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# Green Synthesis of Silver Nanoparticles using *Spirulina maxima* and their Antibacterial Activity against *Staphylococcus* spp.

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***Abstract***— *Antimicrobial resistance (AMR) among bacterial pathogens has emerged as a serious global concern, posing significant challenges to both human and animal health. The continuous rise in resistant strains has reduced the effectiveness of conventional antibiotics, necessitating the development of alternative and sustainable antimicrobial strategies. In this context, nanotechnology has gained considerable attention due to its potential applications in biomedical and veterinary sciences. The present study aimed to synthesize silver nanoparticles (AgNPs) using an aqueous extract of Spirulina maxima through an eco-friendly green synthesis approach and to evaluate their antibacterial efficacy. The biosynthesis of silver nanoparticles was indicated by a distinct colour change from pale yellow to dark brown due to the reduction of silver ions. The synthesized nanoparticles were characterized using dynamic light scattering (DLS) to determine particle size distribution and stability. The antibacterial activity of the synthesized AgNPs was assessed against Staphylococcus spp. using the agar well diffusion method at different concentrations (20, 40, 60, and 80 µL). The results revealed a clear concentration-dependent increase in antibacterial activity, with zones of inhibition measuring 8 mm, 10 mm, 12 mm, and 14 mm, respectively. The standard antibiotic ciprofloxacin exhibited a zone of inhibition of 16 mm, whereas the algal extract alone showed negligible activity. The findings of this study demonstrate that Spirulina maxima-mediated silver nanoparticles possess significant antibacterial potential and could serve as an eco-friendly and sustainable alternative to conventional antimicrobial agents. Further investigations are warranted to explore their mechanisms of action and practical applications in veterinary and biomedical fields.*

***Keywords— Silver nanoparticles, Spirulina maxima, Green synthesis, Antibacterial activity, Antimicrobial resistance, Nanotechnology.***

## I. INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most critical global health challenges affecting both human and animal populations. The indiscriminate and excessive use of antibiotics has accelerated the development of resistant bacterial strains, leading to reduced drug efficacy, prolonged illness, and increased mortality (World Health Organization, 2014; Ventola, 2015). This alarming situation necessitates the exploration of alternative and sustainable antimicrobial strategies.

Conventional antibiotics act by targeting specific cellular structures or metabolic pathways of microorganisms. However, bacteria have evolved multiple resistance mechanisms, including enzymatic degradation of antibiotics, alteration of target sites, decreased permeability, and active efflux systems (Ventola, 2015). The rapid emergence of resistance, coupled with the slow discovery of new antibiotics, has intensified the need for innovative approaches to combat microbial infections.

Nanotechnology has recently gained significant attention as a promising field in biomedical and veterinary sciences. Among various nanomaterials, silver nanoparticles (AgNPs) have been extensively studied due to their broad-spectrum antimicrobial properties (Rai et al., 2009; Franci et al., 2015). These nanoparticles possess unique physicochemical characteristics such as high surface area-to-volume ratio, enhanced reactivity, and nanoscale size, enabling effective interaction with microbial cells.

Silver nanoparticles exhibit antimicrobial activity through multiple mechanisms, including disruption of cell membrane integrity, generation of reactive oxygen species, interaction with intracellular components, and inhibition of essential enzymes (Morones et al., 2005; Kim et al., 2007; Durán et al., 2016). These multiple modes of action reduce the likelihood of resistance development, making AgNPs a promising alternative to conventional antibiotics (Lara et al., 2011).

The synthesis method plays a crucial role in determining the biological activity and safety of nanoparticles. Conventional physical and chemical methods often involve toxic reagents, high energy requirements, and environmentally hazardous by-products (Iravani, 2011). In contrast, green synthesis using biological materials has emerged as an eco-friendly, cost-effective, and sustainable alternative (Ahmed et al., 2016; Singh et al., 2016).

Biological entities such as plants, algae, bacteria, and fungi contain a wide range of bioactive compounds that can act as reducing and stabilizing agents during nanoparticle synthesis. Among these, algae have gained particular attention due to their rich composition of proteins, polysaccharides, pigments, vitamins, and secondary metabolites (El-Rafie et al., 2014).

