



# Biogenic synthesis of silver nanoparticles using *Spirulina maxima* extract and their bactericidal activity

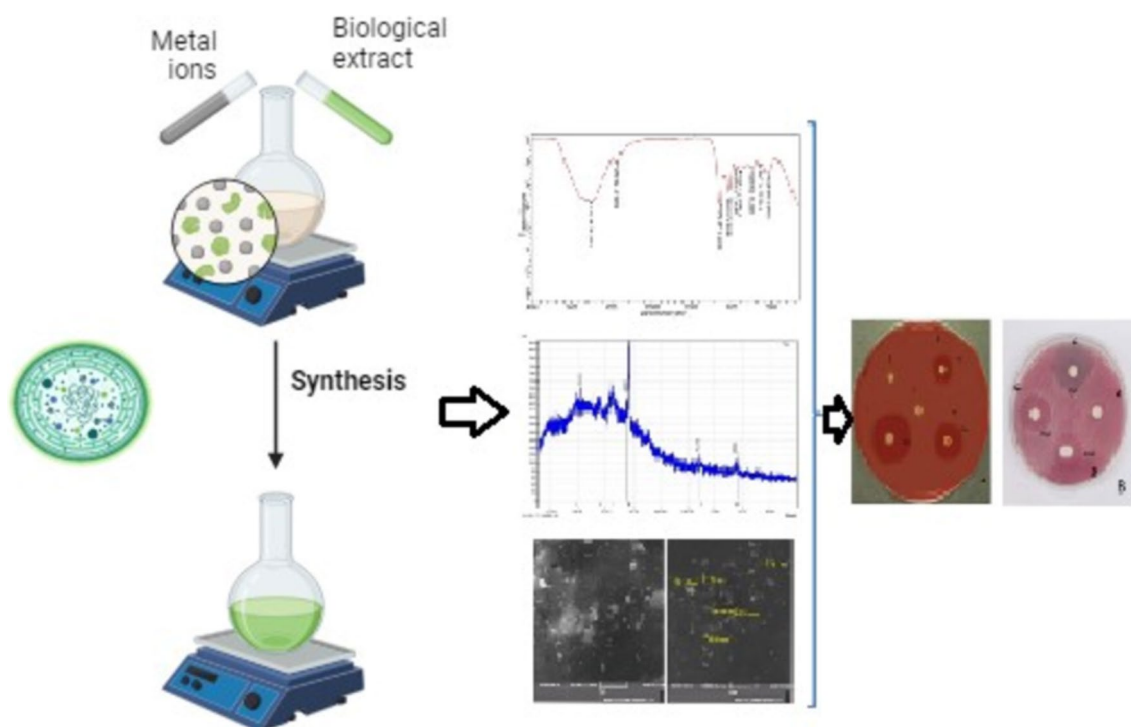
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## Abstract

This study reports a biogenic synthesis protocol for silver nanoparticles (AgNPs) utilizing *Spirulina maxima* extract as a natural reducing agent, offering an economically viable and environmentally sustainable approach. The biosynthesis pathway eliminates conventional chemical reagents while maximizing process sustainability. Comprehensive characterization of the synthesized nanostructures was performed using UV-spectrophotometry, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and X-ray diffraction (XRD) analysis. FTIR spectroscopic studies revealed the role of proteinaceous compounds and biomolecules in AgNP formation and stabilization. The biosynthesized AgNPs demonstrated notable antimicrobial efficacy against two pathogenic bacteria: *Enterococcus faecalis* and *Staphylococcus aureus*, producing inhibition zones of 3.2 and 2.5 mm, respectively. This investigation establishes *Spirulina maxima*-mediated synthesis as an efficient and cost-effective route for producing antimicrobial silver nanostructures, advancing sustainable nanomaterial production methodologies.

## Graphical Abstract



Extended author information available on the last page of the article

**Keywords** Green synthesis · Silver nanoparticles · Anti-bactericidal activity · Silver nanoparticles (AgNPs) · Spirulina

## 1 Introduction

A nanoparticle is an extremely small particle with dimensions ranging from one to one hundred nanometers [1]. At nanometric scales beyond visual perception, materials manifest distinct physicochemical characteristics that substantially deviate from their conventional structure [2]. As a material approaches the quantum scale, its properties change due to the increased specific surface area, leading to performance that is dominated by surface atoms [3, 4]. Nanoparticles, with their minute size, possess a large surface area to volume ratio compared to larger materials such as powders, plates, and sheets [5]. Their small size allows nanoparticles to exhibit unique photonic, mechanical, and chemical properties, including quantum phenomena [6].

Green chemistry has gained prominence in numerous research fields for its role in enhancing and protecting the environment [7]. The pharmaceutical application of nanoparticles (NPs) presents significant potential for developing new biomedical sciences [8]. The confluence of industrial expansion and population growth has intensified the discharge of toxic pollutants and atmospheric contaminants, undermining ecosystem stability [9]. To counteract this and safeguard the environment, the development of natural product nanoparticles is underway [10]. Biomolecules' excellent compatibility with nanotechnology facilitates the synthesis of genuine and highly efficient metal nanoparticles using biological molecules [11].

Silver has long been valued for its medicinal properties, particularly its antibacterial effects [12]. Silver nanoparticles (AgNPs) and their derivatives exhibit broad-spectrum antibacterial activity against sixteen bacterial species due to their high toxicity [13]. Nanotechnology is continuously evolving the properties of particles, such as size and morphology, enabling the integration of nanomaterials into the production of future high-quality materials across nearly every industry [14]. Nanotechnologies have enabled the development of targeted drug carriers based on nanoparticles [15]. Various metal nanoparticles are being synthesized using fungi such as *Aspergillus terreus*, *Paecilomyces lilacinus*, *Fusarium*, *Penicillium* sp., and medicinal herbs [16], including green tea, neem, starch, aloe vera, and lemon [17].

