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Green Synthesis of Bioactive Silver Nanoparticles using *Padina boergesenii* Allender & Kraft and their Antagonistic Activity

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Abstract: Bacterial infections and rising antibiotic resistance present major challenges to global health, while fungal diseases in agriculture contribute significantly to crop loss and food waste. This underscores the urgent need for multifunctional antimicrobial materials applicable to both medical and agricultural sectors. Silver nanoparticles (Ag-NPs) synthesized using seaweed extracts have shown promise as effective antibacterial and antifungal agents. In this study, bioactive Ag-NPs were synthesized using *Padina boergesenii* seaweed collected from Mandapam, Gulf of Mannar, Rameshwaram, Tamil Nadu, India. Phytochemical screening confirmed the presence of bioactive compounds in the seaweed extract. The synthesized Ag-NPs were characterized using spectroscopic techniques and scanning electron microscopy (SEM) to determine their physicochemical properties. Antimicrobial activity was assessed against clinically relevant bacterial strains and plant-pathogenic fungi. The Ag-NPs exhibited significant antibacterial effects, with inhibition zones measuring 19 mm against *Escherichia coli*, 9 mm against *Klebsiella pneumoniae*, 13 mm against *Enterococcus faecalis*, and 11 mm against *Staphylococcus aureus*. Antifungal assays revealed inhibition zones of 17 mm against *Fusarium oxysporum* and 16 mm against *Rhizoctonia solani*. These findings highlight the potential of *Padina boergesenii*-mediated silver nanoparticles as effective agents for both antibiotic therapy and fungicidal applications.

Keywords: Antibacterial, Antifungal, Bioactive compounds, Seaweed, Silver nanoparticles

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Introduction

Seaweeds, also known as marine photosynthetic eukaryotic organisms, are a rich source of various structural bioactive compounds. They contain ten times higher amounts of phytocompounds compared to terrestrial plants (Ghaliaoui and Sneha *et al*., 2024). The bioactive constituents found in seaweeds have multiple applications in pharmacology, agriculture, and cosmetics (Sadeghi *et al*., 2024). The use of macroalgae in biomedical applications is attributed to their bioactive compounds, which include proteins, amino acids, fatty acids, flavonoids, pigments, and vitamins (Jang *et al*., 2024). *Padina boergesenii* Allender & Kraft is a small brown alga which belongs to the family Dictyotaceae that is particularly beneficial among marine algae. It has garnered significant attention in the cosmetic and biomedical fields (Cholaraj and Venkatachalam, 2024). Previous studies have highlighted the biological activities of bioactive compounds derived from seaweeds, especially their benefits for skin care (Patel *et al*., 2025). Seaweeds are also essential resources in sustainable agriculture, promoting plant growth, nutrient uptake, and stress resilience. They act as bio-stimulants, supplying absorbable micro-nutrients and enhancing crop resistance to microbial infections and pests (Sekar *et al*., 2025). Additionally, seaweeds improve soil fertility, contributing to productive agricultural practices. They contain significant organic matter that acts as a chelator for soil minerals. In the context of plant disease control, seaweed extracts play a vital role in managing bacterial and fungal diseases (Sundhar *et al*., 2023; Cornish *et al.,* 2024). These extracts serve as alternative resources for synthetic pesticides and fertilizers, promoting sustainable agriculture (Zokm *et al*., 2022). Moreover, seaweed-mediated nanoparticle synthesis has gained considerable attention among material scientists and biomedical researchers due to their metal chelating and pharmaceutical properties (Chanthini *et al*., 2024; Baskar *et al*., 2024). The selection of *Padina boergesenii* for this study is aimed at the green synthesis of silver nanoparticles, which hold antimicrobial and agricultural significance. Seaweed extracts contain various phenolic compounds, alkaloids, enzymes, and terpenoids that function as reducing agents and stabilizers during the synthesis of nanoparticles (Badr *et al*., 2024; Elsayed *et al*., 2024). Recent research has shown that silver nanoparticles synthesized using seaweed extracts demonstrate remarkable anti-bacterial, antifungal, and pesticidal properties (Jaison *et al*., 2024; Viswanathan *et al*., 2024). These bioactive silver nanoparticles effectively attach to bacterial cell walls, neutralizing their surface charge and ultimately leading to the destruction of the bacterial cells (Bibi *et al*., 2025). Additionally, the generation of reactive oxygen species (ROS) disrupts the bacteria's defense mechanisms and damages their cell membranes. Due to their biocompatibility and effectiveness, bioactive silver nanoparticles are considered highly valuable for biomedical and agricultural applications (Nanda *et al*., 2024). The outer coating of marine polysaccharides enhances both the antimicrobial and pesticidal effectiveness of these silver nanoparticles (Lewinska *et al*., 2024). Furthermore, the secondary metabolites found in seaweeds facilitate cellular internalization and contribute to the cell damage mechanisms of silver nanoparticles against infectious pathogens (Singh *et al.*, 2024). The current study focuses on the eco-friendly synthesis of silver nanoparticles mediated by *Padina boergesenii* extract for applications in controlling bacterial infections and phytopatho-genic fungi. The antibacterial efficacy of these bioactive silver nanoparticles was tested against human pathogenic bacterial strains. In addition, the antifungal potential was evaluated against plant-infecting fungal species using an agar well diffusion assay. The bioactive compounds present in *Padina boergesenii* were identified and quantified using various standard methods. In the future, the bioactive silver nanoparticles produced in this study are expected to be an effective and low-cost option for antimicrobial and agricultural applications.

