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RESEARCH PAPER

Porphyridium cruentum as a biological component for the green synthesis of metal nanoparticles and for the evaluation of their antimicrobial activity

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Abstract

Silver nanoparticles are an alternative to new-generation antimicrobial agents with their antimicrobial activity. Iron and zinc nanoparticles can potentially be used as UV protection in various applications. Nowadays, green synthesis of nanoparticles as a sustainable alternative attracts attention. Microalgae are promising in nanoparticle synthesis among biological sources due to their high biomass productivity and heavy metal accumulation ability. The present study aimed to investigate the potential of synthesizing intracellular silver, zinc and iron nanoparticles from *Porphyridium cruentum* microalgae. For nanoparticle synthesis, the effects of metal solution concentration and amount of biomass on particle size were investigated. The nanoparticles were characterized by dynamic light scattering, UV-vis spectrophotometry, and antimicrobial activity test. Silver nanoparticles of 169.7 nm, zinc nanoparticles of 189 nm, and iron nanoparticles of 356.7 nm were characterized by DLS. 169.7 nm silver nanoparticles were synthesized with 9.83 mM $AgNO_3$ concentration and 0.19 mg/ml biomass: metal solution mixing ratio. The surface plasmon resonance band of silver nanoparticles was observed in the 300-350 nm wavelength range. According to the antibacterial activity results of silver nanoparticles, inhibition zone diameters were obtained as 10.83±0.76 mm and 11.33±0.57 mm against *Escherichia coli* and *Staphylococcus aureus* bacteria, respectively.

Introduction

Nanoparticles, as one of the nanomaterials, are defined as solid colloidal particles sized in the range of 10 nm to 1000 nm (McNamara & Tofail, 2017; Prabha et al., 2016). Biological routes have gained considerable attention in the field of green chemistry for the synthesis of nanoparticles, as they offer environmentally friendly, cost-effective, rapid, and straightforward alternatives to conventional methods due to the absence of complex chemical reactions. The green synthesis of metal nanoparticles using biological metabolites is particularly advantageous because the abundant intracellular components act as reducing and stabilizing agents, effectively converting metal ions into nanoparticles (Vijayaram et al., 2024). These green-synthesized nanoparticles show superior performance in removing synthetic dyes, chemicals, antibiotics, and heavy metals compared to their conventionally produced counterparts. In addition, their biofunctional properties, such as antibacterial, antiviral, antifungal, and antioxidant activities make them highly promising for applications in cosmetics and biomedicine, including UV protection, wound healing, regenerative medicine, diagnostics, biosensing, and immunotherapy. Beyond biomedical applications, they are increasingly being used in environmental remediation, photocatalytic reduction, energy storage, sensing, and food packaging (Aigbe & Asibate, 2024; Cepoi et al., 2021; Dağlıoğlu &

Yılmaz Öztürk, 2019; Jeon et al., 2021; Kandav & Sharma, 2024; Patel et al., 2015; Vijayaram et al., 2024).

Natural resources such as bacteria, fungi, algae, plant extracts, enzymes, and natural polymers are utilized for the biosynthesis of nanoparticles. Although plant extracts and bacteria are the most common resources for metal nanoparticle synthesis, has gained increasing attention to the usage of microalgae for the reduction of metal ions to nanoparticles via biological pathways in recent years. Because microalgae have the ability to accumulate toxic metals and bioremediate them by converting more stable form (Patel et al., 2015). The ability of microalgae to efficiently reduce metal ions comes from the specific intracellular and extracellular metabolites (Yedoti & Supraja, 2024). Within the extracellular pathway, the reduction of metal ions occurs by the reductase enzymes and electron shuttle quinones, whereas the intracellular reduction mechanism has been carried out through the NADH-dependent reductase enzyme as an electron supplier. Proteins and polysaccharides work as stabilizers and capping agents of metal nanoparticles. Most metal nanoparticles that have high surface energy tend to agglomerate. Therefore, capping agents are essential to avoid agglomeration of colloidal dispersions (Dağlıoğlu & Yılmaz Öztürk, 2019; Deb & Sudrathar, 2024; Patel et al., 2015; Restrepo & Villa, 2021). Most microalgae produce these polymers intracellularly or extracellularly during their life cycle. *Porphyridium cruentum*, which is rich in natural bioactive compounds such as proteins, phycoerythrin, polyunsaturated fatty acids, high-quality sulphated polysaccharides, and floridean starch, has a higher capacity as a source of valuable metabolites (Tounsi, 2024). Thus, additional stabilizers may not be required for the nanoparticle synthesis via microalgae.

