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Investigating the antioxidant and antimicrobial properties of silver nanoparticles synthesized using the alcoholic extract of *Spirulina subsalsa* algae

Mona Babakhani[1], Sepideh Khaleghi[2], Shadi Hajrasouliha[3]✉

[1]Department of Biotechnology, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran. Email: monababakhani7@gmail.com
[2]Department of Biotechnology, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran. Email: sp.khaleghi@gmail.com
[3]Herbal Pharmacology Research Center, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran. Email: sh.hajrasouliha@iautmu.ac.ir
Corresponding Author, Herbal Pharmacology Research Center, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran. Email: sh.hajrasouliha@iautmu.ac.ir

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Abstract

Objective: Silver nanoparticles (AgNPs) are valuable commercial nanomaterials due to their unique biological properties. As bacteria become more resistant to synthetic antibiotics, exploring natural alternatives such as essential oils, plant extracts, and mineral substances has become essential. Algae, which are diverse and widespread, are noteworthy for their antibacterial and antioxidant properties. This study focused on creating AgNPs using Spirulina algae alcoholic extract and examining their antibacterial and antioxidant properties.

Materials and Methods: AgNPs were synthesized by the green method and evaluated by Fourier Transform Infrared Spectroscopy (FT-IR), Dynamic Light Scattering (DLS), and Transmission Electron Microscopy (TEM). The green synthesis was evaluated at 400-450 nm using a spectrophotometer. Then, the total phenol and flavonoid content of AgNPs were measured. The Diphenyl Picryhydrazyl (DPPH) test was used to evaluate the antioxidant properties. The antimicrobial activity of nanoparticles against Staphylococcus aureus and Pseudomonas aeruginosa was investigated by Minimum Inhibitory Concenteration (MIC) and Minimum Bacteriocidal Concentration (MBC) tests.

Results: The FT-IR results demonstrate the successful synthesis of AgNPs with unique covalent bonding. DLS analysis also confirms spherical nanoparticle morphology and uniform 15-20 nm distribution. In MIC/MBC results, the antibacterial effect of AgNPs was observed within the first few hours (1 to 5 hours), reaching its peak at 24, 48, and 72 hours. Nanoparticles significantly inhibited the growth and survival of both gram-positive and Gram-negative bacteria.

Conclusion: Green-synthesized AgNPs have antibacterial effects in addition to antioxidant capabilities. As they can kill both gram-positive and gram-negative bacteria, these nanoparticles might be a great alternative to conventional antibiotics.

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Introduction

Silver nanoparticles (AgNPs) are one of the most critical commercial nanomaterials with valuable biological properties such as antimicrobial and antioxidant. Several biomimetic systems and routes to synthesizing AgNPs exist. On the other hand, natural antimicrobial substances such as essential oils and extracts of plants, animals, and minerals have gained significant importance due to the pathogenic resistance of bacteria to synthetic antibiotics [1].

Synthesis of nanoparticles by the biological method is a process that takes place through enzymatic and non-enzymatic biological mechanisms. Green technology is an eco-friendly process that minimizes waste and conserves natural resources. The AgNPs can be antibacterial agents to suppress resistant bacteria, involving oxidative stress induction, DNA replication inhibition, or interaction with enzymes and proteins. AgNP's bioactivity depends on size, shape, surface coatings, and solubility. AgNPs with an average diameter of 5 nm can increase the antibacterial activity of all antibiotics [2]. The mechanism of AgNPs' antibacterial activity is attributed to the continual release of silver ions. Silver ions adhere to or pass through the bacterial cell wall and cytoplasmic membrane, causing their structural changes, the proteins' inactivation, and DNA damage, leading to bacteria death [3]. AgNPs can also exhibit antioxidant activity through their ability to scavenge free radicals [4].

Recently, *Spirulina subsalsa*, one of the most important cyanobacterial algae, was studied the most. Also, the World Health Organization has approved this alga as the best food. *Spirulina* algae have been noticed because it is rich in protein and has high digestibility. It can provide all the essential amino acids for humans and animals. The presence of carotenoids, phycocyanin pigments and phenolic and tocopherol compounds makes Spirulina a natural antioxidant and free radical scavenger [5]. *Spirulina* is a multi-celled, flagellated, and spiral-shaped blue-green algae. *Spirulina* species diversity is limited (about 15 species), of which two species, *Spirulina platensis*, and *Spirulina maxima*, are more critical [6]. Since algae are widely distributed and have a wide variety of characteristics as an essential source of antimicrobials and antioxidants, more research is being done on this issue. Therefore, in the present study, the aqueous and alcoholic extracts of *Spirulina* are examined to determine their antimicrobials and antioxidant potential. The ingredients of spirulina alcoholic extract, including phycocyanins, chlorophyll a, and other bioactive compounds, contribute to its color, flavor, and potential health benefits. Spirulina also contains zinc, iron, magnesium, potassium, phosphorus, calcium, sulfur, selenium, cobalt, chromium, and manganese [7].

Staphylococcus aureus, a bacterium commonly present on human skin and mucous membranes, can lead to a range of infections, from minor skin ailments to severe conditions like bacteremia, endocarditis, and osteomyelitis. The emergence of antibiotic-resistant strains such as MRSA has heightened the challenge of effectively treating Staphylococcus aureus infections, creating significant medical hurdles [8]. The incidence of Staphylococcus aureus bacteremia (SAB) has been increasing, presenting complications and elevated risks for conditions like infective endocarditis and osteomyelitis. Managing these infections has become increasingly complex due to the growing prevalence of methicillin-resistant Staphylococcus aureus (MRSA) and the diminishing efficacy of treatments like vancomycin. Addressing these challenges associated with Staphylococcus aureus infections necessitates the development of new antibiotics and innovative treatment strategies [9, 10].