Materials and Methods

Materials

Acetone, silver nitrate, nutrient agar, and double-distilled (DD) water were purchased from Himedia Chemicals, India. The following microbial strains were used to test antibacterial activity: *Escherichia coli* (MTCC1687), *Enterococcus faecalis* (MTCC29212), *Klebsiella pneumoniae* (MTCC4030), and *Staphylococcus aureus* (MTCC 96). These bacterial strains were collected from the ATCC, Mumbai.

Collection of Seaweed

Seaweed *Padina boergesenii* Allender & Kraft (family Dictyotaceae) was collected from the coastal regions of the Gulf of Mannar, Mandapam, India during low tide in June–August 2024. The species was identified by seaweed experts at CMFRI, Mandapam. The samples were rinsed in seawater to remove debris and epiphytes. Then washed with tap water and then with distilled water to eliminate any remaining particles. The seaweed was spread out on blotting paper to absorb excess moisture and was shade dried in a dark room for 5 to 7 days. After drying, the seaweed was ground into a powder.

Preparation of Seaweed Extract

To prepare the seaweed extract, 1 g of seaweed powder was placed into a 250 ml conical flask and dissolved in 100 ml of DD water. Then heated in a water bath for 20 min at 50 °C. After heating, the seaweed extract was separated through filter paper (Whatman No. 1) and the supernatant was stored at 4 °C for future use.

Phytochemical Analysis

The phytochemical studies of the seaweed *Padina boergesenii* were carried out based on the methodology established by Sobuj *et al.* (2024) with some modifications. The presence of alkaloids was determined using Dragendorff's test, while flavonoids were identified through the alkaline reagent test. Tannins were detected using ferric chloride, and saponins were evaluated with a foam test. Terpenoids were identified using the Salkowski test. Additional phytochemicals, such as phenols, steroids, and quinones, were also tested according to standard procedures. The presence of proteins was confirmed using the Biuret test.

Quantification of Alkaloids

A solution of bromocresol green (BCG) at a concentration of 0.1 mM was prepared by dissolving 69.8 mg of BCG in 3 ml of 2N NaOH and then diluting it to a final volume of 1,000 ml. To this solution, 1 ml of seaweed extract and 1 ml of phosphate buffer (pH 4.7) was added. After thoroughly mixing, 2 ml of chloroform was added to the mixture. A blank control, prepared in the same manner but without the seaweed extract, was used to measure the absorbance of the resulting complex in chloroform at 470 nm. The assay results were expressed as Atropine equivalents, using Atropine as the standard substance.

Quantitative Estimation of Flavonoids

The aluminum chloride technique was employed to determine the total flavonoid content. 1 ml of distilled water was mixed with 1 ml of the algal extract. After 5 min, 0.3 ml of 10% aluminum chloride and 0.3 ml of 5% sodium nitrite were added. Following an incubation period of 6 min at room temperature, 1 ml of 1 M sodium hydroxide was added to the mixture. Distilled water was used to adjust the final volume to 10 ml. The absorbance of the reaction mixture was read at 510 nm. The results are reported as catechin equivalents (mg of catechin per gram of dry extract), with catechin serving as the standard reference material.

Quantitative Estimation of Saponins

0.35 ml of vanillin (8% in ethanol) and 0.8 ml of methanol were added to 1 ml of the extract. After thoroughly mixing, 1.25 ml of a 72% sulfuric acid solution was added. The mixture was stirred and then heated in a water bath at 60°C for 10 min. After heating, the absorbance was measured at 544 nm and compared to a blank for the reagent. The results of the assay were compared with equivalents of diosgenin, which served as the standard material.

Biosynthesis of Silver Nanoparticles

A simple green synthesis method was employed to produce bioactive silver nanoparticles. In a 250 ml beaker, 80 ml of a 0.1 M $AgNO_3$ solution was stirred vigorously using a magnetic stirrer for 15 min. During this time, 20 ml of *Padina boergesenii* aqueous extract was gradually added to the solution. The reaction mixture was stirred for a while and then left in the dark at 37 °C for 48 h. The formation of silver nanoparticles was indicated by a noticeable colour change from pale yellow to brown. To isolate the nanoparticles, ultracentrifugation was conducted for 15 min at 12,000 rpm. The nanoparticles were washed three times with HPLC-grade distilled water and then allowed to dry for 6 h at 50 °C (Viswanathan *et al.*, 2024).

