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Green Synthesis, Characterization, and Antimicrobial Activity of Silver Nanoparticles Derived from *Sargassum* sp. (Ag-SE)

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ABSTRACT

The growing threat of antibiotic-resistant bacteria has led to a search for alternative antimicrobial agents, including nanotechnology. This study successfully synthesized silver nanoparticles (AgNPs) using aqueous extracts of Sargassum sp., a brown macroalgae species rich in bioactive compounds. The formation of the AgNPs was indicated by a visible color change from pale yellow to brown and was further confirmed by UV–Vis spectroscopy. This spectroscopy showed characteristic surface plasmon resonance peaks between 400 and 450 nm across concentrations ranging from 1 to 4 mM. Fourier-transform infrared (FT-IR) analysis revealed functional groups, such as hydroxyl (3888 cm^{-1}), carbonyl (around 3500 cm^{-1}), and aromatic C=C bonds (1635 cm^{-1}), which suggest a role in reducing and stabilizing the nanoparticles. We evaluated the antibacterial activity of the AgNPs against Escherichia coli, and Staphylococcus aureus using the disc diffusion method. At a concentration of 3 mM, the AgNPs inhibited E. coli with a maximum zone of inhibition of 3.90 mm and followed by S. aureus (3.35 mm). Although the inhibition zones were relatively narrow (<2 mm), the results demonstrate the AgNPs' selective antibacterial potential. The antibacterial mechanism likely involves membrane disruption, oxidative stress via reactive oxygen species (ROS) generation, and Ag^{+} ion release. These findings highlight Sargassum sp. as an eco-friendly and effective source for the green synthesis of AgNPs with promising applications in antimicrobial therapies.

INTRODUCTION

Based on their major pigment content, marine macroalgae are generally classified into three groups: *Chlorophyta* (green algae), *Phaeophyta* (brown algae), and *Rhodophyta* (red algae). These macroalgae are recognized as a rich source of bioactive compounds, including pigments, proteins, and polysaccharides. These compounds have demonstrated a wide range of biological activities, such as antibacterial, antihypertensive, and antioxidant effects (Wen *et al.,* 2024. Brown algae (*Phaeophyta*) are the most abundant group of macroalgae in marine ecosystems and play a crucial role in the global carbon cycle (Summer *et al*., 2024). Their abundance and biochemical diversity have attracted significant scientific interest, particularly for their potential application in nanoparticle synthesis, given their renewable nature and eco-friendly profile.

Brown macroalgae, such as species from the Sargassum genus, are rich in polysaccharides (e.g., alginate, fucoidan), proteins, vitamins, enzymes, and secondary metabolites, which serve as natural reducing and stabilizing agents in the biosynthesis of metal nanoparticles. These characteristics make brown macroalgae a promising alternative to conventional chemical and physical methods, which often involve toxic reagents and high energy consumption. Despite seaweed's wide industrial applications, its use in green nanoparticle synthesis, particularly for antibacterial applications, remains relatively unexplored. Only a few studies have investigated the synthesis of silver nanoparticles (AgNPs) from macroalgae for biomedical applications (Thiurunavukkarau *et al*., 2022).

Nanotechnology is a rapidly advancing field with transformative potential in various sectors, including medicine, due to its ability to design materials at the molecular and atomic levels. Metal nanoparticles, particularly silver (Ag), gold (Au), and platinum (Pt), have been extensively studied for their unique physicochemical properties and wide-ranging applications. Silver nanoparticles, in particular, have garnered attention for their broad-spectrum antimicrobial properties. Their small size and high surface-area-to-volume ratio enhance their interaction with microbial membranes, leading to increased antibacterial efficacy (Arroyo *et al*., 2020).

Previous studies have demonstrated the effectiveness of AgNPs against various pathogenic microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Candida albicans*, and *Aspergillus niger*. AgNPs have also been shown to be effective against viruses such as HIV and hepatitis B virus (HBV) (Mohanta *et al*., 2021). However, the conventional synthesis of these nanoparticles often involves hazardous chemicals, raising concerns about environmental safety and sustainability. Therefore, there is a growing demand for green synthesis approaches that employ natural biological resources, such as plant extracts, algae, bacteria, and fungi, as eco-friendly alternatives.

