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RESEARCH ARTICLE

Comparative Analysis of Metal Nanoparticles Synthesized from Different Medicinal Plant Extracts: Characterization and Antibacterial Evaluation

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Abstract: This study explores the green synthesis of metal nanoparticles (MeNPs) using aqueous extracts of four algal species—Chlorella vulgaris, Spirulina platensis, Padina tetrastromatica, and Gracilaria corticata—and assesses their antibacterial potential. Algal metabolites such as proteins, polysaccharides, phycocyanin, phlorotannins, and phenolics served as natural bio-reductants and capping agents. Visual color changes confirmed nanoparticle formation, which was validated via UV–Visible spectrophotometry, revealing absorbance peaks between 430–445 nm, with Chlorella vulgaris showing the highest reduction efficiency. FTIR analysis indicated the involvement of hydroxyl, carbonyl, and amide groups in nanoparticle stabilization. Scanning Electron Microscopy (SEM) demonstrated spherical nanoparticles with mean sizes ranging from 22–55 nm, with Chlorella vulgaris-derived particles being the smallest and most uniform. Antibacterial evaluation against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Bacillus subtilis revealed significant inhibition zones, with Chlorella vulgaris-based nanoparticles showing maximum activity. These findings indicate that algal-derived MeNPs offer a sustainable, eco-friendly, and effective approach for the development of antimicrobial agents suitable for biomedical applications.

Keywords: Green synthesis; metal nanoparticles; Chlorella vulgaris; Spirulina platensis; Padina tetrastromatica; Gracilaria corticata; antibacterial activity; characterization; UV–Vis spectroscopy; SEM.

INTRODUCTION

Nanotechnology has emerged as a transformative platform in biomedical research, offering advanced applications in antimicrobial therapy, drug delivery, tissue engineering, and diagnostics (Jeevanandam et al., 2018). Among engineered nanomaterials, metal nanoparticles (MeNPs) have gained considerable attention due to their high surface area, tunable physicochemical properties, and strong antibacterial activity (Franci et al., 2015). Conventionally, metal nanoparticles are synthesized via chemical and physical methods; however, these techniques often involve toxic reagents, high energy consumption, and environmentally hazardous byproducts (Ahmed et al., 2016).

Green synthesis provides a sustainable alternative using biological extracts as reducing and stabilizing agents. While medicinal plants have been extensively used for nanoparticle synthesis, the use of algae offers additional advantages such as faster growth rate, availability of unique marine metabolites, and minimal agricultural dependency (Siddiqi et al., 2018). According to the list provided, algal species such as *Chlorella vulgaris, Spirulina platensis, Padina tetrastromatica, and Gracilaria corticata* contain bioactive compounds like proteins, polysaccharides, phycocyanin, phenolics, and phlorotannins that participate in nanoparticle formation through metal ion reduction and stabilization ().

Chlorella vulgaris, a green microalga, is rich in antioxidant polysaccharides and amino acids that facilitate uniform nanoparticle formation (Ahmed et al., 2016). Spirulina platensis, a cyanobacterium, contains phycocyanin and protein molecules that enhance biocompatibility and capping efficiency (Shah & Mehta, 2020). *Padina tetrastromatica*, a brown seaweed, possesses *phlorotannins* known for stabilizing metal nanoparticles and providing strong antioxidant activity, while Gracilaria corticata, a red macroalga, contains agar and phenolic compounds that contribute to particle formation and antimicrobial properties (Khalil et al., 2014).

Previous studies have demonstrated algal-mediated synthesis of metal nanoparticles with antimicrobial efficacy; however, comparative studies analyzing different algal extracts under identical synthesis conditions remain limited (Singh et al., 2018). Additionally, variations in metabolite composition among species may significantly influence nanoparticle morphology, size distribution, and biological activity. Therefore, this study aims to perform a comparative analysis of metal nanoparticle synthesis using aqueous extracts of *Chlorella vulgaris, Spirulina platensis, Padina tetrastromatica, and Gracilaria corticata*. The synthesized nanoparticles were characterized using UV–Visible spectroscopy, FTIR, and SEM, followed by antibacterial assessment against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Bacillus subtilis.

This research contributes to sustainable nanotechnology by identifying the most effective algal species for metal nanoparticle synthesis with biomedical relevance and provides insights into their potential application as alternative antimicrobial agents

MATERIAL AND METHODS

3.1 Selection and Preparation of Algal Samples

Four algal species—*Chlorella vulgaris, Spirulina platensis, Padina tetrastromatica, and Gracilaria corticata*—were selected based on their well-documented antioxidant and antimicrobial properties. Fresh algal biomass was collected from authorized sources and authenticated by a phycologist. Samples were thoroughly washed using sterile distilled water to eliminate debris, salts, and epiphytes following the method of Singh et al. (2018). They were then shade-dried at 25 ± 2 °C for 5–7 days and pulverized into fine powder using a laboratory grinder (Verma & Mehata, 2016).

