Tertadesmus obliquus-mediated silver nanoparticles: A green route to potent antibacterials

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Abstract Using algae for nanoparticle biosynthesis offers a green, cost-effective alternative to other chemical and physical methods. This study uses the cell-free supernatant of *Tetradesmus obliquus*. to produce silver nanoparticles (AgNPs) sustainably, achieving notable stability and antimicrobial activity. An initial experimental design was used to optimize the production process to maximize nanoparticle yield. The study examined the effects of key variables, including the ratio of extract to silver nitrate solution, the type of capping agent, and the medium pH. The custom design included 12 experiments with varying values for each variable, and we measured absorbance at 420 nm during both short- and long-term assessments to evaluate yield and stability. The results showed that SDS was the most effective stabilizer, resulting in an eightfold increase in nanoparticle production compared to sodium citrate after 2 weeks. At pH 9, with SDS, a rapid synthesis with maximum production was achieved even at lower $AgNO_3$ concentrations. AgNPs (3.90-10.83 nm average diameter, charge= -23.3 mV) were characterized using Zeta potential, FT-IR, XRD, and TEM. Performing disc diffusion assay, agar plates showed significant inhibition zones against *Escherichia coli*, *Salmonella enterica*, and Staphylococcus aureus at concentrations of 25, 50, and 100 mg/mL, respectively. These findings highlight the potential of algal-synthesized AgNPs as effective antimicrobial agents.

Keywords: Algae-mediated biosynthesis; Sustainable biosynthesis; Eco-friendly biosynthesis; Process optimization; Algae-derived nanoparticles.

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1 Introduction

Recently, nanotechnology has had a significant impact on various fields such as agriculture, medecine, and industrial processes. Among nanoparticles having multible applications, silver nanoparticles are well known for being used in many fields[1] . But there are many issues with producing them with chemical and physical mehods which produce toxic byproducts, high energy requirement and high cost. On the other hand, microorganisms are considered as great alternative as they are eco-friendly, cost effective and biocompatible [2]. Algae are considered highly efficient microbiological systems for producing AgNPs due to their rich content of bioactive metabolites, such as phenolics, proteins, and polysaccharides [3]. These metabolites act as reducing agents with high affinity for electrons, reducing silver ions (Ag^+) to Ag^0. Different algal species, including macroalgae such as Sargassum and Ulva, and microalgae such as Chlorella and Scenedesmus, have been used for AgNPs biosynthesis, demonstrating differences in nanoparticle yield, size, and morphology across algal species [4]. The algal cell-free supernatant (CFS) is considered much easier than other methods, without the complications of extraction techniques, making it more applicable [5]. They both belong to genus of green microalgae that has a very unique metabolic activity that provides it with a lot of biomolecules, including vitamins, polysaccharides, proteins and lipids [6]. Despite their role in nanotechnology, they are is also used in biotechnology for biofuel production, wastewater treatment, and as a protein source in animal feed [7]. Although the production of AgNPs has been studied a lot, enhancing the biosynthesis process by optimizing parameters affecting the process such as ph, ratio between reducing agent and salt precursor and using different capping agents remains not properly investigated [8].

AgNPs have tremendous physical and chemical properties that greatly affect the functionality of them such as surface plasma resonance (SPR) and antibacterial activity, these characters is affected mainly by the biosynthesis process including the microorganism and parameters used[9]. AgNPs are widely used in biomedical applications, consumer products, and environmental remediation due to their broad-spectrum antimicrobial and catalytic properties . The antibacterial mechanism of AgNPs involves multiple pathways: disruption of cell membranes, generation of reactive oxygen species (ROS), and inhibition of DNA replication[10]. Gram-negative bacteria such as E. coli are generally more resistant than Gram-positive bacteria like S. aureus due to their outer membrane, but AgNPs functionalized with targeted capping agents can enhance efficacy against both types[11].

This study was driven by a goal to refine a truly green and sustainable method for creating silver nanoparticles (AgNPs). We harnessed the natural power of *Tetradesmus obliquus* algae, using its cell-free supernatant as the foundation for biosynthesis. To make the process as efficient as possible, we designed a custom experimental approach to pinpoint the ideal conditions for maximizing both the yield and stability of the resulting nanoparticles.

We focused on understanding how key factors—such as the type of capping agent used, the pH of the reaction mixture, and the ratio of algal extract to silver nitrate solution—affect the outcome. The silver nanoparticles we create will undergo comprehensive analysis using various methods: TEM to examine size and morphology, UV-Vis and XRD to verify the synthesis, FTIR to identify the natural compounds that cap them, and zeta potential to assess their stability. A fundamental aspect of our research will involve directly comparing how different synthetic capping agents, both anionic and non-ionic, improve the stability of our algae-based nanoparticles. Ultimately, we will evaluate our optimized AgNPs for their potential as antibacterial agents against various pathogenic bacteria. Our goal is not only to validate their effectiveness but also to investigate how they interact with and compromise bacterial cell walls. By systematically linking algal synthesis to capping agent optimization, this research lays a sustainable pathway to producing high-performance nanomaterials with promising applications in biomedicine.