This study aims to explore the potential of silver nanoparticles synthesized with brown macroalgae, *Sargassum* sp., as a natural and sustainable antibacterial agent. By utilizing *Sargassum* as a bio-reducing agent, the study aims to contribute to the development of eco-friendly nanomaterials for biomedical applications, particularly in addressing the growing challenge of antibiotic-resistant pathogens.

LITERATURE REVIEW

AgNP has been known and used in various industries, especially the food industry as an antifungal agent (Vinci and Rapa, 2019), in the textile industry as a fabric coating with properties such as anti-stain and antibacterial activity (Soo et al., 2020), in the cosmetics industry as an optically active and antibacterial agent (Arroyo et al., 2020), in solar energy harvesting to enhance or improve the light absorption of photovoltaic cells (PV cells), in water treatment as an antimicrobial agent and catalyst for the degradation of chemical waste, in environmental protection, in agriculture as an environmentally friendly pesticide, and in healthcare as an antimicrobial agent (Gong et al., 2024). Additionally, the unique structural and physicochemical properties of AgNP enable them to penetrate and target abnormal cells, opening the door to new medical applications such as cancer therapy and drug delivery (Gul et al., 2021).

AgNP is most commonly used in the food packaging industry due to its thermal stability, antimicrobial properties, and ability to improve the mechanical properties of films through the orientation of molecular structures within the film matrix (Rozilah et al., 2020). The incorporation of AgNP is found to be ideal for integration into polymer matrices to form unique nanocomposites suitable for food packaging applications (Wu et al., 2021). AgNP incorporated into seaweed films exhibit higher mechanical, physical, antioxidant, and antimicrobial activity compared to films developed without nanoparticle incorporation (Sudhakar et al., 2022).

METHOD

Synthesis of Silver Nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) were synthesized from an aqueous extract of *Sargassum* sp. following the previously described method of Ponnusamy *et al*. (2024), with some modifications. First, a 1-4 mM solution of $AgNO_3$ was prepared and stored in the dark. The solution was stirred at 500 rpm and 60°C for 20 minutes, and then 1% aqueous extract of *Sargassum* sp. was added. The silver nanoparticles can be identified by their reddish-brown color. For confirmation, the nanoparticles must be further characterized.

Characterization of Silver Nanoparticles (AgNPs)

The silver nanoparticles were characterized using UV-Vis spectrophotometry and Fourier transform infrared spectroscopy (FTIR), following the method of Shao *et al*. (2018) with modifications. UV-Vis spectrophotometry was monitored with a Genesys 150 UV-Visible Spectrophotometer at wavelengths from 300 to 700 nm with a spectral resolution of 1 nm, using an aqueous extract of *Sargassum* sp. as a blank. The FTIR measurements were taken with a Shimadzu IRTracker 100 FTIR spectrometer.

Antibacterial Test of Silver Nanoparticles (AgNPs)

The antibacterial activity of the synthesized silver nanoparticles (AgNPs) was evaluated using the disc diffusion method with modifications based on Premkumar *et al*. (2018). Four bacterial strains—, *E. coli*, and *S. aureus*—were cultured in Tryptic Soy Broth and incubated at 37 °C for 24 hours. Mueller-Hinton agar (MHA) was prepared, sterilized, and poured into sterile Petri dishes under aseptic conditions. After incubation, the bacterial suspensions were adjusted to the 0.5 McFarland standard and spread evenly into the surface of the solidified MHA plates. Sterile paper discs (6 mm) soaked in various concentrations of AgNPs (e.g., 25, 50, 75, and 100 µg/mL) were placed on the agar surface. One disc contained distilled water and served as the negative control, and one disc contained a standard antibiotic and was used as the positive control. The plates were incubated at 37 °C for 24 hours, then the antibacterial activity was assessed by measuring the diameter of the inhibition zones formed around each disc.