Characterization of the Silver Nanoparticles

The formation and characteristic absorbance of the seaweed-mediated AgNPs were examined using UV-VIS spectroscopy. The functional groups associated with the seaweed extract-mediated AgNPs were identified through FTIR spectroscopy (Shimadzu, Japan). The morphology of the synthesized nanoparticles was evaluated using SEM imaging (ZOEL SEM 500). The crystalline structure of the AgNPs was assessed using powderXRD (Hamouda *et al.*, 2024). Additionally, the hydrodynamic particle size and zeta potential of the nanoparticles were measured using the DLS technique (ZetaSizer NanoR-S90, UK).

Antibacterial Activity of Silver Nanoparticles

The antibacterial potential of synthesized Ag-NPs was evaluated against human pathogenic bacterial strains, including *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Enterococcus faecalis*, through the well diffusion assay. Bacterial strains were sub-cultured in the nutrient broth for 24 h at 37 °C. A fully grown bacterial culture was then uniformly swabbed onto a nutrient agar plate. The plate was prepared by creating 5 mm wells using sterile 1 ml micropipette tips. Different concentrations of Ag-NPs (25-100 µg/ml) were added to the wells and incubated overnight. Gentamicin, a commercial antibiotic, was used as a positive control. After incubation, the plates treated with nanoparticles were observed, and the inhibition was calibrated using the antibiotic zone scale.

Antifungal Activity of Silver Nanoparticles

The antifungal potential of seaweed-mediated Ag-NPs was assessed via the well diffusion method against fungal strains. The experiment was conducted in Petri plates containing 20 ml of PDA at 37ºC. The fungal strains were grown overnight in PDA liquid medium on a rotary shaker at 120 rpm. The fungal strains were treated with various concentrations of Ag-NPs (25 to 100 µg/ml) and incubated at 37 °C overnight under anaerobic conditions. Following the incubation period, the zones of fungal growth inhibition were measured using an antibiotic zone scale, with gentamicin serving as a positive control (Singh *et al.*, 2024).

Statistical Analysis

All data are expressed as mean ± standard error (SE) from three replicates. Statistical significance was determined using Student's t-test and two-way ANOVA, with a significance level set at $p \leq 0.05$.

Results and Discussion

Phytochemical analysis of Padina boergesenii seaweed extract

The results of the phytochemical analysis indicate significant levels of alkaloids, flavonoids, and saponins in the seaweed extract. The visual colour changes observed during the analysis confirm the presence of these compounds (Fig. 1) . Specifically, the total percentage of alkaloid compounds is measured at 0.806 µg/mg, flavonoids at 0.194 µg/mg, and saponins at 0.767 µg/mg (Table 1). Seaweed containing alkaloids holds various pharmaceutical benefits, including anticancer, anti-inflammatory, and anti-diabetic properties. Table 2 illustrates a list of the phytochemicals identified in the seaweed extract. Among the seven bioactive compounds, three primary compounds were highlighted based on their peak areas and UV-vis absorbance. These major phytoconstituents, alkaloids, saponins, and flavonoids, are particularly abundant in the seaweed extract. Most of the phytocompounds identified (Fig. 2) in the extract exhibit antimicrobial activity against various disease-causing pathogens. Some studies have reported that flavonoids derived from seaweed possess significant antibacterial and antifungal effects (Patel *et al.*, 2024; Cadar *et al.*, 2025). Saponins also play a leading role in the silver nanoparticles (Ag-NPs) reduction. The

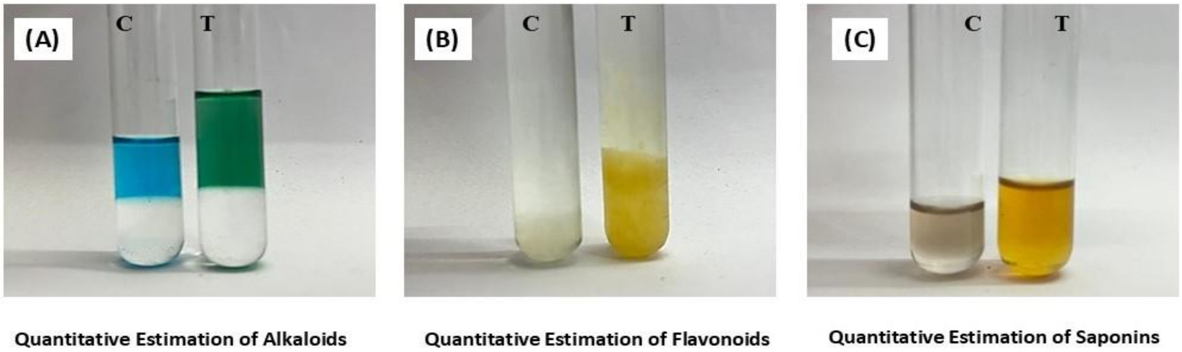

Fig. 1 A-C: Quantification of alkaloids, flavonoids, and saponins in the *Padina boergesenii* seaweed extract.