2 Materials and Methods

2.1 Chemicals

All chemicals were purchased from sigma Aldrich. For preparing Bold's Basal medium, sodium nitrate ($NaNO_3$), calcium chloride dihydrate ($CaCl_2·2H_2O$), magnesium sulfate heptahydrate ($MgSO_4·7H_2O$), dipotassium phosphate (K_2HPO_4), monopotassium phosphate (KH_2PO_4), sodium chloride (NaCl), disodium ethylenediaminetetraacetate dihydrate ($Na_2EDTA·2H_2O$), ferric chloride hexahydrate ($FeCl_3·6H_2O$), boric acid (H_3BO_3), manganese chloride tetrahydrate ($MnCl_2·4H_2O$), zinc chloride ($ZnCl_2$), sodium molybdate dihydrate ($Na_2MoO_4·2H_2O$), cupric chloride dihydrate ($CuCl_2·2H_2O$), and cobalt chloride hexahydrate ($CoCl_2·6H_2O$) were used. Sulbin® 750 mg vial Batch No. 767 labelled to contain 500 mg ampicillin and 250 mg sulbactam.

For the production of nanoparticles and using various capping agents: sodium dodecyl sulfate (SDS), polyethylene glycol 400 (PEG400), polyethylene glycol 6000 (PEG 6000), sodium citrate dihydrate and silver nitrate ($AgNO_3$) were used.

2.2 Algae Cultivation

Tetradesmus obliquus (tarp.) were obtained from algological collection of the National Institute of Oceanography and Fisheries. Expanding the cultivation volume to six liters was imperative to ensure sample quantity of algal samples for the commencement of the nanoparticles production process

2.3 Nanoparticles preparation

Algal cultures were collected through centrifugation at 4000 rpm for a duration of 10 minutes, and the resulting supernatant was gathered. This cell-free extract was subsequently combined with a silver nitrate ($AgNO_3$) solution and enriched with 1% (w/v) of the chosen capping agent. The reaction mixture was left to incubate overnight under regulated conditions overnight without light source to avoid photoreduction, resulting in the formation of silver nanoparticles. (**Figure 1**).

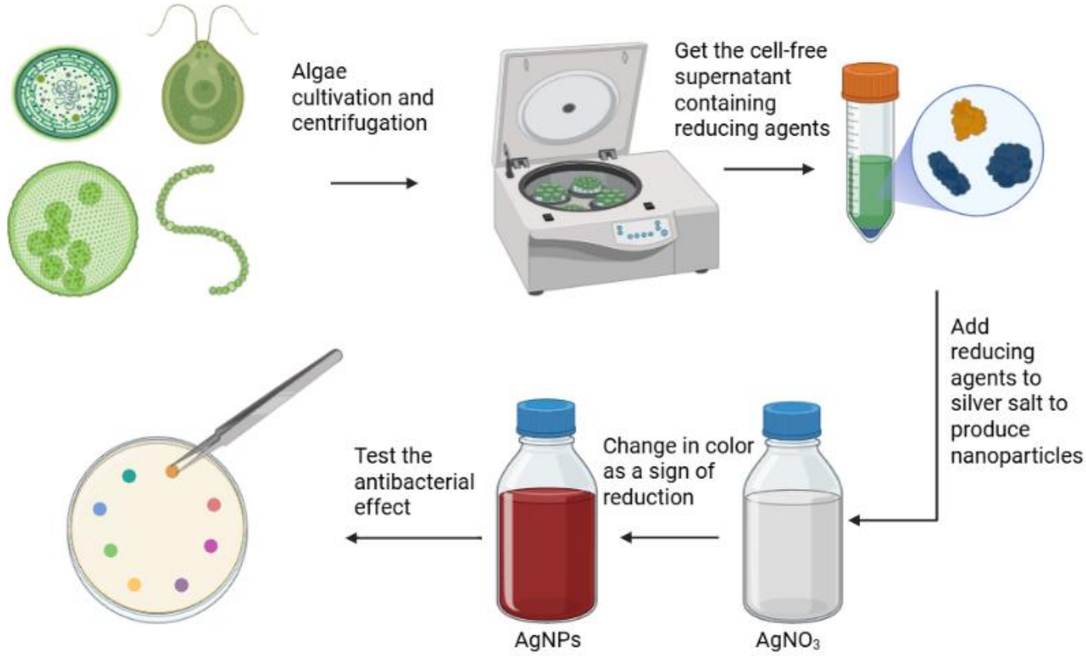

Figure 1. General scheme of Ag-NPs-mediated *Tetradesmus obliquus* cell-free supernatant as source of reducing agents in biosynthesis of nanoparticles

2.4 Condition Optimization

For optimization of the biosynthesis of silver nanoparticles (AgNPs) using *Tetradesmus obliquus* cell-free extract (CFE), a total of 12 experiments were made by varying three key parameters: the extract-to-$AgNO_3$ ratio, type of capping agent, and pH level.

2.4.1 Ratio of CFE to $AgNO_3$: Three different ratios of CFE to 50 mM $AgNO_3$ solution were tested in order to identify the optimum proportion for nanoparticle formation.

2.4.2 pH Level: The influence of pH on nanoparticle yield and stability was examined at three levels (pH 5, 7, and 9).

2.4.3 Capping Agents: To assess their stabilizing effect, four capping agents were evaluated at a concentration of 1% (w/v): sodium dodecyl sulfate (SDS), sodium citrate, polyethylene glycol (PEG 400), and polyethylene glycol (PEG 6000).

