

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING MARINE BACTERIA EXTRACT AND THEIR CHARACTERIZATION

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Science in Microbiology

By

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SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

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BONAFIDE CERTIFICATE

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ABSTRACT

Green synthesis of silver nanoparticles using marine microorganisms has gained profound interest because of their potential to synthesise nanoparticles of various sizes, shapes and morphology. The synthesis of metal nanoparticles using biological methods is an expanding research area due to the potential applications in nanomedicines. Marine environments are considered a good source of metal-tolerant microorganisms due to the continuous release of the metals into marine environments by volcanoes, natural weathering of rocks and also by numerous anthropogenic activities, such as mining, combustion of fuels, industrial and urban sewage and agricultural practices. Recently, they are being explored as potential sources of metal-tolerant microorganisms with the ability to synthesise metallic nanoparticles. The aim of our present study is to develop an eco-friendly, cost-effective, and simple method to biosynthesize silver nanoparticles. The present study aims at exploiting marine microbial diversity for the biosynthesis of metal nanoparticles and their characterization. The biosynthesized nanoparticles will be characterized by various spectroscopic analyses including Fourier-transform infrared spectroscopy, Scanning electron microscopy, X-ray diffraction analysis, UV-Vis, Dynamic Light Scattering, and Zeta sizer.

Keywords: Nanoparticles, Metabolites, Biosynthesis and Antioxidant assay.

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ABBREVIATIONS

Ag NO₃ - Silver nitrate

Ag- Silver

Ag NPs - Silver nanoparticles

Au NPs - Gold nanoparticles

Pt NPs - Platinum nanoparticles

UV-vis- Ultraviolet-visible Spectrophotometer

FTIR - Fourier transform infrared

SEM - scanning electron microscope

TEM - Transmission electron microscope

SPR - surface plasmon resonance

ROS - reactive oxygen species

PHBV - Poly(3-hydroxybutyrate-co-3-hydroxy valerate)

PET- Polyethylene terephthalate

LAF- Laminar Air Flow

BS - *Bacillus subtilis*

DLS - Dynamic Light Scattering

DPPH - 2,2-diphenylpicrylhydrazyl

BSA - Bovine Serum Albumin

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CHAPTER - 1

INTRODUCTION

The oceans, seas, and other saltwater bodies that make up more than 70% of the Earth's surface are referred to as the marine environment. It contains a wide variety of habitats, including open waters, rocky coasts, sandy beaches, seagrass meadows, and coral reefs. A wide variety of plant and animal species make a home in the marine environment, which is essential to the functioning of the Earth's ecosystem. By acting as a carbon dioxide sink and controlling global temperature and precipitation, it also significantly affects the climate and weather on Earth. The maritime ecosystem has been significantly impacted by human activities including overfishing, pollution, and climate change, which has resulted in biodiversity loss, habitat degradation, and other detrimental effects. The oceans, seas, and other bodies of water are called the marine environment (Hoang & Pham, 2021). For the sake of the planet's health and the welfare of both current and future generations, it is crucial to preserve and safeguard the marine environment. The plant life that can be found in oceans, seas, and other saltwater bodies is referred to as marine flora. Despite the fact that water dominates the marine environment, a variety of plant life has adapted to survive there. Seaweed, a type of algae that can be found in a number of various shapes, from small and basic to vast and sophisticated, is one of the most well-known varieties of marine flora (Yong *et al.*, 2022).

Microorganisms known as marine bacteria live in saltwater environments such as the ocean. These bacteria are crucial to the functioning of the marine ecosystem because they cycle nutrients, decompose organic materials, and produce oxygen. The role that marine microorganisms play in the carbon cycle is one of its most significant activities. Additionally, marine microbes are essential to the nitrogen cycle (Baharum *et al.*, 2010). They are in charge of transforming nitrogen gas into forms of nitrogen, such as ammonia and nitrate, that may be utilised by other species. The growth of plants and algae in the ocean depends on a process known as nitrogen fixation (Zehr & Capone, 2020). Microorganisms known as marine bacteria are found in salty environments such as the ocean. They display a wide range of traits that enable them to live and flourish in this setting. Marine bacteria come in a wide variety, each having special traits and functions

in the marine ecosystem (Baharum *et al.*, 2010). The following are some of the major types of marine bacteria:

1. Cyanobacteria- also known as blue-green algae are autotrophic bacteria that can perform photosynthesis.
2. Proteobacteria- breaking down organic matter and cycling nutrients,
3. Bacteroidetes- break down complex organic matter, such as carbohydrates and proteins, Actinobacteria- nutrient cycling and are also known for producing antibiotics,
4. Firmicutes- the ability to form spores,
5. Spirochetes- play important roles in the carbon and nitrogen cycles.

A rod-shaped, gram-positive, facultatively anaerobic bacteria known as *Bacillus subtilis* can be found in soil, dust, and numerous food products. It is well recognised for its capacity to produce endospores, which are extremely resilient to heat and other environmental stresses and enable the bacteria to endure in challenging circumstances. *Bacillus subtilis* is excellent for the bioremediation of contaminated soils and streams because it can break down a variety of organic contaminants. Amylases, proteases, and lipases are only a few of the enzymes and other substances that *Bacillus subtilis* may create and are helpful in commercial bioprocessing. Animal studies have indicated that *Bacillus subtilis* has the potential to act as a probiotic, improving gut health and immunological function. However, its application as a probiotic in humans remains to be researched (Velmurugan *et al.*, 2014)

1.1 NANOTECHNOLOGY:

The research and development of objects in the nanoscale, frequently alluded to as ranging between 1 and 100 nanometers in size, is known as nanotechnology. At this scale, materials frequently show distinctive characteristics that are different from those of their bulk counterparts. These qualities can be taken advantage of for a variety of

applications in industries like electronics, health, energy, and materials science. Nanotechnology entails the creation of tools and methods for manipulating and analysing materials at the nanoscale, as well as their design, synthesis, and characterisation. While there are numerous potential advantages of nanotechnology, there are also worries about its effects on the environment and human health (Mattsson *et al.*, 2018)

1.2 NANOPARTICLES:

Nanoparticles are particles with sizes ranging from 1 to 100 nanometers, which are typically smaller than the wavelength of visible light. They can be made from a variety of materials, including metals, metal oxides, and polymers, and have unique properties that differ from their bulk counterparts (Wei *et al.*, 2015).

1.3 TYPES OF NANOPARTICLES:

Some examples of nanoparticles and their applications include:

- **Silver nanoparticles (Ag NPs):** are commonly used in antimicrobial coatings for medical devices and textiles, due to their ability to kill bacteria and other microorganisms.
- **Gold nanoparticles (Au NPs):** have unique optical properties, making them useful for imaging and sensing applications. They are also used in cancer therapy, as they can be targeted to cancer cells and used to deliver drugs or heat to destroy tumours.
- **Platinum nanoparticles (Pt NPs):** They are nanoparticles made from platinum, a rare and expensive metal that is commonly used in a variety of industrial and biomedical applications due to its unique properties. They are also being investigated for use in diagnostic imaging and biosensors.

1.4 DIFFERENT KINDS OF SYNTHESIS OF NANOPARTICLES:

In the context of nanoparticle synthesis, a top-down approach refers to a fabrication method that starts with a larger bulk material and then reduces its size to create nanoparticles. Here's how a top-down approach can be applied in nanoparticle

synthesis: starting with bulk material, size reduction, nanoparticle formation, size control and characterization, further processing and applications (Tran *et al.*, 2013).

1.5 SILVER NANOPARTICLES:

1.5.1 Characteristics of silver nanoparticles:

Silver nanoparticles exhibit unique characteristics that make them highly desirable for a wide range of applications. Here are some of the key characteristics of silver nanoparticles:

- **Size-dependent properties:** The properties of silver nanoparticles can vary depending on their size. This can result in unique optical, electronic, and catalytic properties that are different from bulk silver.
- **Antimicrobial properties:** Silver nanoparticles have been shown to exhibit broad-spectrum antimicrobial activity against a wide range of bacteria, viruses, and fungi.
- **Optical properties:** They can display unique colours ranging from yellow to brown, depending on their size and the surrounding environment. These colours arise from the interaction of light with the nanoparticles at the nanoscale, a phenomenon known as localised surface plasmon resonance (LSPR).
- **High thermal and electrical conductivity:** Silver nanoparticles can exhibit high thermal and electrical conductivity, making them suitable for applications in electronics, conductive inks, and other technologies that require efficient heat and electrical transfer.
- **Stability and durability:** Silver nanoparticles are generally stable and durable, allowing them to withstand various environmental conditions and maintain their properties over time (Wei *et al.*, 2015).