Silver nanoparticles primarily bind to the outer cell membrane and penetrate deeply into the cell, disrupting organelles by interacting with DNA and proteins, ultimately leading to cell death [18]. This research aims to develop a green synthesis method for silver nanoparticles (AgNPs) using *Spirulina maxima* extract and to evaluate the bactericidal

activity of the synthesized nanoparticles against *Staphylococcus aureus* and *Enterococcus faecalis*. The study seeks to provide a cost-effective and environmentally friendly alternative to chemical synthesis methods and explore the potential biomedical applications of biogenic AgNPs. Apart for the antimicrobial properties. One significant application is the integration of AgNPs into gauze bandages to create antimicrobial wound dressings. This involves embedding the gauze with AgNPs and assessing their efficacy in preventing bacterial infections in wounds. We will evaluate the antibacterial performance of AgNP-coated gauze against common wound pathogens, including *Staphylococcus aureus* and *Enterococcus faecalis*, under simulated wound conditions. Additionally, we will investigate the potential of AgNP-coated gauze to promote wound healing through in vitro and in vivo studies. These studies will assess the impact on cell proliferation and wound closure rates, providing a comprehensive understanding of the therapeutic benefits of AgNPs in wound care.

Moreover, we propose expanding our research to include the development of antibacterial coatings for medical devices, such as catheters, surgical instruments, and implants. This application leverages the potent antibacterial properties of AgNPs to prevent biofilm formation and reduce the risk of hospital-acquired infections. The long-term antibacterial effects of AgNP coatings will be studied to ensure their efficacy and safety in medical environments. Additionally, we will explore the potential of AgNPs as carriers for targeted drug delivery. This involves loading therapeutic agents onto the AgNPs and evaluating their controlled release properties and efficacy in targeting specific cells or tissues. By expanding the scope of our research to these applications, we aim to demonstrate the broader applicability and novelty of our biogenic AgNPs, contributing significantly to the field of nanotechnology and biomedical science.

## 2 Silver nanoparticles and need for green synthesis

Silver nanoparticles (AgNPs) were selected for this study due to their extensively documented broad-spectrum antimicrobial properties, which exhibit significantly higher potency compared to other metallic nanoparticles like gold or zinc. Additionally, silver (Ag) is a fundamental element with both thermal and electrical potential, and it is non-toxic [19]. Modern applications of silver span the textile, plastic, and therapeutic

industries, as well as in surgical instruments, dental restorations, coated water filters, sanitizers, detergents, soap, and wound dressings, leading to an increased demand [20].

The therapeutic applications of silver have expanded considerably, encompassing psychiatric disorders, convulsive conditions, and infectious diseases, thereby altering its environmental distribution dynamics [21]. Silver nanoparticles exhibit exceptional physicochemical attributes, notably their maximized surface-to-volume proportions and heightened reactive capacity, facilitating their efficacy in antimicrobial applications. Their pathogen-control mechanism operates through dual pathways: compromising membrane integrity and interrupting vital cellular processes, resulting in broad-spectrum antimicrobial activity.

Green synthesis, a method utilizing safe, natural herbs, is emerging as an alternative to traditional physiochemical methods [22]. This approach offers multiple advantages, such as the use of phytochemicals and antioxidants as natural reducing agents, cost efficiency, and the elimination of hazardous substances, high pressure, and energies [23]. Additionally, various cytochemical synthesis methods can produce nanoscale silver [24], which, unlike regular metals, can be shaped as needed and has unique features including pH and dissolved ion permeability [25]. The increased surface area per mass of AgNPs improves contact time, driving their demand across industries such as healthcare, food packaging, textiles, and cosmetics [26]. Our research aligns seamlessly with this technological progression, focusing on the sustainable and effective use of AgNPs in biomedical applications. By harnessing their superior antimicrobial capabilities and integrating green synthesis methods, we aim to contribute to the development of safe, eco-friendly medical technologies. The comprehensive understanding of AgNPs' interactions with biological systems will pave the way for innovative solutions in combating microbial resistance and improving patient outcomes. Thus, the inclusion of AgNPs in our research underscores their scientific significance and aligns with our goal of advancing biomedical science through cutting-edge, sustainable materials.

## 2.1 *Spirulina maxima*

A tiny, filamentous cyanobacterium known as spirulina gets its name from the spiral or helical shape of its filaments [27]. This single-cell protein (SCP) has been used as a protein supplement and nutraceutical in humans with no negative side effects [28]. It is an important human staple meal. It also includes vitamins like B12 and provitamin A ( $\beta$ -carotenes),

minerals like iron, and a lot of protein (up to 70%) [29]. It also has a high concentration of tocopherols, phenolic acids, and  $\gamma$ -linolenic acid [30]. The absence of cellulose cell walls allows spirulina to be readily digested [31]. Numerous toxicological tests have validated the safety of spirulina [32]. Spirulina is quite simple to grow, but it only thrives in lakes that are highly alkaline and have a controlled pH level, as well as in sizable outdoor ponds [33]. Only a few places on earth, notably Greece, Japan, India, the US, and Spain, offer the perfect sunny climate for this alga's cultivation [34].