The biosynthesized nanoparticles were monitored for yield and stability using UV–Vis absorbance measurements taken immediately (day 1), after one week, and after two weeks. These analyses allowed the determination of optimal synthesis conditions for stable and uniformly dispersed AgNPs.

2.4 Nanosilver mediated-*Tetradesmus obliquus* Characterization

Following a duration of two weeks, 100 mL of the reaction mixture was precisely extracted, then lyophilized, and the resultant dry product was measured. The morphology, crystallinity, and electrical charge of the resulting optimal nanoparticles were analyzed using transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and zeta potential measurements.

2.5 Antibacterial effect

The antibacterial properties of the biosynthesized AgNPs were assessed using the disc diffusion method against three pathogenic bacterial strains, namely *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus*. Under sterile conditions, the bacteria were inoculated onto LB agar plates at a 0.5 McFarland standard, followed by the application of sterile discs with a diameter of 3 mm that were infused with three varying concentrations of AgNPs (25, 50, and 100 mg/mL), along with silver nitrate, cell-free supernatant, and Sulbin commercial antibiotic serving as negative and positive controls, respectively. After a 24-hour incubation, the inhibition zones around all discs were measured to analyze the results.

3 Results

The present research focused on cultivating *Tetradesmus obliquus*, and the growth medium provided the cell-free supernatant. The cell-free supernatant (CFS) facilitated the synthesis of AgNPs from a 50 mM AgNO3 solution. A tailored experimental design was implemented for an optimization study to statistically assess and analyze the primary effects and interactions of various factors influencing the yield and stability of AgNPs. The AgNPs were produced under the optimal conditions and were characterized using various analytical techniques to assess their size, shape, and charge. Lastly, the research investigated the antibacterial properties of the produced AgNPs..

3.1 Algae cultivation

Tetradesmus obliquus was successfully grown in Bold's Basal Medium (BBM) [12]. After an incubation period of 7 to 10 days, clear green colonies with smooth, circular shapes were observed. Microscopic analysis verified the presence of characteristic diamond forms that measured between 2 and 10 µm in diameter, aligning with the morphology of *Tetradesmus obliquus.* shown in **Figure 2**. To generate a sufficient biomass for further applications, the culture volume was scaled up systematically, ultimately achieving a final volume of 6 L. This resulted in a consistent biomass yield of *Tetradesmus obliquus*. that was appropriate for subsequent experiments involving nanoparticle biosynthesis.

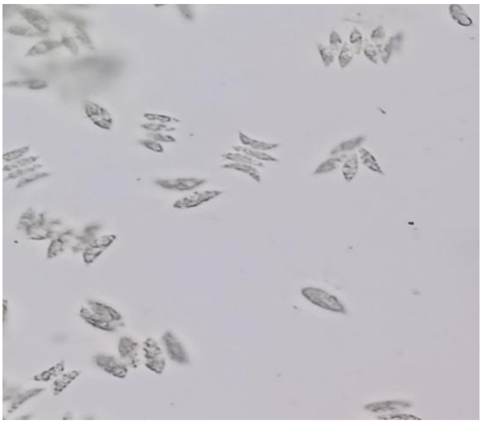

Figure 1. Tetradesmus examination under light microscope

3.2 Optimization of the biosynthesis process

As the biosynthesis reaction proceeded, the solution slowly transformed into a reddish-brown, signifying the ongoing reduction of silver ions and the creation of silver nanoparticles (AgNPs), a distinct color noted in previous studies. The UV-Visible absorption spectra of the reaction mixtures consistently displayed a notable peak near 420 nm, validating the successful production of AgNPs. (**Figure 3A**).

Preliminary experiments revealed that the conditions under which the reaction occurred had a major impact on the yield and stability of AgNPs. In particular, the pH level of the solution and the specific type of capping or stabilizing agent were identified as key factors (**Figure 3A**). Clearly, synthesizing AgNPs with SDS at an alkaline pH resulted in sharper and more clear plasmon resonance peaks compared to those coated with citrate or in acidic environments, resulting in enhanced nanoparticle formation.

To systematically enhance the biosynthesis process, an experimental design was made, consisting of 12 experimental runs that tested three key variables: concentration of silver nitrate, pH level, and incubation period. The absorbance at 420 nm was selected as the main response, assessed in the short term (overnight), after 1 week, and after extended incubation (two weeks). To guarantee precision, samples with high concentrations were diluted, and dilution factors were recalibrated when interpreting the results.

The analysis of model fitting and prediction (**Figure 3B**) revealed a significant correlation between the experimental values and the predicted outcomes. The 3D response surface plots (**Figures 3C–F**) additionally demonstrated the interactive influences of silver nitrate concentration, pH, and incubation time on the yield of AgNPs. Together, these findings confirm that optimizing reaction conditions is crucial for obtaining a high yield and stable biosynthesis of AgNPs..

The predicted versus actual plot shows strong correlation, which signals the significance of the built prediction model.