Table 1: Total amount of alkaloids, flavonoids, and saponins in the *Padina boergesenii* seaweed extract

Estimation	Control (μg/mg)	Test (μg/mg)
Alkaloids	0.214	0.806
Flavonoids	0.064	0.194
Saponins	0.435	0.767

Table2: Qualitative Phytoconstituents in the *Padina boergesenii* seaweed extract.

S. No.	Test	Presence	Absence
1	Alkaloids	+++	
2	Carbohydrate	++	
3	Glycoside		-
4	Saponin	+++	
5	Protein	+	
6	Amino Acid		-
7	Phenol	+	
8	Flavonoid	+++	
9	Terpenoid	++	
10	Steroid		-

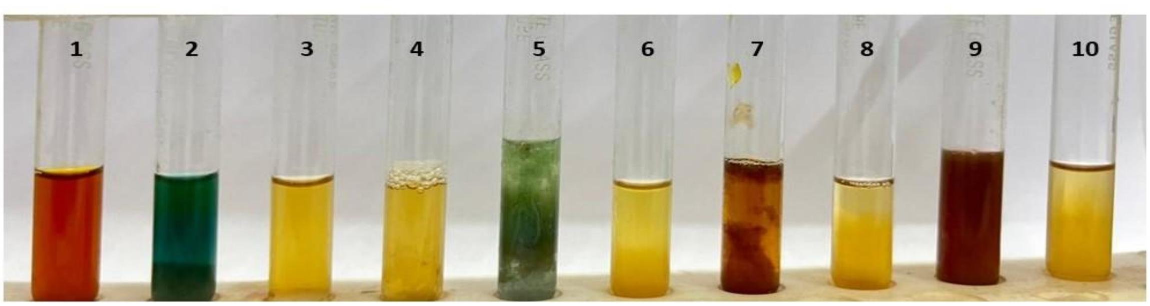

Fig. 2: Identification of phytoconstituents in the *Padina boergesenii* seaweed extract.

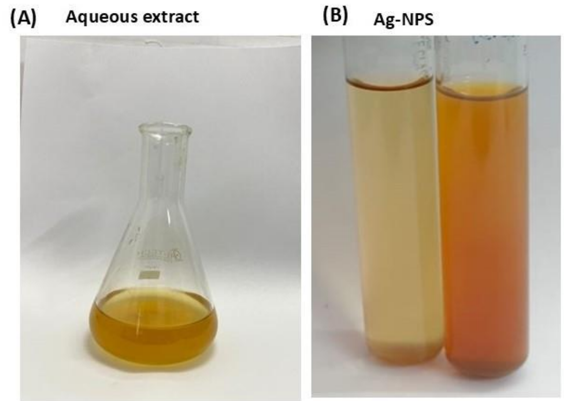

Fig. 3: Synthesis of seaweed-mediated bioactive silver nanoparticles.

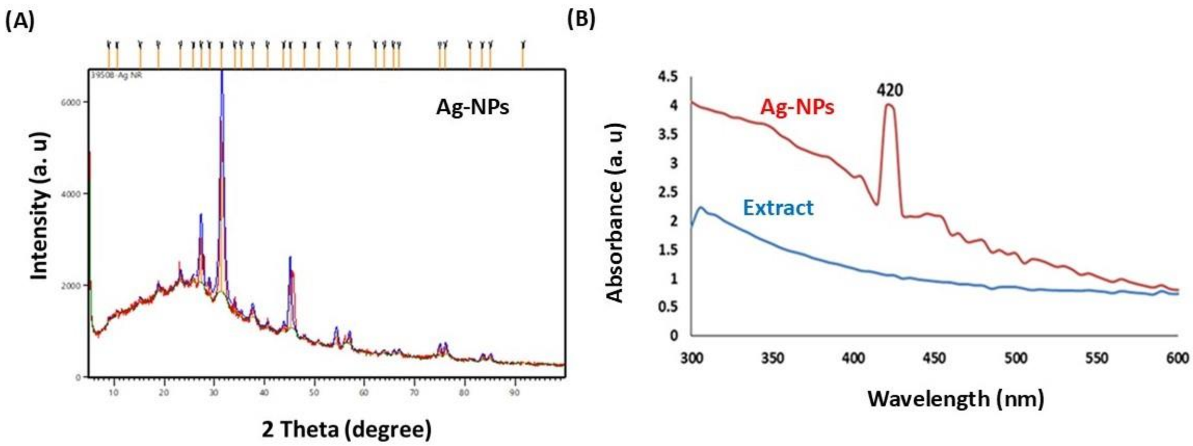

Fig. 4: (A) Powder XRD and (B) UV-vis spectroscopy analysis of Ag-NPs.