1.6 SYNTHESIS OF NANOPARTICLES:

Nanoparticles, defined as particles with at least one dimension less than 100 nanometers, have garnered significant attention in recent years due to their unique properties and potential applications in various fields such as biomedicine, electronics, and energy. The synthesis of nanoparticles, which refers to the production of these small-scale particles from various materials, has become an increasingly important area of research as scientists and engineers seek to develop new and improved methods for creating nanoparticles with precise sizes, shapes, and compositions. The field of nanoparticle synthesis is multidisciplinary, encompassing materials science, chemistry, physics, and engineering, and involves a wide range of techniques such as chemical precipitation, sol-gel processing, and physical vapour deposition (Keat *et al.*, 2015). In this context, the synthesis of nanoparticles represents a crucial area of research with significant potential for the development of novel materials and applications that can benefit society. Since our project deals with synthesised nanoparticles in the medical field, there are few applications for it (Haider and Kang., 2015)

Nanoparticles synthesis has significant potential for various applications in the medical field, including:

1. Drug delivery: Nanoparticles can be used as carriers to deliver drugs and other therapeutic agents to specific cells and tissues in the body, improving efficacy and reducing the toxicity of the drugs.
2. Imaging: Nanoparticles can be used as contrast agents in medical imaging, including magnetic resonance imaging (MRI), computed tomography (CT), and ultrasound imaging.
3. Diagnostics: Nanoparticles can be used in diagnostic tests to detect diseases and infections, including cancer, HIV, and tuberculosis.
4. Therapeutics: Nanoparticles can be used to treat a wide range of medical conditions, including cancer, diabetes, and cardiovascular disease.

5. Tissue engineering: Nanoparticles can be used in tissue engineering to create scaffolds for tissue regeneration and to improve the delivery of therapeutic agents to damaged tissues.
6. Biosensors: Nanoparticles can be used in biosensors to detect and monitor biomolecules and other analytes in biological samples.

Overall, the use of nanoparticle synthesis in the medical field has the potential to revolutionize healthcare by improving the accuracy, efficacy, and safety of medical treatments and diagnostics. However, there are still challenges that need to be addressed, including the potential toxicity of nanoparticles and the need for more robust and reliable methods of nanoparticle synthesis (Morley *et al.*, 2007).

1.7 MARINE BACTERIA BASED SYNTHESIS OF Ag NPs:

Marine bacteria have been shown to have the ability to synthesise nanoparticles, including Ag NPs, using silver nitrate (Ag NO_3) as a precursor. This approach has several advantages, including the use of environmentally friendly and renewable sources of materials and the potential for the development of new antibacterial agents.

The process of marine bacteria-based synthesis of silver nanoparticles typically involves the following steps:

1. Collection and isolation of marine bacteria with nanoparticle synthesis capability.
2. Culturing of the bacteria in a suitable growth medium to encourage the growth and proliferation of the bacteria.
3. The addition of Ag NO_3 to the bacterial culture, triggers the reduction of Ag^+ ions to Ag nanoparticles by the bacterial enzymes.
4. Isolation and purification of the synthesised nanoparticles using various techniques such as centrifugation, filtration, and dialysis.

5. Characterization of the nanoparticles using analytical techniques such as UV-vis spectroscopy, X-ray diffraction, and transmission electron microscopy to determine their size, shape, and other properties.

The marine bacteria-based synthesis of silver nanoparticles has the potential for numerous applications, including antibacterial coatings for medical devices, water treatment, and food preservation. The use of marine bacteria as a source of nanoparticles has attracted significant interest in recent years due to its potential for sustainable and environmentally friendly production of nanoparticles. (El-Nour *et al.*, 2010)

1.8 SECONDARY METABOLITES FROM MARINE BACTERIA:

Marine bacteria are known to produce a wide range of secondary metabolites that have various biological activities, including antimicrobial, anticancer, anti-inflammatory, and antiviral properties. These secondary metabolites are produced by marine bacteria in response to various environmental stress factors, such as nutrient deprivation, high salinity, and changes in temperature and pH. Here are some examples of secondary metabolites produced by marine bacteria (Andryukov *et al.*, 2019).

1. Prodigiosin - This is a red pigment produced by various marine bacteria, including *Vibrio* species. It has been shown to have antibacterial, and antifungal properties.
2. *Pseudoalteromonas* lipopolysaccharides (LPSs) - These are complex polysaccharides produced by the marine bacterium *Pseudoalteromonas*. They have been shown to have anti-inflammatory and anti-tumour properties.
3. Violacein - This is a purple pigment produced by various marine bacteria, including *Janthinobacterium* and *Pseudoalteromonas* species. It has been shown to have antibacterial, antifungal, antiviral, and anticancer properties.
4. Halocins - These are bacteriocins produced by halophilic bacteria, which are bacteria that can thrive in high-salt environments. They have been shown to have antimicrobial properties and may be useful in the development of new antibiotics.

Overall, secondary metabolites produced by marine bacteria have great potential for use in the development of new drugs and other bioactive compounds.

1.9 APPLICATIONS:

Silver nanoparticles (Ag NPs) have been extensively studied for their antimicrobial activity due to their unique physicochemical properties, such as the high surface area to volume ratio, small size, and ability to generate Reactive Oxygen Species (ROS). Here are some applications of silver nanoparticles in antimicrobial activity

1. Wound healing: Ag NPs are effective in promoting wound healing by inhibiting the growth of bacteria and reducing inflammation. They can be used in wound dressings or incorporated into gels or creams for topical application.
2. Water treatment: Ag NPs can be used in water treatment to disinfect water by killing bacteria and other microorganisms. They can be incorporated into water filters or used in water purification systems.
3. Food packaging: Ag NPs can be incorporated into food packaging materials to prevent the growth of bacteria and extend the shelf life of food products.
4. Medical devices: Ag NPs can be incorporated into medical devices, such as catheters, implants, and prosthetics, to prevent infections.
5. Textile industry: Ag NPs can be incorporated into fabrics to impart antimicrobial properties and prevent the growth of bacteria and fungi.
6. Air purification: Ag NPs can be used in air purifiers to remove bacteria and other microorganisms from the air.

It is important to note that the use of Ag NPs in antimicrobial applications should be carefully regulated to avoid potential environmental and health risks (Yaqoob *et al.*, 2020)

1. Cosmetics: Ag NPs can be incorporated into cosmetic products, such as creams and lotions, to provide antioxidant activity and protect the skin from oxidative stress.
2. Food industry: Ag NPs can be used in the food industry as food additives to prevent the oxidation of food products and increase their shelf life.
3. Biomedical applications: Ag NPs can be used in biomedical applications, such as drug delivery systems and implants, to provide antioxidant activity and prevent oxidative stress.
4. Environmental remediation: Ag NPs can be used in environmental remediation to remove pollutants from the environment by scavenging free radicals and preventing oxidative damage.
5. Agriculture: Ag NPs can be used in agriculture as a plant growth regulator and antioxidant to improve crop yields and protect plants from environmental stress.

It is important to note that the use of Ag NPs in antioxidant applications should be carefully regulated to avoid potential environmental and health risks.

CHAPTER - 2

REVIEW OF LITERATURE

Silver and silver salts have been used for as long as there has been human civilization, but the creation of silver nanoparticles (Ag NPs) has only lately come to light. They have especially been employed as antibacterial, antifungal, and antioxidant in agriculture and medicine. It has been demonstrated that Ag NPs arrest the growth and multiplication of many bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Citrobacter koseri*, *Salmonella typhii*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Vibrio parahaemolyticus* and fungus *Candida albicans* by binding Ag/Ag⁺ with the biomolecules present in the microbial cells. Ag NPs may generate free radicals and reactive oxygen species, which lead to apoptosis and cell death by blocking cell replication. Ag NPs permeate into cells and rupture the cell wall because they are smaller than microorganisms, as demonstrated by SEM and TEM images of the suspension containing nanoparticles and pathogens. Additionally, it has been demonstrated that smaller nanoparticles are more poisonous than larger ones. Ag NPs are also utilised in packaging to stop germs from contaminating food goods. Ag NPs toxicity is influenced by the size, concentration, pH of the media, and length of pathogen exposure. (Siddiqi *et al.*, 2018) Against a variety of bacteria, silver nanoparticles (Ag NPs) have demonstrated strong bactericidal capabilities (Wei L *et al.*, 2018) They are produced from a variety of angles, frequently to examine their morphology or other physical traits. Though they did so unintentionally, several authors have mistakenly employed the chemical approach (Zaheer Z and Rafiuddin., 2012) for green synthesis. Ag NPs are environmentally beneficial due to their use in electronics, catalysis, and medicines, and limiting the growth of microorganisms in biological systems (Lokina S *et al.*, 2014). Although numerous reviews on the creation and characterisation of silver nanoparticles have been published, there are very few studies on their environmentally friendly production, biocidal capabilities, and mechanism of action (Ahamed M *et al.*, 2010).