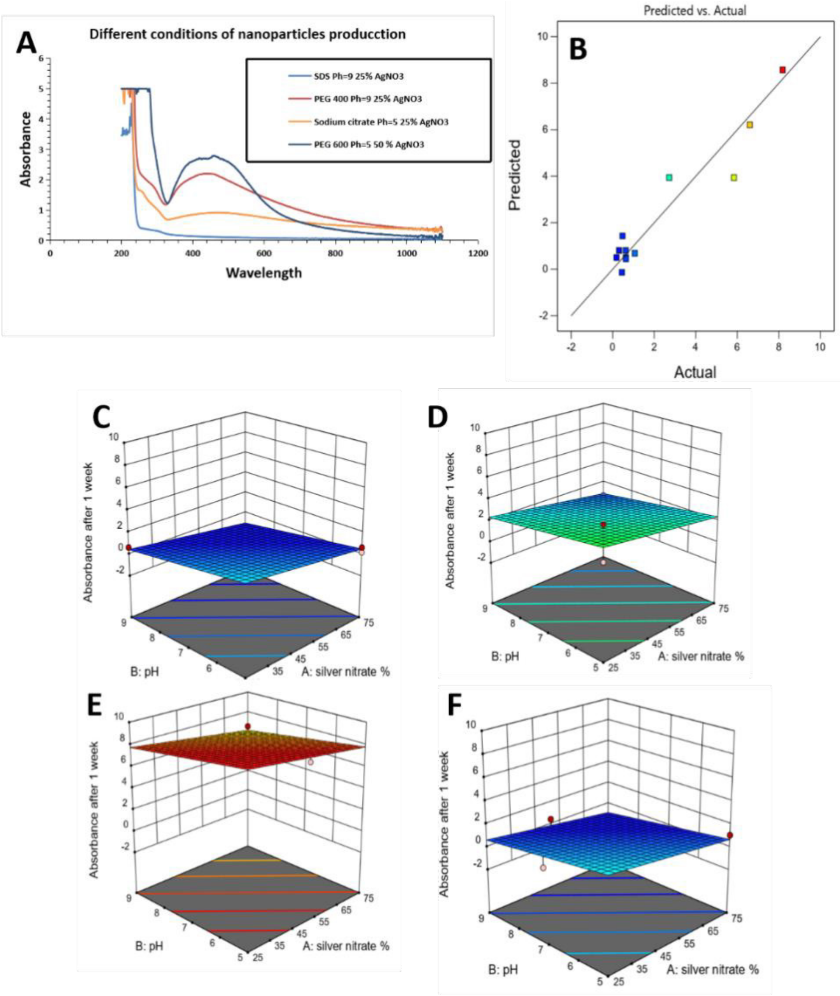

Figure 3. Optimization of silver nanoparticle (AgNP) biosynthesis. (A) UV–Visible absorption spectra of AgNPs synthesized using different capping agents and pH values. A characteristic surface plasmon resonance (SPR) peak is observed at ~420 nm. Insect: photograph of AgNP suspension showing reddish-brown color. (B) Correlation between predicted and actual absorbance values at 420 nm, showing good model agreement. (C–F) Response surface plots showing the effects of silver nitrate concentration, pH, and incubation time on AgNP yield (absorbance at 420 nm, overnight). Red points indicate experimental runs.actual and predicted UV-Vis absorbances of optimized AgNPs. C, D, E, F The 4 capping agents 3D surfaces show their relationship with other factors according to production overnight. C-PEG 400. D-Sodium citrate. E- SDS. F- PEG 6000.

The 3D surface plot illustrates how the impact of silver nitrate ratio changes based on various capping agents. A strong relationship between the predicted data and actual outcomes indicates the model's predictive significance, as shown in **Figure 4.A**. The 3D representations from the two-week model demonstrated a yield increase that corresponded positively with the ratio of silver nitrate, regarding of the capping agent employed. The use of SDS produced AgNPs with the highest yield and stability, as depicted in **Figure 4.E**.