seaweed extract containing saponins acts as a surfactant in the synthesis of Ag-NPs. Furthermore, the presence of alkaloids enhances the antibacterial efficacy of Ag-NPs against human infectious bacteria. A combination of bioflavonoids and silver ions effectively targets and kills plant pathogenic fungi (Pallath *et al.*, 2024; Almeida *et al.*, 2024). Overall, the findings confirm that *Padina boergesenii* seaweed is rich in bioactive compounds, making it a promising marine resource for the development of new therapeutic products and agricultural applications.

UV-Vis Spectroscopy Analysis of Ag-NPs

The formation of Ag-NPs mediated by seaweed was confirmed using UV-vis spectroscopy. A visual color change from pale yellow to brown indicates the reduction of silver nitrate to Ag-NPs. The results of the UV-VIS analysis, shown in Figure 3B, reveal a characteristic peak at 420 nm, which confirms the surface plasmon resonance of silver ions. Notably, the seaweed extract itself did not exhibit any absorbance in this region. The spectrum of the fabricated Ag-NPs aligns perfectly with previous reports (Hamouda *et al.*, 2024).

Powder XRD Analysis of Silver Nanoparticles (*Ag-NPs*)

The crystal nature of the synthesized silver nanoparticles was evaluated using powder X-ray diffraction (XRD) analysis. The X-ray diffraction spectrum of the bioactive Ag-NPs is shown in Figure 4A. The diffraction peaks observed at 2θ values of 25.73º, 27.46º, 32.53º, and 46.24º