Pseudomonas aeruginosa poses a formidable challenge in healthcare settings, as it is capable of causing a variety of infections, including respiratory tract infections, pneumonia, and chronic infections commonly observed in intensive care unit (ICU) patients [11, 12]. These bacteria are frequently encountered in hospital environments and are resistant to antibiotics, presenting multifaceted challenges for eradication [13]. The clinical implications of antibiotic-resistant Pseudomonas aeruginosa infections are significant, as they complicate the treatment of infections acquired both in the community and healthcare settings [14]. A major concern is their ability to form biofilm communities, which adhere to surfaces and exhibit resistance to antibiotic treatments, further complicating clinical management scenarios.

This research aims to make AgNPs using *Spirulina* algae extract and investigate its antioxidant and antibacterial properties. It also wants to investigate whether the antibacteria *Staphylococus aureus* and *Pseudomonas aeruginosa* and antioxidant activities of *Spirulina subsalsa*

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phytochemicals with AgNPs can help clarify its medicinal value and side effects.

Materials and methods

Filamentous cyanobacteria (*Spirulina subsalsa* strain Nish-1: IBRC-M 50004) was obtained from the Microbial Bank of Iranian Biological Resource Center. Ag nitrate, tris-acetate-ethylenediaminetetraacetic acid (EDTA). Spectroscopic analyses were performed via a NanoDrop 2000c UV–Vis spectrophotometer (Thermo scientific), Fourier transform infrared (FT-IR) spectrometer (Perkin-Elmer 843), X-ray diffraction (XRD) devices. Dynamic light scattering (DLS) tests were carried out using NanoBrook 90 Plus (Brookhaven, USA) and Transmission electron microscopy (TEM) images were taken via Philips EM 208S (Netherlands).

Preparation of *Spirulina* extracts

The method of boiling and passing through a sieve was used to prepare the extract. This study used three types of aqueous, ethanolic, and methanolic *Spirulina* extracts to make AgNPs. Briefly, 300 grams of *Spirulina* algae were mixed with 300 cc of solvents (water, 70% ethanol, and methanol) and boiled for 25 minutes. Then it was centrifuged at 9000 rpm for 10 minutes to remove suspended particles in the mixture and passed through a filter paper (Whatman No. 1). Ultimately, the supernatant was kept at -4°C for further analysis [15].

To check the total phenolic contents and flavonoid, 10 ml of solvents (distilled water/70% ethanol/methanol) was added to 0.5 grams of algae powder. Then extraction was done in an ultrasonic machine at 60°C for half an hour. Next, the solution was filtered, and the volume was brought to 10 mL [15].

Total flavonoids of *Spirulina* extracts

Total flavonoids were measured by the aluminum chloride colorimetric method and based on the protocol of Chang et al. 2002 [16]. After preparing a 10 mg/mL concentration of the extract, 0.5 mL was dissolved in 1.5 mL of methanol in three test tubes. Then 0.1 mL of 10% aluminum chloride and 0.1 mL of 1 M potassium acetate were added. Finally, 2.8 mL of distilled water was added to the solution and kept at room temperature for 30 minutes. After keeping the samples at room temperature for 30 minutes, the optical absorbance of the mixture was read at 415 nm. Quercetin was used to draw a standard curve, and the results were expressed in milligrams of quercetin per gram of extract (16).

Total phenolic content of *Spirulina* extracts

The Folin-Ciocalteu method was used to estimate the total phenolic contents of *Spirulina subsalsa* extracts. An equal volume of each extract was mixed with Na2Co3 and Folin-Ciocalteu reagent (half of them), and the absorbent value was recorded at 720 nm after 30 min. The phenolic content was defined as Gallic acid equivalent (µg/mL) [17].

Antioxidant activity of AgNP-Sp DPPH radical scavenging assay

The antioxidant activity of the extracts was evaluated by DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Brand-Williams et al., 1995) [18]. Briefly, 3500 µL of 60 µM methanolic solution of DPPH was mixed with 100 µL of *Spirulina* extract with different concentrations. The transformation between the oxidized (purple) and reduced (yellow) form of DPPH was followed by recording the decrease in absorbance at 517 nm with a Thermo Scientific Genesys S10 spectrophotometer using 10 mm polystyrene cuvettes. The final absorbance values were recorded at steady state. DPPH residual is the ratio of final absorption (A) and initial absorption (A0) (DPPH% = A / A0). The effective concentration (EC50), i.e., the mass of plant extract required to degrade 50% of primary DPPH, was calculated using the DPPH% versus antioxidant concentration curve. Ascorbic acid (AA) was used as a control and ascorbic acid equivalent data (AAEQ) was calculated from the obtained EC50 numbers (AAEQ = EC50, AA / EC50, Alga). The EC50 of AA was found to be 10.4 µg. All chemicals are from VWR International and used in analytical grade. The accuracy of the method is 5% [18].