Metallic nanoparticles are often created using one of two techniques: "top-down" (slicing or sequential cutting of bulk material to obtain nano-sized particles) or "bottom-up" (building material from the bottom up: atom by atom, molecule by molecule, or cluster by cluster). When creating nanoparticles in a homogeneous system, the "bottom-up" method is typically preferable since it uses catalysts (such as enzymes and reducing agents) to create nanostructures that they can control. The size reduction to the nanoscale is accomplished by specialised ablations, such as thermal decomposition, mechanical grinding, etching, cutting, and sputtering, but the "top-down" technique often works with the material in its bulk form. Surface structural flaws are the fundamental drawback of the top-down strategy. Metallic nanoparticles' physical characteristics and surface chemistry are significantly impacted by such imperfections. For the synthesis of Ag NPs, a variety of techniques are available, including chemical, physical and biological approaches (Zhang Q *et al.*, 2011).

Chemical reduction, electrochemical, irradiation-assisted chemical, and pyrolysis processes are different types of chemical synthesis. Metal precursors, reducing agents, and stabilising or capping agents are necessary for the synthesis of Ag NPs in solution. Ascorbic acid, alcohol, borohydride, sodium citrate, and hydrazine compounds are often utilised as reducing agents. (Zhang W *et al.*, 2007). According to Sotiriou and Pratsinis (Sotiriou GA and Pratsinis SE., 2010), flame aerosol technology was used to create the Ag NPs supported on nanostructured SiO₂, allowing for precise control of the silver concentration and size. Additionally, flame spray pyrolysis was used to create silver/silica nanoparticles with a rather limited size distribution (Sotiriou GA *et al.*, 2011).

Physical procedures, however, typically process quickly and don't require dangerous or highly reactive chemicals. Arc discharge (Tien DC *et al.*, 2008), physical vapour condensation (Abou El-Nour KMM., 2010) the energy ball milling method (Wright R *et al.* 2011) and direct current magnetron sputtering (Asanithi P *et al.*, 2012) are a few examples of these techniques. Physical approaches have an additional benefit over chemical methods because Ag NPs have a restricted size distribution, while the primary drawbacks include high energy consumption (Asanithi P *et al.*, 2012).

Considering the numerous benefits that biological synthesis of Ag NPs from the herbal extract and/or microorganisms provides over chemical and physical techniques of synthesis, this method has been proposed as an alternative. Additionally, it is generally known that these approaches are straightforward, affordable, environmentally benign, and simple to scale up for high yields and/or production. In the field of nanotechnology, the biosynthesis of metal and metal oxide nanoparticles utilising biological agents such as bacteria, fungi, yeast, plant, and algae extracts has grown in popularity. (Siddiqi, K.S., Husen, A. & Rao., 2018).

The possibility of producing Ag NPs using bacteria has recently come to light (Gopinathan P *et al.*, 2013). To create Ag NPs inside the cells, for instance, *Pseudomonas stutzeri* AG259—isolated from a silver mine—was utilised (Klaus T *et al.*, 1999). Additionally, several gram-positive and gram-negative bacterial strains, including *A. calcoaceticus*, *B. amyloliquefaciens*, *B. flexus*, *B. megaterium*, and *S. aureus*, have been employed to produce Ag NPs both extracellularly and intracellularly (Nanda A and Saravanan., 2009). The shapes of these Ag NPs include spheres, discs, cuboids, hexagons, and triangles. Cells, aqueous cell-free extract, or culture supernatant have all been used to create them. The extracellular biogenesis of Ag NPs (5-50 nm) has been established by Saifuddin *et al.*, 2009 by combining *B. subtilis* culture supernatant with microwave irradiation in water. According to Shahverdi *et al.* (Shahverdi AR *et al.*, 2007), *K. pneumoniae*, *E. coli*, and *Enterobacter cloacae* culture supernatants were used to quickly biosynthesis Ag NPs (within 5 min). In the presence of aqueous solutions of Ag⁺ ions, (Saravanan *et al.*, 2011) also reported an extracellular production of Ag NPs using a *B. megaterium* culture supernatant. The combination of a *Bacillus flexus* group bacterial strain S-27 and 1 mM Ag NO₃ in an aqueous medium led to the rapid production of Ag NPs. The colourless supernatant solution changed from colourless to yellow and then brown. As a result of the silver nanoparticles' surface plasmon resonance (SPR), their UV-vis spectrum showed a strong peak at 420 nm. Although their progressive deterioration cannot be stopped, anisotropic nanoparticles of 12 and 65 nm size were stable in the dark for 5 months at ambient temperature. They had a face-centred cubic structure and were crystalline. Gramme-positive and gramme-negative bacteria that are resistant to many drugs were discovered to be

sensitive to these nanoparticles. The concentration of the reacting elements determines the intensity of the colour and the speed of the reaction. (Priyadarshini S *et al.*, 2013).

It has been widely studied how pathogenic and non-pathogenic fungi produce Ag NPs (Durán N *et al.*, 2005). According to one study, when fungi are present, silver ions are reduced extracellularly to produce stable Ag NPs in water (Kathiresan K *et al.*, 2009). The extracellular production of Ag NPs by the thermophilic fungus *Hemichytrium* sp. has also been documented by Syed *et al.*, 2010. In an Erlenmeyer flask set at 50 °C with 100 mL of a 1 mM Ag NO₃ solution suspended in it, the mixture was shaken for 96 hours at pH 9 while being watched for any colour changes. Due to the creation of Ag NPs, the solution's colour changed from yellow to brown (Ahmad A *et al.*, 2003). The extracellular production of Ag NPs from *Humicola* sp. is a straightforward procedure. A TEM image revealed evenly distributed nanoparticles, the majority of which were spherical and ranged in size from 5 to 25 nm. They have a face-centred cubic structure and are crystalline. Peaks at 1644 and 1523 cm⁻¹ in the IR spectrum of the Ag NP suspension were attributed to the amide I and II bands of protein, which correspond to the -C=O and N-H lengths, respectively. (Chwalibog A *et al.*, 2010).

The production of Ag NPs from the yellow exotic oyster mushroom *Pleurotus cornucopiae* var. *citrinopileatus* has been documented by (Owaid *et al.*, 2015). The dried basidiocarps were pulverised, and then the supernatant was boiled in water and freeze-dried. At 25 °C, 1 mM Ag NO₃ was mixed with various hot water extract quantities of this lyophilized powder, and the mixture was then incubated for 24, 48, and 72 hours. An absorption peak at 420 and 450 nm in the UV-vis range was visible as the colour changed from yellow to yellowish brown, which is a hallmark of spherical silver nanoparticles. The poly-dispersed character of nanoparticles is suggested by the width of the absorption peak. For various groups, the IR spectrum of Ag NPs showed absorption maxima at 3304, 2200, 2066, 1969, 1636, 1261, 1094, and 611(nm). Even though scholars have mentioned the polysaccharide and protein content of the mushroom, they have overlooked its stretching frequencies in the IR spectrum. The peak at 3304, on the other hand, has been attributed to the (OH) of carboxylic acid, and the peaks at 2200 and 1969 cm⁻¹ have been linked to unsaturated aldehydes. Unsaturated

alkaloids are the cause of the other peaks below 1500 cm⁻¹. Ag NPs appeared to be spherical with an average size between 20 and 30 nm according to the high-resolution transmission electron micrograph and field emission scanning electron. (Sanghi R and Verma P., 2009) (Al-Bahrani *et al.*, 2017) publication on the biogenic production of Ag NPs from *Pleurotus ostreatus*, a tree oyster mushroom, is recent. 1 mM Ag NO₃ and dried aqueous mushroom extract (1-6 mg/mL) were combined and incubated in the dark for 6–40 hours. The creation of silver nanoparticles was indicated by the hue changing from pale yellow to dark brownish yellow. At 420 nm, a prominent and wide absorption band was visible in the UV-vis spectra. They are poly-dispersed nanoparticles with an average size of 28 nm and a size range of 10–40 nm. Numerous fungi have been employed for the extracellular and intracellular production of Ag NPs, including *Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium oxysporum*, *Fusarium acuminatum*, *Fusarium culmorum*, *Fusarium solani*, *Metarhizium anisopliae*, *Phoma glomerata*, *Phytophthora infestans*, *Trichoderma viride*, and there are many sizes of these nanoparticles. (Vigneshwaran N *et al.*, 2007).