Currently, spirulina is primarily sold as a nutritional additive in the form of beverages or tablets and can be obtained in health food stores [35]. Spirulina is well known for its numerous health benefits [36]. It boosts antioxidant and anti-inflammatory properties, lowers blood cholesterol and triglyceride levels, improves symptoms of allergic rhinitis, and has anticancer properties [37]. There are numerous other advantages to consuming spirulina [38] (Table 1).

## 3 Materials and methods

### 3.1 Sample collection

*Spirulina maxima* was purchased from Oferr Nalayan Spirulina Research Center, Natham, Tamil Nadu—600130, India.

### 3.2 Synthesis of silver nanoparticles (AgNPs) by *Spirulina maxima*

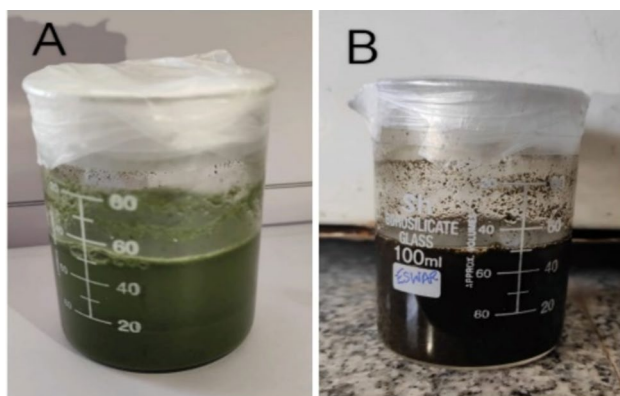
The Spirulina-NPs (nanoparticles) were produced by combining 5 g of freshly cleaned *Spirulina maxima* biomass with 100 mL of 1 mM aqueous silver nitrate in a 100 mL glass beaker for 24 h at pH 7 and 25 °C, facilitating the reduction of silver metal ions to nanomaterial (Fig. 1) [39].

### 3.3 Centrifugation

The obtained silver nanoparticles were centrifuged for 25 min at 10,000 rpm and 8 °C [39], separating the solvent from the pellet. To obtain pure silver nanoparticles (AgNPs) powder, the pellet was rinsed numerous times with 90% ethanol and distilled water.

**Table 1** Comparison of using *Spirulina maxima* vs Silver nitrate extracts

Method	Material	Findings	Novelty	Study
Chemical synthesis	Silver nitrate	Moderate antibacterial activity	Chemical reagents used	Ref. [39]
Biogenic synthesis	<i>Spirulina maxima</i> extract	High antibacterial activity, eco-friendly	Green synthesis, no chemical waste	Current study



**Fig. 1** The biotransformation of  $\text{AgNO}_3$  to silver nanoparticles (AgNPs) by *Spirulina maxima* (A) and the color change (B)

### 3.4 Important features

**Enhanced biogenic synthesis efficiency:** Our method utilizes *Spirulina maxima* extract which has a higher content of bioactive compounds like peptides and proteins that facilitate more efficient reduction and stabilization of AgNPs compared to the methods described in Ref. [39]. **Eco-friendly process:** Unlike the previous methods which might involve additional chemical reagents or harsh conditions, our synthesis process is completely green, relying solely on natural extracts without the need for external stabilizers or reductants.

**Optimized parameters:** We have optimized the synthesis parameters, including pH and temperature, to achieve better control over the size and shape of the AgNPs, which are critical for their biological activity and application in biomedical fields.

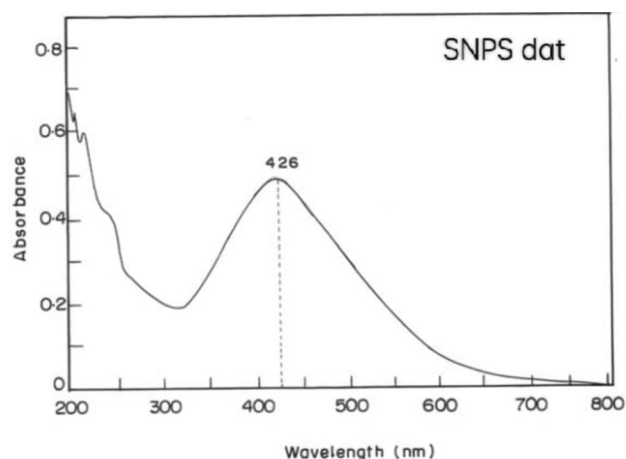
### 3.5 Characterization analysis

#### 3.5.1 UV-vis spectrometry

The reduction of  $\text{AgNO}_3$  to silver nanoparticles (AgNPs) and the subsequent development of silver NPs in an aqueous medium are evidenced by the UV-Vis spectrum of  $\text{AgNO}_3$  and the change in color of the mixture from green to a dark brown. The highest absorption is observed around 426 nm.

#### 3.5.2 Fourier transform infrared (FT-IR)

FTIR analysis showed an absorption peak at  $2929\text{ cm}^{-1}$ . Different compounds such as esters, amines, aldehydes, alkenes, alkanes, and carboxylic acids were identified through the peaks obtained from the FTIR curve. The focus on the absorption peak at  $2929\text{ cm}^{-1}$  in the FTIR analysis is significant as it indicates the presence of specific functional groups, such as aliphatic CH stretching, which play a crucial



**Fig. 2** UV-spectrum reported after the reaction of 1 mM  $\text{AgNO}_3$  solution with 5 g *Spirulina maxima* wet algae at pH 7 and  $26^\circ\text{C}$  and formation of Silver Nanoparticles (AgNPs)

role in the stabilization and capping of silver nanoparticles. This peak is directly associated with the organic compounds derived from *Spirulina maxima* that are involved in the synthesis and stabilization of the nanoparticles.

