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Antibacterial Activity of Silver Nanoparticles Synthesized by Hexane Extract of some freshwater algae Against multi-Drug Resistance Bacteria

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Abstract

Objectives

Antibiotic-resistant bacteria have become a worldwide concern due to the unintentional use of antibiotics, which has resulted in bacterial strains resistant to many or all available antibiotics. The primary and secondary metabolites found in algae play a major role in the conversion of silver nitrate to silver nanoparticles (AgNPs).

Materials and methods

Hexane extract of some freshwater algae was used in the process of making these nanoparticles. The reaction solution's color changing from yellow to dark brown due to the surface plasmon resonance's excitation serves as evidence for this. AgNPs were identified using UV-Vis spectroscopy, proteins and phenols were found to play a significant role in the formation of AgNPs, according to research done using Fourier Transformation-infrared (FTIR) to identify the effective algae group that contributes to the formation of those nanoparticles. A scanning electron microscope (SEM) was used to characterize the shapes and sizes of the synthesized AgNPs, which included spherical, rod-like, and hexagonal structures. Vitek Compact 2 system-diagnosed Multi-Drug Resistant (MDR) bacteria were used to test AgNPs' antibacterial activity.

Results

A study was conducted on the antibacterial effectiveness of biosynthetic silver nanoparticles against selected isolates of MDR bacteria. The results showed that silver nanoparticles prepared from hexane extract of the isolated algae at a concentration of 100% showed greater inhibition than crude extract of all types of pathogenic bacteria, with statistically significant differences ($P<0.05$).

Conclusions

The silver nanoparticles prepared from hexane extract was more effective against G-ve and G+Ve MDR bacterial isolates (*E. coli, P. aeruginosa*, *S. aureus*, *K. Pneumoniae, and E. faecalis*) at concentrations 100 µg/mL than those prepared without silver nanoparticles hexane extract, the extract from *Cladophora neglecta* silver nanoparticles demonstrated highly significant inhibition in all species of bacteria.

Keywords: Antibacterial activity, *Cladophora glomerata*, freshwater algae, MDR, Silver nanoparticles

Paper Type: Research Paper.

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Introduction

Nanotechnology is a multidisciplinary scientific field that uses a set of tools and techniques derived from engineering, physics, chemistry and biology (Mohammadabadi et al. 2009, Heidarpour et al. 2011, Mohammadabadi & Mozafari 2018). Advances in nanoscience and nanotechnology have routinely enabled the fabrication and identification of submicron bioactive carriers. The delivery of bioactive substances to target sites in the body and their release behavior are directly affected by particle size (Mortazavi et al. 2005, Zarrabi et al. 2020). Compared to micrometer-sized carriers, nanocarriers provide more surface area and have the potential to increase solubility, increase bioavailability, improve controlled release, and enable precise targeting of entrapped substances (Heidarpour et al. 2011, Mohammadabadi & Mozafari 2019). Nanoparticles are being studied for their antimicrobial (antibacterial) activity; the most common types of metallic nanoparticles used for antiviral activity is Ag nanoparticles (Lok et al. 2006). Silver nanoparticles have a direct interaction with the microbial surface proteins, the size of the nanoparticles plays a significant role in the interaction; the smaller the size, the more interaction and inhibition occur (Li et al. 2022).

The emergence and spread of multi-drug resistant (MDR) bacterial pathogens have substantially threatened the current antibacterial therapy (Boucher 2020). MDR bacterial infections often lead to increased mortality, longer lengths of stay in hospitals, and higher costs of treatment and care (Morris & Cerceo 2020). However, the indiscriminate use of antibiotics has led to a significant increase in the emergence of drug resistance in pathogenic bacteria (Hernández-González, et al. 2021). Green synthesis of metal nanoparticles using Algae extracts had a universal interest due to their physiochemical and their implementation in different fields of biotechnology. These methods had attention in the last decade because these metal nanoparticles are mediated by eco-friendly Algae extracts with low toxicity to humans. Metal nanoparticles mediated by Algae extracts are characterized by high productivity in addition to their stability in size and shape as well as having good antimicrobial activity (Semchuk et al. 2021). Algae extracts have an important role in reducing and stabilizing metal nanoparticles as they reduce toxicity compared with using other methods in synthesizing nanoparticles, algae extracts have secondary metabolites that play an important role in the manufacturing of metal nanoparticles such as polyphenols (Yadi et al. 2018). Silver nanoparticles show great attention because of their special characteristics like shape and size. In recent years, green synthesis of silver nanoparticles by using plant extracts has been studied and invested for a wide range of metabolites, including antioxidant and antibacterial activities. Silver nanoparticles made from plant extracts have numerous implantations as a result of their unique characteristics higher than in their bulk form. Silver nanoparticles have been used for nearly 120 years and are called colloidal silver (Joudah & Hamim 2023). The freshwater algae (*Chlorella vulgaris, Cladophora glomerata, Spirogyra neglecta,* and *Spirulina platensis*) belonging to cyanobacteria and Chlorophyta algae, spread to water marshes in Iraq. It is present in Puddles and swamp water, the algae are used in the treatment of Skin diseases, respiratory tract infections, and diseases of the urinary system (Hlail 2023). The emergence of antibiotic-resistant bacteria is one of the biggest problems facing humanity, antibiotic resistance bacteria first arise in hospitals, and then spread everywhere (Vivas et al. 2019). This is due to the misuse of antibiotics, which will eventually result in a shortage of antibiotics for medical treatment (Hamdani et al. 2020). The current study aims to produce AgNPs composed of hexane extract from some freshwater algae (*Chlorella vulgaris*, *Cladophora glomerata*, *Spirogyra neglecta*, and *Spirulina platensis*) as antimicrobial agents since that is the first time that hexane extract of this Algae was tested as an antimicrobial agent to inhibit MDR human pathogenic bacteria.

Materials and Methods

Preparation of Algae extracts: In April 2023, water samples were randomly taken from a number of water bodies in Thi-Qar Provence, southern Iraq, at four separate locations of water marshes (Al-Islah, Al-Chibayish, Al-Shatrah, and Al-Battha'a). Using a plankton collection net, samples were taken 30 cm below the water's surface and placed in plastic containers that had been sterilized. Using Stein's 1975 dilution method, samples were sent right away to the College of Agriculture laboratory at Misan University for the purpose of isolating and culturing freshwater algae species. 20 gm of dried powder was mixed with 200 mL of hexane by Soxhlet continuous extraction, the solution was filtered by using Whatman No.13 filter paper then the filtrate was concentrated under reduced pressure on a rotary evaporator at 50°C and dried at 25°C, the extract was collecting in sterilized glass tubes and kept at 4 °C until used.

