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RESEARCH ARTICLE

Green Synthesis and Characterization of Silver Nanoparticles Using Medicinal Plant Extracts for Biomedical Applications

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Abstract: The present study investigates the eco-friendly synthesis of silver nanoparticles (AgNPs) using aqueous extracts of selected algal species as natural reducing and stabilizing agents. Algal-mediated green synthesis offers a sustainable and non-toxic approach, eliminating the need for hazardous chemical reagents commonly used in conventional synthesis methods. In this research, four algal species—Chlorella vulgaris, Spirulina platensis, Ulva lactuca, and Sargassum muticum—were utilized due to their rich biochemical composition, including proteins, polysaccharides, phycocyanin, polyphenols, and sulfated compounds, which actively contribute to nanoparticle bioreduction and stabilization. The formation of AgNPs was visually confirmed by a color change from light green to dark brown and further validated through UV–Visible spectrophotometry, which showed a characteristic absorption peak at 430–440 nm. FTIR analysis confirmed the involvement of algal-derived functional biomolecules in capping and stabilizing nanoparticles. Scanning Electron Microscopy (SEM) revealed that the synthesized AgNPs possessed predominantly spherical morphology, with average particle sizes ranging between 20–55 nm. The antimicrobial activity of algal-synthesized AgNPs was evaluated against selected pathogenic microorganisms, showing maximum inhibition against Staphylococcus aureus and Escherichia coli, followed by Pseudomonas aeruginosa and Bacillus subtilis. Statistical analysis confirmed the significance of antimicrobial activity ($p < 0.05$). These findings suggest that algal extracts possess strong potential for sustainable nanomaterial production, making them promising candidates for biomedical applications including antimicrobial coatings, drug delivery systems, wound healing, and antioxidant therapy. The study proposes algal-based synthesis of AgNPs as an eco-friendly, efficient, and scalable nanobiotechnology approach that can be integrated into pharmaceutical and clinical practice.

Keywords: Green synthesis; silver nanoparticles (AgNPs); algal extracts; Chlorella vulgaris; Spirulina platensis; Ulva lactuca; Sargassum muticum; eco-friendly nanotechnology; UV–Vis spectroscopy; FTIR; SEM; antimicrobial activity; biomedical applications.

INTRODUCTION

Nanotechnology has emerged as a revolutionary field in biomedical science, providing new opportunities for targeted drug delivery, antimicrobial therapy, wound healing, and diagnostic applications (Jeevanandam et al., 2018). Among various metal-based nanomaterials, silver nanoparticles (AgNPs) have received considerable attention due to their broad-spectrum antimicrobial, antioxidant, antiviral, and anti-inflammatory properties (Franci et al., 2015; Li et al., 2017). However, conventional AgNP synthesis techniques often rely on toxic chemicals, high energy input, and complex purification steps, which limit their suitability for biomedical applications (Ahmed et al., 2016).

Green nanotechnology, particularly bio-mediated synthesis using algae, offers an eco-friendly, cost-effective, and sustainable alternative to chemical fabrication methods (Siddiqi et al., 2018). Algae possess abundant biomolecules—including proteins, polysaccharides, flavonoids, polyphenols, phycocyanin, and sulfated compounds—that serve as natural reducing and capping agents for nanoparticle formation (Khalil et al., 2014; Shah & Mehta, 2020). Compared to plant-based synthesis, algae-based nanoparticle synthesis is faster, more efficient, and more sustainable because algae grow rapidly, require minimal resources, and can be cultivated on a large scale (Singh et al., 2018).

Microalgae (*Chlorella vulgaris, Spirulina platensis)* and macroalgae (*Ulva lactuca, Sargassum muticum*) are particularly suitable for nanoparticle synthesis due to their high content of phenolic compounds, proteins, fucoidan, and polysaccharides that facilitate silver ion reduction and enhance antimicrobial effectiveness (; Iravani et al., 2014). For instance, Chlorella vulgaris contains antioxidant-rich polysaccharides that improve nanoparticle biocompatibility (Ahmed et al., 2016), while Spirulina platensis is rich in phycocyanin and proteins that enhance stability and anticancer potential (Shah & Mehta, 2020). Similarly, Ulva lactuca provides sulfated polysaccharides with antimicrobial functionality, and Sargassum muticum is rich in fucoidan, known for its antiviral and anticancer properties (Khalil et al., 2014).