#### 3.5.3 X-ray diffraction (XRD)

For XRD examination, the bio-reduced  $\text{AgNO}_3$  solution was deposited onto a glass substrate. To obtain a pure nanoparticle pellet, the sample of silver nanoparticle materials was centrifuged for ten minutes at 15,000 rpm at  $8^\circ\text{C}$  [40].

The diameter of the produced silver nanostructures was determined using the Scherrer equation. The observed peak positions ( $2\theta^\circ$ ) are 32.22, 38.33, 64.63, and 77.94, corresponding to the crystallographic planes (111), (200), (220), and (311).

$$D = k\lambda/(\beta\cos\theta)$$

The crystalline size of the nanoparticles was determined using the Debye-Scherrer equation [41], where  $D$  represents the nanoparticle size,  $K$  is the Scherrer constant (0.98),  $\lambda$  is the wavelength ( $1.54\text{ \AA}$ ), and  $\beta$  is the full width at half maximum (FWHM), with  $\theta$  being the angle of diffraction. The particle sizes of nano silver produced by *Spirulina maxima* in aqueous solution were measured as 20.5, 12.6, 17.5, and 11.9 nm, respectively.

#### 3.5.4 Scanning electron microscope (SEM) analysis

SEM is a crucial tool for detecting very small particles [42], including silver nanoparticles (AgNPs), to determine their sizes. For this study, characterization was conducted using the Tescan Vega 3 machine. Figure 2 depicts the histogram

showing the size distribution of the synthesized silver nanoparticles. It is evident that nanoparticles produced from *Spirulina maxima* had diameters ranging from 50 to 90 nm.

### 3.6 Bactericidal activity

The antimicrobial activity of biogenically synthesized silver nanostructures was investigated against two reference pathogens: *Staphylococcus aureus* and *Enterococcus faecalis*. Bacterial growth inhibition was quantified at three AgNP concentrations (40, 60, and 80  $\mu$ l) throughout a 24-h exposure period.

## 4 Results and discussion

### 4.1 UV-visible spectrophotometer

This investigation demonstrates the extracellular biosynthesis of silver nanoparticles using *Spirulina maxima* biomass. The resultant SNPs exhibited characteristic green–brown coloration in aqueous media, attributed to surface plasmon resonance phenomena. UV–visible spectroscopic analysis confirmed the bioreduction of silver ions, evidenced by a distinct chromatic transition. The progressive transformation to dark brown coloration signified the conversion of ionic silver (Ag<sup>+</sup>) to silver oxide (Ag<sub>2</sub>O) and subsequent nanoparticle formation. Spectrophotometric measurements revealed a characteristic absorption maximum at 426 nm.

In the study by Al-Katib et al. [43], which involved the green synthesis of silver nanoparticles from the cyanobacterium *Gloeocapsa* sp., absorption peaks were observed in the range of 400–450 nm, similar to the findings in this study. Comparable results were reported by Muthusamy et al. [44], who focused on synthesizing silver nanoparticles from *Spirulina platensis*. Their UV–Vis spectroscopy analysis revealed an absorbance peak at 450 nm, which aligns with the absorption characteristics observed in our study due to the close relationship between *Spirulina platensis* and *Spirulina maxima*. Additionally, Ameen et al. [45] conducted a study involving *Spirulina platensis*, where the reduction of silver ions to Ag was initially identified by a color change in the solution from pale yellow to dark brown. UV–Vis spectroscopy in this study indicated a peak at 417 nm. These consistent UV–Vis spectroscopy findings across different studies underscore the reliable optical properties of silver nanoparticles synthesized from various cyanobacteria species, reflecting their potential for diverse applications.

### 4.2 Fourier transform infrared (FT-IR)

Fourier Transform Infrared (FT-IR) spectroscopy enables characterization of functional moieties and

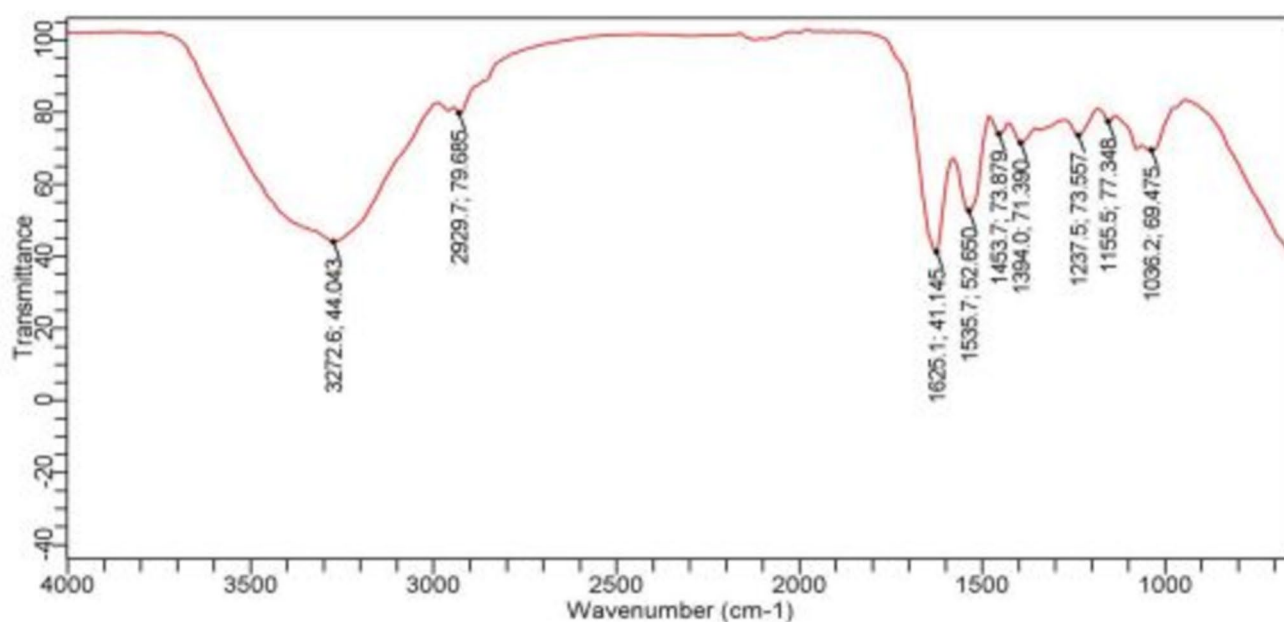
metal–biomolecule interactions. Analysis of the synthesized Ag nanomaterial revealed distinct absorption bands: a peak at 2929  $\text{cm}^{-1}$  indicating –OH stretching modes, prominent signals at 1625 and 1237  $\text{cm}^{-1}$  attributed to carboxyl C–O stretching and amine N–H vibrations, respectively. Additional N–H stretching modes of primary and secondary amines were detected at 1036  $\text{cm}^{-1}$ . The spectral analysis confirmed biomolecule-mediated reduction, evidenced by the transformation of Ag ions through polyol oxidation, resulting in unsaturated carbonyl formation (peak at 2930  $\text{cm}^{-1}$ ). These identified functional groups participate in nanoparticle stabilization and surface modification (Fig. 3, Table 2).