In previous reports, various microalgae have been investigated for the biogenic nanoparticle synthesis. *Euglena* sp., *Amphora* sp., *Botryococcus braunii*, *Chlorella* sp., *Chlamydomonas* sp., *Anabaena* sp., *Synechocystis* sp., *Synechococcus* sp., *Spirulina platensis, Phaeodactylum tricornutum, Galdieria sulphuraria, Schizochytrium* sp., *Chaetoceros calcitrans, Chlorella salina, Isochrysis galbana, Tetraselmis gracilis, Scenedesmus* sp. are some suggested microalgae and cyanobacteria species for metal nanoparticle synthesis (Çalışkan et al., 2020, 2022; Dahoumane et al., 2016; Gallón et al., 2019; Merin et al., 2010; Mutaf et al., 2020, 2023; Senapati et al., 2012; Wishkerman et al., 2017).

The rise of multidrug-resistant bacterial strains, exacerbated by the inappropriate use of antibiotics, and the adverse side effects of conventional treatments, has made the development of new-generation antibacterial agents essential (Shumi et al., 2023). In this context, metal nanoparticles, including silver, zinc, iron, gold, and selenium, have demonstrated significant antibacterial properties and offer promising solutions for treating antibiotic-resistant bacteria and improving wound healing (Sharifiaghdam et al., 2021). Research suggests that metal oxide nanoparticles, such as zinc oxide, iron oxide, and nickel oxide, are emerging as potential nano-antibiotics. Silver nanoparticles, another class of metal nanoparticles, have inherent antibacterial activity. Studies have shown that they have potent antibacterial effects against pathogens such as *Escherichia coli, Staphylococcus aureus*, *Vibrio cholerae*, and *Bacillus subtilis*. These nanoparticles exert their antibacterial effect primarily by disrupting the microbial cell wall and membrane, compromising cell integrity, and inducing oxidative stress. The efficacy of nanoparticles is influenced by their size and shape (Vijayaram et al., 2024). In addition to bacterial infections, degenerative diseases such as diabetes, ageing, Parkinson's disease, cancer, atherosclerosis and cardiovascular disease are associated with oxidative stress caused by free radicals. Consequently, antioxidant agents, potentially in the form of metal nanoparticles, offer protection against these reactive diseases. Recent studies highlight the potent antioxidant activities of metal nanoparticles synthesized by green methods, positioning them as promising candidates for next-generation antioxidants (Dhandapani et al., 2020; Rani et al., 2023). However, concerns remain regarding the cytotoxicity of metal nanoparticles, especially with long-term use. Limited studies have investigated their interaction with healthy human cells, as these nanoparticles may induce oxidative stress and cellular damage, and their safety and toxicity profiles require further investigation (Sharifiaghdam et al., 2021; Vijayaram et al., 2024).

P. cruentum has the high potential to be used in many industries, especially food and cosmetics, with its sulphated exopolysaccharide, and its carbohydrate, lipid, and protein content and especially intracellular phycoerythrin pigment. In addition to its current areas of use, *P. cruentum* has the potential to reduce metal ions to nanoparticles thanks to its rich intracellular metabolites. The current study aims to investigate the synthesis potential of silver, zinc, and iron nanoparticles through intracellular metabolites of the microalgae *P. cruentum*. The effects of metal solution concentration and amount of wet biomass on particle size were investigated. To the best of the authors' knowledge, this study is the first to investigate the synthesis of intracellular metal nanoparticles from *P. cruentum* by optimizing the important parameters for nanoparticle synthesis.

Materials and Methods

Materials

Porphyridium cruentum UTEX161 was purchased from the University of Texas Culture Collection, and the modified BG11 (supplemented with 15 g/L sea salt) culture media was used (Ruiz-Ruiz et al., 2013; Stanier et al., 1971). All chemicals, reagents, and solvents used in this study were purchased from Sigma-Aldrich, USA, and Merck & Co. Inc., Germany.

***Porphyridium cruentum* biomass production**

P. cruentum microalgae were cultivated photoautotrophically with a 150 mL working volume in BG11 supplemented with a sea salt culture medium. Cultivation was carried out at 23±1 ℃ temperature, 70 µE m^{-2} s^{-1} light intensity, and 120 rpm orbital shaking. Daily analyses were performed by measuring optical density at 760 nm and microscopic cell counting to determine the cell growth cycle. Biomass was harvested on about the 20th day of cultivation when the culture was in the late exponential phase. Harvesting was performed at room temperature by centrifugation (NF400, Nüve) for 10 min at 4000 rpm. Obtained wet biomass was used to synthesize silver, iron, and zinc nanoparticles.