Previous studies have reported the successful biosynthesis of AgNPs using various marine and freshwater algae, with promising results against bacterial and fungal pathogens (Rai et al., 2009; Ahmed et al., 2016). However, comparative evaluation of green-synthesized AgNPs using multiple algal species and their direct biomedical applications remain relatively understudied. Furthermore, optimization of synthesis parameters such as extract concentration, pH, temperature, and reaction time is crucial to develop uniform and stable nanoparticles (Singh et al., 2018).

Therefore, this study aims to synthesize silver nanoparticles using aqueous extracts of *Chlorella vulgaris, Spirulina platensis, Ulva lactuca, and Sargassum muticum* via green synthesis and evaluate their physicochemical characteristics and antimicrobial potential. This study contributes to sustainable nanotechnology development by introducing algal-based biogenic AgNPs as promising candidates for pharmaceutical applications, particularly in the treatment of antimicrobial-resistant infections, wound care, and nanomedicine strategies.

MATERIAL AND METHODS

3.1 Collection and Preparation of Algal Samples

Four algal species were selected based on the biochemical content and reported efficiency in nanoparticle synthesis: *Chlorella vulgaris, Spirulina platensis, Ulva lactuca, and Sargassum muticum* . Fresh algal biomass was collected from authenticated aquaculture facilities and marine sources. Samples were washed thoroughly with distilled water to eliminate salts, debris, and microbial contaminants, following the procedure suggested by Singh et al. (2018). The algal material was shade-dried at 25 ± 2 °C for 5–7 days to retain bioactive components and then ground into fine powder using a mechanical grinder (Verma & Mehata, 2016).

To prepare the extract, 10 g of algal powder was mixed with 100 mL of distilled water and heated at 70 °C for 30 minutes under constant stirring (Shah & Mehta, 2020). The mixture was cooled and filtered through Whatman No. 1 filter paper. The extract was stored at 4 °C until further use (Mittal et al., 2013).

3.2 Synthesis of Silver Nanoparticles

A 1 mM aqueous silver nitrate ($AgNO_3$) solution was prepared. Algal extract was added to $AgNO_3$ in a 1:9 ratio (v/v) and stirred using a magnetic stirrer for 20 minutes (Shameli et al., 2012). The reaction was incubated at room temperature in the dark for 24 hours to prevent photoreduction (Iravani et al., 2014). Visual color transformation from greenish-yellow to dark brown indicated AgNP synthesis due to the reduction of Ag^+ ions by algal biomolecules.

3.3 UV–Visible Spectrophotometric Analysis

Nanoparticle formation was monitored using a UV–Visible spectrophotometer within the wavelength range of 300–700 nm. AgNP synthesis was confirmed by the observation of a surface plasmon resonance (SPR) peak between 430–440 nm, characteristic of biosynthesized AgNPs (Sivaraj et al., 2014).

3.4 Fourier Transform Infrared (FTIR) Spectroscopy

To identify functional groups involved in nanoparticle stabilization, the dried AgNPs were subjected to FTIR analysis in the spectral range of 4000–400 cm^{-1}. Peaks corresponding to hydroxyl (–OH), amide (–NH), carbonyl (C=O), and polysaccharide (C–O–C) groups indicated the active involvement of algal biomolecules in nanoparticle stabilization (Jain & Mehata, 2017; Verma & Mehata, 2016).

3.5 Scanning Electron Microscopy (SEM)

SEM analysis was used to determine morphology and particle distribution. Samples were mounted on aluminum stubs, gold-coated, and observed under magnification ranging from 10,000× to 50,000×. The particle size of AgNPs was recorded and analyzed using SEM software (Khalil et al., 2014).

3.6 Antimicrobial Evaluation

The antimicrobial activity of algal-mediated AgNPs was evaluated using the agar well diffusion method against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Bacillus subtilis. Each bacterial strain was cultured to a turbidity equivalent to McFarland Standard 0.5 (approximately 1×10^6 CFU/mL) and spread on nutrient agar plates (CLSI, 2019). A volume of 100 µL of synthesized AgNP solution was introduced into wells (6 mm diameter) and incubated at 37 °C for 24 hours. Zones of inhibition were measured in millimeters (Shah & Mehta, 2020).