Green synthesis of AgNPs

The biosynthesis of high purity green AgNPs (AgNP-Sp) was carried out by precipitation method with the

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regeneration of silver ions and by *Spirulina* algae. First, 170 mg of silver nitrate (AgNO3) 99.9% (Merck, Germany) was dissolved in 100 cc of distilled water. 10 ml of each of the aqueous, ethanolic and methanolic extracts were synthesized separately in separate containers with 90 ml of AgNO3 with a concentration of 0.01 mM. During the synthesis process, Ag+ ions are exposed to the regenerating compounds of the extract, and in this way the reduction of AgNO3 salt begins. The complete recovery of Ag+ ions to AgNPs was done by changing the color of the environment. After one hour passed from the reaction time and the color of the solution changed, the sediment was washed three times with distilled water. All washing steps were centrifuged at 13000 rpm for 15 minutes (Eppendorf 5804R, Germany). The product was incubated at 37 °C for 4 hours. The obtained dry powder was used for FT-IR and DLS analysis [19].

Characterization of AgNP-Sp

AgNP-Sp was scanned for structure and size using UV-vis spectroscopy (Agilent, Germany) at 300–700 nm. FTIR analysis of AgNP-Sp was conducted using Fourier transform infrared spectroscopy (FTIR) (PerkinElmer, USA), with pulses ranging from 500 to 4000 cm−1. Nanoparticles' size distribution, diagram, and zeta potential were determined by Dynamic Light Scattering (DLS) (PSS NICOMP 380, USA) technique [20].

Antibacterial Activity of AgNP-Sp

The antibacterial activity of AgNP-Sp was evaluated against one Gram-positive, *Staphylococcus aureus* (ATCC 33591), and one Gram-negative, *Pseudomonas aeruginosa* (ATCC 25668) bacteria, purchased from the Microbial Bank of Iranian Biological Resource Center. Bacteria were cultured in Mueller Hinton Broth (MHB) (HiMedia, India) overnight at 37 °C. The prepared homogeneous bacterial suspension with standard turbidity equivalent to 0.5 McFarland (about 1.5×10^8 CFUs/ml) was diluted by 1:100 with MHB to obtain 0.5×10^6 CFUs/ml. Antibacterial effects of AgNP-Sp were investigated using the methods of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) [21, 22].

MIC test

The MIC was investigated by microdilution method, using a 96-well microplate. *S. aureus* and *P. aeruginosa* were exposed to different concentrations of the three aqueous, ethanolic, and methanolic extracts of AgNP-Sp (1, 2, 4, 6, and 8 µg/ml) separately. Then, the effects of AgNP-Sp on bacterial growth were investigated. Shortly, three replicates of AgNP-Sp serial dilutions were prepared by the MHB medium in the final volume of 200 µl. Then all the wells were inoculated with 5 µl bacterial suspension and incubated at 37°C for 24 hours. Three wells contained 195 µl of medium and 5 µl of bacterial suspension without AgNP-Sp treatment (as negative control). Antibiotic Vancomycin was used for *S. aureus* and Gentamicin for *P. aeruginosa* (as positive controls). Eventually, the wells' absorbance was read using a microplate reader (BioRad, model 550, USA) at 630 nm [21, 22].

MBC test

The MBC of AgNP-Sp was determined using 10 µl of MIC concentration and less cultured on Mueller Hinton Agar (HiMedia, India) for 24 hours. The plates were examined for bacterial growth, and the first dilution on which no growth was observed was reported as an MBC value [22].

Statistical Analysis

The data are presented as the Mean±SD and statistically evaluated using GraphPad Prism 8.0 software (GraphPad Software, La Jolla, CA, USA). One-way analysis of variance (ANOVA) was performed for the antimicrobial test results. P value ≤ 0.05 was considered statistically significant.

Results

Total phenolic and flavonoid content and antioxidant activity of the extracts

The total phenols extracted from *Spirulina* algae were evaluated in three solvents (water, ethanol, and methanol). The aqueous *Spirulina* extract changed color after adding the Folin-Cicalto reagent and sodium carbonate. Nevertheless, no color change occurred for ethanolic and methanolic extracts. Excessively, the drastic green color and intense light interference caused errors in evaluating higher concentrations. The total phenolic content in the *Spirulina* extracts were calculated based on the absorbance values and

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from the equation of the line obtained from the standard graph of the Gallic acid. The standard curve with regression equation ($y = 0.0112x$, $R^2 = 0.9985$) was obtained using the absorbance against the Gallic acid concentration (µg/mL). Ethanol and methanolic extracts had no total phenolic and flavonoid content, and only the aqueous extract of algae had total phenolic content. Aqueous extract, like ethanolic and methanolic extracts, lacked total flavonoid content. On the other hand, no antioxidant properties were observed for any of the extracts. The total phenolic content of aqueous extract of *Spirulina* was found to be 32.67 ± 0.81 mg GAE /g DW, whereas the difference was significant ($P < 0.001$).