Comparative FTIR analyses from literature corroborate these findings. Al-Katib et al. [43] reported characteristic bands (400–4000  $\text{cm}^{-1}$ ) for AgNPs synthesized using *Gloeocapsa* sp., with amine-associated peaks at 3339, 3267, and 2917  $\text{cm}^{-1}$ , and carbonyl signatures at 2359  $\text{cm}^{-1}$ . Additional vibrational modes included C–H stretching (799, 756  $\text{cm}^{-1}$ ) and C=O stretching (683, 670  $\text{cm}^{-1}$ ). Similarly, Muthusamy et al. [44] identified absorption bands at 3474  $\text{cm}^{-1}$ , with COOH and NH group signatures at 1745 and 1242  $\text{cm}^{-1}$ , respectively, and amine stretching at 1051  $\text{cm}^{-1}$ . Bankar et al. [46] observed characteristic peaks in banana peel-mediated AgNP synthesis: N–H/C=O stretching at 2353  $\text{cm}^{-1}$ , amide-associated C=O/C=N stretching at 1640  $\text{cm}^{-1}$ , and aliphatic/aromatic C–H deformation vibrations at 1445–1454  $\text{cm}^{-1}$ . These collective spectroscopic observations demonstrate the diverse functional groups mediating AgNP formation and stabilization.

### 4.3 X-ray diffraction (XRD)

XRD investigation of silver nanoparticles (SNPs) revealed significant peaks corresponding to (111), (200), (220), and (311) Rayleigh reflections, with a lattice constant (*a*) of 4.09 Å. Notably, no peaks attributable to silver oxides (Ag<sub>2</sub>O or Ag<sub>2</sub>O) or AgCl were observed (Fig. 4). This XRD pattern conclusively demonstrates that the SNPs were synthesized in an aqueous solution by *Spirulina maxima* through the reduction of metal ions, confirming their crystalline nature. The mean crystallite diameter of the silver crystal lattice was determined using the Scherrer equation from the full width at half maximum (FWHM) of the peak intensities. The diameters of the silver nanoparticles (AgNPs) were measured as 20.5, 12.6, 17.5, and 11.9 nm, with a mean value of 15.6 nm across all four peaks. The larger Bragg peaks indicate a reduction in silver particle size (Table 3).

In the study by Hanna et al. [47], X-ray diffraction (XRD) analysis was employed to identify the lattice peaks for silver nanoparticles (AgNPs) synthesized from the cyanobacteria *Desertifilum tharense* and *Phormidium ambiguum*. The diffractogram of *Desertifilum tharense* AgNPs revealed five



**Fig. 3** FTIR analysis of silver nanoparticles (AgNPs) synthesized by *Spirulina maxima*

**Table 2** The major biomaterials play a major role in bio reduction of silver ions (Ag<sup>+</sup>) into silver nanoparticles (AgNPs) (AgO) present in the *Spirulina* strain is identified by FTIR spectroscopy

Wavenumber	Intensity	Appearance	Group	Compound class
1036.19992	69.47456	Strong	C–O stretching	Ester
1155.47473	77.34772	Strong	C–N stretching	Amine
1237.47616	73.55674	Strong	C–N stretching	Aromatic amine
1394.02435	71.39026	Medium	C–H bending	Aldehyde
1453.66176	73.87915	Medium	C–H bending	Alkane
1535.66319	52.64960	Strong	N–O stretching	Nitro compound
1625.11930	41.14512	Medium	C=C stretching	Alkene
2929.68754	79.68520	Strong	O–H stretching	Carboxylic acid
3272.60262	44.04347	Medium	N–H stretching	Amine salt