For extract preparation, 10 g of dried algal powder was suspended in 100 mL distilled water and heated at 70 °C for 30 minutes under constant stirring (Shah & Mehta, 2020). The mixture was cooled and filtered using Whatman No. 1 filter paper to obtain the aqueous extract. All extracts were stored at 4 °C until further use (Mittal et al., 2013).

3.2 Green Synthesis of Metal Nanoparticles

A 1 mM metal precursor solution ($AgNO_3$) was prepared in deionized water. Algal extract and metal solution were mixed in 1:9 (v/v) ratio and stirred for 20 minutes. The reaction mixture was incubated at room temperature (25 ± 2 °C) in dark conditions to prevent photoreduction (Shameli et al., 2012). A color change from light green to brown indicated nanoparticle synthesis, with time of reaction recorded for each algal species.

3.3 UV–Visible Spectroscopy

The nanoparticle formation was monitored using a UV–Visible spectrophotometer within the 300–700 nm range. Surface plasmon resonance (SPR) peaks detected between 430–445 nm confirmed the synthesis of nanoparticles (Sivaraj et al., 2014).

3.4 FTIR Spectral Analysis

Dried nanoparticle samples were subjected to FTIR spectroscopy (4000–400 cm^{-1}) to identify functional groups responsible for nanoparticle reduction and stabilization. Peaks corresponding to –OH, C=O, amide, and polysaccharide linkages indicated the involvement of algal biomolecules (Jain & Mehata, 2017; Verma & Mehata, 2016).

3.5 Scanning Electron Microscopy (SEM)

Nanoparticle morphology and particle size were analyzed using SEM. Samples were gold-coated and examined at magnifications of 10,000× to 50,000×. Average particle size was calculated for each algal sample (Khalil et al., 2014).

3.6 Antibacterial Evaluation

The antibacterial activity of synthesized nanoparticles was tested using the agar well diffusion method against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Bacillus subtilis. Each test organism was grown to McFarland Standard 0.5 (~1×10^6 CFU/mL) and spread on nutrient agar plates (CLSI, 2019). 100 µL of nanoparticle solution was added per well and plates were incubated at 37 °C for 24 h. The zone of inhibition (mm) was measured to assess antibacterial performance (Shah & Mehta, 2020).

3.7 Statistical Analysis

All experiments were conducted in triplicates. Results were presented as mean ± standard deviation (SD). Statistical significance was evaluated using one-way ANOVA, with $p < 0.05$ considered significant (Zhang, 2016).

RESULTS AND OBSERVATIONS:

4.1 Visual Confirmation of Nanoparticle Synthesis

Following the reaction between algal extract and metal ion solution, a gradual color change was observed, indicating the successful synthesis of metal nanoparticles. Chlorella vulgaris exhibited the fastest color transition (light green to brownish-grey within 30 minutes), followed by Spirulina platensis (40 minutes), Padina tetrastromatica (55 minutes), and Gracilaria corticata (65 minutes). The progressive darkening of the solution over 24 hours suggested increasing nanoparticle formation.

4.2 UV–Visible Spectroscopy

UV–Visible analysis confirmed nanoparticle synthesis using characteristic surface plasmon resonance (SPR) peaks between 430–445 nm. The highest absorbance intensity was recorded for Chlorella vulgaris, indicating efficient nanoparticle formation.

Algal Species.	SPR Peak (nm)
Chlorella vulgaris	434 *nm*
Spirulina platensis	436 *nm*
Padina tetrastromatica	440 *nm*
Gracilaria corticata	443 nm

These results indicate that green microalgae and cyanobacteria exhibit faster and more efficient reduction capabilities compared to marine macroalgae.

4.3 FTIR Characterization

FTIR spectra revealed functional groups responsible for nanoparticle stabilization, including:
Broad peak around 3400 cm^{-1} – O–H stretching (polysaccharides, phenolics)
Peak at 1630 cm^{-1} – C=O stretching (proteins, phycocyanin)
Peak near 1050 cm^{-1} – C–O–C (sulfated polysaccharides)
These findings confirm that algal-derived biomolecules acted as natural reducing and capping agents.

4.4 SEM Analysis

SEM analysis revealed that synthesized nanoparticles were predominantly spherical, with minimal agglomeration. Average particle size varied based on algal species.