Identification of bacterial isolate with the VITEK 2 system: The VITEK-2 system, which has a high sensitivity of 98% when used to diagnose gram-positive and negative isolates, was employed in this study. The device comprises 64 biochemical tests that are used to diagnose bacteria, and the results of the examination take eight hours or less. It also includes a test of antibiotic sensitivity in bacteria (Putra et al. 2020).

Synthesis of silver nanoparticles: Green synthesis of silver nanoparticles was done according to (Alnuaimi et al. 2019). The colors of *Cladophora glomerata, Chlorella vulgaris, Spirogyra neglecta,* and *Spirulina platensis* changed from green to dark brown, green to pale brown, and green to yellow, respectively, to show the formation of silver nanoparticles. After centrifugation the produced AgNPs at 3000 rpm, they were washed with double-distilled water.

Characterization of AgNPs: Using a UV-Vis spectrophotometer, the range of absorbance for synthesized AgNPs made from hexane extract of (*Chlorella vulgaris, Cladophora glomerata, Spirogyra neglecta,* and *Spirulina platensis*) was measured. The dried AgNPs were measured at room temperature using the FTIR-LiTaO3 Detector 8-Perkin Elmer machine, USA, and the

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surrounding area (450–4000 cm^{-1}). A Leo 1455vp (Germany) scanner was used for the SEM (Scanning Electron Microscope) analysis.

***In vitro* antibacterial activity of silver nanoparticles:** Using an agar well diffusion method with some modifications, the antibacterial activity of AgNPs mediated by using hexane extract of freshwater algae (*Chlorella vulgaris, Cladophora glomerata, Spirogyra neglecta,* and *Spirulina platensis*) was assessed against five isolates of MDR Gram-negative and Gram-positive human pathogenic bacteria isolated from urine and blood sample from out visit to bacteriological laboratory at the Child and Maternity Hospital in Misan Province, In brief Muller-Hinton agar medium wells containing 100 µg/mL of synthesized AgNPs were swabbed with the tested bacterial suspension, which contained roughly 1X106 CFU/mL. By measuring the inhibition zone's diameter in millimeters, antibacterial activity was determined. Independent sample t-test was used for statistical analysis, with a p-value of less than 0.05.

Results and discussion

Characterization of AgNPs: The color change of the reaction mixture from green to dark brown in *Cladophora glomerata*, The color change from green to pale brown in *Chlorella vulgaris*, The color change from green to dark yellow in *Spirogyra neglecta*, and the color change from green to yellow in *Spirulina platensis* indicated the formation of AgNPs due to the reduction of silver ions to AgNPs, mediated by biomolecule founded in Algae extracts of *Chlorella vulgaris, Cladophora glomerata, Spirogyra neglecta, Spirulina platensis* (Islam et al. 2018). The results of chromatic changes of hexane extract from the algae under study are shown in Figure1 (A, B, C, and D). The color changed due to the plasmon resonance surface of sedimented silver nanoparticles because of coherent and collective surface electron oscillation (Yu et al. 2019). The color changes in solutions are caused by the algal component extracts reducing, capping, and stabilizing silver nanoparticles from silver nitrate (Shirley & Jarochowska 2022).

UV spectroscopy (UV spectrum) was used to examine the samples' optical absorption properties at room temperature. The samples' absorption spectra are shown in Figure 2, with particular emphasis on the 400 nm wavelength range absorption band. By means of the collective oscillation of conduction electrons on their surface, metallic nanoparticles are able to absorb visible electromagnetic waves. The phenomenon is called the surface plasmon resonance event. This phenomenon has the benefit of being observable with a UV-visible spectrometer, making it a useful marker for the presence of metallic nanoparticles (Biliuk et al. 2020). An electromagnetic field is generated by light, and this leads to the fluctuation of NMNP that is restricted by plasma. From ultraviolet (UV) to infrared (IR) wavelengths, plasmonic nanoparticles can absorb a broad range of electromagnetic waves (Semchuk et al. 2021).

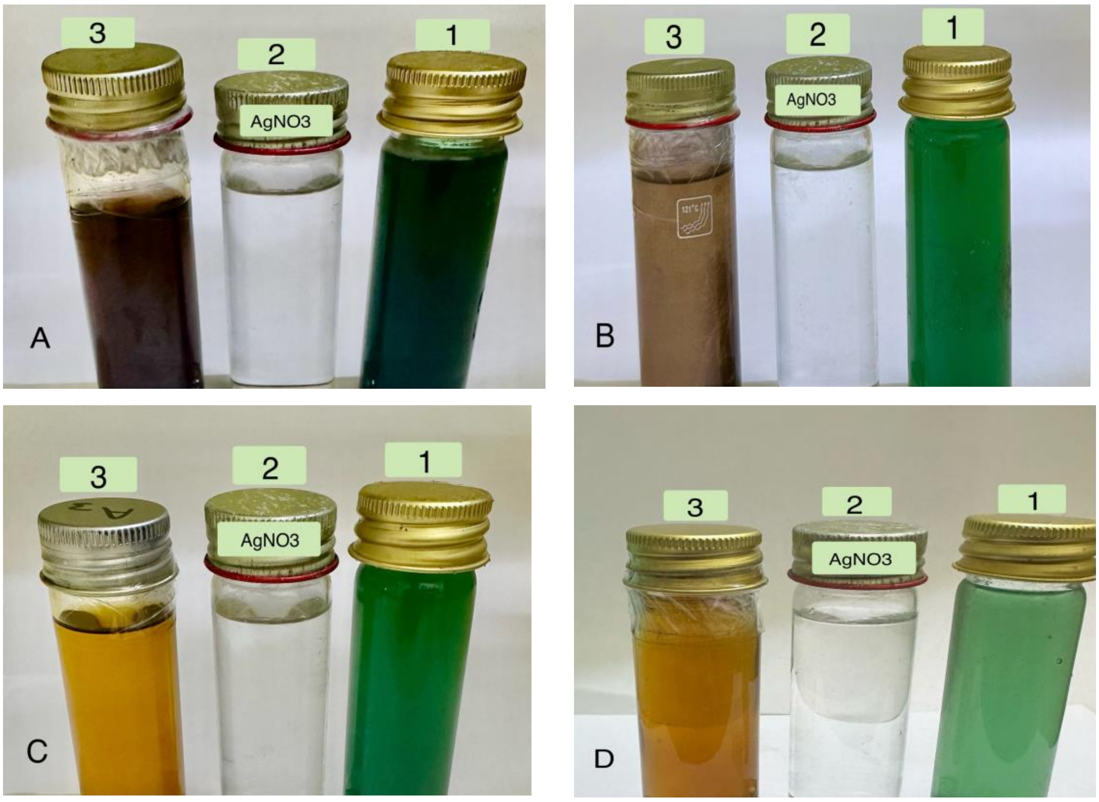

Figure 1. Color changed of hexane extract indicating the formation of silver nanoparticles: 1A: *Cladophora glomerata* extract, 1B: *Chlorella vulgaris* extract, 1C: *Spirogyra neglecta* extract and 1D: *Spirulina platensis* extract. 2: ($AgNO_3$) solution and 3: AgNPs

The UV-vis absorption spectra of the samples revealed a prominent absorption peak at a wavelength of around 400 nm, which can be attributed to the surface plasmon resonance of the silver nitrate nanomaterial (Biliuk et al. 2020). Fourier Transform Infrared Spectroscopy (FTIR) is a technique used to analyze the interaction between matter and infrared light by measuring the absorption and transmission of infrared radiation.