Confirmation of the synthesis of AgNP-Sp

This study used FTIR spectroscopic analysis for AgNP-Sp aqueous, ethanolic, and methanolic extracts of *Spirulina* algae. The graphs of AgNP-Sp aqueous, ethanolic, and methanolic extracts can be seen in **Figure 1**, respectively. The main peaks for AgNP-Sp aqueous extract can be seen in **Figure 1a**, which examination and comparison with other related studies show that the peak at 1126 cm^{-1} is related to the vibrations of the C-OH side group. The observed peaks at 1456, 1516, and 1629 cm^{-1} indicate the aromatic stretching of C-C, C=C, and C=O bonds, respectively. Also, the absorption band of 2924 cm^{-1} is related to the C-H stretching of CH2 groups. The peak at 3292 cm^{-1} indicates O-H stretching. In **Figure 1b**, the AgNP-Sp ethanolic extract had main peaks at 1379 cm^{-1} corresponding to the symmetric CH3 bending mode. The C=O carbonyl stretch of aliphatic esters was observed from 1735-1735 cm^{-1}. Also, the absorption band in the 3430 cm^{-1} is related to the stretching of the hydroxyl group. The peaks of AgNP-Sp methanolic extract (shown in **Figure 1c**) closely resemble the AgNP-Sp ethanolic xtract, as seen in FTIR spectroscopy.

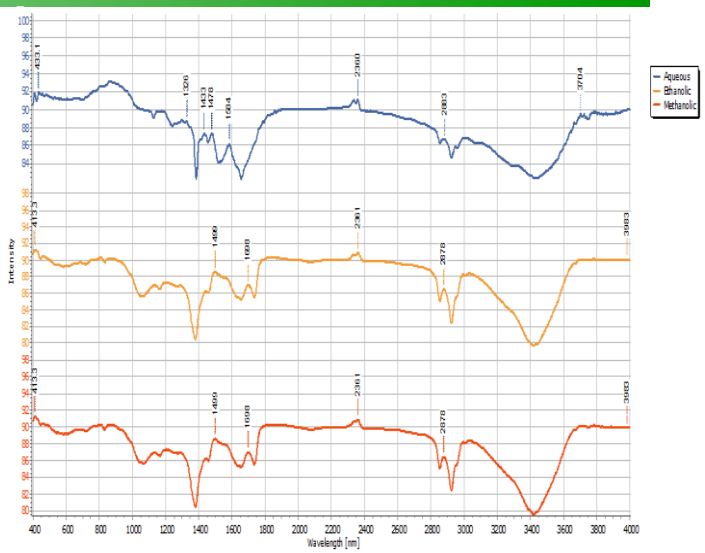

Figure 1. FTIR spectroscopic analysis of AgNP-Sp extracts. a. main peaks for AgNP-Sp aqueous extract, **b.** main peaks for AgNP-Sp ethanolic extract, and **c.** main peaks for AgNP-Sp methanolic extract

Confirming the size of AgNP-Sp (DLS analysis)

The graphs of DLS spectroscopic analysis for biosynthesized AgNP-Sp aqueous, ethanolic, and methanolic extracts are in **Figure 2**. The diameter of AgNP-Sp aqueous extract in a 100% dispersion state was between $1e^{-6}$ and $1e^{-7}$. Similarly, the diameter of AgNP-Sp ethanolic and methanolic extracts in a 100% dispersion state was obtained between $1e^{-7}$ and $1e^{-8}$, respectively. So, the size of the AgNP-Sp aqueous extract was between 175-192 nm. The size of AgNP-Sp ethanolic extract was between 12-90 nm, while the size of AgNP-Sp methanolic extract was between 17-106 nm

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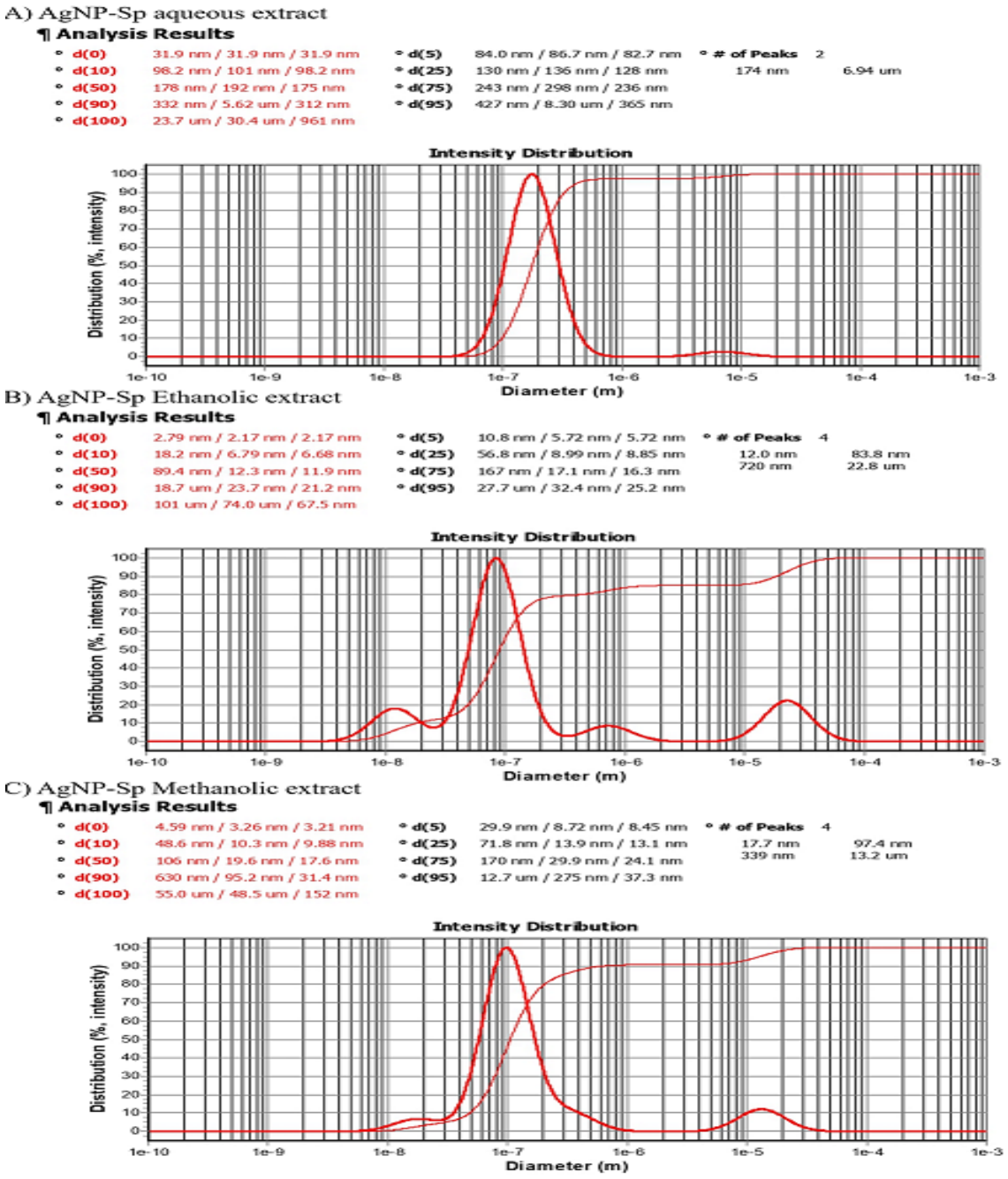