Determination of intracellular metabolite concentrations

To obtain intracellular metabolites of microalgae, wet biomass was washed with distilled water and used for total protein, total carbohydrate, and total pigment (chlorophyll and carotenoid) analysis. The total protein measurement was performed by the Lowry protein assay (Lowry et al., 1951). Total carbohydrate content was measured by the phenol-sulfuric acid method (Dubois et al., 1956). Total carbohydrate and total protein content were calculated using the calibration curve. For pigment measurement, 5 mL of methanol was added to the 5 mL culture in a ratio of 1:1 (v:v). The sample was vortexed for 1 min and kept at 60 °C for 30 min. It was vortexed again and centrifuged at 4100 rpm for 10 min. The absorbance of supernatant was measured by UV-Vis spectroscopy (Optizen POP, MECASYS) at wavelengths of 470 nm, 645 nm, and 662 nm (Mhatre et al., 2018). The amount of total carotenoid and total chlorophyll was calculated using the formulae by Mhatre et al. (2018) as described in equations (1) – (4).

$$Chlorophyll_a(mg/L) = (11.75 \times A_{662}) - (2.35 \times A_{645}) \quad (1)$$

$$Chlorophyll_b(mg/L) = (18.65 \times A_{645}) - (3.96 \times A_{662}) \quad (2)$$

$$Total\ chlorophyll\ (mg/L) = Chlorophyll_a + Chlorophyll_b \quad (3)$$

$$Total\ carotenoid(mg/L) = (1000 \times A_{470}) - (2.27 \times Chlorophyll_a) - [81.4 \times (Chlorophyll_b/227)] \quad (4)$$

Green synthesis of silver, zinc and iron nanoparticles

For nanoparticle synthesis experiments, the effect of two parameters, metal solution concentration and amount of wet biomass, on nanoparticle size was investigated. The investigated metal solution concentrations were 1-10 mM for $AgNO_3$, 0.25-5 mM for $ZnSO_4$*$7H_2O$, and 1-15 mM for $FeSO_4$*$7H_2O$ (Salem et al., 2019; Soleimani & Habibi-Pirkoohi, 2017). The amount of wet biomass was studied in the range of 0.1-5 mg/mL (Jena et al., 2015; Kathiraven et al., 2015; Sharma et al., 2014).

The wet biomass was suspended with 10 mL of metal solution at the appropriate concentration, and then the total volume of metal solution was made up to 20 mL working volume. To allow the reduction reaction to take place, incubation was carried out in a shaking incubator for 24 h at 25±1℃ with an orbital shaking speed of 200 rpm. After incubation, color changes were observed, and the samples were centrifuged at 4100 rpm for 5 min to remove the metal solution. As a negative control, cell-free metal solution and metal-free wet biomass were incubated under the same conditions (Jena et al., 2015).

In the intracellular synthesis of nanoparticles from microalgae, enzymes in the cell wall and cell membrane, and carboxyl groups, polyphosphates, enzymes, and polysaccharides in the cytoplasm play a fundamental role. Metal ions are reduced to metal nanoparticles within the cell wall, cell membrane and cytoplasm (Senapati et al., 2012; Wishkerman et al., 2017). Intracellularly synthesized nanoparticles have been collected into the extracellular environment for characterization after incubation. To obtain intracellularly synthesized nanoparticles, 3 mL of distilled water was added to the biomass, and cell disruption was performed by ultrasonication (Bandelin, Germany) at 90% amplitude for 9 cycles. Cell debris was then removed by centrifugation, and the nanoparticles that entered the extracellular environment were stored and characterized.

Physical characterization of nanoparticles

Dynamic light scattering (DLS)

The hydrodynamic diameter and polydispersity index of synthesized metal nanoparticles were measured with a Malvern, Zeta Sizer Nano-ZS instrument to determine optimum synthesis parameters. The measurements were performed in duplicates at room temperature.

UV-Visible (UV-Vis) Spectroscopy

During the formation of silver nanoparticles, the color of the suspension changes from clear to brown as an indicator of the reduction reaction (Murillo-Rábago et al., 2022). For FeNPs, the color of the solution changes from yellow to dark brown, while reduced zinc nanoparticles are light yellow in color (Fakhari et al., 2019; Saif et al., 2016). Since each metal nanoparticle has its own unique surface plasmon resonance, the color changes of the suspension differ after the reduction. The color change that gives a preliminary idea after nanoparticle formation needs to be quantitatively confirmed by UV-Vis spectroscopy. For this purpose, the surface plasmon bands of the nanoparticles were confirmed by spectroscopic measurements. Metal

nanoparticles' surface plasmon resonance bands were evaluated with UV-Vis spectroscopy at 300-700 nm.