FTIR for $AgNO_3$Ps that add to *Cladophora glomerata* algae: The peaks at (3436.65, 2078.95, 1637.26, 1384.79, and 687.44) cm^{-1} are related to different types of functional groups, including alcohol and hydroxy compound H-bonded OH stretch, alkynes C≡C stretch, alkene C=C stretch, alcohol and hydroxy compound O-H bend, and alcohol and hydroxy compound OH out- of plane bend) respectively, as shown in Figure 3. These results correspond with (Singh et al. 2019).

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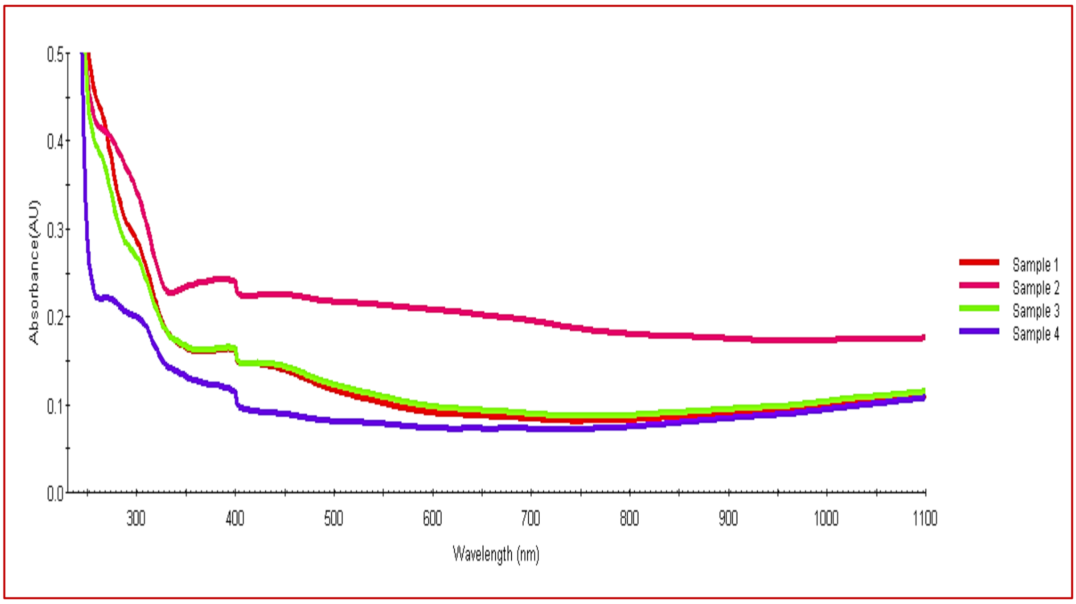


Figure 2. Results of UV-VIS analysis of AgNPs composed by hexane extract of (*Cladophora glomerata, Chlorella vulgaris, Spirogyra neglecta, and Spirullina platensis*) algae

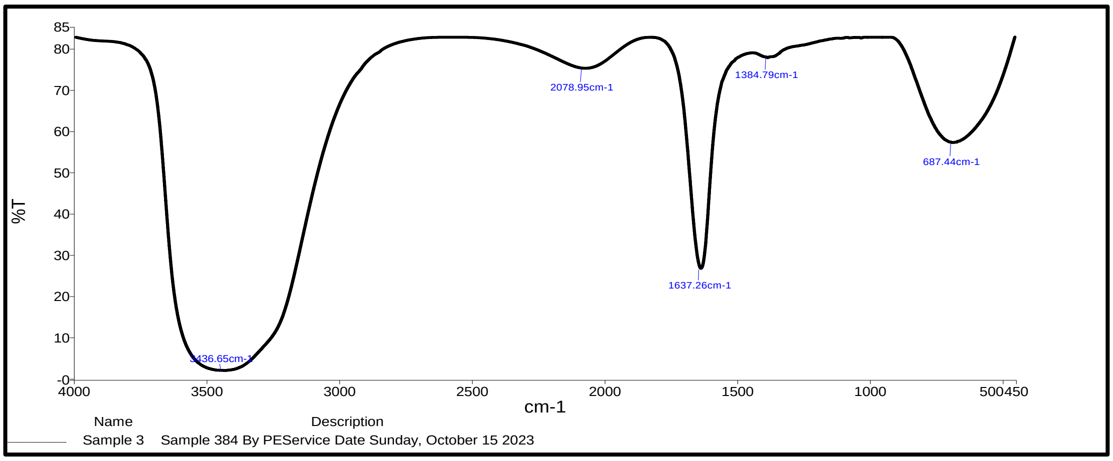


Figure 3. Results of FTIR spectra analysis of AgNPs composed by hexane extract of *Cladophora glomerata* Algae

FTIR for AgNO$_3$Ps that add to *Chlorella vulgaris* algae: The peaks at (3437.44, 2075.20, 1637.30, 1384.76, and 687.51) cm^{-1} are related to different types of functional groups, including alcohol and hydroxy compound H-bonded OH stretch, alkynes C≡C stretch, alkene C=C stretch, alcohol and hydroxy compound O-H bend, and alcohol and hydroxy compound OH out- of plane bend) respectively, as shown in Figure 4. These results correspond with (Bērziņš et al. 2021).