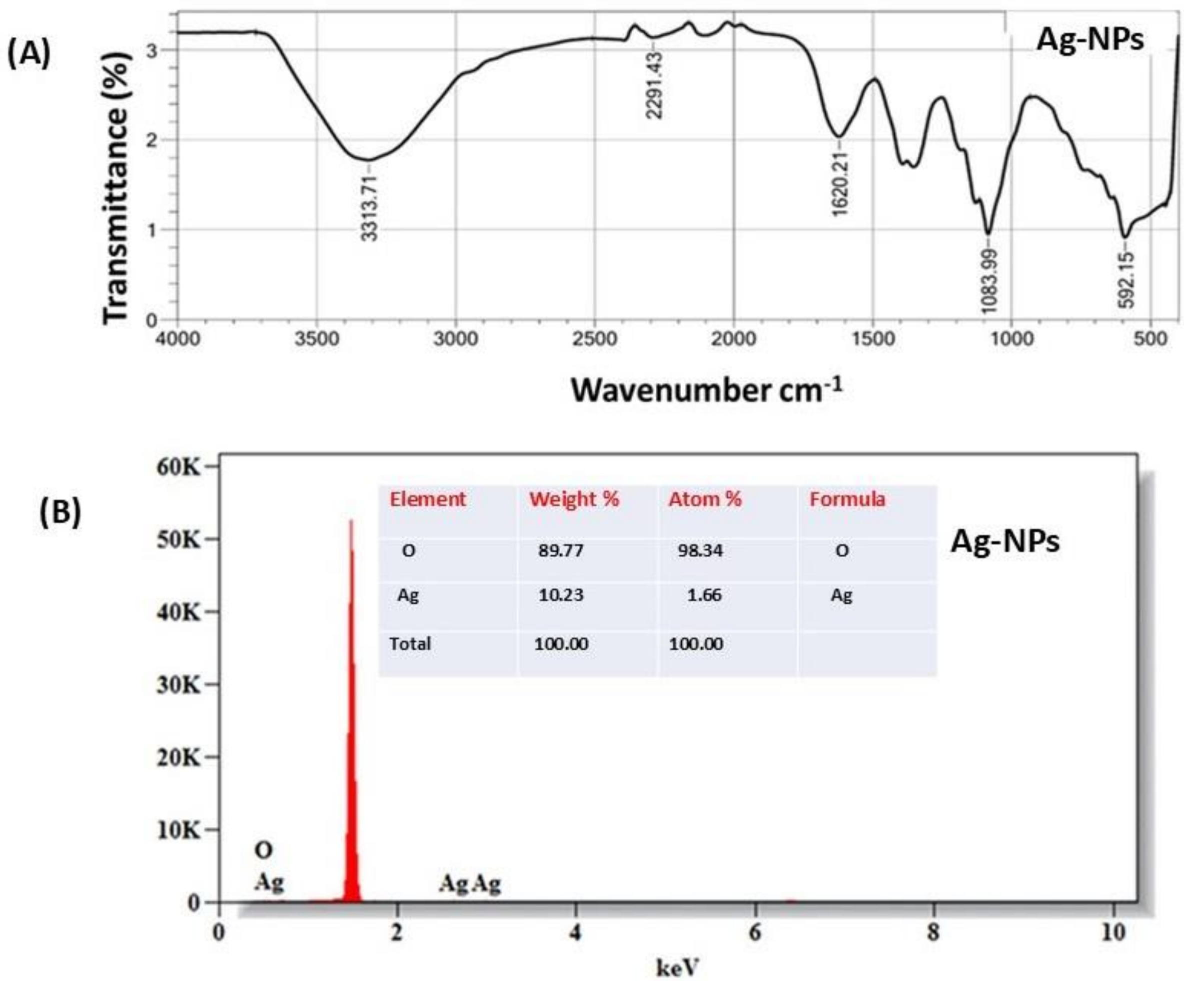

Fig. 5: (A) FTIR spectroscopy and (B) EDX analysis of Ag-NPs.

correspond to the crystal planes 111, 200, 311, and 222, respectively, which closely align with the standards of JCPDS:12-793 for cubic crystalline structures. The sharpness of the diffraction peaks in the Ag-NPs indicates uniform crystallinity, confirming the effective reduction of silver nitrate to form silver nanoparticles.

FTIR and EDX Analysis of Ag-NPs

The results of FTIR analysis are illustrated in Figure 5A. Spectra of Ag-NPs display intensive peaks at 592.15 cm^{-1}, corresponding to the Ag-O stretching vibration associated with silver and oxygen binding. There are also significant absorption peaks around 1083.39 cm^{-1}, which indicate the C–O stretching from the seaweed extract. Peaks observed at approximately 1620.21 cm^{1} are attributed to C=C stretching from carboxyl groups found in the seaweed extract. A smaller peak at 2291.43 cm^{-1} represents the aliphatic C–H stretching vibration. Additionally, the broad peak located around 3313.71 cm^{-1} indicates the hydroxyl groups in the Ag-NPs. The EDX analysis results depicted in Figure 5B confirm the presence of only two elements in the Ag-NPs: silver and oxygen. The diffraction peaks verified the purity of the synthesized Ag-NPs. The weight percentages of the elements are as follows: silver (Ag) is 10.23%, while oxygen constitutes 89.77%.