Plant-related components such as leaves, stems, roots, shoots, flowers, barks, seeds, and their metabolites have all been employed effectively to produce nanoparticles (Husen A., 2017). Beg *et al.*, 2017 research on the green production of Ag NPs from *Pongamia pinnata* seed extract are recent. An absorption maximum at 439 nm proved that nanoparticles were formed. The zeta potential of the evenly distributed nanoparticles, which had an average size of 16.4 nm, was equal to or less than 23.7 mV, supporting their stability and dispersion. Ag NPs and human serum albumin did not interact significantly, according to the research. Karatoprak *et al.* (Karatoprak GS *et al.*, 2017) have described the green synthesis of Ag NPs from the medicinal plant extract *Pelargonium endlicherianum* in a fairly recent article. Gallic acid, apocynin, and quercetin from the plant function as reducing agents to create silver nanoparticles. Moldovan *et al.* reported on the phytomediated production of spherical Ag NPs from *Sambucus nigra* fruit extract. (Moldovan B *et al.*, 2016) They were crystallised, according to an XRD examination. Investigating the *in vivo* antioxidant activity of Wistar rats revealed excellent results. It implies that modifying Ag NPs with natural phytochemicals may shield cell proteins from the formation of ROS. Additionally, an aqueous leaf extract of

Artocarpus altilis was used to create Ag NPs. They had a weak antibacterial and antioxidant effect. Based on the occurrence of a sharp peak at 420 nm in the UV-vis region of the spectrum, it has been determined that *Thalictrum foliolosum* root extract mediates Ag NPs formation (Hazarika SN *et al.*, 2016). The face-centred cubic geometry of the 15–30 nm monodispersed spherical nanoparticle. According to Logaranjan *et al.*, 2016 the regulated production of Ag NPs from Aloe vera plant extract and their antibacterial effectiveness.

Due to their special properties, silver nanoparticles are of particular interest in contemporary nanotechnology research. These properties allow for the incorporation of silver nanoparticles into a wide range of applications, including antiseptics in the medical industry, cosmetics, food packaging, bioengineering, electrochemistry, catalysis, and environmental uses. Noble nanoparticles have differing catalytic activity as compared to their bulk components, and as a result, nano-catalysis, which employs nanoparticles as catalysts in a variety of processes, has recently attracted a lot of attention. For instance, well-known catalysts in the conversion of H_2O_2 to oxygen include gold, silver, platinum, and metal ions (Merga *et al.* 2007). The catalytic potential of silver nanoparticles was found to be greater than that of gold and platinum nanoparticles in the emission system of chemiluminescence from luminol- H_2O_2 in the study by Guo *et al.*, 2008. Moreover, the use of silver nanoparticles immobilised on silica spheres can improve the catalysis of the reduction of dyes by sodium borohydride (NaBH_4). The pace of the reaction was nearly motionless in the absence of silver nanoparticles acting as catalysts, indicating that very little or even no reduction of the dyes had taken place (Guo *et al.* 2008). Numerous medical applications impregnated with Ag NPs, such as catheters and cardiovascular and bone implants, have been recognised for preventing the formation of biofilm and reducing the risk of pathogenic invasion due to the significant antimicrobial properties of Ag NPs against a wide range of microorganisms (Tran *et al.* 2013). Due to its high vulnerability to wear and tear, ultrahigh molecular weight polyethylene has often been utilised extensively as an insert for artificial joint replacement, but its applicability is rather constrained (Morley *et al.* 2007). However, the inclusion of silver nanoparticles considerably reduces the disadvantage of polymer wear and tear. Additionally, it is loaded with polymethyl methacrylate, which is commonly used in bone cement, and is

widely employed as a synthetic joint replacement due to the strong antibacterial qualities of silver nanoparticles (Alt *et al.* 2004). Xing *et al.*, 2010 concluded from their thorough investigation of both osteoblast (bone cells) and fibroblast (skin cells) cultured on such scaffolds that (poly-(3-hydroxybutyrate-co-3-hydroxy valerate) PHBV nanofiber scaffolds containing Ag NPs tend to aid in bone and skin tissue regeneration. Therefore, by manufacturing the surface of the bone implant devices and scaffolds with silver nanoparticles, the risk associated with implantation surgery can be reduced (Xing *et al.* 2010). Additionally, nano-crystalline dressings containing silver nanoparticles are used to treat wounds or hospital-acquired infections while reducing the inflammatory response (Fong and Wood., 2006). For instance, traditional surgical meshes are utilised for tissue therapy and to bridge serious wounds, but they are extremely susceptible to pathogenic invasions. Thus, the addition of silver nanoparticles to these meshes improves their efficacy. As opposed to typical fluorescent dyes, silver nanoparticles' plasmonic capabilities make them broadly applicable in bio-imaging for monitoring dynamic events over time without photo-bleaching. In order to help eliminate unwanted or damaged cells, the conjugation of cells to the target cells results in the conversion of light energy to thermal energy, which subsequently causes the target cells to be thermally ablated (Loo *et al.* 2005). Additionally, silver nanoparticles' plasmonic capabilities can be used for bio-sensing, which effectively detects a variety of proteins that conventional biosensors cannot. Silver nanoparticles are widely used for identifying numerous abnormalities and diseases in the human body system due to their special ability, such as tumour cells or cancer. According to Morley *et al.*, 2007, the size, shape, and dielectric potential of the surrounding medium, all influence the plasmonic characteristics of silver nanoparticles.

In general, silver nanoparticles are an excellent choice for a wide range of applications in many industries, particularly in the biomedical sector for diagnosis, drug delivery, cell imaging, and implantation. However, according to several studies, silver nanoparticles have negative effects on both humans and the environment. In vitro, toxicity testing on rat liver cells was done as part of a toxicology study on silver nanoparticles, and the results showed that even at low exposure levels (10–50 g ml), silver nanoparticles caused oxidative stress and stopped mitochondrial function. However, greater dosages of Ag NPs (>1.0 mg L⁻¹) displayed considerable cytotoxicity and led to aberrant cellular

morphology, cellular shrinkage, and the acquisition of an irregular shape (McAuliffe and Perry., 2007). In particular, exposure to silver nanoparticles causes glutathione levels to drop, ROS levels to rise, lipids to oxidise, and ROS-responsive genes to express themselves more, which results in DNA damage, apoptosis, and necrosis (Haider and Kang., 2015). Due to the large surface area to volume ratio, silver nanoparticles can easily enter human bodies through ingestion, inhalation, or skin contact. As a result, their penetrating potential is greatly increased, giving them the ability to enter the circulatory system and even move unrestrictedly throughout the human body (Sung *et al.*, 2008). The male reproductive system is thought to be hazardous as a result of silver nanoparticles purportedly passing past the blood-testes barrier and settling in the testes, according to an earlier study. Additionally, according to McAuliffe and Perry., 2007, silver nanoparticles can have a deleterious effect on peripheral blood mononuclear cells' ability to proliferate and express cytokines. In contrast, Kim *et al.* concluded in their gastrointestinal toxicology investigation that silver nanoparticles do not cause genetic toxicity in male and female Sprague-Dawley rat bone marrow when administered in vivo. In his study, over the course of 28 days, neither the body weight of male nor female rats significantly changed in response to dosages of Ag NPs (size: 60 nm). However, it was discovered that values for alkaline phosphatase and cholesterol were changed after exposure to more than 300 mg of Ag NPs, leading to minimal liver injury (Kim *et al.*, 2008). Therefore, additional research is needed to determine the actual toxicity of silver nanoparticles to humans and animals in - vivo conditions.

CHAPTER-3

AIM AND SCOPE OF THE STUDY

3.1 AIM:

- Biosynthesis of silver nanoparticles from marine microorganism *Bacillus subtilis*.
- Characterisation of silver nanoparticles by UV-vis, Zeta Potential, and a Particle size analyzer.

3.2 SCOPE:

- Collection of marine bacteria
- Subculturing of microorganisms
- Synthesis of silver nanoparticles by bioreduction
- Conduction of a comparative study on various parameters using spectrophotometer
- Checking antioxidant activity using DPPH assay.

CHAPTER - 4

MATERIALS AND METHODS

4.1 SAMPLE COLLECTION:

At a depth of two to ten meters, seawater was collected from Kovalam Beach on the East Coast of Tamil Nadu. The sample was taken in PET bottles and kept chilled in an ice bag at 4°C. On the same day that the sample was taken, it was processed. The acquired material was used for a spread plate approach followed by serial dilution.