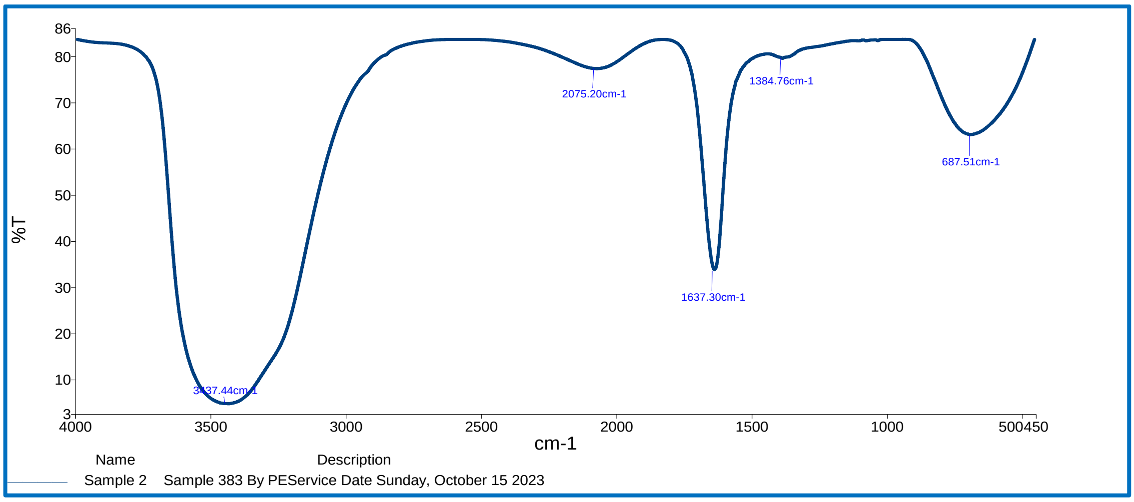


Figure 4. Results of FTIR spectra analysis of AgNPs composed by hexane extract of *Chlorella vulgaris* Algae

FTIR for $AgNO_3$Ps that add to *Spirogyra neglecta* algae: The peaks at (3437.16, 2067.76, 1637.15, 1384.76, and 684.56) cm^{-1} are related to different types of functional groups, including alcohol and hydroxy compound H-bonded OH stretch, alkynes C≡C stretch, alkene C=C stretch, alcohol and hydroxy compound O-H bend, and alcohol and hydroxy compound OH out- of plane bend) respectively, as shown in Figure 5. These results correspond with (Butova et al. 2023).

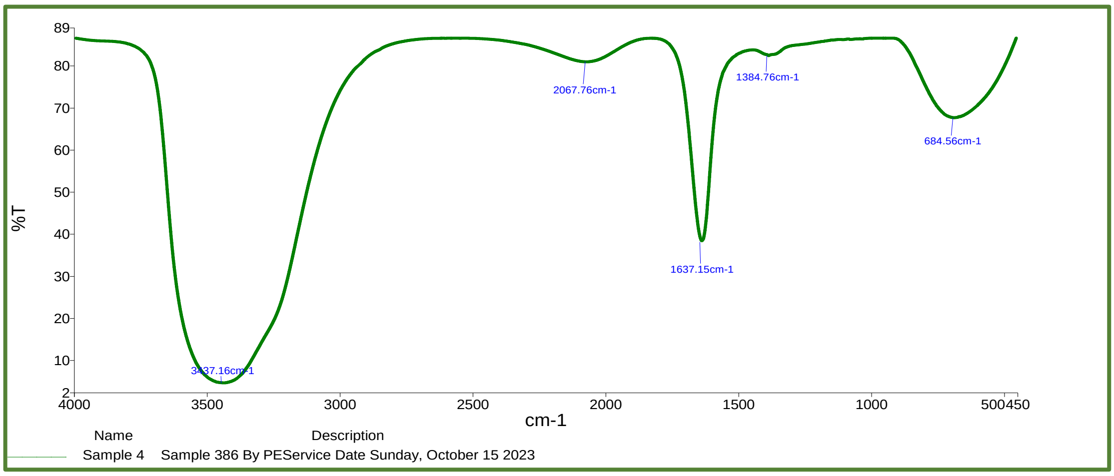


Figure 5. Results of FTIR spectra analysis of AgNPs composed by hexane extract of *Spirogyra neglecta* Algae

FTIR for $AgNO_3$Ps that add to *Spirullina platensis* algae: The peaks at (3437.14,2070.38, 1637.37, 1384.58, and 687.88) cm^{-1} are related to different types of functional groups, including alcohol and hydroxy compound H-bonded and OH stretch, alkynes C≡C stretch, alkene C=C stretch, alcohol and hydroxy compound O-H bend, and alcohol and hydroxy compound OH out-of plane bend) respectively, as shown in Figure 6. These results correspond with (Contreras et al. 2022).

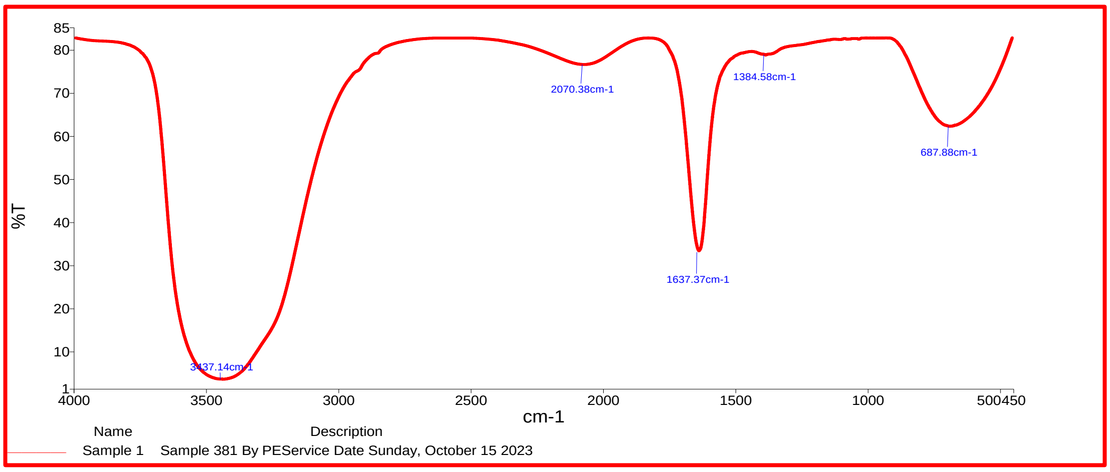


Figure 6. Results of FTIR spectra analysis of AgNPs composed by hexane extract of *Spirullina platensis* Algae

The scanning electron microscopy (SEM) photographs showed the presence of silver nitrate nanoparticles ($AgNO_3$Ps) in various types of algae (*Cladophora glomerata, Chlorella vulgaris, Spirogyra neglecta, and Spirullina platensis*). The SEM images clearly showed varied morphologies of the nanoparticles, including spherical, rod-like, and hexagonal structures (Mar et al. 2018). The structure comprises clusters of granules that are oriented in a regular pattern and vary in size. Additionally, there is a uniform distribution of $AgNO_3$Ps, which have varying magnification powers (10µ, 1µ, 500nm, and 200nm) as shown in Figure 7.