Data Analysis

Experimental data were analysed by Origin and Two-ways Analysis of Variance (ANOVA) followed by Duncan's test in IBM SPSS 25.0 software

RESULT AND DISCUSSION

Synthesis of Silver Nanoparticles

The synthesis of silver nanoparticles (AgNPs) using the aqueous extract of *Sargassum* sp. and silver nitrate ($AgNO_3$) was successfully carried out at room temperature, as indicated by a noticeable color change in the reaction mixture. Initially, the solution was pale yellow, gradually turning brown after a few hours of incubation. This visual transformation is a primary indicator of AgNP formation (Figure 1). This brown color arises from the surface plasmon resonance (SPR) phenomenon, which is commonly observed in silver nanoparticles when conduction electrons on the nanoparticle surface oscillate collectively upon exposure to light. This observation aligns with the findings of Valsalam *et al*. (2019), who reported similar color changes during green synthesis of AgNPs. Their study showed that bioactive compounds in the algal extract reduce Ag^+ ions to elemental Ag^0. Polysaccharides, polyphenols, and proteins in *Sargassum* sp. are considered to act as reducing and stabilizing agents during nanoparticle formation. This visual cue is simple and effective for preliminary confirmation of nanoparticle synthesis before further characterization by techniques such as UV–Vis spectrophotometry, FTIR, or TEM. Thus, the observed color change not only serves as an early indication of successful nanoparticle synthesis, but also highlights the potential of marine macroalgae as an eco-friendly and efficient platform for green nanotechnology.