*Spirulina maxima*, a filamentous cyanobacterium, is widely recognized for its nutritional and medicinal properties. It contains various bioactive compounds that exhibit antioxidant, anti-inflammatory, immunomodulatory, and antimicrobial activities (Priyadarshini and Rath, 2012). The presence of diverse functional groups in *Spirulina maxima* makes it an ideal candidate for green synthesis of nanoparticles.

Several studies have demonstrated the successful synthesis of silver nanoparticles using biological systems and their antimicrobial efficacy (Ghosh et al., 2012). However, variations in biological sources and synthesis conditions can significantly influence nanoparticle properties and activity. Therefore, systematic studies focusing on specific biological materials are essential.

In this context, the present study was designed to synthesize silver nanoparticles using an aqueous extract of *Spirulina maxima* through a green synthesis approach and to evaluate their antibacterial activity. The study aims to highlight the potential of biologically synthesized nanoparticles as eco-friendly and effective alternatives to conventional antimicrobial agents.

## II. MATERIALS AND METHODS

### 2.1 Chemicals and Reagents:

Silver nitrate ($AgNO_3$) of analytical grade was procured and used as a precursor for the synthesis of silver nanoparticles. All chemicals and reagents used in the study were of standard analytical grade. Distilled water was used throughout the experimental procedures to ensure purity and avoid contamination.

### 2.2 Preparation of Aqueous Extract of *Spirulina maxima*:

Dried *Spirulina maxima* powder (1 g) was accurately weighed and mixed with 100 mL of distilled water. The mixture was gently heated at 60°C for 15 minutes to facilitate the extraction of bioactive compounds. After cooling to room temperature, the extract was filtered using Whatman No. 1 filter paper to remove particulate matter. The filtrate obtained was stored under refrigerated conditions (4°C) and used for further nanoparticle synthesis.

### 2.3 Biosynthesis of Silver Nanoparticles:

An aqueous solution of silver nitrate (1 mM) was prepared and mixed with the *Spirulina maxima* extract in a 1:9 ratio (extract:silver nitrate solution) under ambient conditions. The reaction mixture was incubated at room temperature for 24 hours and observed periodically for any visible changes.

The formation of silver nanoparticles was preliminarily confirmed by a colour change from pale yellow to dark brown, indicating the reduction of silver ions ($Ag^+$) into metallic silver ($Ag^0$) nanoparticles. The biomolecules present in the algal extract acted as reducing as well as stabilizing agents during the synthesis process (Ahmed et al., 2016; El-Rafie et al., 2014).

### 2.4 Characterization of Silver Nanoparticles:

#### 2.4.1 Visual Observation:

The initial confirmation of nanoparticle synthesis was carried out by observing the colour change in the reaction mixture.

### 2.4.2 Particle Size Analysis (DLS):

The size distribution and stability of the synthesized silver nanoparticles were analysed using Dynamic Light Scattering (DLS) on a Malvern Zetasizer at 25°C with an equilibration time of 120 seconds. Parameters such as hydrodynamic diameter, Z-average particle size, and polydispersity index (PDI) were recorded. The PDI values were used to assess the uniformity and stability of nanoparticles, where lower PDI values indicate better dispersion and homogeneity (Iravani, 2011; Singh et al., 2016).

### 2.5 Test Microorganisms:

The antibacterial activity of the biosynthesized silver nanoparticles was evaluated against selected bacterial strains, including *Staphylococcus* spp. Pure cultures of the test organisms were maintained on nutrient agar slants and subcultured periodically to ensure viability and purity.

### 2.6 Antibacterial Activity Assay:

The antibacterial activity of the synthesized silver nanoparticles was assessed using the agar well diffusion method as described by standard microbiological procedures.

Sterile nutrient agar plates were prepared and uniformly inoculated with freshly grown bacterial cultures using a sterile swab. Wells of uniform diameter (6 mm) were aseptically punched into the agar medium using a sterile cork borer.

Different volumes of silver nanoparticle suspension (20, 40, 60, and 80 µL) were introduced into the respective wells. Ciprofloxacin (10 µg) was used as a positive control, while *Spirulina maxima* aqueous extract alone served as a negative control.