Figure 2. *Size distribution of AgNP synthesized by Spirulina extracts by DLS analysis. a. AgNP-Sp aqueous extract size was between 175-192 nm, b. AgNP-Sp ethanolic extract size was between 12-90 nm, and c. AgNP-Sp methanolic extract size was between 17-106 nm.*

Antibacterial Activity of AgNP-Sp

The antibacterial effects of AgNP made from aqueous, ethanolic, and methanolic extracts of Spirulina algae tests were conducted at various time intervals using the MIC and MBC methods. Figure 3 displays the survival rates of Staphylococcus aureus and Pseudomonas aeruginosa after being treated with varying concentrations (1, 2, 4, 6, and 8) for 1, 2, 3, 4, 5, 24, 48, and 72 hours.

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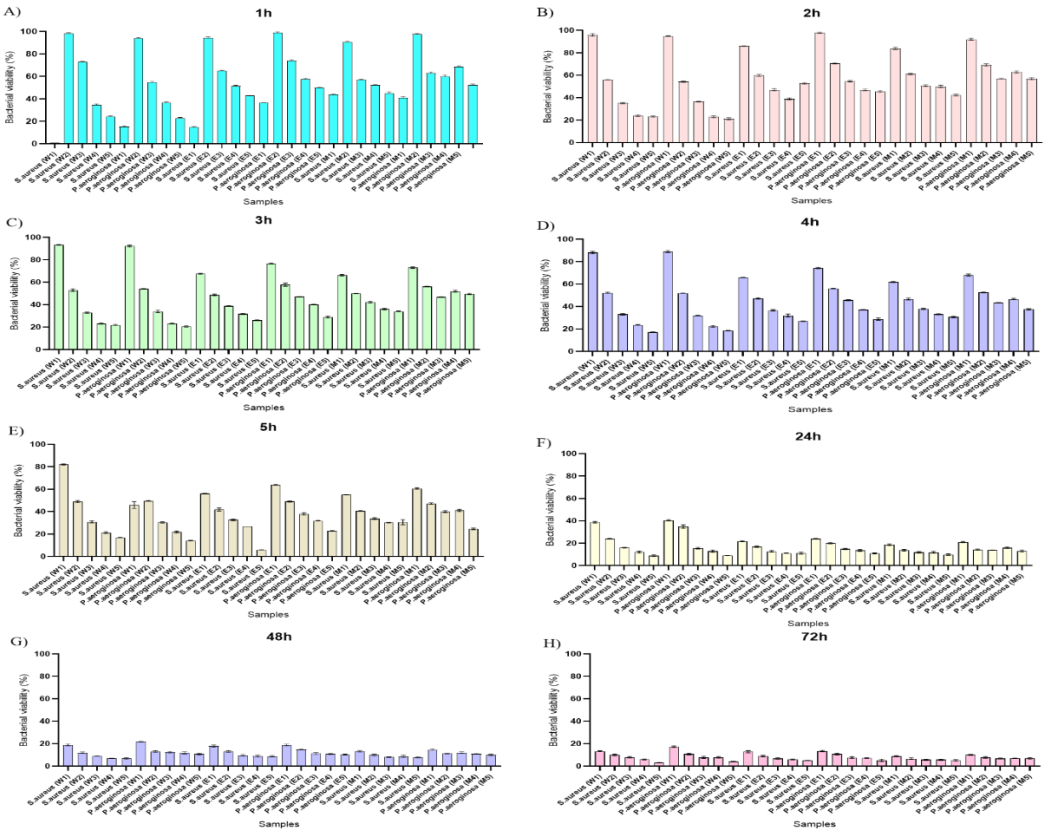