Investigation of bio-functional properties of nanoparticles

Antimicrobial activity of nanoparticles

Well diffusion method was used to investigate the antimicrobial activity of metal nanoparticles against gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* bacteria. Metal solution and gentamicin (Biochrom AG,10 mg/mL) were used as the negative and positive control, respectively. For the inhibition tests, the Mueller Hinton broth culture medium was used. Bacterial cultures were incubated in broth culture media at 37°C for 18 h. The cultures incubated overnight were adjusted to McFarland 0.5 turbidity. The culture was then inoculated onto the agar using the spread plate technique. The nanoparticle solution containing 70 µg nanoparticles and negative and positive control samples were inoculated onto Oxoid antimicrobial susceptibility discs. Cultures were incubated at 37 ˚C for 24 h. After the incubation, inhibition zone diameters were measured (Mutaf et al., 2023; Subhapriya et al., 2018).

Antioxidant activity of nanoparticles

Considering the nanoparticle sizes, PDI values, agglomeration tendencies, and antimicrobial activities, it was determined that AgNPs were more stable and had higher biotechnological use potential than ZnNPs and FeNPs. Therefore, the antioxidant activity of the synthesized silver nanoparticles was also evaluated. The antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Butylated hydroxyanisole (BHA) and pure water were used as a positive control and negative control, respectively. The DPPH stock solution was prepared by dissolving methanol to a concentration of 0.1 mM. 500 µl of DPPH solution was added to 500 µL of colloidal nanoparticle suspension for activity determination. The same procedure was followed for the positive and negative control solutions. The samples were incubated in the dark for 30 min. After the reaction, the absorbance of the samples was measured at a wavelength of 517 nm, and the radical scavenging activity of the samples was expressed as % inhibition. The following formula was used to calculate the % inhibition (Al-Salhi et al., 2019):

$$\%DPPH\ free\ radical\ scavenging\ activity = \left[(A_{control} - A_{sample})/(A_{control})\right] \times 100 \quad (5)$$

$A_{control}$ is the absorbance of DPPH solution without sample and A_{sample} is the absorbance of sample solution.

Statistical analysis

Nanoparticle size measurements by DLS were performed in duplicate (n=2). Analyses of intracellular metabolites, as well as antimicrobial and antioxidant activity assays, were conducted in triplicate (n=3) and are presented as mean ± standard deviation. For antioxidant activity and antibacterial activity assays, statistical analysis was performed using GraphPad Prism version 8.0 for Windows. Statistically, differences between mean values were determined using one-way ANOVA ($p < 0.05$; mean ± SD).

Results and Discussion

Intracellular protein, carbohydrate, and pigment content of *P. cruentum*

For the biological synthesis of nanoparticles, primary and secondary metabolites are very important in reducing metal ions to metal nanoparticles and maintaining the stability of the synthesized nanoparticles (Singh et al., 2016). Since *P. cruentum* microalgae is rich in both intracellular and extracellular metabolites, it has a high potential to reduce metal ions (Cepoi et al., 2021; Jeon et al., 2021; Mutaf et al., 2023). The amounts of total carbohydrate, protein, chlorophyll, and carotenoids were determined as intracellular metabolites. Table 1 shows that total carbohydrate content was measured as 18%, while total protein content was calculated as approximately 13% of biomass. The total carbohydrate content was lower than in previous reports. In previous studies, the analyses were carried out using dry biomass. It is thought that this difference is caused by the fact that the analysis was carried out using wet biomass in the current study. (Aguilar-Ruiz et al., 2022; Fuentes et al., 2000; Sánchez-Saavedra et al., 2018). Total chlorophyll and carotenoid pigment contents of microalgae have been calculated as 8.477 ± 0.271 mg/L and 870.5471 ± 45.44 mg/L, respectively. Total protein and pigment contents as chlorophyll and carotenoid match those observed in earlier studies (Aguilar-Ruiz et al., 2022; Gallego et al., 2019; Rinawati et al., 2020; Sánchez-Saavedra et al., 2018). The rich intracellular carbohydrate, protein, and pigment content is promising that *P. cruentum* will provide the necessary reducing environment for the reduction of metal ions to metal nanoparticles.