4.2 MEDIA PREPARATION:

The glassware were all thoroughly sterilised. A 500ml flask was filled with 250ml of distilled water. In addition, 4g of agar agar was added after carefully dissolving 42.9g of Zobell Marine agar in distilled water. The conical flask was completely covered and autoclaved for two hours at 121 degrees. It was removed from the autoclave after two hours and evenly distributed over ten petri plates. The petri dishes spent 15 minutes in the UV. The plates were taken in order to subculture.

4.3 SPREAD PLATE TECHNIQUE:

For serial dilution, 7 test tubes were taken. In each test tube, 9 ml of distilled water was added. To produce a 10^{-1} dilution, one ml of the seawater used as the sample was added to the first test tube. The dilution is completely blended by repeatedly emptying and filling the pipette. The old pipette tip is taken off the pipette and replaced with a new one. Now, 1 ml of the mixture is taken out of the 10^{-1} dilution and transferred to the second tube. In the second tube, the overall dilution factor is now 10^{-2} . The remaining tube is then put through the same process, adding 1 ml from the first tube to the following 9 ml of diluents. Because six tubes were used, the final dilution of the bacteria/cells will be 10^{-6} (1 in 1,000,000). On a petri dish, the diluted material was spread with Zobell Marine agar. The culture was kept for growth-inducing incubation at 37 °C.

4.4 STREAKING METHOD:

For subculture, culture samples were collected from isolation plates. Under Laminar Air Flow, the streaking was carried out. Before opening the culture, it was ensured that the Laminar Air Flow had been thoroughly cleaned with ethanol and that the spirit lamp was on. Small colonies were streaked from the mother culture after the inoculation loop had been carefully incinerated. Sample cultures were streaked on Zobell Marine agar and incubated at 37 °C. The material was subcultured once more until the contamination was eliminated. Due to its ability to convert Ag NO₃ to Ag NP, this sample was regarded as a promising candidate for the synthesis of silver nanoparticles. For the purpose of preserving the culture for later research, all of these strains were regularly sub-cultured. Before subculturing, all glassware was sterilised.

4.5 IDENTIFICATION OF BACTERIA BY GRAM STAINING:

To reduce confusion, the slides were well-cleaned and clearly labelled. A drop of distilled water was placed on the marked slide. The inoculation loop was used to apply a smear to the microscopic slide. The slide was thermally fixed before the staining process was applied. Crystal violet was used as the primary stain. Gram's iodine was added as a mordant. Gram's decolourizer was used to wash off the primary stain in the case of Gram-positive bacteria. Safranin was then used as a counterstain. 1 minute must pass between each stain addition. Using a Leica phase contrast microscope, the slide was examined.

4.6 PREPARATION OF SILVER NITRATE SOLUTION:

A solution of 1 mM silver nitrate was prepared. Under the light-sensitive zone, 0.0017 mg of silver nitrate was carefully weighed. The determined amount of the weighted sample was diluted in 100 ml of distilled water. A falcon tube was used to contain the solution, which was then foil-wrapped. Make sure the solution isn't exposed to extra light because silver nitrates are reduced when exposed to light.

4.7 SYNTHESIS OF SILVER NANOPARTICLES:

Different molarities, such as 0.1 mM, 0.5 mM, 1 mM, 0.005 mM, and 0.001 mM concentrations, were taken by adjusting sample concentration and Ag NO₃ concentration, and the ratio utilised was 2:1. The synthesis was based on molarity using the mechanism of bio-reduction.

TABLE 4.1: Concentrations of Molarity

MOLARITY (mM)	BS SAMPLE (ml)	H₂O (ml)	AgNO₃
0.1	0.2	1.8	1
0.5	1	1	1
1	2	0	1
0.01	0.02	2	1
0.05	0.1	1.9	1

Autoclave water and Ag NO₃ were also added before the sample. To prevent confusion, the test tubes were labelled with the proper molarity. Following the addition, a timer was set to record the time of the colour shift, and both the time and the colour were recorded.

4.8 MECHANISM INVOLVED SYNTHESIS OF SILVER NANOPARTICLES:

Ions that generate nanoparticles. Bacteria frequently produce inorganic substances either inside or outside of the cell. The method for creating nanoparticles intracellularly and extracellularly with different biological agents varies, nevertheless. A special ion transport system within the microbial cell is used by the intracellular procedure.

The intracellular formation of nanoparticles depends on the bacteria's cell walls. The negative charge of the cell wall and the positive charge of the metal ions interact electrostatically to form the mechanism. The enzymes within the cell wall change the ions into nanoparticles, which then disperse beyond the cell wall.

An orderly process for intracellular manufacture of nanoparticles using bacterial sp. involves trapping, bioreduction, and capping in the production of nanoparticles. The

metal ions are retained when they come into contact with the fungal cell surface due to electrostatic interactions. Enzymes present in cell walls change metal ions into metal nanoparticles. The bacterium *Lactobacillus sp.* is where metal ion clusters are initially formed. Nanoclusters are produced as a result of the electrostatic interaction between the bacterial cell and the metal clusters. Later, the tiny nanoclusters transfer after breaking through the bacterial cell wall.

4.9 BULK SYNTHESIS OF SILVER NANOPARTICLE:

By preparing 50 ml of *Bacillus subtilis* samples in a conical flask, the silver nanoparticles were bulk synthesised. In order to raise the sample's temperature, a magnetic stirrer was used. A 1mM silver nitrate solution was then added to the sample to form a 60ml solution. It was ensured that aluminium foil was wrapped around the conical flask. It had been observed that the colour turned yellowish brown.

4.10 CENTRIFUGATION OF SILVER NANOPARTICLES:

The entire synthesised sample was put into a falcon tube and centrifuged for 20 minutes at 6000 rpm at 21°C. The particle was recovered and subjected to further centrifugation while the colourless supernatant solution was discarded. A hot air oven was used to dry the final centrifuged pellet before the sample was given for characterisation.

4.11 CHARACTERIZATION OF SILVER NANOPARTICLES:

The 0.05 molarity of the bulk synthesised Ag NP was provided for characterisation. Before sending the synthesised sample for characterisation, its stability was assessed. The synthesis of silver nanoparticles has been verified by a UV-vis spectrophotometer. The FTIR spectrum shows that the biomolecules in the bacterial endophyte extract are acting as a capping and reducing agent. The size of each individual particle and the size distribution range for the given sample are both determined using the particle size analyzer.

4.11.1. UV-visible spectroscopy:

It was conducted at the Sathyabama University Centre for Ocean Research and Development. The absorption wavelengths were in the 200–600 nm range. This technique is most typically used to characterise metallic nanoparticles by maintaining track of their stability and production. The free movement of the electrons inside these bands produces the surface plasmon resonance absorption bands. The ability of silver nanoparticles to absorb light is influenced by their chemical environment, dielectric medium, and particle size. It is common knowledge to examine and research the surface plasmon peak for a variety of metal nanoparticles. The ability of silver nanoparticles to absorb light is influenced by their chemical environment, dielectric medium, and particle size. The analysis and examination of the surface plasmon peak for multiple metal nanoparticles with diameters ranging from 2-100 nm is well known. UV-vis spectrophotometry was used to locate a surface plasmon resonance peak at the same wavelength and to examine the stability of biologically produced silver nanoparticles for around a year.

4.11.2 DLS (Particle size Analyzer) Analysis and zeta potential:

The DLS Disc Centrifuge Model No. 12000 UHR of DLS Instruments.Inc. was used in the Centre for Ocean Research at Sathyabama University to conduct particle size analyses. A significant challenge in particle size analysis is how to characterise a particle using only a single number. The preferred technique for reporting particle size distributions in the majority of particle sizing techniques is a two-dimensional graph with the particle size on the x-axis and the amount of material on the y-axis.

4.11.3 Antioxidant activity:

1 mM of DPPH was added to 100 ml of ethanol in sterile glassware. The DPPH solution must be stored in an opaque bottle separately to avoid synthesis and variation in results. The DPPH particle had a purple colouration. Using stable radical DPPH, the free radical scavenging activity and standard BSA were assessed. Various Ag NPs concentrations of 200µl, 400µl, 600µl, 800µl, and 1000µl were taken, and 2 ml of newly prepared 0.1 mM DPPH solution (in ethanol) was added. After that, the mixture was left to sit at room temperature in the dark for 30 miles. The colour change demonstrated the silver

nanoparticles' antioxidant properties. In order to determine the absorbance, a UV-vis spectrophotometer was used at 517 nm.

4.11.4 OPTIMIZATION OF SILVER NANOPARTICLES

By altering variables including temperature, pH, and reaction time, silver nanoparticles were produced. Additionally, the impact of pH, temperature, and time on the synthesis of stable silver nanoparticles was examined. Silver nanoparticles that are solid have been synthesised as a result of the experiment. The nanoparticles didn't agglomerate or settle down in any way.