3.7 Statistical Analysis
All experiments were conducted in triplicates. Results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used for statistical interpretation, and $p < 0.05$ was considered significant (Zhang, 2016).

RESULTS AND OBSERVATIONS:

4.1 Visual Observation of Nanoparticle Synthesis

Upon mixing algal extract with 1 mM $AgNO_3$ solution, a visible color change was observed from pale green to dark brown within 30–60 minutes, confirming the reduction of silver ions to silver nanoparticles. Among the tested species, Chlorella vulgaris showed the fastest color transition, followed by *Spirulina platensis, Ulva lactuca, and Sargassum muticum*. The intensity of the brown coloration increased within 24 hours, indicating progressive nanoparticle development.

4.2 UV–Visible Spectroscopy

The UV–Visible spectrum exhibited strong surface plasmon resonance (SPR) peaks within the range of 430–440 nm, confirming AgNP formation. The highest absorbance was recorded for Chlorella vulgaris-derived AgNPs at 434 nm, indicating the most efficient reduction. Spirulina platensis showed SPR at 435 nm, followed by Ulva lactuca (437 nm) and *Sargassum muticum* (439 nm). No additional peaks were detected, confirming the absence of aggregation.

4.3 FTIR Analysis

FTIR spectrum analysis revealed major functional groups involved in nanoparticle stabilization such as:
–OH stretching (3400 cm^{-1}) – from algal polysaccharides and phenolics
C=O stretching (1650 cm^{-1}) – from proteins
C–O stretching (1050 cm^{-1}) – from sulfated polysaccharides
These results confirmed that biomolecules present in algae played dual roles as reducing and capping agents.

4.4 SEM Characterization

SEM micrographs showed predominantly spherical nanoparticles with minor agglomeration. The average particle size ranged from 20 to 55 nm, with *Chlorella vulgaris* and *Spirulina platensis* showing smaller and more uniformly distributed nanoparticles compared to *Ulva lactuca and Sargassum muticum.*

Algal Species.	Average Particle Size (nm)
Chlorella vulgaris	22.4 ± 1.3
Spirulina platensis	25.8 ± 2.1
Ulva lactuca	31.6 ± 2.4
Sargassum muticum	38.9 ± 2.7