Table 1. Intracellular protein, carbohydrate, chlorophyll, and carotenoid content of *P. cruentum*

Intracellular metabolite	Concentration
Total carbohydrate	0.18 ± 0.002 mg /mg dry biomass
Total protein	0.129 ± 0.0123 mg /mg dry biomass
Chlorophyll-a	7.538 ± 0.259 mg/L
Chlorophyll-b	0.94 ± 0.0302 mg/L
Total chlorophyll	8.477 ± 0.271 mg/L
Total carotenoid	870.5471 ± 45.44 mg/L

Physical characterization of synthesized silver, zinc, and iron nanoparticles

Dynamic light scattering (DLS)

Tables 2-4 present the hydrodynamic diameter of silver nanoparticles (AgNPs), zinc nanoparticles (ZnNPs), and iron nanoparticles (FeNPs) by DLS analysis. In terms of particle size and polydispersity index value (PDI), the

most successful nanoparticle synthesis was achieved with experiment 6 for AgNPs (Table 2), experiment 11 for ZnNPs (Table 3), and experiment 10 for FeNPs (Table 4). As shown in Figure 1a,. AgNPs synthesized with a 9.83 mM $AgNO_3$ solution concentration and 0.19 mg wet biomass presented a hydrodynamic diameter of 169.7 nm and PDI of 0.073. ZnNPs synthesized with 2.63 mM $ZnSO_4*7H_2O$ solution concentration and 0.1 mg wet biomass presented a hydrodynamic diameter of 189.1 nm and a PDI of 0.330 (Figure 1b). FeNPs synthesized with a 14.73 mM $FeSO_4*7H_2O$ solution concentration and 0.19 mg wet biomass presented a hydrodynamic diameter of 356.7 nm and PDI of 0.365 (Figure 1c).

Table 2. Experiments for silver nanoparticle synthesis

Experiment	$AgNO_3$ solution concentration (mM)	Wet biomass (mg/mL)	Hydrodynamic diameter (nm)
1	5.5	2.55	162.6
2	5.5	2.55	192.3
3	5.5	0.1	163.0
4	1.17	0.19	183.7
5	10	2.55	185.1
6	9.83	0.19	169.7
7	1	2.55	239.6
8	9.8	4.91	201.4
9	5.5	5	192.2
10	5.5	2.55	184.5
11	5.5	2.55	183.0
12	1.17	4.91	221.1
13	5.5	2.55	176.7

When the DLS analysis results are evaluated, Figure 1 shows that the metal solution concentration and the amount of biomass as parameters affect the particle size for all metal ions. In particular, the amount of biomass significantly affects both the particle size and the PDI of the nanoparticles. It can be seen that as the amount of biomass decreases for the production of AgNPs, ZnNPs, and FeNPs, the particle size decreases, and the PDI enters the desired range. The PDI indicates the average particle diameter and quality of nanoparticles according to their size distribution and gives an idea of the agglomeration tendency of nanoparticles. PDI values of 0.2 and below are an acceptable nanoparticle range (Danaei et al., 2018).

Table 3. Experiments for zinc nanoparticle synthesis

Experiment	$ZnSO_4$ solution concentration (mM)	Wet biomass (mg/mL)	Hydrodynamic diameter (nm)
1	2.63	5	261.2
2	5	2.55	340.8
3	2.63	2.55	228.4
4	0.25	2.55	273.7
5	0.34	0.19	586.0
6	2.63	2.55	273.9
7	4.91	0.19	573.9
8	2.63	2.55	371.0
9	2.63	2.55	293.3
10	4.91	4.91	317.0
11	2.63	0.1	189.1
12	2.63	2.55	301.9
13	0.34	4.91	243.5

When comparing the ability of metal ions to be reduced to metal nanoparticles and their ability to maintain their stability, AgNPs maintained their stability longer than ZnNPs and FeNPs. No agglomeration was observed in AgNPs stored at +4 ˚C, while ZnNPs and FeNPs began to agglomerate after two weeks. The PDI value of silver nanoparticles (0.073) confirms that they do not have agglomeration tendency.