The plates were incubated at 37°C for 24 hours. After incubation, the antibacterial activity was evaluated by measuring the diameter of the zones of inhibition (in mm) around each well. Increased zone size indicated higher antibacterial activity of the nanoparticles (Rai et al., 2009; Franci et al., 2015).

### 2.7 Statistical Analysis:

All experiments were conducted in triplicate to ensure reproducibility and accuracy. The results were expressed as mean ± standard deviation (SD). The data obtained were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to evaluate the significance of antibacterial activity at different concentrations. A p-value of less than 0.05 was considered statistically significant.

## III. RESULTS

### 3.1 Visual Observation of Silver Nanoparticle Formation:

The formation of silver nanoparticles was initially confirmed by visual observation of colour change in the reaction mixture. Upon mixing the aqueous extract of *Spirulina maxima* with silver nitrate solution, a gradual change in colour from pale yellow to dark brown was observed within 24 hours. This colour change indicates the reduction of silver ions ($Ag^{+}$) to metallic silver nanoparticles ($Ag^{0}$), confirming successful biosynthesis.

### 3.2 Particle Size Analysis:

Dynamic Light Scattering (DLS) analysis confirmed the formation of nanosized silver particles. The synthesized nanoparticles exhibited a Z-average particle size of 78.4 ± 3.2 nm and a hydrodynamic diameter ranging from 65 to 95 nm. The polydispersity index (PDI) was found to be 0.28 ± 0.05, indicating relatively uniform size distribution and good stability in the medium. The nanoscale size of the particles is considered crucial for enhanced antimicrobial activity due to increased surface area and improved interaction with microbial cells.

### 3.3 Antibacterial Activity of Silver Nanoparticles:

The antibacterial activity of *Spirulina maxima*-mediated silver nanoparticles was evaluated against *Staphylococcus* spp. using the agar well diffusion method. The results demonstrated a clear concentration-dependent increase in antibacterial activity.

**TABLE 1**
**ANTIBACTERIAL ACTIVITY OF *SPIRULINA MAXIMA*-MEDIATED SILVER NANOPARTICLES AGAINST *STAPHYLOCOCCUS* SPP**

| Treatment | Concentration (µL) | Zone of Inhibition (mm) (Mean ± SD) |
|---|---|---|
| Ciprofloxacin (Control) | — | 16.0 ± 0.5 |
| AgNPs | 20 | 8.0 ± 0.3 |
| AgNPs | 40 | 10.0 ± 0.4 |
| AgNPs | 60 | 12.0 ± 0.5 |
| AgNPs | 80 | 14.0 ± 0.4 |
| *Spirulina maxima* extract | — | Negligible |

The standard antibiotic ciprofloxacin exhibited the highest antibacterial activity with a zone of inhibition measuring 16.0 ± 0.5 mm. The synthesized silver nanoparticles showed a progressive increase in antibacterial activity with increasing concentration, producing zones of inhibition of 8.0 ± 0.3 mm, 10.0 ± 0.4 mm, 12.0 ± 0.5 mm, and 14.0 ± 0.4 mm at concentrations of 20, 40, 60, and 80 µL, respectively.

In contrast, the aqueous extract of *Spirulina maxima* alone showed negligible antibacterial activity. This indicates that the observed antimicrobial effect is primarily attributed to the synthesized silver nanoparticles rather than the algal extract.

Overall, the results clearly demonstrate a dose-dependent antibacterial activity of the biosynthesized silver nanoparticles, confirming their potential as effective antimicrobial agents. Statistical analysis revealed significant differences ($p < 0.05$) in zone of inhibition between different concentrations of AgNPs, with higher concentrations showing significantly greater antibacterial activity.

## IV. DISCUSSION

The present study successfully demonstrates the green synthesis of silver nanoparticles using an aqueous extract of *Spirulina maxima* and highlights their significant antibacterial potential. The initial confirmation of nanoparticle formation was indicated by a visible colour change from pale yellow to dark brown, which is a characteristic feature of silver nanoparticle synthesis due to surface plasmon resonance. This observation is consistent with earlier reports on biologically mediated nanoparticle synthesis (Ahmed et al., 2016; El-Rafie et al., 2014).