The morphology of the acquired nanoparticles is contingent upon the composition of the material and the specific circumstances employed during the preparation process. The software (Image J 1.47) was utilized to determine the average particle size (Kusumaningrum et al. 2019). The Tables 1 illustrated the differences in particle size and characteristics for the examined samples. The granular sizes for silver nitrate fed to the algae (*Cladophora glomerata, Chlorella vulgaris, Spirogyra neglecta, and Spirullina platensis*) were as follows: (13.81354, 9.18145, 716.94268, and 61.97411 nm), respectively. The variation in granular sizes can be attributed to the distinct biological makeup of the algae being examined. The magnitude of its reaction to the impact of silver nitrate nanoparticles incorporated into it (Khoshnamvand et al. 2020). The hexane extracts of *Cladophora glomerata*, *Chlorella vulgaris*, *Spirogyra neglecta*, and *Spirulina* *platensis* were used to synthesize AgNPs, which were then tested against MDR pathogenic bacteria to determine the zone of inhibition that was produced.

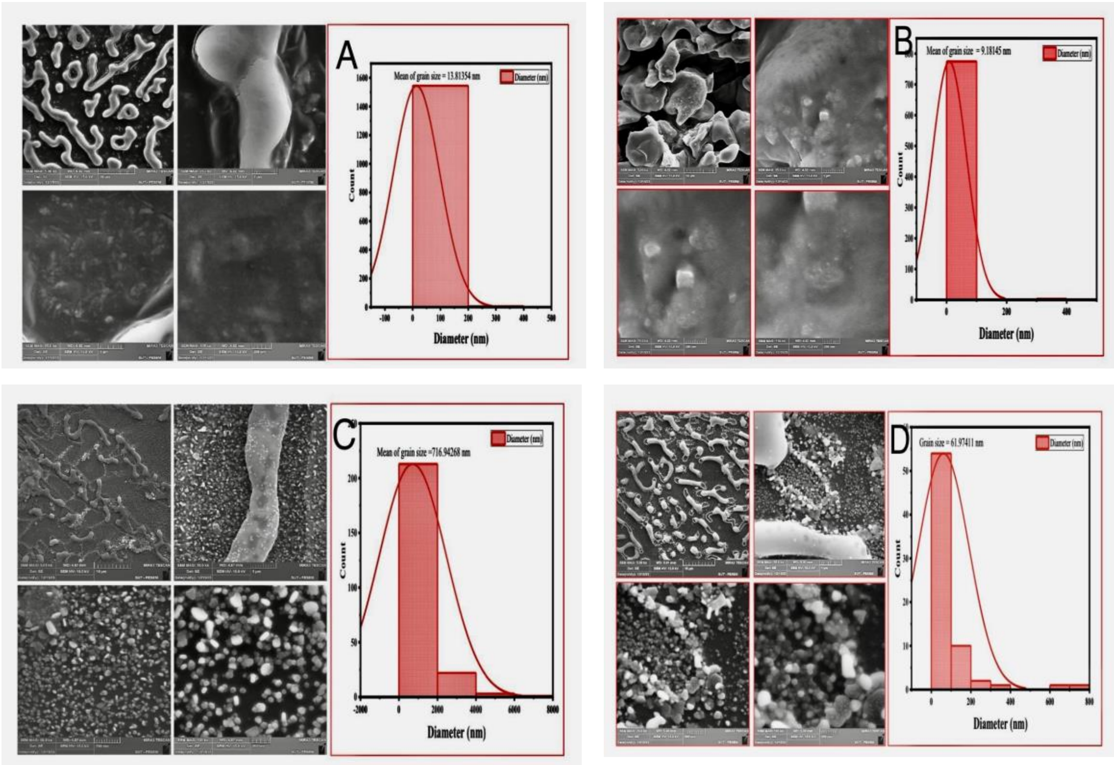


Figure 7. SEM image of AgNPs composed by hexane extract of (A-*Cladophora glomerata*, B-*Chlorella vulgaris, C-Spirogyra neglecta, and* D-*Spirullina platensis*) algae

Table 1. Mean of diameter and Standard Deviation for AgNO3Ps that add to algae in this study

Diameter (nm)	N total	Mean	Standard Deviation	Sum	Minimum	Median	Maximum
Cladophora glomerata	1554	13.81354	83.41216	21466.24204	1.27389	3.82166	3001.27389
Chlorella vulgaris	782	9.18145	57.14331	7179.89268	1.32643	2.65287	1366.2035
Spirogyra neglecta	240	716.94268	1741.59126	172066.24204	1.27389	28.02548	21775.7961
Spirullina platensis	69	61.97411	128.07531	4276.21369	0.13248	5.57102	701.53885

The antibacterial activity of crude extracts and silver nanoparticles was evaluated. Of the tested multidrug resistant bacteria, the extract from *Cladophora glomerata* silver nanoparticles demonstrated highly significant inhibition in all species of bacteria. Table 2 displays the statistically significant (P<0.05) outcomes of *Chlorella vulgaris* AgNPs against various microorganisms, including *E. coli* (AgNPs, 43.8; crude, 19.3 mm), *S. aureus* (AgNPs, 42.8; crude, 21.8 mm), *P. aeruginosa* (AgNPs, 42.1; crude, 18.3 mm), *E. faecalis* (AgNPs, 41.5; crude, 19.3 mm), and *K. pneumonia* (AgNPs, 40.5; crude, 21.0 mm) as shown in Figure 8. Table 3 displays the statistically significant (P<0.05) outcomes of the *Cladophora glomerata* extracts against the following microorganisms: *E. coli* (AgNPs, 44.1; crude, 23.5 mm), *S. aureus* (AgNPs, 43.1; crude, 19.8 mm), *P. aeruginosa* (AgNPs, 42.5; crude, 21.0 mm), *E. faecalis* (AgNPs, 41.5; crude, 18.6 mm), and *K. pneumonia* (AgNPs, 40.1; crude, 19.6 mm) as shown in Figure 9. As indicated in Table 4, *Spirullina platensis* extracts demonstrated statistically significant (P<0.05) outcomes against the following pathogens: *E. coli* (AgNPs, 31.1; crude, 20.6 mm), *S. aureus* (AgNPs, 32.5; crude, 19.5 mm), *P. aeruginosa* (AgNPs, 30.8; crude, 18.5 mm), *E. faecalis* (AgNPs, 30.8; crude, 16.0 mm), and *K. pneumonia* (AgNPs, 30.8; crude, 19.5 mm) as shown in Figure 10. Table 5 displays the statistically significant (P< 0.05) outcomes obtained from *Spirogyra neglecta* extracts against the following microorganisms: *E. coli* (AgNPs, 37.5; crude, 20.8 mm), *S. aureus* (AgNPs, 37.1; crude, 20.3 mm), *P. aeruginosa* (AgNPs, 35.8; crude, 18.3 mm), *E. faecalis* (AgNPs, 35.1; crude, 19.6 mm), and *K. pneumonia* (AgNPs,36.1; crude, 15.3 mm) as shown in Figure 11.

