hurt people. This approach is not only budget-friendly but also safe for humans and the planet. By using natural systems, we can produce and modify these tiny materials without causing harm, which is a big plus. Overall, this technology has a lot of potential for treating diseases like cancer in a more targeted and environmental friendly way (Saravanan et al., 2021).

Author Contributions

R.T. (Rajasekar Thirunavukkarasu): Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Supervision.

M.B. (Magalakshmi Boopathi): Methodology, Validation, Investigation, Writing – review & editing.

V.T. (Vaishnavi T): Investigation, Data curation, Formal analysis.

R.M. (Radhakrishana M): Investigation, Resources, Data curation.

R.K.V. (Ramesh Kumar V): Formal analysis, Software, Visualization.

A.S. (Arumugam Suresh): Resources, Methodology, Validation.

S.S. (Shobana Sampath): Investigation, Data curation.

Mohammad Ahmad Wadaan (M.A.W) & Sandhanasamy Devanesan (S.D): Formal analysis, Writing – review & editing.

D.M. (Dhinakaran M): Resources, Supervision.

A.K. (Ali Kudamba): Supervision, Project administration, Writing – review & editing.

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Data availability

This published article contains all of the data created or examined during this investigation. Upon a reasonable request, the corresponding author will make the datasets created and/or examined during the current work available.

Declarations

Ethics approval

Not applicable

Consent to participate

Not applicable

Clinical Trial Number

Not applicable

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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