4.11.4.1 Based on reaction time:

The biosynthesis of Ag NPs was measured in a UV-visible spectrophotometer periodically in order to optimise the reaction time. At room temperature, the reaction mixture consists of 1 ml of 1mM Ag NO₃ and 3 ml of BS bacteria (0.05 conc.) as a function of reaction time ranging from 0 min to 2 hrs. 7 test tubes were taken in total, and the sample was placed to the microtiter plate for plate reading at the appropriate moment.

TABLE 4.2: Synthesis of Ag No₃, with the influence of time.

MOLARITY	TIME	OBSERVATION
0.05	0th second	Yellow colour
0.05	5th minute	Yellow colour
0.05	10th minute	Yellow colour
0.05	20th minute	Yellow colour
0.05	40th minute	Yellow colour
0.05	1st hour	Yellow colour
0.05	2nd hour	Yellow colour

4.11.4.2 Based on Temperature

To examine the effect of temperature on the biosynthesis of Ag NO₃, reaction mixtures were maintained at five different temperatures: 12° C, 20° C, 37° C, 70° C, and 100° C. Colour changes in test tubes were recorded every 5 minutes. Before adding the silver nitrate solution, the temperature was monitored with a thermometer.

TABLE 4.3: Synthesis of Ag NO₃, with the influence of temperature

MOLARITY	TEMPERATURE	OBSERVATION
0.05	11°C (fridge)	No change
0.05	20°C (AC)	No change
0.05	37°C (Room temp)	Brown colour
0.05	70°C (Hot air oven)	Brown colour
0.05	100°C (water bath)	Light change to Brown

4.11.4.3 Based on pH:

The effect of pH on the production of Ag NPs was assessed by maintaining the pH of the reaction medium (which was obtained by 0.05 concentration of BS bacteria (1ml) and 1mM of Ag NO₃ (1ml)) at 3, 6, 7, 9, 12. HCL and NaOH were used to adjust the pH. The colour change was observed.

Table 4.4: Synthesis of Ag NO₃ by varying pH

MOLARITY	pH	OBSERVATION
0.05	3	No change
0.05	6	No change
0.05	7	No change
0.05	9	Yellow colour
0.05	12	Yellow colour

CHAPTER 5

RESULTS AND DISCUSSIONS

5.1 ISOLATION AND IDENTIFICATION OF MARINE BACTERIA:

The bacteria were effectively isolated from seawater using a spread plate technique followed by repeated dilution. The bacteria were then subcultured using the quadrant streaking approach (Fig 5.1A). The bacteria's development ceased after 24 hours. Gram staining was used to identify the bacteria in the subcultures (Fig 5.1B). *Bacillus subtilis* has been found to be a spore-forming gram-positive bacteria.

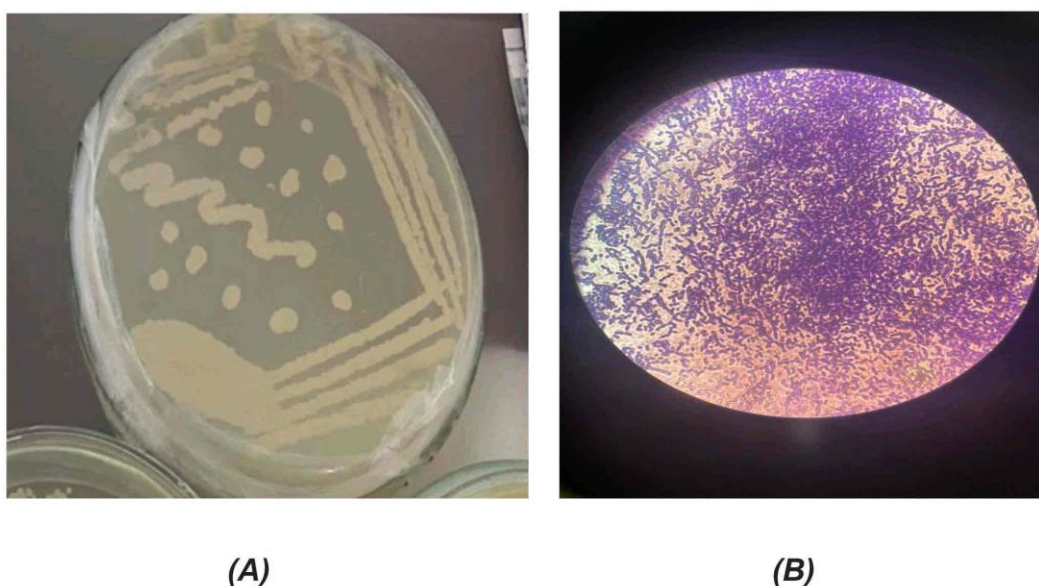


FIGURE 5.1: Isolation (A) and Identification of bacteria by gram staining (B)

5.2 SYNTHESIS OF SILVER NANOPARTICLES AND CHARACTERIZATION:

The first indicator of nanoparticle formation is a colour change from colourless solution to yellowish brown, which is supported by UV-vis spectra. After being exposed to *bacillus subtilis* biomass, the colour of the treated solution changed from colourless to yellowish brown, indicating that the concentration of aqueous silver nitrate ions was reduced. The colour was stable and constant at 0.05mM concentration. This concentration was bulk synthesised, and the outcome was the same as previously observed. The characteristic yellowish-brown colour is caused by the stimulation of surface plasmon oscillations in

silver metal nanoparticles. Thus, UV-vis spectroscopy makes it easier to monitor the reduction of silver nitrate ions in biomass.

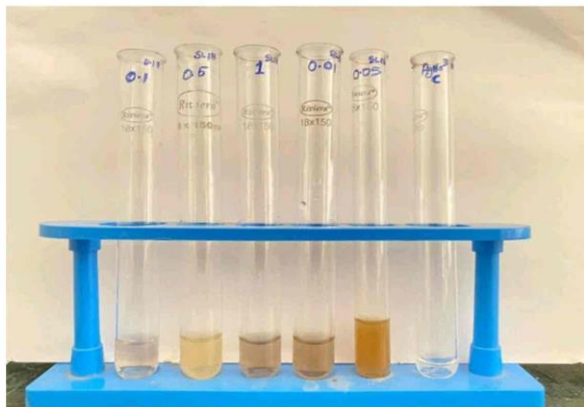


FIGURE 5.2: Synthesis of silver nanoparticles based on different concentrations of molarity



FIGURE 5.3: Bulk synthesis of silver nanoparticles with 0.05 molarity, formation of silver nanoparticles by colour change.

5.3 UV-VIS SPECTROPHOTOMETER:

As shown in Figure 5.4, a sample of the aqueous component was collected and the UV-visible spectrum of the solution was measured. The reduction of Ag ions in the solution by the supernatant of the test bacteria and the creation of silver nanoparticles were studied using UV-visible spectroscopy. The UV-Visible spectra of these samples

were acquired using a 1 nm resolution Ultraviolet-Visible Spectrophotometer. Throughout the reaction period, the surface plasmon band in the silver nanoparticle solution remains close to 440 nm, indicating that there is no evidence of aggregation and that the particles are dispersed throughout the aqueous solution. The presence of silver nanoparticles became apparent by the peak at 440 nm.

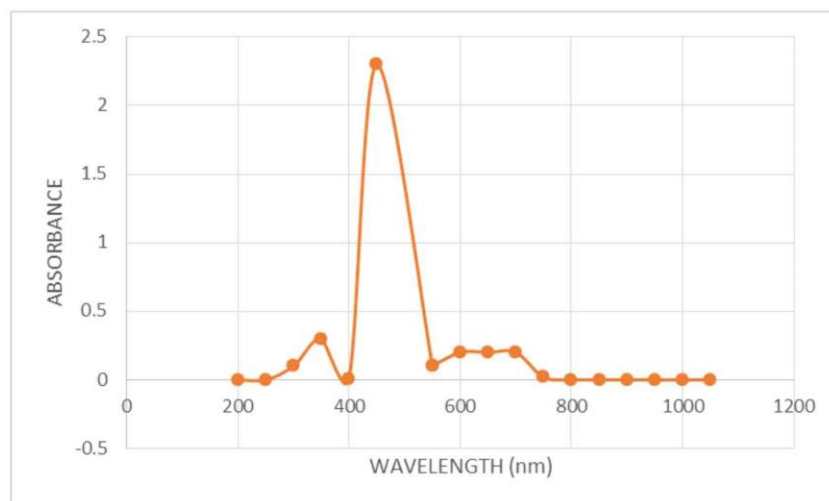


FIGURE 5.4: UV-vis spectrum of silver nanoparticles

5.4 PARTICLE SIZE AND ZETA POTENTIAL ANALYSIS:

The image (Fig: 5.5) shows the DLS size distribution image of silver nanoparticles formed during biosynthesis. The size distribution of Ag NPs is observed to range from 10 to 400 nm. According to DLS data, the Z-average was 387.8 nm. The average size of the synthesised Ag NPs was 0.769 Pdl value. The identification of an individual peak showed that the silver nanoparticles synthesised were high-grade. The zeta potential of biosynthesised Ag NPs was observed to be at an elevated peak at 32.8 mV (Fig: 5.6). The surfaces of the nanoparticles are thought to be negatively charged and disseminated throughout the liquid. The fact that the value is negative shows how strongly the particles are attracted and repulsed by one another.