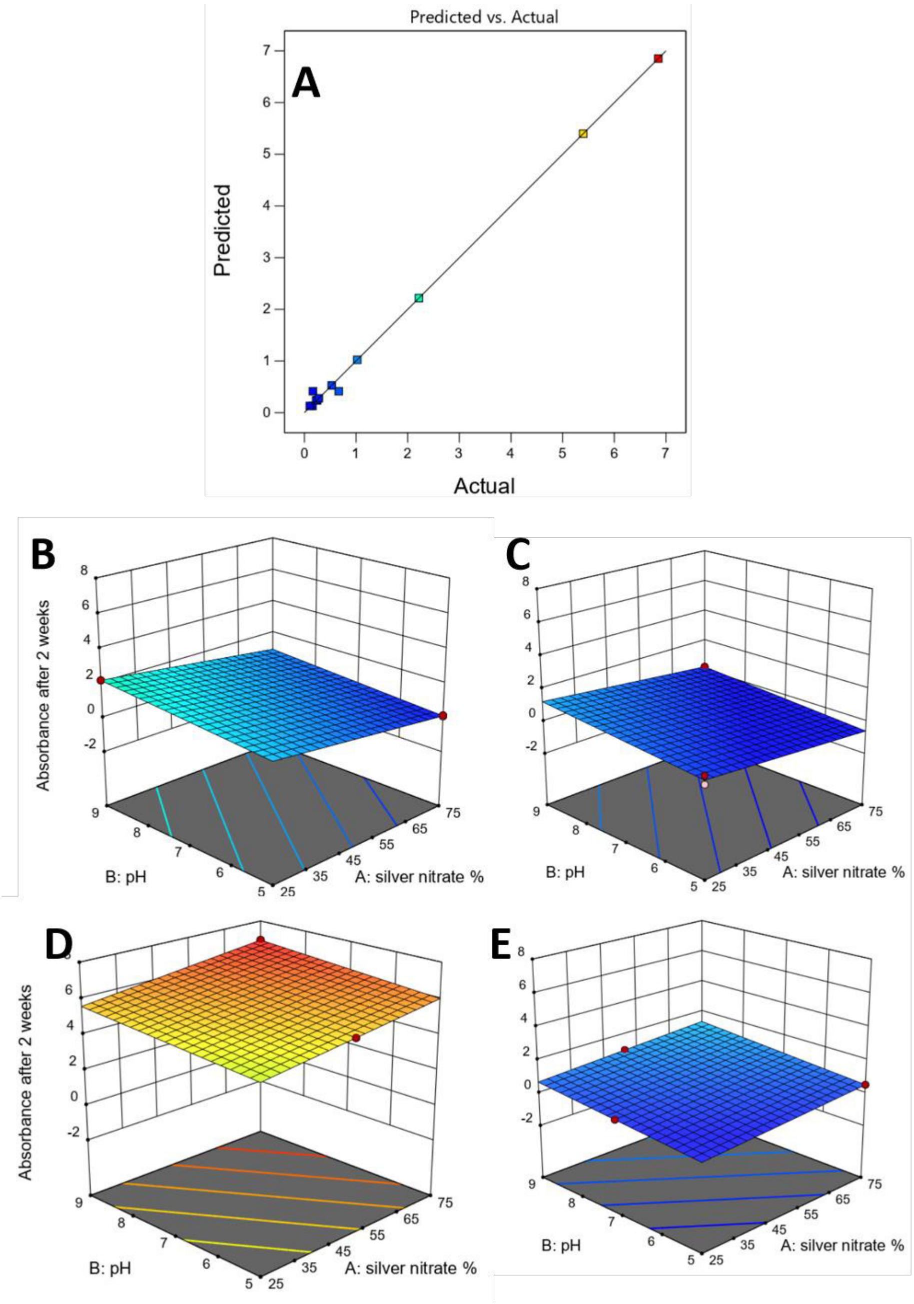

Figure 4. A. Deviance between actual and predicted UV-Vis absorbances of optimized AgNPs. **B,C,D,E** The 4 capping agents 3D surfaces show their relationship with other factors according to production overnight. **B**-PEG 400. **C**-Sodium citrate. **D**- SDS. **E**- PEG 6000.

All optimal conditions proposed by RSM support using lower concentrations of silver nitrate and higher pH, making the production process more commercially viable for generating

AgNPs from silver nitrate (at low concentrations) while utilizing algal cell-free supernatant as a reducing agent (which is easy to obtain), keeping in mind the possibility of further diluting AgNPs for antibacterial or other applications. The importance of SDS is highlighted because it provides the best stability and yield, which can be explained by its electrostatic repulsion from the negative charge of the sulfate group that prevents the agglomeration of biosynthesized AgNPs. [13],

In contrast, PEG 400 relies on its polymeric properties to use steric hindrance to stop nanoparticle agglomeration, with alkaline pH proving more effective than acidic conditions due to the deprotonation of proteins and other reducing agents in the cell-free supernatant.[14] This deprotonation increases the electron density of the reducing agents, enhancing their ability to donate electrons to Ag+, converting them to Ag0. The desirability function was optimized to ensure maximum yield and stability. This function indicates several solutions with a high desirability level of 0.921. All suggested solutions recommend using 21.4% to 26% AgNO3, with a pH range of 7.9 to 8.9, and unanimously propose using SDS as the capping agent. The chosen optimized conditions (solution 1) consisted of mixing 25 mL of 50 mM AgNO3 with 75 mL of CFS. One gram of SDS was added to the solution, and the pH was adjusted to 7.9 using 1 mM KOH. The DoE generated 30 solutions over two weeks for determining the optimal conditions for producing AgNPs, with some reaching a desirability of 0.92.

3.3 Characterization of the Biosynthesized AgNPs

The biosynthesized silver nanoparticles (AgNPs) were characterized using Fourier-transform infrared spectroscopy (FT-IR), zeta potential analysis, X-ray diffraction (XRD), and transmission electron microscopy (TEM) to confirm their formation, stability, crystallinity, and morphology.

3.3.1 FT-IR Analysis. The spectrum of the cell-free algal supernatant exhibited characteristic absorption bands, which were then compared to the spectrum of the silver nanoparticles. Key shifts and changes in intensity were observed in the nanoparticle sample; notably, the bands in the regions of 1636 cm^{-1} and 1073 cm^{-1}, corresponding to the amide I (C=O stretching of proteins) and C-O stretching vibrations of polysaccharides, respectively, were prominently altered. This suggests that proteins and polysaccharides present in the algal supernatant play a crucial role as reducing and capping agents, effectively facilitating the biosynthesis and stabilization of the silver nanoparticles. (**Figure 5A**).