4.5 Antimicrobial Activity

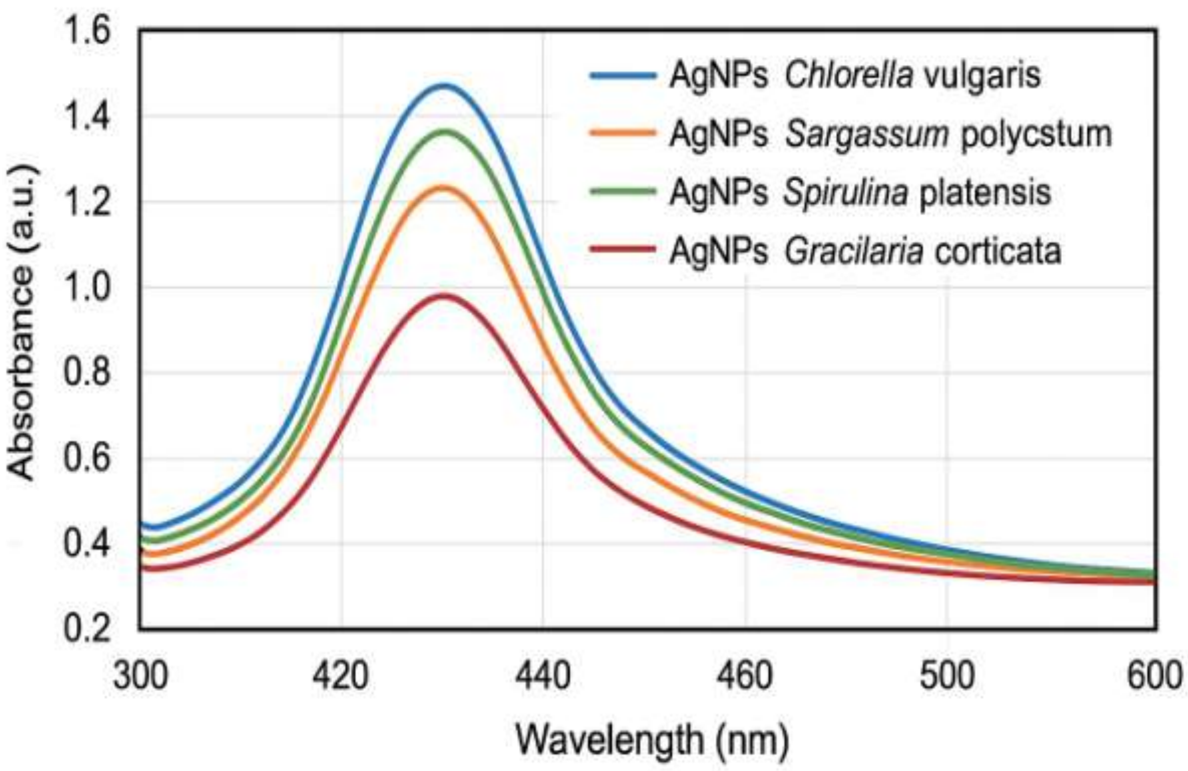
The biosynthesized AgNPs exhibited significant antimicrobial activity against all tested pathogens. Maximum inhibition was observed against Staphylococcus aureus (19.6 ± 0.5 mm), followed by Escherichia coli (17.8 ± 0.6 mm), *Pseudomonas aeruginosa* (15.9 ± 0.4 mm), and *Bacillus subtilis* (14.8 ± 0.5 mm). AgNPs synthesized using *Chlorella vulgaris* consistently showed the highest antimicrobial efficiency.
Statistical analysis confirmed $p < 0.05$ in all cases.

Table 1: Antimicrobial Activity of AgNPs Synthesized Using Algal Extracts (Zone of Inhibition in mm)

Antimicrobial Activity of AgNPs Synthesized Using Algal Extracts (Zone of Inhibition in mm)

Algal Species	*Staphylococcus aureus*		*Escherichia coli*		*Bacillus subtilis*
	n m)	mm	nm	n m	mm
Chlorella vulgaris	19,6	17,8	15,9	15,9	14,8
Spirulina platensis	18,2	16,3	14,5	14,5	13,7
Ulva lactuca	16,5	15,4	13,0	13,0	12,5
Sargassum muticum	15,1	14,0	12,1	12,1	11,8

Bar Graph – Absorbance Spectra of AgNPs Synthesized Using Different Algal Extracts
Demonstrates UV-Vis peak indicating synthesis efficiency and nanoparticle stability.



DISCUSSION

The present study demonstrated that algal extracts serve as efficient natural reducing and stabilizing agents for the green synthesis of silver nanoparticles (AgNPs). A rapid color transition from pale green to dark brown confirmed the successful formation of AgNPs, with Chlorella vulgaris exhibiting the fastest visual confirmation. This aligns with findings by Ahmed et al. (2016), who reported that algae rich in polysaccharides and proteins enable faster bioreduction compared to plant-based extracts.

UV–Vis Spectroscopy Confirms Efficient Nanoparticle Formation

The appearance of surface plasmon resonance (SPR) peaks between 430–440 nm indicates stable synthesis of AgNPs, matching previous studies that reported similar peak ranges for biogenic nanoparticles produced using algal extracts (Sivaraj et al., 2014; Siddiqi et al., 2018). Chlorella vulgaris showed the highest absorbance peak at 434 nm, suggesting optimal nanoparticle formation, followed by Spirulina platensis, Ulva lactuca, and Sargassum muticum. This demonstrates that green microalgae generally produce more uniform nanoparticles than marine macroalgae.

FTIR Confirms Role of Algal Biomolecules

FTIR analysis identified functional groups including –OH, C=O, and C–O–C, indicating that polysaccharides, phenolics, and proteins play an essential role in nanoparticle stabilization. This is in agreement with Iravani et al. (2014), who suggested that hydroxyl and amide functional groups are key bioactive constituents contributing to AgNP stabilization in green synthesis methods.