Figure 3. Antibacterial activity of AgNP along with aqueous (W), ethanolic (E), and methanolic (M) extracts of *Spirulina* **algae.** *The survival percentage of Staphylococcus aureus and Pseudomonas aeruginosa treated with different concentrations (1, 2, 4, 6, and 8 µg/ml) of AgNP-Sp in time frames of 2, 1, 3, 4, 5, 24, 48 and 72 hours. M: AgNP-Sp methanolic extract, E: AgNP-Sp ethanolic extract, and W: AgNP-Sp aqueous extract. Numbers after letters: concentration of extracts (µg/ml).*

The survival rate of both strains decreased by 60%, 75%, and 80%, respectively, when exposed to 1 µg/mL AgNP synthesized with aqueous, ethanolic, and methanolic extracts. When using 2 µg/mL of AgNP synthesized with ethanolic and methanolic extract, there was a reduction of approximately 80% and 85% in both strains' viability. Additionally, when using 2 µg/mL of AgNP synthesized with aqueous extract, there was a 75% and 55% decrease in the viability of Gram-positive and Gram-negative strains, respectively. When 4 µg/mL of AgNP, synthesized with aqueous and ethanolic extracts, were used, there was an 85% decrease in both strains. With the same concentration of methanolic extract, there was a nearly 90% decrease in Staphylococcus aureus and an 85% decrease in Pseudomonas. Then, a concentration of 6 µg/ml of AgNP synthesized with aqueous extract decreased the viability of both Aureus and Pseudomonas by more than 85%. The same concentration for ethanolic and methanolic extracts reduced the viability of Aureus by approximately 90% and Pseudomonas by 85%. The most effective concentration was 8 µg/ml of AgNP synthesized with aqueous extract, which reduced over 90% in both strains' viability after 24 hours. The same concentration was effective for ethanolic and methanolic extracts, with a decrease in viability of nearly 90% for both bacteria.

Discussion

Algae are diverse and found all over the world. Each group has unique characteristics worth investigating in various scientific fields, which is vital. Algae have medicinal properties and have been a source of medicine for thousands of years. They have been used as food and medicine for over two thousand years (23). Today, algae's secondary metabolites are used worldwide to create medicine. Primary and secondary metabolites of algae are used in various industries like agriculture, pharmaceuticals, and medicine. Algae can be used in pharmaceutical and medical science as anti-cancer, anti-virus, and antifungal agents (24).

Studies have shown that bacteria are becoming more resistant to synthetic antibiotics (25). Therefore, researchers are turning to natural compounds with diverse bioactivities such as essential oils and extracts from various organisms,

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including plants, animals, fungi, algae, plankton, and bacteria, to create natural antimicrobial substances (26). Additionally, living organisms like bacteria, fungi, algae, and plants have been used to synthesize nanoparticles, which are the basis of nanotechnology and have the potential to fight pathogens in medicine (27).

To better understand these natural compounds, studies were conducted to investigate the antioxidant and antibacterial properties of extracts and its ability to synthesize silver nanoparticles (3, 4, 28, 29). This information is crucial in determining the medicinal value and potential side effects.

Spirulina is a type of blue-green algae (Cyanobacteria) that grows in shallow and alkaline areas of lakes and seas. It is commonly consumed as food in Mexico and Africa due to its nutritional value. *Spirulina* comprises 62% amino acids, vitamin B12, and photosynthetic pigments like xanthophyll and carotene. Its soft wall contains sugar and protein compounds, easily digestible compared to other algae. Furthermore, *Spirulina* is known for its anti-viral, anti-cancer, and immune system-strengthening properties without causing any adverse effects on human cells. Studies have shown that a type of polysaccharide in *Spirulina* can increase the enzyme activity of the cell nucleus and restore and modify DNA. *Spirulina* strengthens and stimulates the immune system and boosts the body's ability to produce new blood cells (30, 31).

This research analyzed the antioxidant and antibacterial effects of silver nanoparticles with *Spirulina* extracts (AgNP-Sp). We created aqueous, ethanolic, and methanolic extracts of the *Spirulina* and measured the amount of phenolic content, total flavonoids, and antioxidant properties using the DPPH free radical method. The unique ability of *Spirulina* was used to synthesize metal nanoparticles to produce AgNP-Sp, which were examined using FTIR and DLS techniques. To test the antibacterial properties of the nanoparticles, they were used on Gram-positive bacteria Staphylococcus aureus and Gram-negative bacteria Pseudomonas aeruginosa. The MIC and MBC values were estimated for the percentage of bacteria survival.

We evaluated the total phenols content of Spirulina extracts using water, ethanol, and methanol. However, we did not observe any color change after adding the Folin-Cicalto reagent and sodium carbonate to the ethanolic and methanolic extracts. Additionally, evaluating higher concentrations was challenging due to the dominant green color and intense light interference, causing some errors. The Spirulina extract turned a different color during the phenol test, but there was no color change in the ethanolic and methanolic extracts. This may be because the phenolic compounds in those extracts were converted into glycosidic compounds. It is also possible that proteins in the aqueous environment caused the color change in the aqueous extract. According to Agostini et al., flavonoids and phenolic compounds can easily dissolve in water or polar solvents because they are attached to a sugar group. Improper drying of leaves can cause the loss of phenolic compounds in the cell membrane. The length of the extraction process also affects the total phenol content (32). found that longer extraction time results in higher phenol levels as the solvent penetrates cell walls and dissolves more phenolic compounds (5).

The results of the FTIR test indicate that proteins from the extracts play a crucial role in stabilizing AgNPs. Additionally, the DLS test showed that the size of the AgNP-Sp ethanolic extract was smaller than those synthesized with other extracts. These tests confirm the successful synthesis of AgNPs.