Table 2. Antibacterial zone of inhibition (mm) of crude and silver nanoparticles' hexane extracts of *Chlorella vulgaris* at 100 mg/mL concentration

Bacteria	**Inhibition Zone of *Chlorella vulgaris* hexane extract Mean ± SD**		***p. value***
	Crude Extract	**AgNPs Extract**	
E. coli	19.3 ± 1.52	43.8 ± 3.88	0.001**
S. aureus	21.8 ± 2.84	42.8 ± 2.84	0.001**
P. aeruginosa	18.3 ± 2.08	42.1 ± 2.46	< 0.001**
E. faecalis	19.3 ± 1.75	41.5 ± 4.33	0.001**
K. pneumonia	21.0 ± 2.29	40.5 ± 2.17	< 0.001**
Control	0.00 ± 0.00	0.00 ± 0.00	---

*** P<0.05, **P<0.01**

Table 3. Antibacterial zone of inhibition (mm) of crude and silver nanoparticles' hexane extracts of *Cladophora glomerata* at 100 mg/mL concentration

Bacteria	Inhibition Zone of *Cladophora glomerata* hexane extract Mean ± SD		*p. value*
	Crude Extract	AgNPs Extract	
E. coli	23.5 ± 2.29	44.1 ± 1.44	< 0.001**
S. aureus	19.8 ± 1.25	43.1 ± 0.76	< 0.001**
P. aeruginosa	21.0 ± 2.00	42.5 ± 0.50	< 0.001**
E. faecalis	18.6 ± 1.25	41.5 ± 2.78	< 0.001**
K. pneumonia	19.6 ± 0.57	40.1 ± 2.75	< 0.001**
Control	0.00 ± 0.00	0.00 ± 0.00	---

*** P<0.05, **P<0.01**

Table 4. Antibacterial zone of inhibition (mm) of crude and silver nanoparticles' hexane extracts of *Spirulina platensis* at 100 mg/mL concentration

Bacteria	Inhibition Zone of. *Spirulina platensis* hexane extract Mean ± SD		*p. value*
	Crude Extract	AgNPs Extract	
E. coli	20.6 ± 2.08	31.1 ± 5.00	0.028*
S. aureus	19.5 ± 0.50	32.5 ± 4.09	0.005**
P. aeruginosa	18.5 ± 0.86	30.8 ± 4.01	0.004**
E. faecalis	16.0 ± 2.64	30.8 ± 3.68	0.006**
K. pneumonia	19.5 ± 1.32	30.8 ± 4.64	0.015*
Control	0.00 ± 0.00	0.00 ± 0.00	---

*** P<0.05, **P<0.01**

In the present study, both the crude and AgNP extracts of all selected algae, *Cladophora glomerata*, *Chlorella vulgaris, Spirogyra neglecta, and Spirullina platensis*, showed good inhibition against MDR bacteria activity. Comparing AgNP extract to crude extract, however, the former demonstrated somewhat greater inhibition against bacterial strains. SEM analysis has confirmed that AgNPs synthesized from *Cladophora glomerata* have a good antimicrobial effect against both gram-positive and gram-negative pathogens by disrupting the bacterial cell membrane (Habibullah et al. 2022).

Agricultural Biotechnology Journal; Print ISSN: 2228-6705, Electronic ISSN: 2228-6500

Table 5. Antibacterial zone of inhibition (mm) of crude and silver nanoparticles' hexane extracts of *Spirogyra neglecta* at 100 mg/mL concentration

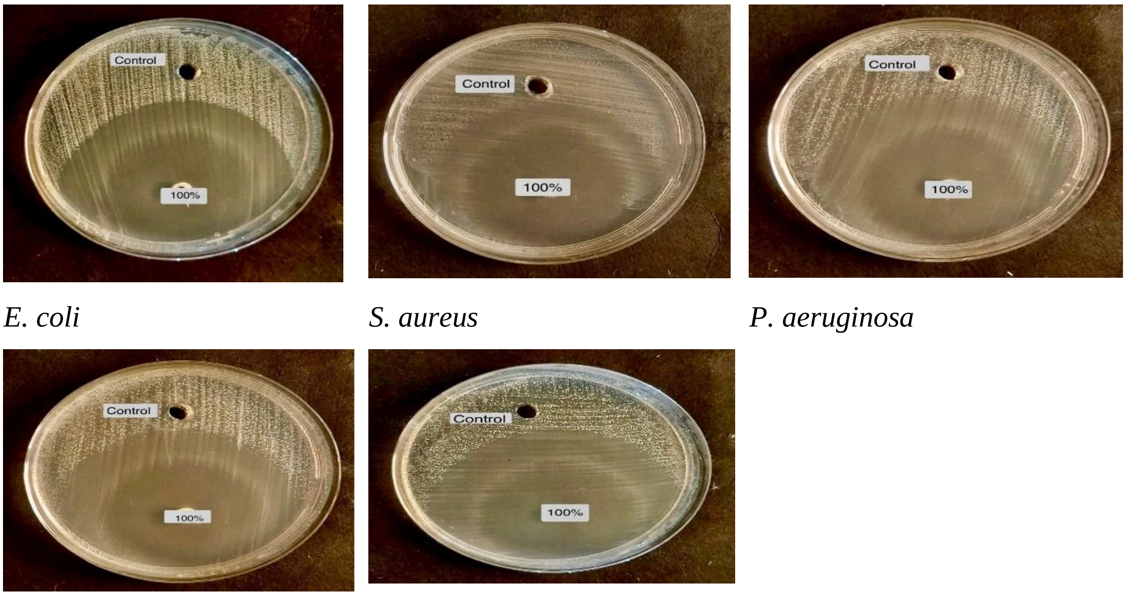
Bacteria	Inhibition Zone of *Spirogyra neglecta* hexane extract Mean ± SD		*p. value*
	Crude Extract	AgNPs Extract	
E. coli	20.8 ± 2.02	37.5 ± 3.12	0.001**
S. aureus	20.3 ± 2.30	37.1 ± 5.92	0.010*
P. aeruginosa	18.3 ± 0.57	35.8 ± 3.01	0.001**
E. faecalis	19.6 ± 2.08	35.1 ± 4.25	0.005**
K. pneumonia	15.3 ± 1.44	36.1 ± 5.05	0.002**
Control	0.00 ± 0.00	0.00 ± 0.00	---

*** P<0.05, **P<0.01**

The pathogenic microbes' cellular structure is damaged by these tiny, readily absorbed silver nanoparticles (AgNPs), which also target the disease site. AgNPs with strong antimicrobial activity against gram-negative bacteria were also found in the Solanaceae family (Fatimah et al. 2023). It has been reported that nanoparticles encased with phytoconstituents can be more effective than unbound glycoproteins. These monomeric glycoproteins were isolated from the antimicrobial activities against bacterial isolates (Ali & Taher 2023). According to a study by (Panzarini et al. 2018), methanol extracts from *Cladophora glomerata* showed notable antimicrobial activity against a variety of bacteria. The mechanisms underlying metallic nanoparticles' antimicrobial activity include proteins that inactivate DNA replication and weaken it (Bhuyar et al. 2020).