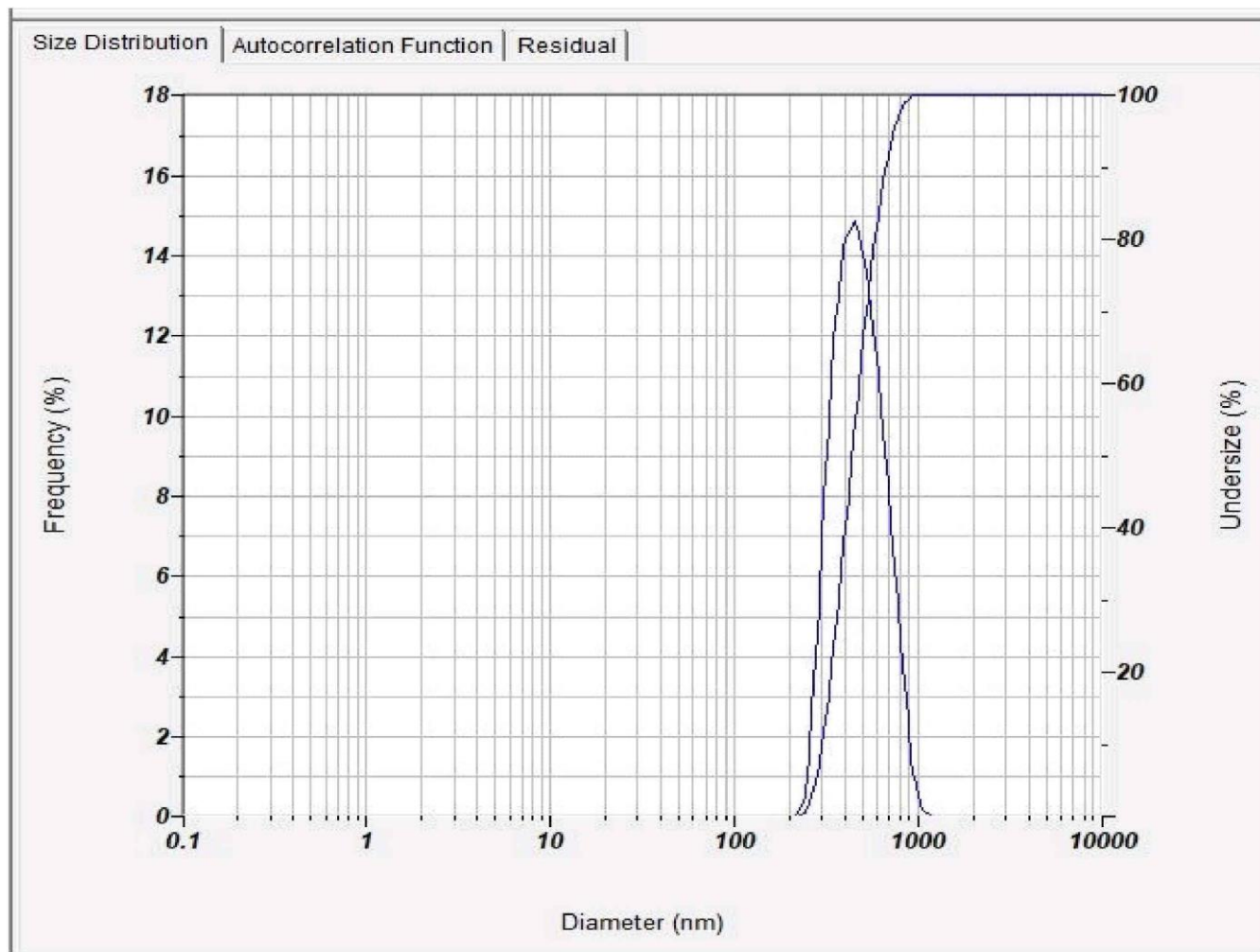


FIGURE 5.5: Particle size analyser

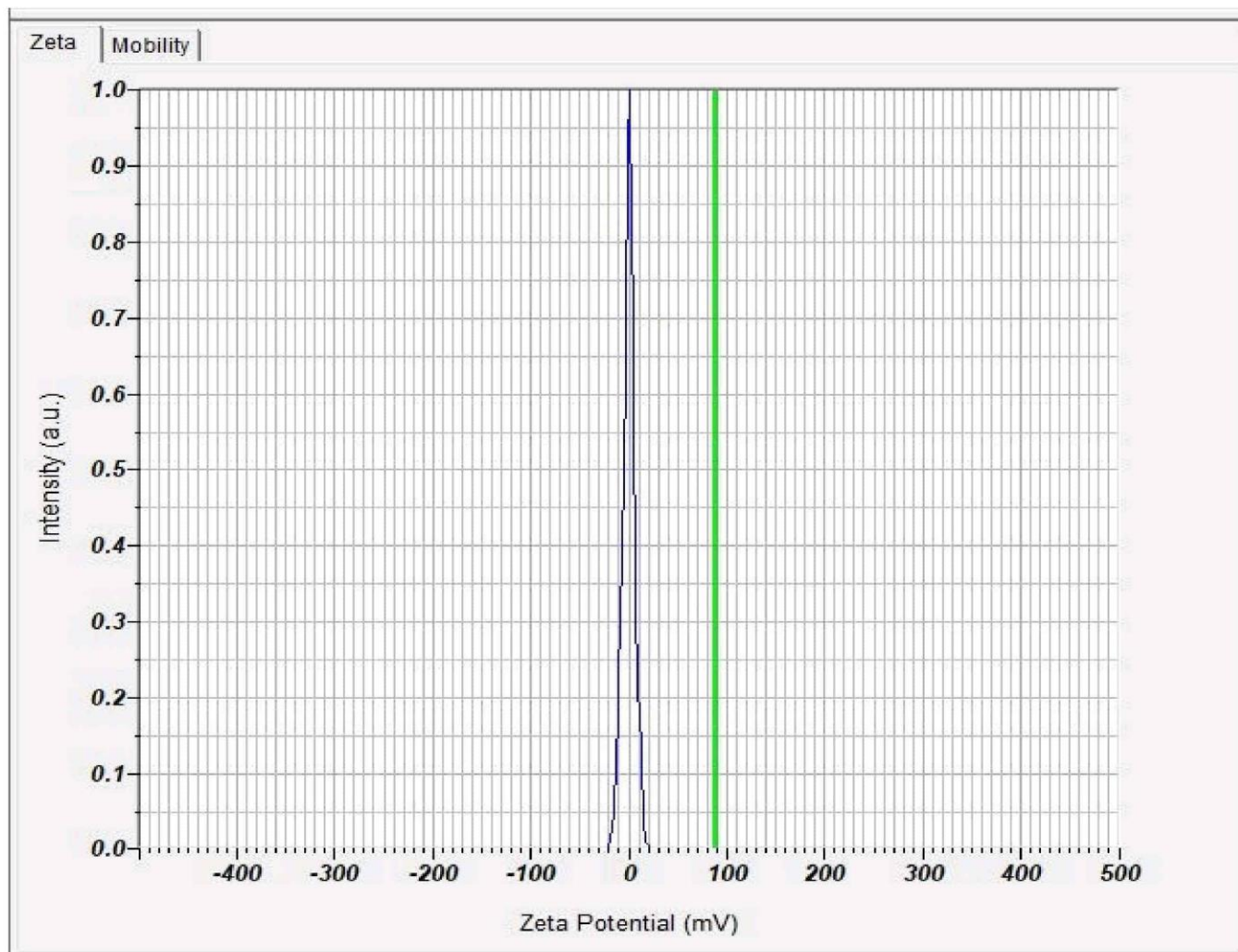


FIGURE 5.6: Zeta potential analyser

5.5: OPTIMIZATION OF SILVER NANOPARTICLES:

5.5.1: Based on reaction time:

The synthesis of silver nanoparticles was done periodically and the colour change was monitored every minute. Time intervals of 0, 5, 10, 20, 40, 1 hour, and 2 hours were used. In the 20th minute, the UV-vis spectrum peak was formed at 412 nm. The colour remained consistent and solid. There were no aggregates produced.