Studies have also been conducted on synthesizing silver nanoparticles from algae and their antibacterial effects (3, 4, 15, 21, 33). According to a 2019 study by Hamouda et al., the synthesized Ag-NPs displayed powerful antibacterial properties against drug-resistant bacteria such as *E. coli*, *Bacillus cereus* and *Staphylococcus aureus* (28). A study conducted by Muthusamy et al. in 2017 used FT-IR spectroscopic analysis to demonstrate that *Spirulina* extract's bioactive molecules can effectively reduce Ag ions. The researchers also tested the synthesized AgNPs against harmful bacteria like Staphylococcus and Klebsiella and found that they significantly slowed down the growth rate of these pathogens (29). Here, AgNPs' antibacterial properties were studied on Gram-positive and Gram-negative strains. The study found that as the concentration of nanoparticles increased, the survival rate of bacterial cells decreased. The most significant death was observed in both strains with an aqueous extract concentration of 8 µg/ml. Additionally, nanoparticles synthesized from the aqueous extract were more effective against gram-negative strains, while those from the ethanolic and methanolic extracts were more effective against gram-positive strains. The longer exposure time to AgNPs leads to decreased bacterial cell survival. After

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72 hours of exposure to 8 µg/ml of AgNP-Sp aqueous extract, bacterial strain survival rates were reduced by 97%.

Conclusion

This study explores the potential applications and health benefits of *Spirulina* algae and the use of silver nanoparticles in the pharmaceutical and medical industry. Specifically, it aims to investigate the green synthesis of AgNP-Sp extract and their effectiveness in providing antioxidant and antibacterial properties against two strains of bacteria (*S. aureus* and *P. aeruginosa*). The study found that biosynthesized AgNP-Sp did not have antioxidant effects. However, it was discovered that increasing the concentration of biological nanoparticles reduces the viability of both bacterial strains. Furthermore, bacterial cell viability decreased significantly with longer exposure to different concentrations. The most effective concentration was 8 µg/ml AgNP-Sp aqueous extract at different intervals. These findings suggest potential use in diagnosing and treating disease-related pathogens in the medical and pharmaceutical industries, pending further testing.

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Declaration

Ethical Approval

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Competing interest:

Not applicable

Authors Contribution:

Shadi Hajrasouliha conceived and designed the study and wrote the manuscript, Mona Babakhani performed the expriments and acquired the funding, Sepideh Khaleghi analyzed interpreted the data.

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Availability of data and materials

All data available from corresponding author by request

References

1. Wei L, Lu J, Xu H, Patel A, Chen Z-S, Chen G. Silver nanoparticles: synthesis, properties, and therapeutic applications. Drug discovery today. 2015;20(5):595-601. https://doi.org/10.1016/j.drudis.2014.11.014
2. Ismail GA, Allam NG, El-Gemizy WM, Salem MA. The role of silver nanoparticles biosynthesized by Anabaena variabilis and Spirulina platensis cyanobacteria for malachite green removal from wastewater. Environmental technology. 2021;42(28):4475-89. https://doi.org/10.1080/09593330.2020.1766576.
3. Yin IX, Zhang J, Zhao IS, Mei ML, Li Q, Chu CH. The antibacterial mechanism of silver nanoparticles and its application in dentistry. International journal of nanomedicine. 2020:2555-62.
4. Ansar S, Tabassum H, Aladwan NS, Naiman Ali M, Almaarik B, AlMahrouqi S, et al. Eco friendly silver nanoparticles synthesis by Brassica oleracea and its antibacterial, anticancer and antioxidant properties. Scientific Reports. 2020;10(1):18564.
5. Kumar A, Ramamoorthy D, Verma DK, Kumar A, Kumar N, Kanak KR, et al. Antioxidant and phytonutrient activities of Spirulina platensis. Energy Nexus. 2022;6:100070. https://doi.org/10.1016/j.nexus.2022.100070
6. Prüser T, Braun P, Wiacek C. Microalgae as a novel food. Potential and legal framework. Ernahr Umsch. 2021;68:78-85.
7. Zhao Y, Han C, Wu Y, Sun Q, Ma M, Xie Z, et al. Extraction, structural characterization, and antioxidant activity of polysaccharides from three microalgae. Science of The Total Environment. 2024:172567.
8. Taylor TA, Unakal CG. Staphylococcus aureus infection. 2017.
9. Rasmussen RV, Fowler Jr VG, Skov R, Bruun NE. Future challenges and treatment of Staphylococcus

[DOI: 10.61186/pbp.6.1.32]