3.3.1 TEM Analysis. TEM imaging showed that the biosynthesized AgNPs were mostly spherical in shape, with sizes varying from 3.90 to 0.83 nm (Figure 5B). The nanoparticles showed good dispersion with minimal aggregation, indicating that the metabolites from *Tetradesmus obliquus*. acted as capping and stabilizing agents. The size of the particles obtained in this study was notably smaller than those reported in other biosynthetic methods, such as 55.06 ± 9.67 nm [15], 73.13 nm [16], and 79 nm[1], showing the efficiency of algal biomolecules in regulating nanoparticle nucleation and growth. Besides that, the achieved small size distribution results in a greater surface area-to-volume ratio, enhancing interactions between nanoparticles and cells, promoting silver ion release, and increasing the generation of reactive oxygen species (ROS). This physicochemical characteristic is related to the significant antibacterial activity in the agar diffusion assays, where AgNPs synthesized with *Tetradesmus obliquus*. demonstrated good efficacy. Overall, these characterization results validate the successful biosynthesis of stable, crystalline, and uniformly distributed AgNPs using *Tetradesmus obliquus*. extract, indicating that the small size and biogenic capping play crucial roles in the observed antibacterial effectiveness.

3.3.1 XRD Analysis. The crystalline structure and phase purity of the biosynthesized silver nanoparticles were confirmed by X-ray diffraction (XRD) analysis. The XRD pattern exhibited distinct diffraction peaks at 2θ values of approximately 29.08°, 37.81°, 44.02°, 64.13°, and 77.26°, which can be indexed to the (110), (111), (200), (220), and (311) lattice planes of face-centered cubic (fcc) silver, respectively (JCPDS card no. 04-0783). The absence of extraneous peaks indicates the high purity of the synthesized AgNPs.. (**Figure 5C**).

*3.3.1 Zeta Potential Analysis***.** Zeta potential measurements revealed a surface charge of approximately –23.3 mV, suggesting good colloidal stability of the biosynthesized AgNPs (**Figure 5D**). The negative charge likely results from the adsorption of algal metabolites on the nanoparticle surface, which provides electrostatic repulsion and prevents aggregation.

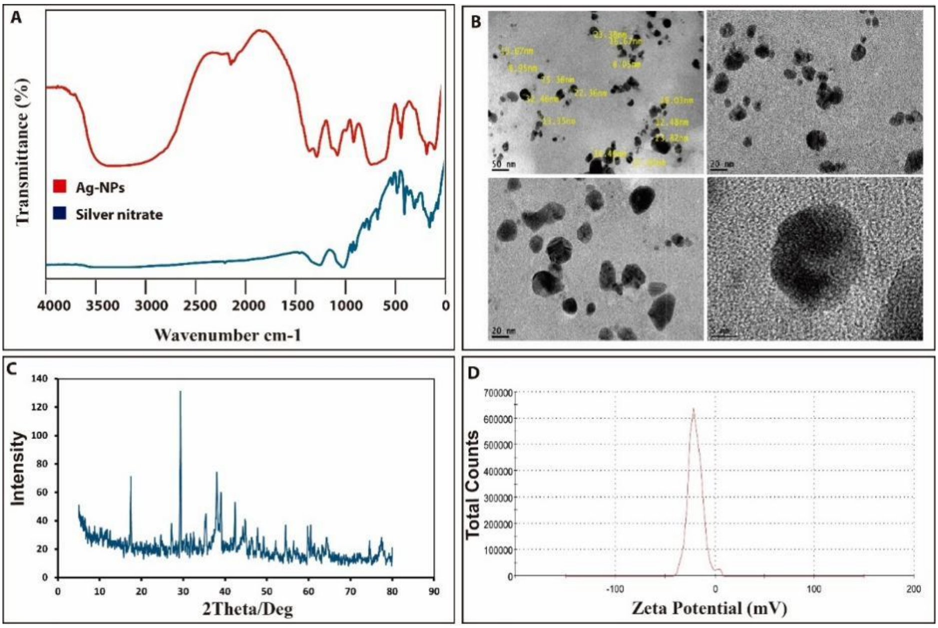

Figure 5. A. FT-IR of algae supernatant vs AgNPs with optimal conditions. **B.** TEM analysis of biosynthesized silver nanoparticles (AgNPs). Transmission electron microscopy images showing spherical AgNPs synthesized using *Tetradesmus obliquus* cell-free extract. The nanoparticles ranged in size from 8.05 to 32.46 nm and appeared well-dispersed with minimal aggregation, confirming the stabilizing role of algal metabolites as natural capping agents. **C.**XRD analysis showing strong evidence of AgNPs formation. **D.** Zeta potential

3.4 Antibacterial activity

The antibacterial effect of silver nanoparticles (AgNPs) synthesized were tested against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella enterica* at concentrations of 25, 50, and 100 mg/mL. A distinct effect was noted, signifying heightened antibacterial activity at all levels (see **Figures 6 and 7**). Among the bacteria tested, all of them showed large clear zones, but, E.coli had the largest one, followed by S.aureus , then S.typhi , which aligns with the higher resistance observed in Gram-positive bacteria due to their thicker peptidoglycan cell wall. both positive and negative control tests demonstrated that the observed activity was due to the biosynthesized AgNPs: the cell-free algal extract alone resulted in no inhibition, and SDS (used as a control for the capping agent) also showed no antibacterial effect, eliminating any potential antimicrobial influence from these components. Subtilin, used as a positive antibiotic control, resulted wide inhibition zones for all bacterial strains, confirming the validity of the assay. Importantly, the smaller size of SDS-capped AgNPs greatly improved their antibacterial effectiveness by allowing deeper penetration into bacterial cell envelopes. These results confirm that green-synthesized AgNPs using *Tetradesmus obliquus.* extract shows considerable and broad-spectrum antibacterial properties, showing great effectiveness of uncapped AgNPs.