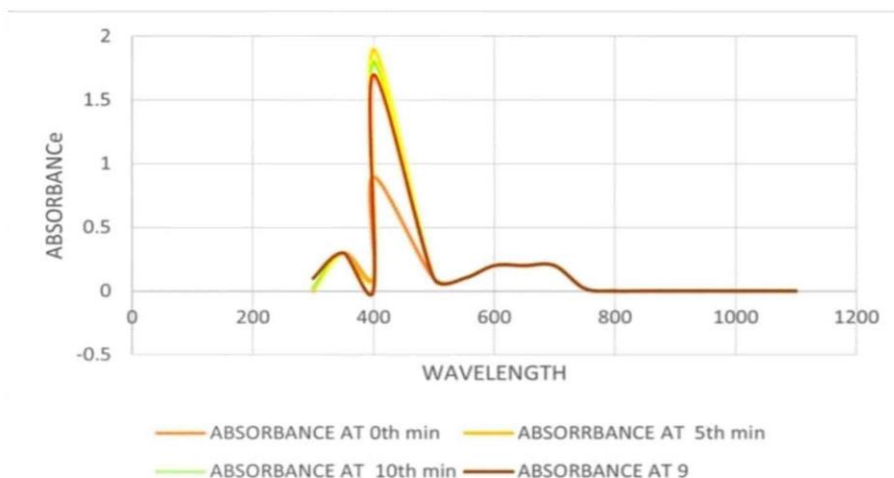


FIGURE 5.7: graph plotted from the results obtained from optimization of silver nanoparticles based on reaction time.

5.5.2 Based on temperature:

Silver nanoparticle synthesis was carried out at various temperatures. However, at 0°C or 4°C, the colour did not change, indicating that these temperatures were not suitable for the production of silver nanoparticles. At room temperature, the colour changed and silver nanoparticles were produced. At 22°C, there was a very minimal colour shift. At 100°C, the solution evaporated. As a result, the highest peak in the synthesised Ag NP was observed at ambient temperature.

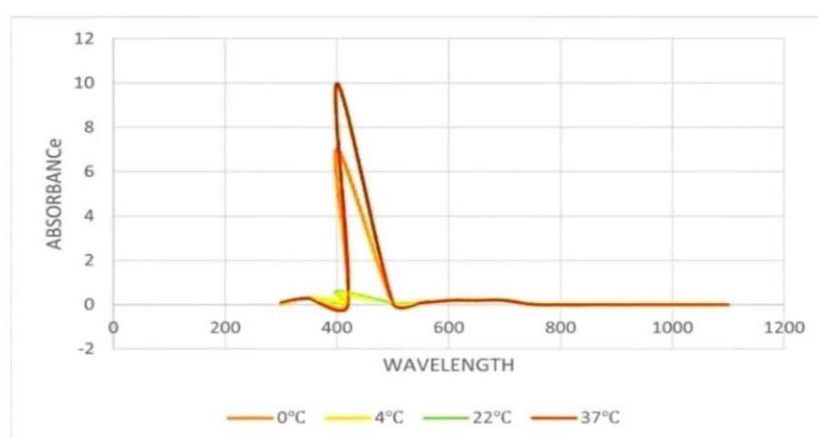


FIGURE 5.8: graph plotted from the results obtained from optimization of silver nanoparticles based on temperature

5.5.3 Based on pH:

Optimised conditions were used to produce Ag NPs. Following the addition of aqueous silver nitrate, the solution's colour changed from clear to brownish-yellow after 30 minutes. When the colour of brown changes, Ag NPs develop. At pH 3, there is no colour change, indicating that Ag NPs do not develop in an acidic environment. At pH 9, colour changes were rapid, and the peak of silver absorbance shifted to 500 nm due to Ag NPs agglomeration. Aggregation was observed in the sample at pH 9 immediately after adding the silver nitrate to the reaction mixture. The pH of 6 was nearly neutral; no colour change occurred. The formation of Ag NPs, which are silver nanoparticles, was confirmed by the characteristic UV-vis absorption peak at 420 nm.



FIGURE 5.9: graph plotted from the results obtained from optimization of silver nanoparticles based on pH

5.6 ANTIOXIDANT ACTIVITY

The antioxidant activity of Ag NPs was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical scavenger. In this experiment, eliminating the H-atom from the antioxidant molecule causes DPPH to turn a pale yellow rather than violet. The assay reaction mixture contained 2 mL (0.1 mM) of DPPH and 1 mL of Ag NPs at various concentrations (10, 20, 30, 40, and 50 mg/mL) and was incubated at 37°C with vigorous

shaking for 0.5 hours. The absorbance of the combination was measured at 517 nm, with BSA serving as the positive control.



FIGURE 5.10: Colour change from violet to pale yellow indicating antioxidant activity

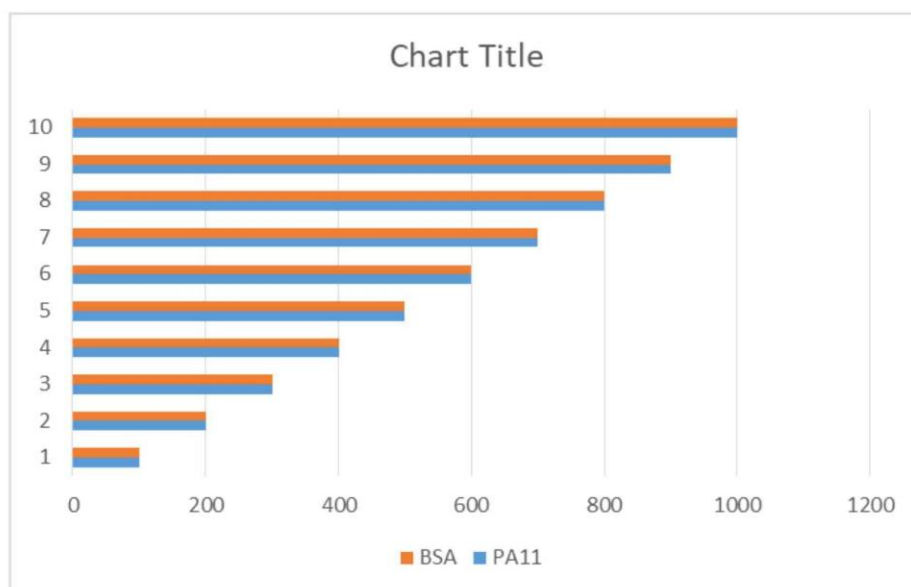


FIGURE 5.11: The graph plotted from the results obtained in antioxidant activity

CHAPTER-6

SUMMARY AND CONCLUSION

The current study uses the marine bacteria *Bacillus subtilis* to biosynthesize silver (Ag NO₃) nanoparticles. This study, soil and water samples from Kovalam, India, to isolate and identify the bacteria. The silver nanoparticles' characterisation was done by UV-visible spectroscopy. The size of the silver nanoparticles was reported by the DLS analysis. Zeta Potential analysis was used to estimate the particle distribution in a solution. According to reaction time, temperature, and pH, the silver nanoparticles were optimised depending on how the colour of the solution changed. Furthermore, the DPPH assay was done for the radical scavenging of the antioxidant in the sample solution.

The use of nanoparticles in the pharmaceutical industry has been a major research topic for the past few decades. In addition to being employed as an adjuvant in vaccines, an anti-diabetic medication, and biosensors, Ag NPs are primarily used in antibacterial and anticancer therapy. They are also applied to the promotion of bone and wound healing. Agriculture is one of the major sectors where the use of AgNO₃ is employed. It is used to lessen the impact of pests on crops and has been tested as an insecticide. It requires nutrient augmentation and insect control. Ag NPs have also been known to be used in the textile industry, food packaging and air purification.

The results of this study indicate the potential of marine bacteria *Bacillus subtilis* extract as a green and sustainable source of silver nanoparticles. The synthesized silver nanoparticles were characterized using various techniques such as UV-Vis spectroscopy, particle size analyzer and Zeta analysis which confirmed the successful formation of nanoparticles with a size range of 1-440 nm. The nanoparticles exhibited excellent stability, high purity, and antibacterial activity against the gram-positive bacteria *Bacillus subtilis*. Further research may involve in-vivo toxicity and biocompatibility testing of these nanoparticles in addition to optimizing the production parameters.

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