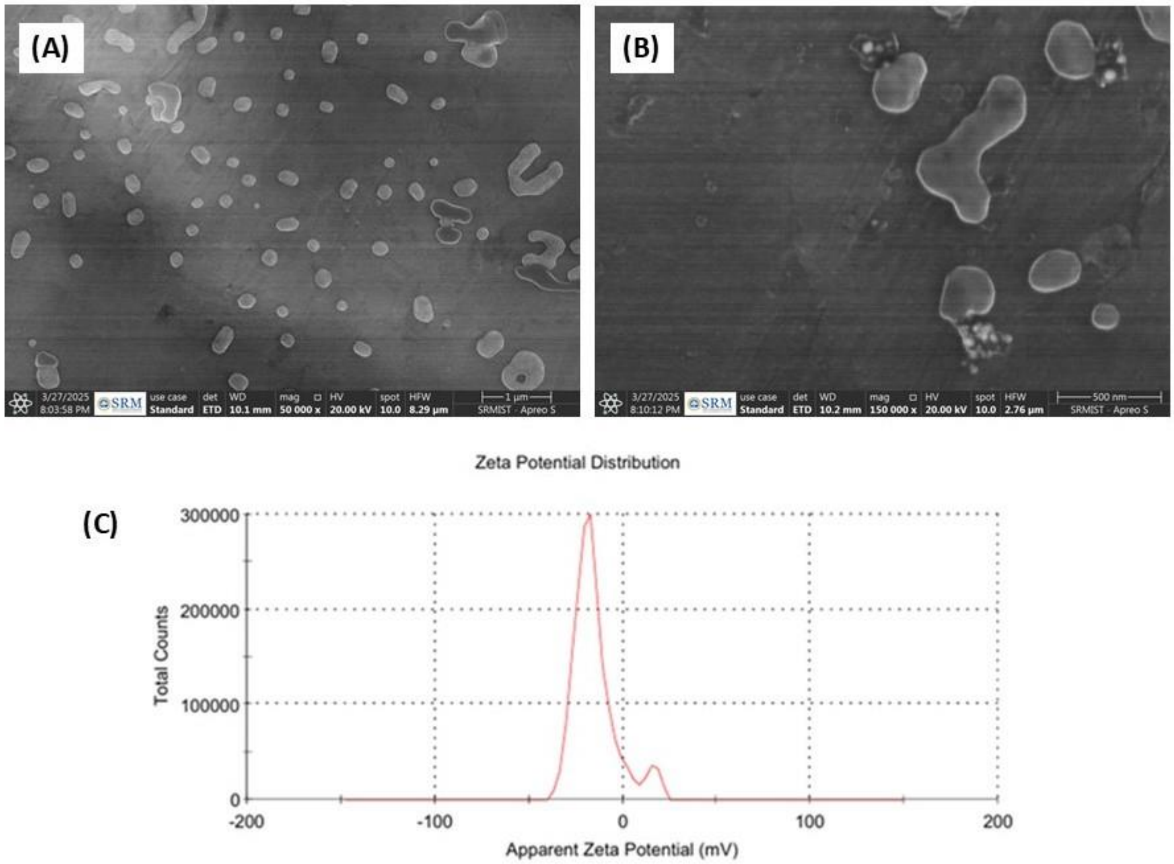

Fig. 6: (A-B) Scanning Electron Microscopy images and (C) zeta potential analysis of Ag-NPs.

SEM and Zeta Potential Analysis of Silver Nanoparticles (Ag-NPs)

The morphology and zeta potential of nanomaterials are crucial factors in the biomedical importance of Ag-NPs. The charge of the nanoparticles significantly influences their cellular internalization and interaction with cell membranes (Nanda *et al*., 2024; Sati *et al*., 2025). Figures 6A and 6B illustrate the crystal morphology of Ag-NPs as observed through scanning electron microscopy. The microscopic images revealed that Ag-NPs typically have a spherical shape, though they exhibit some variation in their crystal structure, including the presence of mildly agglomerated polygonal particles. The average size of the Ag-NPs is less than 200 nm, making them highly suitable for antimicrobial applications.

The results from the zeta potential analysis indicate that the synthesized Ag-NPs possess a charge of -34 mV, demonstrating good stability in aqueous conditions (Fig. 6C). The negatively charged Ag-NPs are likely to be attracted to positively charged microbial cells, enhancing their potential efficacy. Furthermore, the presence of seaweed biomolecules modifies the surface charge of the Ag-NPs, thereby improving their functionality.

Antibacterial activity of Ag-NPs against human infectious pathogens

The produced silver nanoparticles (Ag-NPs) were tested for their antibacterial effectiveness against four human pathogenic bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterococcus faecalis.* A well diffusion assay was conducted to evaluate the antibacterial activity of the bioactive Ag-NPs at various concentrations, as illustrated in Figure 7. The results demonstrated that the Ag-NPs effectively and dose-dependently inhibited the growth of the bacterial strains, with

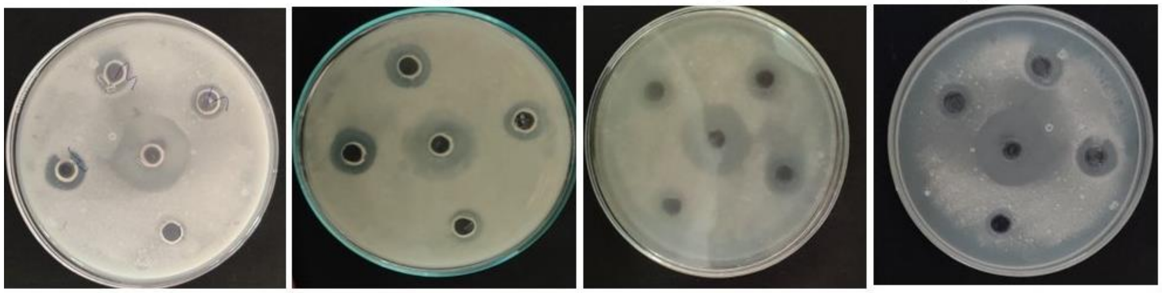

Fig. 7: Antibacterial efficacy of bioactive Ag-NPs against human pathogenic bacterial strains.

Table 3: Antibacterial efficacy of bioactive Ag-NPs against human pathogenic bacterial strains

Pathogens	Zone of inhibition (mm)				
	Gentamicin	**25 µg/ml**	**50 µg/ml**	**75 µg/ml**	**100 µg/ml**
E. coli	25	0	0	0	19
E. faecalis	20	7	7	8	9
K. pneumoniae	29	3	5	9	13
S. aureus	18	5	8	9	11

the highest level of growth inhibition observed at a concentration of 100 µg/ml. Silver nanoparticles (Ag-NPs) exhibited superior antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, with inhibition zones measuring 18 mm and 22 mm, respectively. When compared to the commercial antibiotic gentamicin, Ag-NPs demonstrated a significant ability to suppress bacterial growth. The notable zones of inhibition were observed for all tested bacterial strains, including *Klebsiella pneumoniae*, *Escherichia coli*, *Enterococcus faecalis*, and *Staphylococcus aureus* (Table 3). The measured inhibition zones for Ag-NPs were 19 mm for *K. pneumoniae*, 9 mm for *E. coli*, 13 mm for *E. faecalis*, and 11 mm for *S. aureus*. The antibacterial activity of silver nanoparticles (Ag-NPs) is believed to be due to their electrostatic interaction with the lipopoly-saccharides in the bacterial cell wall (Hafeez *et al*., 2024; Gupta *et al*., 2024). This interaction alters the integrity of the phospholipid bilayer, facilitating the entry of nanoparticles into the internal organelles of the bacteria. Furthermore, silver ions and bioactive compounds released from seaweed through hydrolysis contribute to the promotion of reactive oxygen species (ROS)-mediated death pathways in bacterial cells (Singh *et al*., 2024; Wu *et al*., 2025).