The release of silver ions (Ag +) in the cells and attached bioactive components is what gives AgNPs their antimicrobial activity (Abd El Aty et al. 2020). The antimicrobial activity of synthesized silver nanoparticles against *E. coli, S. aureus, K. pneumoniae, E. faecalis, and P. aeruginosa* was reported by (Ghajiri et al. 2023). The precise process has not been fully explained, but the mechanisms involved include changes in cell membrane permeability (Shareef et al. 2024), the production of a class of free radicals that cause cell membrane damage (Drummer et al. 2021), and indulgence of the single proton (H^+) attractive force that causes cell membrane damage (Liu 2023). Furthermore, bacterial growth was significantly retarded by the effect of silver nanoballs on various bacteria strains, including *E. Coli, S. typhimurium, P. aeruginosa,* and *B. subtilis,* as measured by the formation of colony forming units (cfu) and growth rate at different concentrations of 40 mg/mL (Zhao et al. 2022). Given that Ag^{2+} forms complexes with DNA and

RNA and interacts with nucleosides specifically, its positive charge may have strong toxicity or antimicrobial properties (Holder & Schaak 2019). Furthermore, it has been documented that negatively charged microbial cells and positively charged NP are attracted to each other electrostatically (Lyonnais et al. 2021). Ag^{2+} ions exhibit cytoplasmic and cell wall binding through their affinity for sulfur proteins and electrostatic attractions. This leads to a significant increase in permeability and the disintegration of the bacterial casing. Thus, various forms of nanoparticles can be employed for effective agricultural management and the creation of novel insecticides. While the comparative standard drug exhibited greater inhibition, the chemically and biologically synthesized crude extracts of algae and AgNPs are cost-effective and eco-friendly to use.



E. coli *S. aureus* *P. aeruginosa*

K. Pneumoniae *E. faecalis*

Figure 8. Antibacterial activity of AgNPs composed by hexane extract of *Chlorella vulgaris* by (100µg/mL) with control DMSO

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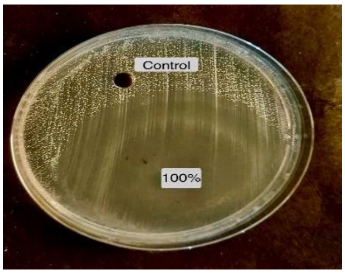
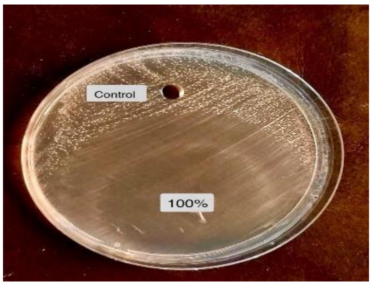
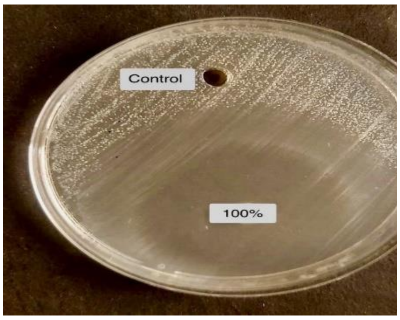
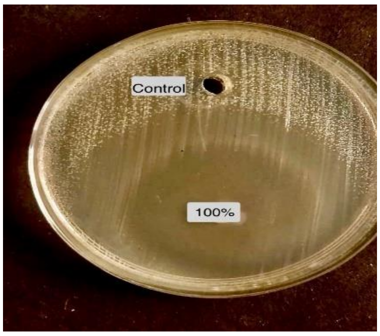




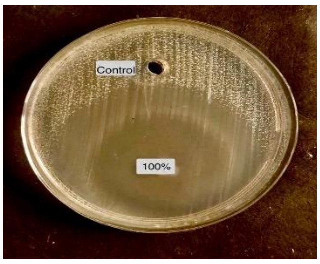
E. coli *S. aureus* *P. aeruginosa*

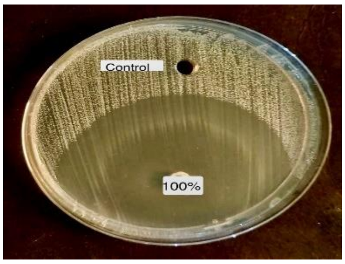


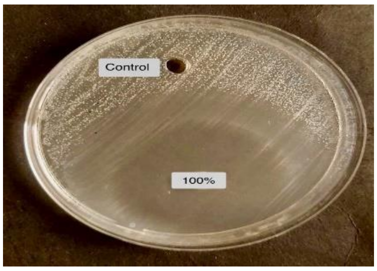


K. Pneumoniae *E. faecalis*

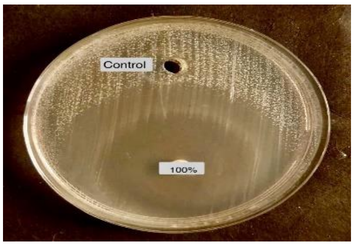
Figure 9. Antibacterial activity of AgNPs composed by hexane extract of *Cladophora glomerata* by (100µg/mL) with control DEMSO







E. coli *S. aureus* *P. aeruginosa*



K. Pneumoniae *E. faecalis*

Figure 10. Antibacterial activity of AgNPs composed by hexane extract of *Spirogyra neglecta* by (100µg/mL) with control DMSO