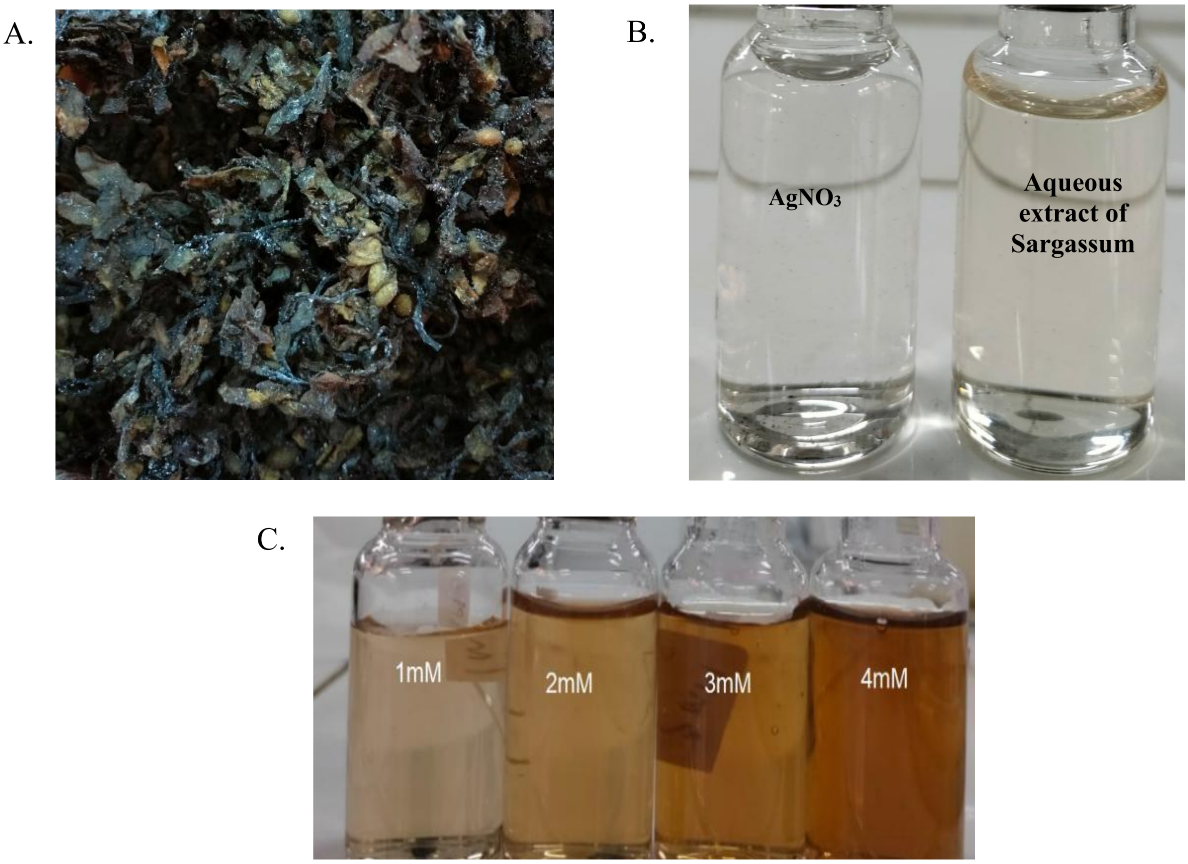

Figure 1. Sargassum sp. (A), AgNO3 and Sargassum extract (B), Synthesized silver-Sargassum extract (Ag-SE) nanoparticles

Uv-Vis spectrophotometric of Silver Nanoparticles

The formation and optical properties of silver nanoparticles (AgNPs) synthesized using Sargassum extract were confirmed by UV–visible spectrophotometry. As exhibited in Figure 2, the UV–Vis absorption spectra of AgNPs at various silver nitrate concentrations (1 mM, 2 mM, 3 mM, and 4 mM) displayed characteristic absorption peaks within the 400–450 nm range. These peaks indicate the surface plasmon resonance (SPR) phenomenon, which is common to silver nanoparticles and occurs when electrons oscillate collectively in response to light exposure. The position and intensity of the absorption bands varied slightly with concentration, potentially reflecting differences in nanoparticle size or aggregation level. The presence of an SPR band within this range aligns with the findings of Thiurunavukkarau *et al.* (2022), who reported that AgNPs synthesized using aqueous extracts of *Sargassum* sp. exhibited a maximum absorption peak around 411 nm. This suggests that the biomolecules in *Sargassum* sp. effectively reduced Ag^+ ions and stabilized the resulting nanoparticles. Thus, UV–Vis spectroscopy supports the successful synthesis of AgNPs and highlights the effectiveness of *Sargassum* sp. as a natural reducing and capping agent in green nanoparticle synthesis