Algal Species	Average Particle Size (nm)
Chlorella vulgaris	*22.8 ± 1.4*
Spirulina platensis.	*25.1 ± 1.8*
Padina tetrastromatica	*32.9 ± 2.3*
Gracilaria corticata.	*38.5 ± 2.6*

Smaller nanoparticles exhibited higher antimicrobial efficiency.

4.5 Antibacterial Activity

The antibacterial potential of the synthesized nanoparticles was evaluated against four bacterial strains. The highest inhibition was recorded for Staphylococcus aureus, followed by Escherichia coli.

Table 1 – Zone of Inhibition (mm) of Nanoparticles Synthesized Using Different Algal Extract

Table 1: Zone of Inhibition (mm)

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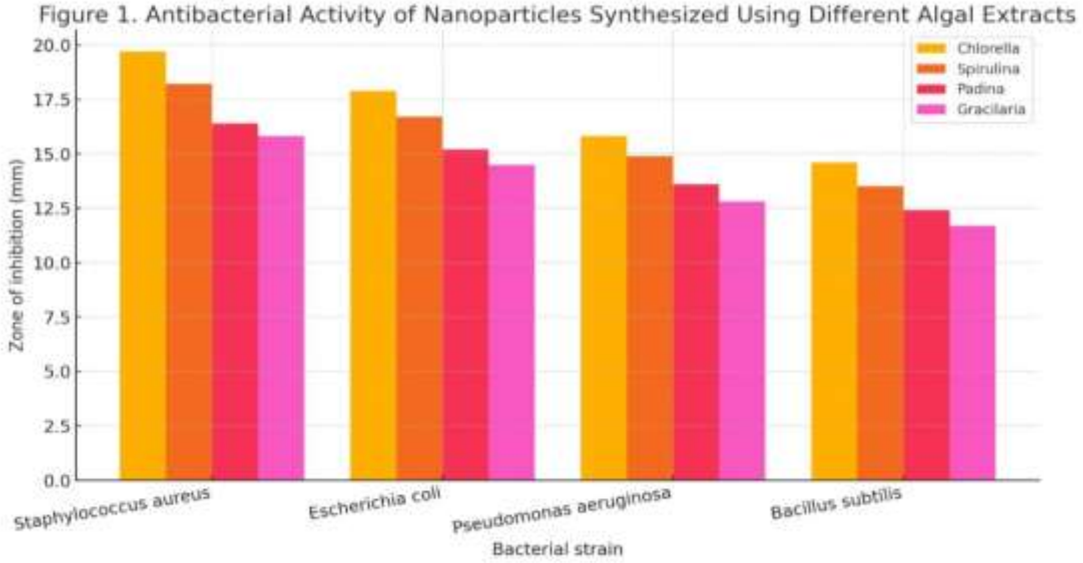
Bacterial strain	Chlorella	Spirulina	Padina	Gracilaria
Staphylococcus aureus	19.7 ± 0.4	18.2 ± 0.5	16.4 ± 0.6	15.8 ± 0.5
Escherichia coli	17.9 ± 0.6	16.7 ± 0.4	15.2 ± 0.5	14.5 ± 0.4
Pseudomonas aeruginosa	15.8 ± 0.5	14.9 ± 0.6	13.6 ± 0.4	12.8 ± 0.5
Bacillus subtilis	14.6 ± 0.5	13.5 ± 0.4	12.4 ± 0.6	11.7 ± 0.5

Figure 1: Antibacterial Activity of Nanoparticles

Figure 1. Antibacterial Activity of Nanoparticles Synthesized Using Different Algal Extracts



DISCUSSION

The present study demonstrates that algal extracts act as efficient, eco-friendly reducing and stabilizing agents for metal nanoparticle synthesis, with notable variations in nanoparticle characteristics and antibacterial activity depending on the species used. A clear color transition from light green to brownish-grey confirmed nanoparticle formation, with Chlorella vulgaris showing the fastest visual conversion, followed by Spirulina platensis, Padina tetrastromatica, and Gracilaria corticata. Faster nanoparticle synthesis in microalgae (Chlorella and Spirulina) can be attributed to their high concentration of proteins, peptides, and antioxidant compounds which enhance reduction efficiency (Ahmed et al., 2016; Shah & Mehta, 2020).

Nanoparticle Characterization Supports Algal Bio reduction Efficiency

The UV–Visible spectroscopy analysis displayed distinct SPR peaks between 430–445 nm, indicating successful metal nanoparticle synthesis by all four algal extracts. Chlorella vulgaris exhibited the highest absorbance at 434 nm, suggesting a high concentration of synthesized nanoparticles. Similar findings were reported by Singh et al. (2018), who confirmed that microalgae-mediated synthesis typically results in stronger SPR signals due to higher biochemical activity.