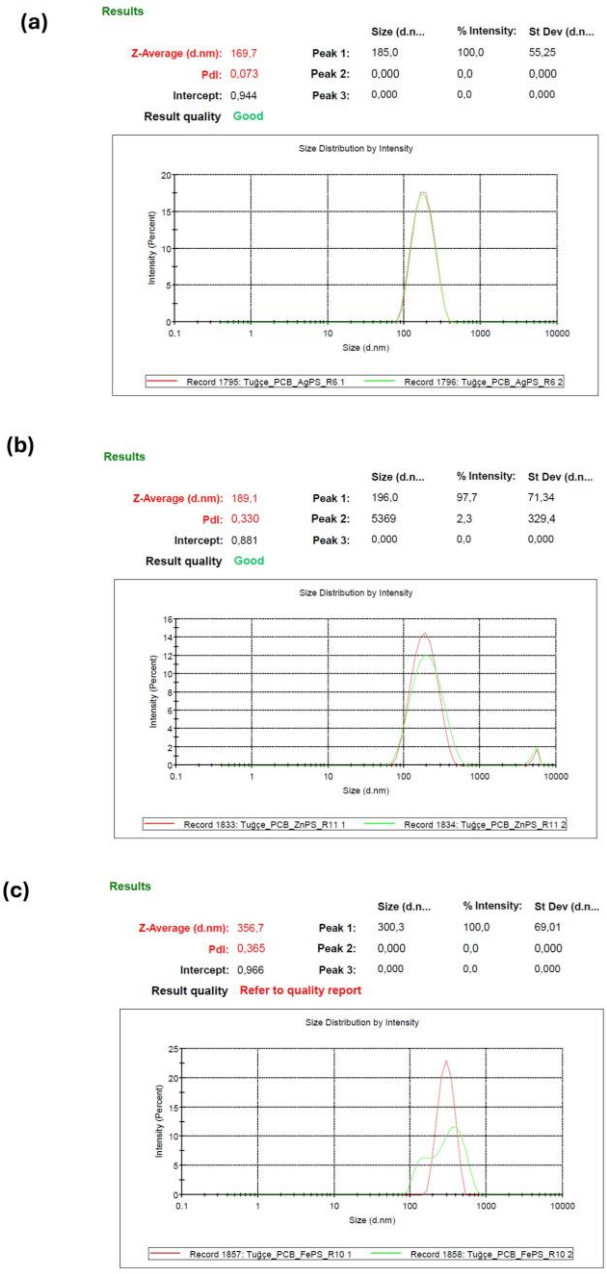

Figure 1. Dynamic light scattering characterization of synthesized **(a)** silver **(b)** zinc **(c)** iron nanoparticles (*n=2*).

Previous studies have indicated that the main factors in the agglomerated sedimentation of FeNPs and ZnNPs are the ionic strength and pH value of the suspension. The high ionic strength of water increases the sedimentation tendency. It has also been stated that an agglomeration tendency increases at pH values near the zero point of charge. Since agglomeration causes the stability of the nanoparticles and the particle surface area to decrease, the solubility decreases. Since the desired colloidal suspension cannot be obtained, the nanoparticles cannot maintain their targeted

properties. Therefore, in order to maintain stability in nanoparticles, it is recommended to add humic acid and citric acid to the suspension, preserve it in sucrose solution, or coat the nanoparticles with chitosan-like coating agents (Caliskan et al., 2022; Domingos et al., 2013;).

Table 4. Experiments for iron nanoparticle synthesis

Experiment	$FeSO_4$ solution concentration (mM)	Wet biomass (mg/mL)	Hydrodynamic diameter (nm)
1	8	2.55	385.5
2	8	2.55	409.8
3	1	2.55	399.6
4	1.27	0.19	368.1
5	1.27	4.91	443.9
6	8	2.55	407.0
7	8	2.55	463.5
8	8	2.55	452.6
9	15	2.55	481.3
10	14.73	0.19	356.7
11	8	0.1	1124
12	8	5	415.0
13	14.73	4.91	429.5

UV-Vis Spectroscopy

In addition to DLS characterization, spectroscopic analysis is another common characterization method that investigates the success of the reduction of metal ions to metal nanoparticles. Each metal nanoparticle gives a characteristic absorption band due to its surface plasmon resonance. Observation of the expected absorption band indicates that the nanoparticles have been synthesised. In this context, the color change in the colloidal suspension over time is an indicator of the reduction reaction. Due to the surface plasmon resonance of the AgNPs, the color of the colloidal suspension gradually changed to brown. As shown in Figure 2a, the AgNPs gave an absorbance peak at 450 nm and a characteristic curve was obtained. According to published studies, AgNPs give a maximum peak in the range of 400-450 nm, due to their surface plasmon resonances. AgNPs synthesized from *Caulerpa racemosa* microalgae extract by Kathiraven et al. (2015) started to give a surface plasmon resonance band at 440 nm wavelength and showed the maximum peak at 413 nm. Jena et al. (2015) also produced AgNPs with *Amphora* sp. extract, stating that the particles gave a maximum peak at 413 nm. In different studies, silver nanoparticles giving peaks at 420 nm (Merin et al., 2010), 430 nm (Gallon et al., 2019), and 450 nm (Mubarak Ali et al., 2011; Soleimani & Habibi-Pirkoohi, 2017) wavelengths were synthesized.