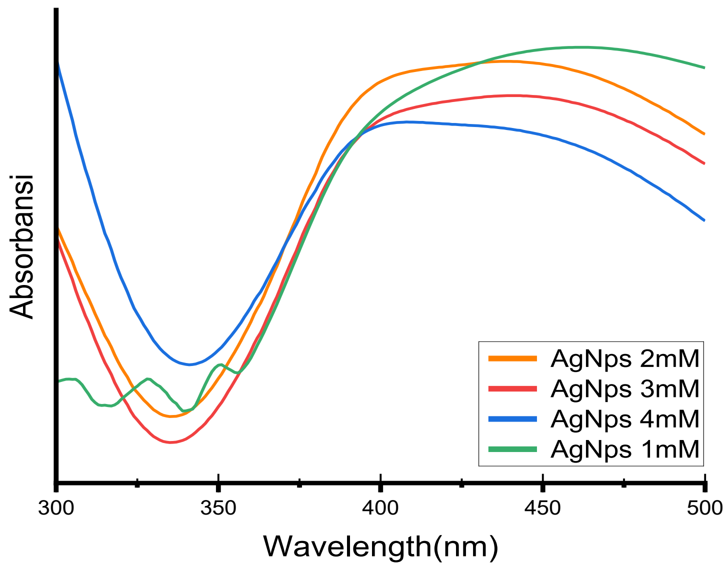

Figure 2. Uv-Vis spectrum of silver-Sargassum extract (Ag-SE) synthesized

FTIR of Silver Nanoparticles

The functional groups involved in the synthesis and stabilization of silver nanoparticles (AgNPs) using *Sargassum* extract were identified through Fourier transform infrared (FTIR) spectroscopy. The resulting spectrum is presented in Figure 3. FT-IR analysis revealed several prominent absorption bands, indicating the presence of bioactive compounds responsible for reducing and capping silver ions. A broad, strong peak was observed at approximately 3888.49 cm^{-1}, corresponding to the O–H stretching vibration of alcohols, indicating the presence of hydroxyl-containing compounds. Additionally, a broad band centered around 3500.80 cm^{-1} was detected, which is typically attributed to overlapping vibrational modes, particularly C=O stretching, which is often associated with carboxylic acids, esters, or conjugated carbonyl groups. Another absorption feature appeared between 3300 and 2500 cm^{-1}, centered around 2627 cm^{-1}, which is consistent with O–H stretching of carboxylic acids. This suggests the involvement of acid groups in nanoparticle stabilization (López-Miranda *et al*., 2021). A strong and sharp peak at 1635 cm^{-1} was also identified, corresponding to C=C stretching in aromatic rings, indicating the presence of phenolic or flavonoid compounds. This aligns with existing literature highlighting seaweeds, including *Sargassum* sp., as rich sources of phenolic compounds and flavonoids (González-Fuentes *et al*., 2020). These compounds play crucial roles in nanoparticle formation by acting as reducing and capping agents. Together, these functional groups confirm that multiple bioactive compounds in the alga extract contribute to the green synthesis and stabilization of AgNPs, supporting the eco-friendly nature of this method

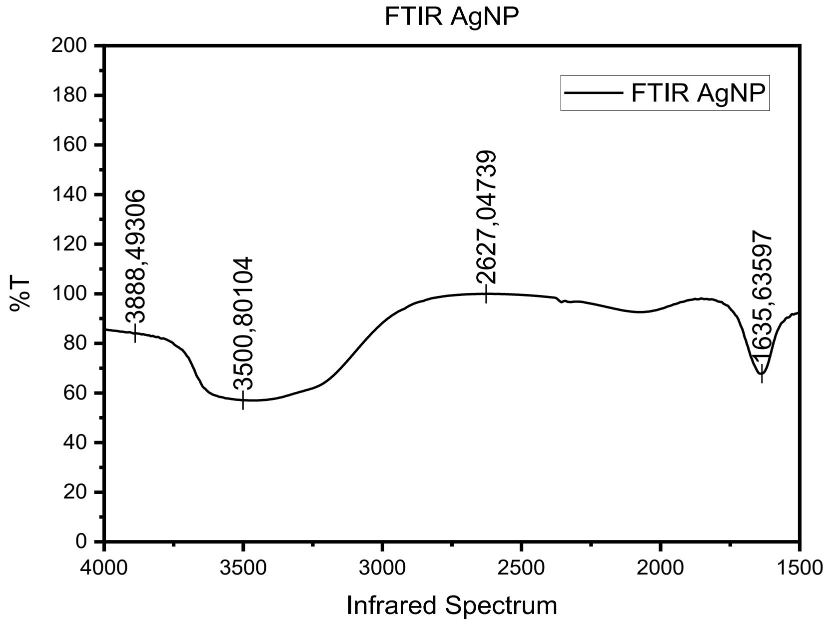

Figure 3. FT-IR of AgNP

Antibacterial Activity of Silver Nanoparticles

Two bacterial strains were used to evaluate the antibacterial activity of silver nanoparticles (AgNPs) synthesized from *Sargassum* sp.: *E. coli*, and *S. aureus*. The assay was conducted in triplicate to ensure reliability. As shown in Figure 4 and table 1, the AgNPs exhibited notable inhibitory effects, particularly at concentrations 3 mM. However, the diameter of the inhibition zones remained relatively narrow, generally less than 2 mm, indicating moderate antibacterial activity. Among the tested bacteria, *E. coli* showed the highest sensitivity to AgNPs, with a maximum inhibition zone of 3.90 mm, and followed by *Staphylococcus aureus* exhibited an inhibition zone of 3.35 mm, both at 3 mM. These results suggest that AgNPs synthesized using *Sargassum* sp. exhibit selective antibacterial activity, with effectiveness varying by bacterial species and nanoparticle concentration. Despite