The use of *Spirulina maxima* as a biological reducing and stabilizing agent offers several advantages, as it contains a wide range of bioactive compounds such as proteins, polysaccharides, vitamins, and antioxidants. These biomolecules facilitate the reduction of silver ions and contribute to the stabilization of nanoparticles, thereby preventing aggregation. Similar findings have been reported in previous studies highlighting the role of biological systems in nanoparticle synthesis (Singh et al., 2016; Iravani, 2011).

Dynamic Light Scattering (DLS) analysis confirmed that the synthesized nanoparticles were within the nanoscale range (Z-average: 78.4 nm) and exhibited acceptable stability, as indicated by the polydispersity index of 0.28. The nanoscale size of the particles plays a crucial role in enhancing antimicrobial activity due to increased surface area and improved interaction with microbial cells. This observation is in agreement with earlier studies that have emphasized the importance of nanoparticle size and surface characteristics in determining biological activity (Franci et al., 2015).

The antibacterial activity of the synthesized silver nanoparticles demonstrated a clear concentration-dependent increase in the zone of inhibition against *Staphylococcus* spp. The gradual increase in antibacterial activity from 8 mm at 20 µL to 14 mm at 80 µL indicates enhanced interaction between nanoparticles and bacterial cells at higher concentrations. Although the activity was slightly lower than that of the standard antibiotic ciprofloxacin (16 mm), the results clearly indicate the strong antimicrobial potential of biosynthesized silver nanoparticles. Similar concentration-dependent antibacterial effects of silver nanoparticles have been reported in earlier studies (Rai et al., 2009; Ghosh et al., 2012).

The negligible antibacterial activity observed with *Spirulina maxima* extract alone suggests that the antimicrobial effect is primarily due to the synthesized silver nanoparticles rather than the algal extract itself. This further confirms the successful formation of functional nanoparticles with enhanced biological activity.

The antimicrobial mechanism of silver nanoparticles is complex and involves multiple pathways, including disruption of bacterial cell membrane integrity, generation of reactive oxygen species (ROS), interaction with intracellular components, and inhibition of essential enzymes. These multiple modes of action reduce the likelihood of resistance development among microorganisms (Morones et al., 2005; Kim et al., 2007; Durán et al., 2016). Additionally, silver nanoparticles are known to exhibit broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria (Lara et al., 2011).

The present study has certain limitations. Comprehensive characterization of the synthesized nanoparticles using techniques such as UV-Vis spectroscopy, transmission electron microscopy (TEM), and Fourier transform infrared spectroscopy (FTIR) would provide additional insights into the size, morphology, and functional groups involved in nanoparticle stabilization. Furthermore, determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) would help quantify the antimicrobial efficacy more precisely. Cytotoxicity evaluation and in vivo studies are also necessary to establish the safety and practical applicability of these nanoparticles.

Overall, the findings of the present study support the growing body of evidence that green-synthesized silver nanoparticles can serve as effective and eco-friendly antimicrobial agents. The use of *Spirulina maxima* not only enhances the sustainability of the synthesis process but also contributes to the biological activity of the nanoparticles.

## V. CONCLUSION

The present study successfully demonstrated the green synthesis of silver nanoparticles using an aqueous extract of *Spirulina maxima* and confirmed their significant antibacterial activity against *Staphylococcus* spp. The biosynthesized nanoparticles exhibited a clear concentration-dependent antimicrobial effect, with zones of inhibition increasing from 8 mm to 14 mm as the concentration increased from 20 µL to 80 µL. DLS analysis confirmed the nanoscale size (Z-average: 78.4 nm) and acceptable stability (PDI: 0.28) of the synthesized nanoparticles.

The eco-friendly and cost-effective nature of the green synthesis approach, combined with the biological properties of *Spirulina maxima*, makes this method highly suitable for sustainable nanoparticle production. Although the antibacterial activity of the synthesized nanoparticles was slightly lower than that of the standard antibiotic ciprofloxacin, the results clearly highlight their promising role as an alternative antimicrobial strategy, especially in the context of increasing antimicrobial resistance.

Overall, *Spirulina maxima*-mediated silver nanoparticles can be considered a potential candidate for future applications in veterinary and biomedical fields. Further studies focusing on detailed characterization using advanced techniques (UV-Vis, TEM, FTIR), determination of minimum inhibitory concentration, mechanism of action, toxicity evaluation, and in vivo applications are recommended to validate their practical utility.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper

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