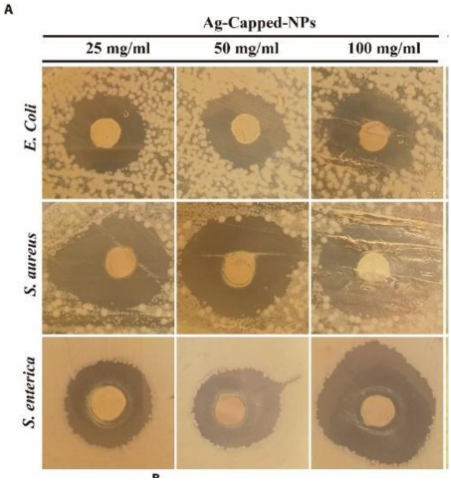

Figure 6. Antibacterial activity of *Monoraphidium sp*–mediated silver nanoparticles (AgNPs). A. treatment using nanoparticle using Agar disc diffusion assay showing inhibition zones against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella enterica* at three concentrations of biosynthesized AgNPs (25, 50, and 100 mg/mL).

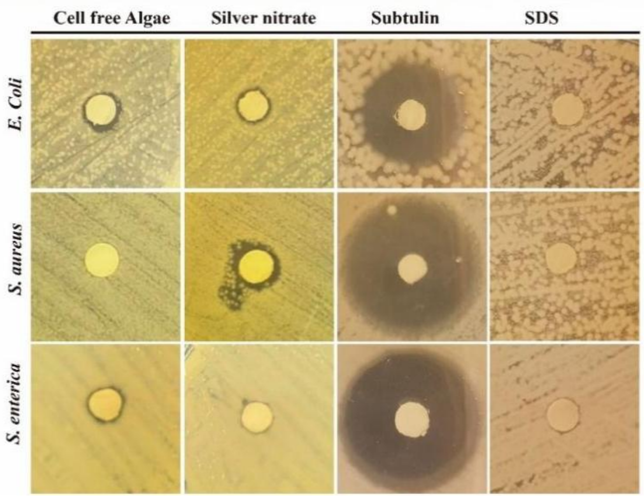

Figure 7. Antibacterial activity of *Monoraphidium sp*–mediated silver nanoparticles (AgNPs). Controls included cell-free algal extract, silver nitrate, subtilin (positive control), and SDS (capping agent control). Dose-dependent inhibition was observed with the biosynthesized AgNPs, with the highest activity at 100 mg/mL. 100 mg/ml SDS capped silver nanoparticles showed the strongest antibacterial activity, while SDS and the cell-free extract exhibited no inhibition.

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Antibacterial effectiveness of AgNPs synthesised by *Tetradesmus obliquus*. increased slightly with concentration against all three bacterial strains tested. The zones of inhibition increased in size as the AgNP concentration (25, 50, and 100 mg/mL) increased. S. aureus exhibited the greatest sensitivity, followed by S. enterica and E. coli. In contrast, the positive control (subtilin) produced the largest zones of inhibition across all strains, whereas both the capping-agent control (SDS) and the cell-free extract showed no detectable antibacterial activity. These results confirm that the inhibition observed was due to the biosynthesized AgNPs, not of the capping agent or the algal filtrate.

4 Discussion

This research introduces an environmentally friendly method for producing silver nanoparticles (AgNPs) utilizing the cell-free supernatant (CFS) derived from Tetradesmus obliquus, and comprehensively assesses how various process parameters affect nanoparticle yield, physicochemical stability, and antibacterial effectiveness. The biosynthesis of nanoparticles utilizing algal extracts has recently attracted significant interest as a viable alternative to traditional chemical or physical methods, mainly due to the presence of diverse biomolecules in algae—such as proteins, polysaccharides, lipids, and pigments—that function as both reducing and capping agents concurrently[3]. By avoiding toxic chemicals and reducing energy consumption, this approach promotes environmental sustainability as well as potential applications in biomedicine.

4.1 Influence of Synthesis Parameters

pH had the most significant impact among the parameters tested. The results suggest that alkaline conditions (approximately pH 9) significantly promoted the formation of nanoparticles, resulting in stable and well-dispersed particles. In an alkaline environment, the hydroxyl and amine groups in biomolecules lose protons, which speeds up the electron transfer to Ag^+ ions and facilitates the nucleation and subsequent growth of silver nanoparticles (AgNPs). Similar results were observed in a recent study involving Sargassum cymosum extract, where alkaline pH not only shortened the reaction time but also produced ultrasmall particles (< 5 nm) with enhanced stability [17] . On the other hand, acidic conditions hindered nanoparticle formation, likely due to the competition with protons that decreases the availability of functional groups needed for reduction.

The concentration of silver nitrate also played a crucial role. Excessively high concentrations frequently resulted in agglomeration and non-uniform particle sizes, while optimized lower concentrations were effective in achieving high yields when combined with alkaline conditions. A similar pattern was noted in the synthesis of AgNPs mediated by Planophila laetevirens, where a moderate concentration of silver ions improved yield while reducing waste and toxicity [18]. These findings emphasize that fine-tuning precursor concentration is essential to balance productivity with particle quality.