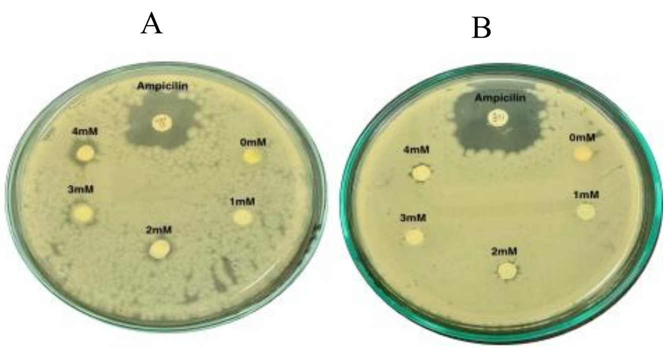

Figure 4. Antibacterial test of AgNP. Staphylococcus aureus (A) and E. coli (B)

the relatively small inhibition zones, the results confirm the potential of AgNPs as antibacterial agents, possibly due to their interaction with bacterial membranes and intracellular components, leading to cell death. Further optimization, such as controlling the size of the nanoparticles and modifying their surfaces, could enhance their efficacy in future applications.

Table 1. Antibacterial test of AgNPs

Konsentrasi	*Staphylococcus aureus*	*Escherecia coli*
0mM	0,00 ± 0,00 [a]	0,00 ± 0,00 [a]
1mM	1,02 ± 1,77 [a]	0,41 ± 0,71 [a]
2mM	1,62 ± 0,94 [a]	1,88 ± 0,34 [a]
3mM	1,92 ± 1,73 [a]	1,52 ± 2,09 [a]
4mM	2,26 ± 1,52 [a]	2,31 ± 2,06 [a]
Ampicilin	12,69 ± 10,61 [b]	17,05 ± 13,64 [b]

The antibacterial mechanism of silver nanoparticles (AgNPs) is multifaceted and primarily attributed to their ability to disrupt bacterial cell integrity and function via direct physical and biochemical interactions. According to Ahmad *et al*. (2020), contact between AgNPs and microbial cells leads to an imbalance in cellular homeostasis. This results in irreversible damage to membrane permeability, oxidative stress, and eventual rupture of the bacterial cell wall. The antibacterial efficacy of AgNPs is strongly influenced by their size, shape, and dispersion in an aqueous medium, especially during the initial interaction phase. This mechanism can be described in three interrelated stages: (1) the AgNPs adhere to the bacterial cell surface, compromising membrane integrity and altering its permeability; (2) the AgNPs penetrate the bacterial cell and interact with intracellular components, including DNA and proteins, often through the generation of reactive oxygen species (ROS), which induce oxidative damage; Third, the gradual release of Ag^{+} ions from the nanoparticles in the surrounding medium enhances the bactericidal effect by further disrupting enzymatic and structural functions within the cell (López-Miranda *et al*.., 2021). These combined actions make AgNPs highly effective antimicrobial agents, which supports their classification as a new generation of bactericidal nanomaterials. They may provide promising alternatives for combating antibiotic-resistant pathogens

CONCLUSION

This study has demonstrated the successful green synthesis of silver nanoparticles (AgNPs) using aqueous extracts of Sargassum sp., as evidenced by a distinct color change, which was further confirmed through UV–Vis and FT-IR analyses. UV–Vis spectra showed characteristic surface plasmon resonance peaks between 400 and 450 nm. FT-IR analysis revealed functional groups, such as hydroxyl, carbonyl, and aromatic compounds, that are responsible for reducing and stabilizing AgNPs. The synthesized nanoparticles exhibited antibacterial activity against *Escherichia coli*, and *Staphylococcus aureus*, with varying degrees of inhibition depending on the concentration and bacterial species. While the inhibition zones were relatively small, the results suggest that Sargassum-mediated AgNPs (Ag-SE) have selective antibacterial properties. The mechanism of action involves the disruption of bacterial membranes, the induction of oxidative stress through reactive oxygen species (ROS), and the release of Ag^+ ions, all of which contribute to bacterial cell death. Overall, this study highlights the potential of *Sargassum* sp. as an eco-friendly and cost-effective source for synthesizing antibacterial silver nanoparticles, offering a promising alternative to conventional antimicrobial agents.

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