While FeNP changed to a dark yellow color after reduction, the color of ZnNPs changed from clear to yellow. Colloidal suspensions containing FeNPs started to give a surface plasmon band below 450 nm and a maximum absorbance peak in the range of 300-350 nm (Figure 2b). According to previous studies, FeNPs give a maximum absorbance peak in the range of 275-300 nm (Bouafia & Laouini, 2020; Karpagavinayagam &Vedhi, 2019). According to the UV-Vis spectrum of ZnNPs shown in Figure 2c, the maximum absorbance was observed in the range of 300-350 nm. According to published studies, ZnNPs give absorbance peaks in the range of 300-380 nm (Agarwal et al., 2019; Dhandapani et al., 2020; Lu et al., 2019).

As a negative control, cell-free metal solution and metal-free wet biomass were incubated under the same conditions. No color change was observed in either control group after incubation.

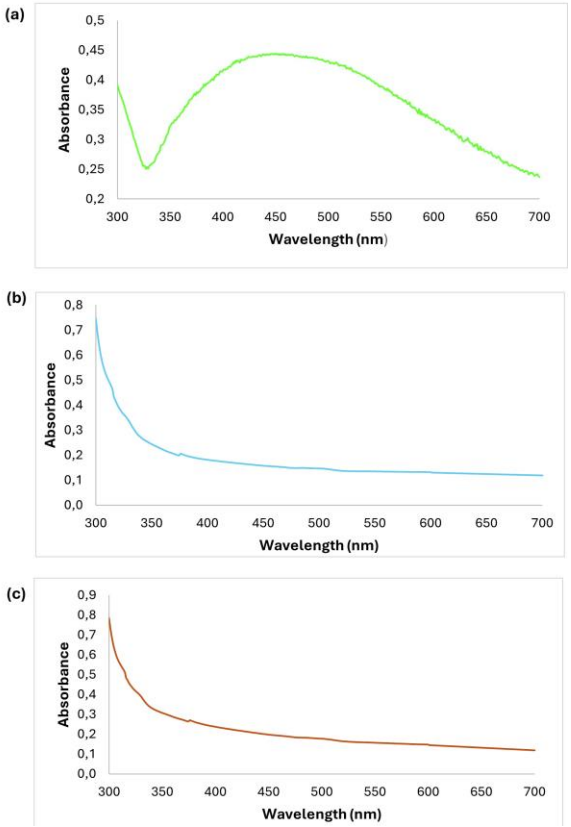

Figure 2. UV-Vis Spectroscopy of **(a)** silver **(b)** iron **(c)** zinc nanoparticles.

Investigation of bio-functional properties of nanoparticles

Antimicrobial activity of nanoparticles

Both *Escherichia coli* and *Staphylococcus aureus* bacteria were inhibited by ZnNPs, FeNPs, and AgNPs, while there was no inhibition with the negative control. Figure 3, inhibition zone diameters of 7.66±0.28 mm, 9.66±0.57 mm, and 10.83±0.76 mm were detected in ZnNPs, FeNPs, and AgNPs against *E. coli* bacteria, respectively. Against *S. aureus* bacteria, inhibition zone diameters of 8.66±0.57 mm, 8.66±0.57 mm, and 11.33±0.57 mm were detected for ZnNPs, FeNPs, and AgNPs, respectively. When the results are analyzed statistically with a one-way ANOVA test, it is seen that there is a significant difference ($p<0.05$) between AgNPs, ZnNPs and FeNPs in terms of antimicrobial activity. Compared to ZnNPs and FeNPs, higher inhibition zone diameters against both bacteria were observed with AgNPs. It can be said that AgNPs are a better alternative to the positive control gentamicin compared to others. The results obtained regarding the antibacterial activity of AgNPs synthesized from *P. cruentum* are similar to

previous studies (Jeon et al., 2021). The bactericidal effect and mechanism of AgNPs has been proposed in prior studies as follows: AgNPs in contact with the bacterial cell interact with the negatively charged cell membrane and affect membrane permeability. Penetrating into the cell, AgNPs can interfere with respiratory reactions, damage energy production metabolism, and thus lead to the death of bacterial cells. The bactericidal effect of AgNPs is mostly concentration-dependent, and increasing concentration also increases the antibacterial effect (Ahmad et al., 2020; Jeon et al., 2021).

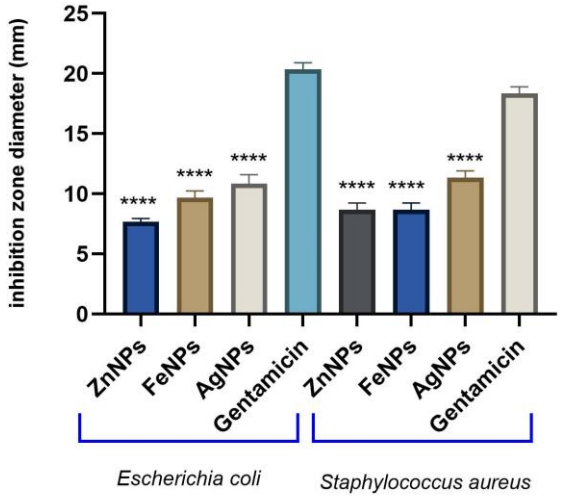

Figure 3. Antibacterial activity of silver, zinc and iron nanoparticles against *Escherichia coli* and *Staphylococcus aureus* bacteria. One-way ANOVA revealed a significant difference among the groups (F=284.9, R^2=0.9907, ****p<0.0001, n=3).