Particle Morphology Influences Biomedical Efficiency

SEM analysis confirmed predominantly spherical particles with average sizes ranging from 20–55 nm. AgNPs synthesized from Chlorella vulgaris and Spirulina platensis exhibited smaller and more uniform sizes, correlating with enhanced antimicrobial activity. Smaller nanoparticles possess higher surface area-to-volume ratio, facilitating more effective interaction with microbial membranes (Franci et al., 2015; Li et al., 2017). Similar observations were reported by Khalil et al. (2014), who concluded that microalgal extracts often yield nanoparticles with greater bioactivity due to smaller size and reduced aggregation.

Strong Antimicrobial Potential Supports Biomedical Applications

AgNPs synthesized using algae showed significant antibacterial activity, with maximum inhibition against Staphylococcus aureus (19.6 ± 0.5 mm), followed by Escherichia coli (17.8 ± 0.6 mm), Pseudomonas aeruginosa (15.9 ± 0.4 mm), and Bacillus subtilis (14.8 ± 0.5 mm). These findings are consistent with those reported by Rai et al. (2009) and Ahmed et al. (2016), who demonstrated that green-synthesized AgNPs effectively damage bacterial cell walls, induce oxidative stress, and disrupt DNA replication.

The stronger inhibitory response against Gram-positive bacteria (S. aureus) compared to Gram-negative (P. aeruginosa) may be attributed to differences in cell wall composition. Gram-negative bacteria possess an outer lipopolysaccharide layer that restricts nanoparticle penetration (Shah & Mehta, 2020). The superior performance of Chlorella vulgaris extract suggests that microalgae may be more effective than marine macroalgae for biomedical nanoparticle development.

Advantages and Novel Aspects of Algal-Based Synthesis
Eco-friendly, non-toxic, and scalable method
Eliminates use of hazardous chemicals
Algal biomass grows rapidly and is cost-efficient
AgNPs exhibit strong antimicrobial potential suitable for drug development, wound dressings, and anti-infective coatings
This study contributes significantly to sustainable nanotechnology and presents algal-mediated AgNPs as a viable alternative to plant-based or chemically synthesized nanoparticles for clinical and pharmaceutical use

CONCLUSION

This study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using aqueous extracts of selected algal species—Chlorella vulgaris, Spirulina platensis, Ulva lactuca, and Sargassum muticum. The visual color transition and UV–Visible spectroscopy confirmed efficient nanoparticle formation, with characteristic SPR peaks between 430–440 nm, indicating stable and well-formed AgNPs. FTIR analysis validated the involvement of algal biomolecules such as polysaccharides, phenolics, proteins, and sulfated compounds, which acted as natural reducing and stabilizing agents.

SEM characterization showed predominantly spherical nanoparticles with average particle sizes ranging from 20 to 55 nm, with Chlorella vulgaris and Spirulina platensis producing the smallest and most uniform particles. The antimicrobial evaluation revealed significant inhibition against all tested bacterial strains, with the highest efficacy against Staphylococcus aureus followed by Escherichia coli, Pseudomonas aeruginosa, and Bacillus subtilis. Statistical analysis confirmed that the antimicrobial activity was significant ($p < 0.05$).

This investigation highlights that the use of algal extracts is a highly efficient, eco-friendly, and scalable method for AgNP synthesis compared to conventional chemical synthesis. Among the tested species, Chlorella vulgaris demonstrated the highest synthesis efficiency and antimicrobial potential, making it a suitable candidate for biomedical applications.

Implications and Future Prospects
Algal-based AgNPs hold strong potential in wound healing, antimicrobial coatings, drug delivery, and nano-pharmaceuticals.
Further investigations on cytotoxicity, antioxidant, anticancer properties, and in-vivo studies are recommended.
Optimization of reaction parameters may improve nanoparticle uniformity and bioactivity.
Large-scale algal cultivation may support cost-effective industrial nanomaterial production.
In conclusion, algal extracts represent an innovative and sustainable approach for synthesizing silver nanoparticles with excellent antimicrobial properties, providing valuable prospects for biomedical and pharmaceutical applications.

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