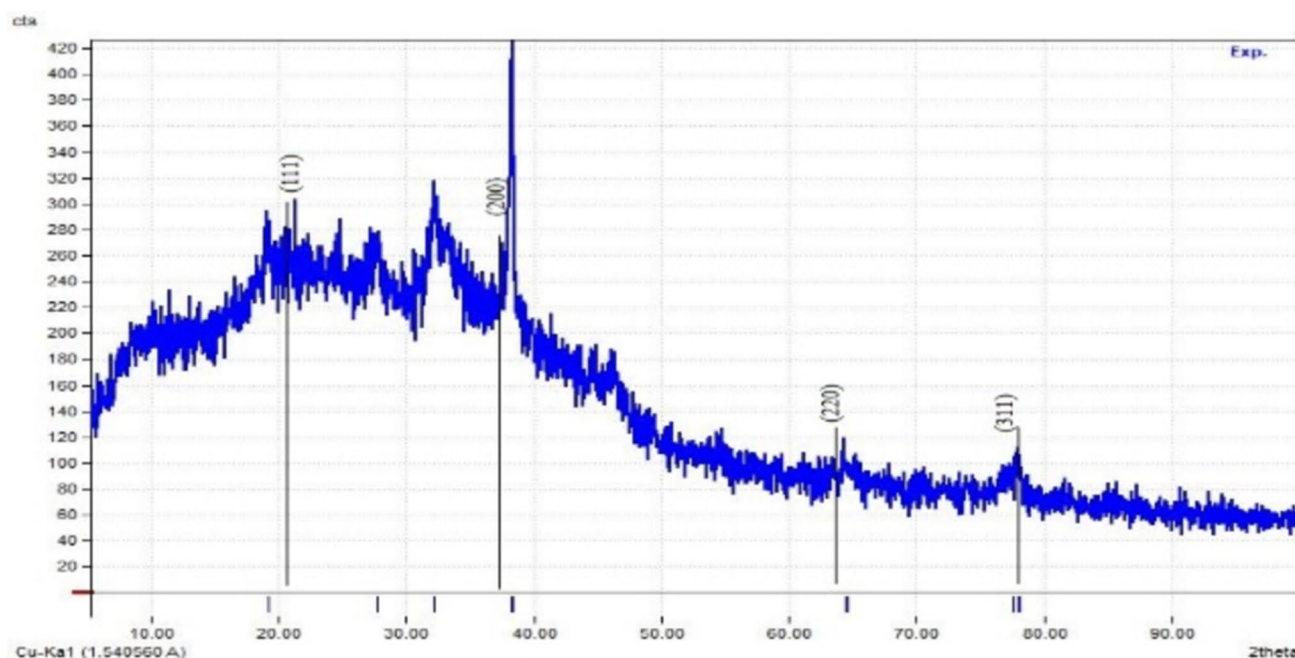
major peaks at 32.19°, 38.089°, 44.2567°, 64.4547°, and 77.4727°, corresponding to the lattice planes (101), (111), (200), (220), and (311), respectively. These peaks confirmed the presence of face-centered cubic (FCC) structures. Similarly, in another study by Muthusamy et al. [44], the XRD pattern of AgNPs synthesized from *Spirulina* microalgae exhibited four distinct diffraction peaks at 32.1°, 45.84°, 54.80°, and 76°, corresponding to the reflections of the silver

FCC structures (111), (142), (220), and (311). Moreover, in a study conducted by Ameen et al. [45], XRD analysis identified recognizable diffraction peaks for the lattice planes (111), (200), (220), and (311) at  $2\theta = 38.11^\circ$ ,  $44.78^\circ$ ,  $64.45^\circ$ , and  $77.32^\circ$ , respectively. These peaks indicate the crystalline nature of the silver nanoparticles, with peak broadening suggesting the nanoscale size of the particles. The consistent identification of these crystalline planes across different studies underscores the reliability of XRD analysis in characterizing the structural properties of silver nanoparticles.

#### 4.4 Scanning electron microscope (SEM) analysis

Scanning electron microscope (SEM) analysis was conducted to investigate the morphology of silver nanoparticles (AgNPs), revealing the biosynthesis of monodisperse spherical AgNPs alongside nanoparticle aggregation (Fig. 5). The size and dispersion of the silver nanoparticles ranged from 50 to 90 nm.

In the study by Hanna et al. [47], silver nanoparticles synthesized from cyanobacteria were characterized by SEM analysis, revealing sizes in the range of 6.24–11.7 nm. In contrast, our study observed significantly larger particles. For instance, SEM analysis of nanoparticles synthesized from *Penicillium oxalicum* in another study [48] showed sizes ranging from 60 to 80 nm, with nearly spherical shapes observed. Additionally, Elumalai et al. [49] reported on silver nanoparticles synthesized from *Euphorbia hirta*, with SEM analysis indicating particle sizes between 40 and 50 nm. These variations in nanoparticle size underscore the



**Fig. 4** XRD image of silver nanoparticles (AgNPs) produced by *Spirulina maxima* strain

**Table 3** Shows the peak orientation of (111), (200), (220) and (311) for the suspension

Peak position ( $^{\circ}2\theta$ )	Peak orientation	FWHM	Size of silver nanoparticles (AgNPs)
32.22	111	0.3533	20.5
38.33	200	0.9291	12.6
64.63	220	1.1418	17.5
77.94	311	1.4682	11.9