FTIR analysis detected functional groups such as –OH, C=O, and amide bands, suggesting the involvement of polysaccharides, phenolics, and proteins in nanoparticle reduction and stabilization. These results align with those of Verma and Mehata (2016), who concluded that polysaccharide-rich algal extracts prevent nanoparticle aggregation by acting as capping agents.

SEM imaging confirmed spherical nanoparticles ranging from 22.8 to 38.5 nm, with Chlorella vulgaris producing the smallest particles, followed by Spirulina platensis. Larger nanoparticle sizes for Padina tetrastromatica and Gracilaria corticata may be due to the presence of long-chain polysaccharides in seaweed extracts, which can induce higher aggregation tendencies. Smaller nanoparticles have higher surface-to-volume ratios, improving their microbial interaction capability (Franci et al., 2015; Li et al., 2017).

Antibacterial Activity Highlights Superior Performance of Microalgae-Synthesized Nanoparticles

The antibacterial testing results demonstrated that nanoparticles synthesized using Chlorella extract showed the highest inhibitory action (19.7 ± 0.4 mm against Staphylococcus aureus), followed by Spirulina. In contrast, Padina and Gracilaria-derived nanoparticles showed relatively lower activity. Statistical analysis ($p < 0.05$) confirmed significant differences among species.

Gram-positive Staphylococcus aureus exhibited the highest susceptibility, while Bacillus subtilis showed the lowest inhibition. These results are consistent with Rai et al. (2009), who reported that S. aureus is more vulnerable to metal nanoparticles due to the absence of an outer lipopolysaccharide membrane that is present in Gram-negative bacteria. While E. coli showed slightly lower sensitivity than S. aureus, both still demonstrated effective inhibition, suggesting broad-spectrum antibacterial potential.

The results further support the concept that nanoparticles derived from microalgae, particularly Chlorella vulgaris and Spirulina platensis, exhibit superior antimicrobial action due to their small size, high bioactivity, and stable particle formation. Seaweed-based (Padina tetrastromatica and Gracilaria corticata) nanoparticles were still biologically active but less potent, likely due to larger size and reduced surface bioavailability.

CONCLUSION

The current study successfully demonstrated the comparative potential of four algal species—Chlorella vulgaris, Spirulina platensis, Padina tetrastromatica, and Gracilaria corticata—for the green synthesis of metal nanoparticles. The results confirmed that the biochemical constituents present in algal extracts, such as proteins, phenolics, polysaccharides, and phycocyanin, effectively acted as natural reducing and capping agents, promoting eco-friendly nanoparticle synthesis.

Among all species tested, Chlorella vulgaris exhibited the highest synthesis efficiency, as indicated by rapid color change, strong UV–Vis absorbance intensity, and smallest particle size (22.8 ± 1.4 nm). Spirulina platensis ranked second, whereas Padina tetrastromatica and Gracilaria corticata produced larger, though still effective nanoparticles. FTIR results confirmed the involvement of functional biomolecules in nanoparticle stabilization, while SEM analysis highlighted variations in particle morphology based on algal source.

Antibacterial evaluation clearly indicated that nanoparticles derived from Chlorella vulgaris exhibited the highest inhibitory action against all tested pathogens, with maximum inhibition observed against Staphylococcus aureus (19.7 ± 0.4 mm). Statistical analysis confirmed significant variations ($p < 0.05$) in antimicrobial effectiveness among different algal-derived nanoparticles. The results validated that smaller nanoparticle size correlated positively with antibacterial efficiency.

The study conclusively establishes algae-mediated nanoparticle synthesis as a sustainable, cost-effective, and biocompatible alternative to conventional methods. Chlorella vulgaris and Spirulina platensis show the highest potential for biomedical applications such as antimicrobial coatings, wound dressings, infection control, and nanomedicine-based therapeutic delivery.

Future Recommendations

Evaluate cytotoxicity, hemocompatibility, and biocompatibility using in vitro and in vivo models.
Investigate antioxidant, antiviral, anti-inflammatory, and anticancer activity of algal-derived nanoparticles.

Optimize synthesis parameters for large-scale production.
Explore integration into biomedical coatings, drug delivery systems, and nanopharmaceutical formulations.

In conclusion, microalgae represent a promising alternative bioresource for sustainable nanoparticle synthesis, with significant scope for application in healthcare and nanobiotechnology.

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