Antifungal Activity of Ag-NPs against Plant Pathogens

In this study, we developed multifunctional materials using bioactive silver nanoparticles (Ag-NPs) mediated by seaweed extract. We assessed the antifungal properties of the Ag-NPs against various plant pathogenic fungi, including *Fusarium oxysporum*, *Aspergillus niger*, *Rhizoctonia solani*, and *Aspergillus fumigatus*, using an agar well diffusion assay. The bioactive Ag-NPs were designed for antifungal applications in pest control. The combination of the seaweed extract from *Padina boergesenii* demonstrated significant efficacy in reducing the growth of plant pathogenic fungi. The synergy between the seaweed extract and silver nanoparticles effectively diminished the proliferation of fungal pathogens through the generation of reactive oxygen species (ROS) that induce cell death (Hafeez *et al*., 2024; Pallath *et al*.,

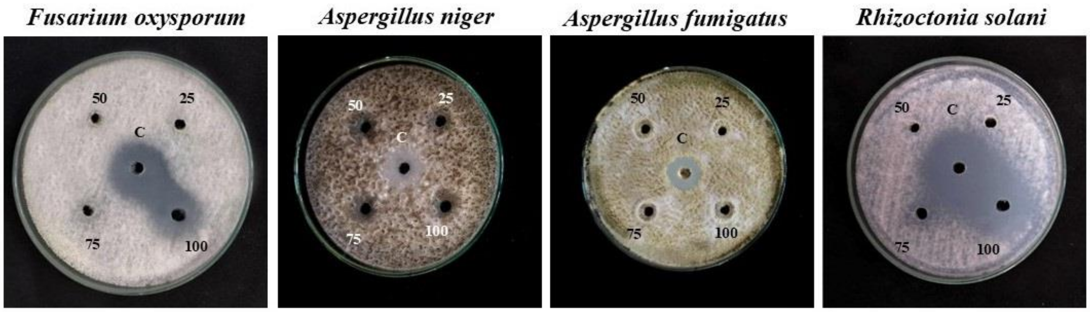

Fig. 8: Antifungal efficacy of seaweed-mediated Ag-NPs against plant pathogenic fungal strains on agar well diffusion assay.

Table 4: Antifungal efficacy of seaweed-mediated Ag-NPs against plant pathogenic fungal strains on agar well diffusion assay

Pathogens	Zone of Inhibition (mm)				
	Gentamicin	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Fusarium oxysporum	20	0	0	0	17
Aspergillus niger	18	4	6	7	9
Aspergillus fumigatus	15	2	3	5	6
Rhizoctonia solani	22	0	3	5	16

2024).

As shown in Figure 8, the bioactive Ag-NPs resulted in the largest zones of inhibition, measuring 17 mm against *Fusarium oxysporum* and 16 mm against *Rhizoctonia solani* (Table 4). Images from the agar well diffusion assay exhibit a significant level of fungal growth inhibition. The Ag-NPs facilitate enhanced penetration into the fungal cell wall, damaging protein synthesis mechanisms. Additionally, the agar plate images demonstrated a dose-dependent reduction in fungal colonies.

Conclusion

This study focused on synthesizing bioactive silver nanoparticles (Ag-NPs) using aqueous extracts from *Padina boergesenii*, exploring their antibacterial and antifungal properties against human infectious bacteria and plant fungi. The antimicrobial tests indicated that the Ag-NPs have a spherical shape, resembling crystals, and possess negative surface charges. Characterization results showed that the synthesized bioactive Ag-NPs are stable in aqueous conditions and nanostructured materials. Their high stability and nanoscale size offer significant advantages for biological applications. The antibacterial assays indicated that the seaweed-mediated Ag-NPs effectively eliminate human pathogens, including *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Staphylococcus aureus*. Meanwhile, the antifungal assays demonstrated that the Ag-NPs can potentially control the growth of plant pathogenic fungal species such as *Fusarium oxysporum*, *Aspergillus niger*, *Rhizoctonia solani*, and *Aspergillus fumigatus*. In the future, these fabricated bioactive Ag-NPs are expected to serve as a low-cost and effective solution for antibacterial and agricultural applications.

Ethical Statement

All experimental procedures involving microbial cultures were conducted in accordance with institutional biosafety guidelines and relevant national guidelines.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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