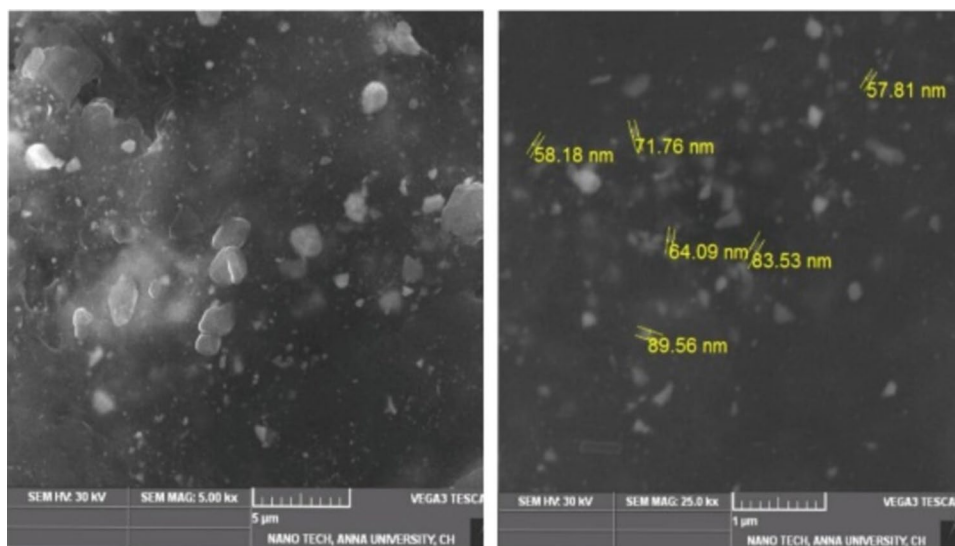
influence of biological sources and synthesis methods on nanoparticle morphology and size distribution.

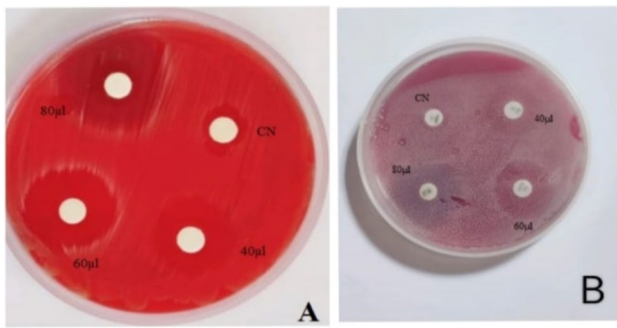
## 4.5 Bactericidal activity

### 4.5.1 *Staphylococcus aureus*

The in vitro antibacterial activity of silver nanoparticles synthesized by *Spirulina maxima* against isolated

**Fig. 5** SEM image of developed SNPs



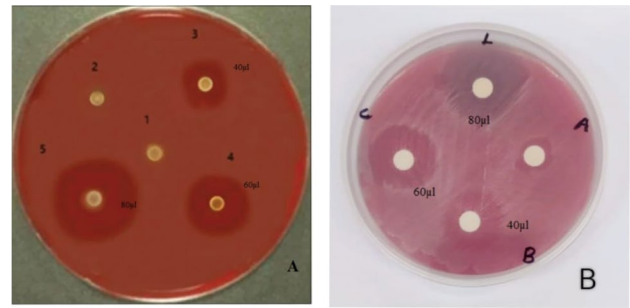


**Fig. 6** Zone of inhibition  $\text{AgNO}_3$  (A) and silver nanoparticles (AgNPs) (B)—*Staphylococcus aureus*

**Table 4** Zone of inhibition of  $\text{AgNO}_3$  and silver nanoparticles (AgNPs) against *Staphylococcus aureus*

S. no	Sample	Zone of inhibition		
		40 µl	60 µl	80 µl
A	$\text{AgNO}_3$	0.8 mm	1.4 mm	1.9 mm
B	Silver nanoparticles (AgNPs)	1.6 mm	2.2 mm	2.5 mm

colonies of *Staphylococcus aureus* was investigated using the Kirby-Bauer disk diffusion method to determine minimal inhibitory concentrations and the zone of inhibition (MIC) [50]. *S. mutans* strains were inoculated onto fully prepared blood agar plates. Blood agar is chosen because it can support the growth of a wider range of bacterial species, including fastidious organisms that require additional nutrients. Blood agar provides a more comprehensive environment, allowing for a more accurate assessment of antibacterial effects on diverse bacterial strains, which may better represent clinical conditions. This choice ensures that the study can evaluate the efficacy of silver nanoparticles against bacteria that might not grow well on Mueller–Hinton agar. After the plates dried, discs are introduced. Subsequently, 40, 60, and 80 µl of silver nanoparticles (AgNPs) were added. One control disc is also placed mentioned as CN. The use of a control (CN) is crucial in accurately interpreting the antibacterial efficacy of silver nanoparticles (AgNPs). The agar plates were then left undisturbed to allow passive diffusion of AgNPs into the agar culture media. Following an incubation period at 36 °C for 24 h, the zones of inhibition were measured in millimeters. The synthesized silver nanoparticles from *Spirulina maxima* exhibited zones of inhibition measuring 1.6, 2.2, and 2.5 mm against *Staphylococcus aureus*, corresponding to the concentrations tested (Fig. 6, Table 4).



**Fig. 7** Zone of inhibition  $\text{AgNO}_3$  (A) and silver nanoparticles (AgNPs) (B)—*Enterococcus faecalis*

**Table 5** Zone of inhibition of  $\text{AgNO}_3$  and silver nanoparticles (AgNPs) against *Enterococcus faecalis*

S. no	Sample	Zone of inhibition		
		40 µl	60 µl	80 µl
A	$\text{AgNO}_3$	0.7 mm	1.6 mm	2.4 mm
B	Silver nanoparticles (AgNPs)	1.6 mm	2.2 mm	3.2 mm

#### 4.5.2 *Enterococcus faecalis*

The antimicrobial activity of biosynthesized silver nanoparticles (AgNPs) was evaluated against *Enterococcus faecalis* by using Kirby-Bauer disk diffusion method to determine zone of inhibition [50]. The bacterial strains were introduced in blood agar plate. After the plates had dried, four discs were placed for 40, 60, 80 µl and control (CN) respectively. After introducing silver nanoparticles (AgNPs) plates are incubated for 24 h at 36 °C. The zone of inhibition of silver nanoparticles (AgNPs) synthesized by *Spirulina maxima* are 1.6, 2.2 and 3.2 mm against *Enterococcus faecalis* with respect to its concentration (Fig. 7, Table 5).