aureus bacteremia with emphasis on MRSA. Future microbiology. 2011;6(1):43-56.
10. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler Jr VG. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical microbiology reviews. 2015;28(3):603-61.
11. Young LS. The clinical challenge of infections due to Pseudomonas aeruginosa. The University of Chicago Press; 1984. p. S603-S7.
12. Pelegrin AC, Palmieri M, Mirande C, Oliver A, Moons P, Goossens H, et al. Pseudomonas aeruginosa: a clinical and genomics update. FEMS Microbiology Reviews. 2021;45(6):fuab026.
13. Reig S, Le Gouellec A, Bleves S. What Is New in the Anti–Pseudomonas aeruginosa Clinical Development Pipeline Since the 2017 WHO Alert? Frontiers in Cellular and Infection Microbiology. 2022; 12:909731.
14. Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clinical microbiology reviews. 2009;22(4):582-610.
15. Mocan A, Vlase L, Vodnar DC, Bischin C, Hanganu D, Gheldiu A-M, et al. Polyphenolic content, antioxidant and antimicrobial activities of Lycium barbarum L. and Lycium chinense Mill. leaves. Molecules. 2014;19(7):10056-73. https://doi.org/ 10.3390/molecules190710056
16. Chang C-C, Yang M-H, Wen H-M, Chern J-C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of food and drug analysis. 2002;10(3). https://doi.org/10.38212/2224-6614.2748
17. Akbarizare M, Ofoghi H, Hadizadeh M, Moazami N. In vitro assessment of the cytotoxic effects of secondary metabolites from Spirulina platensis on hepatocellular carcinoma. Egyptian Liver Journal. 2020;10:1-8.
18. Brand-Williams W, Cuvelier M-E, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology. 1995;28(1):25-30. https://doi.org/10.1016/S0023-6438(95)80008-5
19. El-Sheekh MM, El-Kassas HY. Application of biosynthesized silver nanoparticles against a cancer promoter cyanobacterium, Microcystis aeruginosa. Asian Pacific Journal of Cancer Prevention. 2014;15(16):6773-9. https://doi.org/10.7314/APJCP.2014.15.16.6773
20. Ebrahimzadeh A, Karamian M, Abedi F, Hanafi-Bojd MY, Ghatee MA, Hemmati M, et al. Topically applied luteolin/quercetin-capped silver nanoparticle ointment as antileishmanial composite: Acceleration wound healing in BALB/c mice. Advances in Materials Science and Engineering. 2023;2023:1-11. https://doi.org/10.1155/2023/1878170
21. El-Batal AI, Mosallam FM, El-Sayyad GS. Synthesis of metallic silver nanoparticles by fluconazole drug and gamma rays to inhibit the growth of multidrug-resistant microbes. Journal of Cluster Science. 2018;29(6):1003-15. https://doi.org/10.1007/s10876-018-1411-5
22. Garibo D, Borbón-Nuñez HA, de León JND, García Mendoza E, Estrada I, Toledano-Magaña Y, et al. Green synthesis of silver nanoparticles using Lysiloma acapulcensis exhibit high-antimicrobial activity. Scientific reports. 2020;10(1):1-11. https://doi.org/10.1038/s41598-020-69606-7
23. Rico M, González AG, Santana-Casiano M, González-Dávila M, Pérez-Almeida N, de Tangil MS. Production of primary and secondary metabolites using algae. Prospects and challenges in algal biotechnology. 2017:311-26. http://dx.doi.org/10.1007/978-981-10-1950-0_12
24. Bhowmick S, Mazumdar A, Moulick A, Adam V. Algal metabolites: An inevitable substitute for antibiotics. Biotechnology advances. 2020;43:107571. https://doi.org/10.1016/j.biotechadv.2020.107571
25. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. The Lancet infectious diseases. 2018;18(3):318-27. https://doi.org/10.1016/S1473-3099(17)30753-3

[DOI: 10.61186/pbp.6.1.32]

26. Hutchings MI, Truman AW, Wilkinson B. Antibiotics: past, present and future. Current opinion in microbiology. 2019;51:72-80. https://doi.org/10.1016/j.mib.2019.10.008
27. Mubeen B, Ansar AN, Rasool R, Ullah I, Imam SS, Alshehri S, et al. Nanotechnology as a novel approach in combating microbes providing an alternative to antibiotics. Antibiotics. 2021;10(12):1473. https://doi.org/10.3390/antibiotics10121473
28. Hamouda RA, Yousuf WE, Abdeen EE, Mohamed A. Biological and chemical synthesis of silver nanoparticles: Characterization, MIC and antibacterial activity against pathogenic bacteria. J Chem Pharm Res. 2019;11:1-12.
29. Muthusamy G, Thangasamy S, Raja M, Chinnappan S, Kandasamy S. Biosynthesis of silver nanoparticles from Spirulina microalgae and its antibacterial activity. Environmental Science and Pollution Research. 2017;24(23):19459-64. https://doi.org/10.1007/s11356-017-9772-0
30. Grosshagauer S, Kraemer K, Somoza V. The true value of Spirulina. Journal of agricultural and food chemistry. 2020;68(14):4109-15. https://doi.org/10.1021/acs.jafc.9b08251
31. Karkos P, Leong S, Karkos C, Sivaji N, Assimakopoulos D. Spirulina in clinical practice: evidence-based human applications. Evidence-based complementary and alternative medicine. 2011;2011. https://doi.org/10.1093/ecam/nen058
32. Agostini-Costa TdS, Teodoro AFP, Alves RdBdN, Braga LR, Ribeiro IF, Silva JP, et al. Total phenolics, flavonoids, tannins and antioxidant activity of lima beans conserved in a Brazilian Genebank. Ciência Rural. 2014;45:335-41. https://doi.org/10.1590/0103-8478cr20140030
33. Garibo D, Borbón-Nuñez HA, de León JND, García Mendoza E, Estrada I, Toledano-Magaña Y, et al. Green synthesis of silver nanoparticles using Lysiloma acapulcensis exhibit high-antimicrobial activity. Scientific reports. 2020;10(1):12805. https://doi.org/10.1038/s41598-020-69606-7

[DOI: 10.61186/pbp.6.1.32]