4.2 Role of Capping Agents

The effect of capping agents on the stability and antibacterial properties of silver nanoparticles (AgNPs) was observed. In this study, sodium dodecyl sulfate (SDS) demonstrated the most pronounced stabilizing capability, resulting in smaller and evenly distributed nanoparticles. The negatively charged sulfate group of SDS generates electrostatic repulsion among the particles, which helps minimize aggregation. This observation is consistent with recent research suggesting that ionic stabilizers generally surpass steric (non-ionic) agents in maintaining colloidal stability[19]. Importantly, the stabilization achieved with SDS was also associated with enhanced antibacterial activity, indicating that improved dispersion facilitates better interactions between nanoparticles and cells. A review published in 2024 highlighted that the selection of a capping agent can considerably influence biological activity by altering surface charge, hydrophobicity, and ion release rates [20].

4.3 Structural and Physicochemical Characterization

Characterisation confirmed the production of crystalline, mostly spherical AgNPs measuring 3.9-10.83 nm. These measurements are similar to earlier research involving different algae, which generally produce nanoparticles within the 10–30 nm range that also possess strong antibacterial properties [21]. FT-IR spectra identified functional groups such as hydroxyl, amine, and carbonyl, confirming their roles in both reduction and stabilisation processes. XRD patterns showed evident diffraction at the (111) plane, indicating face-centred cubic silver, consistent with findings in other green synthesis studies [22].

Zeta potential analysis revealed moderately negative values (–15 to –20 mV), suggesting adequate stability due to electrostatic repulsion. This result agrees with recent observations that nanoparticle suspensions with zeta potentials below –15 mV can remain stable for weeks under ambient conditions. Critically, the stability evaluation over two weeks provides valuable insight into long-term applicability, which is often overlooked in studies that evaluate only immediate post-synthesis stability.

4.4 Antibacterial Activity and Mechanism

The biosynthesized silver nanoparticles (AgNPs) demonstrated notable antibacterial effectiveness against both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli, Salmonella enterica) bacteria. In general, Gram-negative bacteria exhibit greater resistance, linked to their thinner peptidoglycan layer and enhanced membrane integrity compared to Gram-positive bacteria. [21]. This structural distinction allows for more straightforward penetration of AgNPs and subsequent intracellular damage.

The structural features facilitate easier entry of AgNPs, leading to subsequent harm within the cells. The antibacterial potency of SDS-capped AgNPs suggests a collaborative effect between improved colloidal stability and stronger interactions between the nanoparticles and cellular membranes. The antibacterial mechanisms of AgNPs comes from several factors, such as membrane disruption through direct nanoparticle attachment that increases permeability and causes leakage of cellular components, the liberation of Ag^+ ions that bind to protein thiol groups and disrupt enzymatic functions, and the generation of reactive oxygen species (ROS) that induce oxidative stress, DNA damage, and ultimately result in cell death. Recent studies highlight that the choice of capping agents can affect these mechanisms, either by enhancing ROS production or by facilitating the release of silver ions[23]. Therefore, the significant antibacterial zones of inhibition observed reflect not only the intrinsic properties of silver but also the optimized synthesis conditions that produced small, stable, bioactive nanoparticles [24].

5 Novelty and Future Perspectives

This study differentiates itself from many previous investigations by integrating a systematic optimization of synthesis parameters while also assessing long-term stability and antibacterial characteristics, providing a more thorough evaluation of biosynthesized AgNPs. The findings indicate that careful modifications in pH, silver nitrate concentration, and the selection of capping agents collaboratively enhance yield, stability, and biological efficacy.

For upcoming studies, it is recommended to include minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assessments for a quantitative comparison with antibiotics. Investigating antibiofilm activity and quorum sensing inhibition would foster a deeper understanding of potential applications against multidrug-resistant bacteria. In addition, assessing cytotoxicity and biocompatibility on mammalian cell lines is essential for determining safety for medical or food-related applications. Finally, conducting large-scale studies will be crucial to verify the feasibility of applying this green synthesis approach in industrial settings.

6 Conclusion

Based on the findings of this research into the antibacterial properties and improvement of nanosilver, several important conclusions have been made. The study looked at how key factors like the concentration of silver nitrate, the pH level, and the selected capping agent affect the biosynthesis of AgNPs using the cell-free supernatant (CFS) from Tetradesmus obliquus. Through careful experimentation, the research offers data-driven ways to optimize the whole process to achieve maximum yield, stability, and biological effectiveness of the nanoparticles. The results showed that lower concentrations of silver nitrate, along with an alkaline pH, create the best conditions for AgNPs biosynthesis. These factors not only increased nanoparticle production but also improved stability over time. Among the various stabilizers tested, sodium dodecyl sulfate (SDS) performed best as an anionic capping agent, producing AgNPs with higher output, longer stability, and strong antibacterial properties compared to other agents. Additionally, antibacterial tests revealed that the synthesized AgNPs have significant inhibitory effects against a wide range of pathogenic bacterial strains. Notably, all three concentrations tested produced strong antibacterial responses, highlighting the potential of biosynthesized AgNPs as effective antimicrobial agents. This combination of optimized synthesis conditions and proven antibacterial effectiveness shows great promise for AgNPs in biomedical applications and food safety. Overall, this research offers a clear path for optimizing AgNP biosynthesis using Tetradesmus obliquus algal cell-free extracts and demonstrates their potential as environmentally friendly, stable, and efficient antimicrobial nanomaterials. Future studies may explore these outcomes further by looking into their molecular antibacterial mechanisms, scaling up production methods, and assessing their safety and practical use in real-world settings.

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