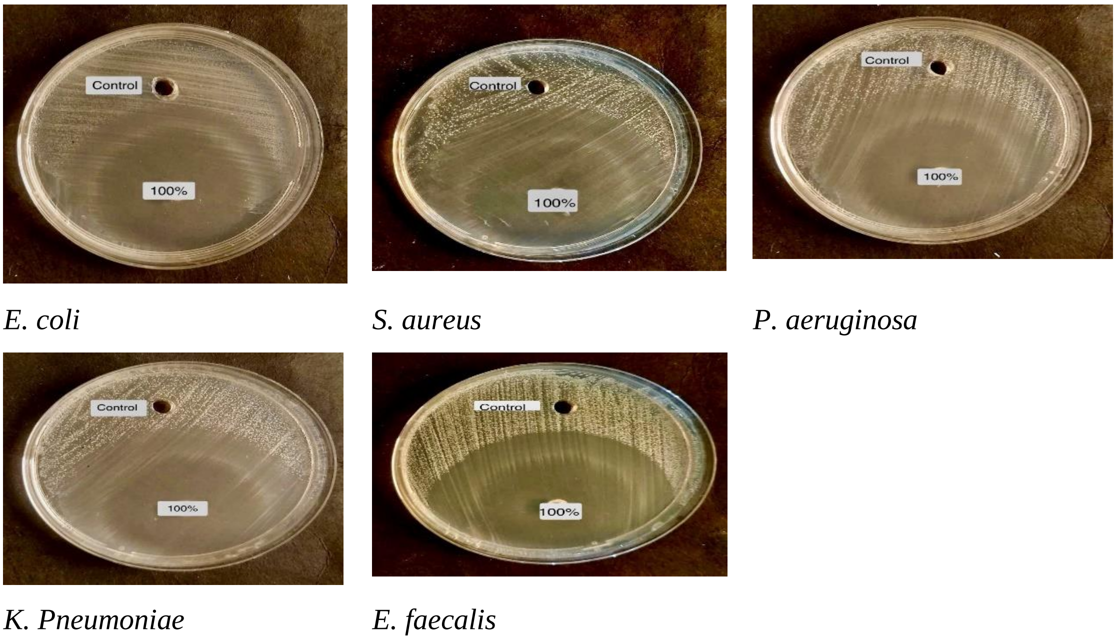


Figure 11. Antibacterial activity of AgNPs composed by hexane extract of *Spirulina platensis* by (100µg/mL) with control DMSO

Conclusions: Silver nanoparticles (AgNPs) could be synthesized using hexane extract from *Chlorella vulgaris, Cladophora glomerata, Spirogyra neglecta,* and *Spirulina platensis*. These nanoparticles were examined using SEM and UV-Vis spectroscopy. AgNPs' antibacterial activity was evaluated at a concentration of 100 µg/mL against MDR bacteria. However, in contrast, AgNPs synthesized from freshwater algae, such as *Chlorella vulgaris, Cladophora glomerata, Spirogyra neglecta,* and *Spirulina platensis*, demonstrated enhanced inhibition against MDR bacteria. This study found a proportionate relationship between crude and AgNP extracts exhibiting good effects. The biological activities of AgNPs on pathogens that have been tested pave the way for the development of novel antimicrobial agents and may hold the key to combating antibiotic resistance. Additionally, nanomaterials in a variety of forms can be employed to manage agricultural practices and create new insecticide formulations. The results of this study have given researchers working in the field of nanomedicine a foundation upon which to synthesis and test the biological activity of compounds isolated from freshwater algae and nanoparticles against other microorganisms. These natural products should be used to create novel antibiotics and therapeutics in nanoform, and those that exhibit microorganism resistance should be substituted, the extract from *Cladophora neglecta* silver nanoparticles demonstrated highly significant inhibition in all species of bacteria.

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فعالیت ضد باکتریایی نانوذرات نقره سنتز شده با عصاره هگزان برخی از جلبک‌های آب شیرین در برابر باکتری‌های مقاوم به چند دارو

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چکیده

هدف: باکتری‌های مقاوم به آنتی بیوتیک به دلیل استفاده ناخواسته از آنتی بیوتیک‌ها به یک نگرانی جهانی تبدیل شده‌اند که منجر به ایجاد سویه‌های باکتریایی مقاوم به بسیاری یا همه آنتی بیوتیک‌های موجود شده است. متابولیت‌های اولیه و ثانویه موجود در جلبک‌ها نقش عمده‌ای در تبدیل نیترات نقره به نانوذرات نقره (AgNPs) دارند.

مواد و روش‌ها: در فرآیند ساخت نانوذرات از عصاره هگزانی برخی از جلبک‌های آب شیرین استفاده شد. تغییر رنگ محلول واکنش از زرد به قهوه ای تیره به دلیل تحریک رزونانس پلاسمون سطحی به عنوان شاهدی برای این امر است. بر اساس تحقیقات انجام شده با استفاده از تبدیل فوریه مادون قرمز (FTIR) برای شناسایی گروه جلبک‌های موثر که در تشکیل آن‌ها نقش دارند، AgNPها با استفاده از طیف سنجی UV-Vis شناسایی شدند، پروتئین‌ها و فنل‌ها نقش مهمی در تشکیل نانوذرات نقره ایفا می‌کنند. نانوذرات یک میکروسکوپ الکترونی روبشی (SEM) برای مشخص کردن شکل‌ها و اندازه‌های نانوذرات نقره سنتز شده، که شامل ساختارهای کروی، میله‌ای و شش ضلعی است، استفاده شد. باکتری‌های مقاوم به چند دارو (MDR) تشخیص داده شده توسط سیستم Vitek Compact 2 برای آزمایش فعالیت ضد باکتریایی AgNPها استفاده شد.

نتایج: مطالعه بر روی اثر ضد باکتریایی نانوذرات بیوسنتزی نقره در برابر جدایه‌های منتخب باکتری MDR انجام شد. نتایج نشان داد که نانوذرات نقره تهیه‌شده از عصاره هگزانی جلبک‌های جدا شده در غلظت ۱۰۰ درصد، مهار بیشتری نسبت به عصاره خام انواع باکتری‌های بیماری‌زا، با تفاوت‌های آماری معنی‌دار نشان دادند (P<۰/۰۵).

نتیجه‌گیری: نانوذرات نقره تهیه‌شده از عصاره هگزانی بر روی جدایه‌های باکتری G-ve و G+Ve MDR (E. coli, P. aeruginosa, S. aureus, K. Pneumoniae, and E. faecalis) در غلظت‌های ۱۰۰ میکروگرم بر میلی‌لیتر موثرتر از عصاره نانوذرات نقره Cladophora neglecta که بدون عصاره هگزان نانوذرات نقره تهیه شده بودند، مهار بسیار قابل توجهی را در همه گونه‌های باکتری نشان داد.

واژه‌های کلیدی: جلبک آب شیرین، فعالیت ضد باکتریایی، کلادوفورا گلومراتا، نانوذرات نقره، MDR

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