Based on growth data, increasing concentrations of silver nanoparticles (AgNPs) effectively inhibited bacterial populations. The maximum zones of inhibition observed for silver nanoparticles (AgNPs) and  $\text{AgNO}_3$  against *Staphylococcus aureus* were 2.5 and 1.9 mm, respectively on 80 µl concentration. Against *Enterococcus faecalis*, the maximum zones of inhibition for silver nanoparticles (AgNPs) and  $\text{AgNO}_3$  were 3.2 and 2.4 mm, respectively on 80 µl concentration. Notably, silver nanoparticles (AgNPs) exhibited greater antibacterial activity compared to  $\text{AgNO}_3$ .

Antimicrobial efficacy studies of biogenic silver nanoparticles demonstrate significant inhibitory effects across diverse bacterial species. Investigation by Hanna et al. [47] revealed that AgNPs derived from *Desertifilum tharense* produced inhibition zones ranging from 9 mm (*Micrococcus luteus*) to 25 mm (methicillin-resistant *Staphylococcus*

*aureus*). Similarly, *Phormidium ambiguum*-mediated AgNPs exhibited zones spanning 9–18 mm against various pathogens, with maximum efficacy observed against *Escherichia coli* [47]. Further validation comes from Muthusamy et al. [44], where *Spirulina*-synthesized AgNPs demonstrated concentration-dependent inhibition against *Staphylococcus* and *Klebsiella* species. The observed dose–response relationship substantiated their antimicrobial potential. Complementary research by Feroze et al. [48] utilizing *Penicillium oxalicum*-derived AgNPs documented substantial growth inhibition against multiple pathogens: *Staphylococcus aureus* (17.5 mm  $\pm$  0.5), *Shigella dysenteriae* (17.5 mm  $\pm$  0.5), and *Salmonella typhi* (18.3 mm  $\pm$  0.6). The antimicrobial mechanism of AgNPs operates through multiple pathways like, compromising bacterial cell membrane integrity, enhancing permeability and triggering cytoplasmic leakage and generating reactive oxygen species (ROS) leads to oxidative damage of cellular components including lipids, proteins, and nucleic acids. This multi-modal mechanism of action, supported by extensive research, establishes AgNPs as potent antimicrobial agents with broad-spectrum activity.

## 5 Conclusion

The present study investigates the synthesis of silver nanoparticles (AgNPs) utilizing *Spirulina maxima* and employs a comprehensive set of analytical techniques for characterization. UV–Vis spectroscopy confirmed the presence of silver nanoparticles, showed the absorbance peak at 426 nm. The color change from green to brown was also visualized. While Fourier Transform Infrared (FTIR) spectroscopy elucidated the protein-capping nature of silver nanoparticles (AgNPs) derived from *Spirulina maxima* biomass. It has proved the presence of various functional groups present discussed in the study. X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM) were employed to analyze the crystal structure and reveal particle characteristics, respectively. The SEM results showed the size of the synthesized nanoparticles were in the range of 50–90 nm.

Beyond synthesis and characterization, the study evaluates the antibacterial efficacy of silver nanoparticles (AgNPs) against *Staphylococcus aureus* and *Enterococcus faecalis*. Results demonstrate a pronounced bactericidal effect, suggesting potential utility in antimicrobial applications. The synthesized silver nanoparticles showed maximum zone of inhibition (ZOI) 2.5 mm for *Staphylococcus aureus* and 3.2 mm for *Enterococcus faecalis*.

Building on this promising antimicrobial activity, the authors propose future investigations into the potential anticancer properties of silver nanoparticles. This multifaceted approach not only highlights current achievements in nanoparticle synthesis from *Spirulina maxima* but

also underscores their broader applications in biomedical research. The study's holistic perspective emphasizes understanding both the physicochemical properties and functional efficacy of biosynthesized nanoparticles for diverse biomedical applications.

## 5.1 Potential applications

**Applications in biomedical devices:** The silver nanoparticles (AgNPs) synthesized in this study have significant potential for use in biomedical devices, such as antimicrobial coatings for medical instruments and wound dressings. The AgNPs could be incorporated into gauze bandages to enhance their antimicrobial properties and promote faster healing.

**Expanded antimicrobial applications:** We are exploring further studies to evaluate the effectiveness of silver nanoparticles against a broader range of pathogens, including antibiotic-resistant strains, which could provide valuable insights into their utility in combating infections in health-care settings.

**Industrial and environmental applications:** Beyond biomedical applications, our study indicates the potential use of silver nanoparticles in environmental applications such as water purification and air filtration, where their antimicrobial properties could help reduce contamination and pollution.

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**Author contributions** Hanisha—Supervision and Validation; Balaganapathy, Eswar, Kathirvelan—Data curation, investigation, Draft preparation; Jothi Ramalingam—Fund acquisition and supervision; Nadeem—Revision and validation; Yuvaraj—Conceptualization, Supervision, Review & Editing.

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**Availability of data and material** Data will be made available on request.

## Declarations

**Conflict of interest** The authors declare no known competing interest.

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