Antioxidant activity of nanoparticles

The decrease in absorbance at 517 nm indicates that the antioxidants have been able to scavenge the DPPH free radicals. It was shown that the synthesized AgNPs had a scavenging potential of 83.59% for DPPH radicals and was comparable to BHA (96.11%) antioxidant (Figure 4). An antioxidant activity of AgNPs is similar to the antioxidant activity of AgNPs synthesized with different precursors in previous studies (Krishnamoorthy et al., 2023).

Among the green synthesized ZnNPs, FeNPs, and AgNPs from *P. cruentum* microalgae, AgNPs have been identified as the nanoparticles with the highest biotechnological application potential in terms of antibacterial and antioxidant activities. The results obtained from the present study indicate that microalgal-derived AgNPs have the potential to be used in industrial areas such as food and cosmetics. However, regulatory agencies require comprehensive data to approve commercial products containing nanoparticles, which poses a significant obstacle to commercialization (Fernandes, 2023). For green synthesis methods to be implemented on an industrial scale, future research should address several critical issues. These include a deeper understanding of the biological synthesis mechanisms, identification of suitable biological and chemical agents, and optimization of process parameters to ensure reproducibility and consistency. Moreover, applicable strategies that address issues such as cost and availability of raw materials, energy consumption, and equipment scalability are needed to transition green synthesis methods from laboratory scale to industrial scale production (Ahmed, 2022; Gupta et al., 2023). Although a large number of studies have been conducted on green synthesis methods, their current limitations restrict their widespread adoption to industry. To overcome these challenges, collaborative efforts focusing on scalability, reproducibility, and economic viability are recommended for future studies.

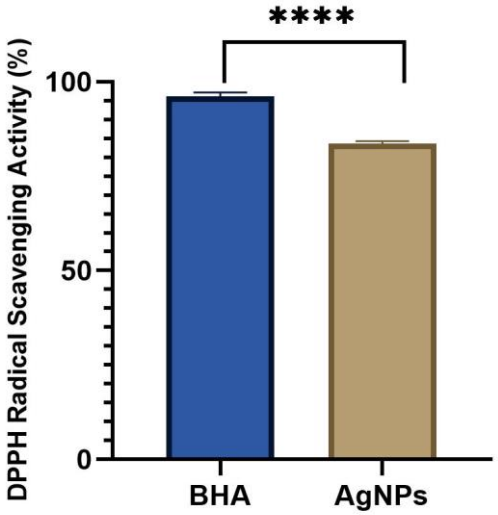

Figure 4. DPPH free radical scavenging activity of AgNPs and BHA antioxidant. The antioxidant activity values of the positive control and the nanoparticles differed significantly (unpaired t-test, t=16.69, df=4, ****p<0.0001, n=3).

Conclusion

This study demonstrated the potential of *Porphyridium cruentum* microalgae as a biological resource for the green synthesis of silver, zinc, and iron nanoparticles. The substantial intracellular metabolite content of *P. cruentum*, encompassing proteins, carbohydrates, and pigments, played a pivotal role in the effective reduction and stabilization of metal nanoparticles. In the context of synthesized nanoparticles, AgNPs have been demonstrated superior characteristics in terms of particle size, stability, antimicrobial and antioxidant activities. The results of the study showed a significant correlation between nanoparticle size and polydispersity, on the one hand, and metal ion concentration and biomass amount, on the other. Furthermore, AgNPs exhibited potent antibacterial properties against both *E. coli* and *S. aureus*, along with substantial radical scavenging activity, underscoring their promise for biomedical and biotechnological applications. Despite the initial indications from laboratory studies, further research is necessary to address the challenges associated with the

large-scale application of green synthesis methods. Such challenges include the assurance of reproducibility, cost-efficiency, and regulatory compliance for industrial applications. This work contributes to the expanding corpus of research that supports the utilization of microalgae in sustainable nanomaterial production, thereby opening new avenues for the development of eco-friendly nanotechnology.

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Author Contributions

Conceptualization: TMK, GC, SSO, ME, Data Curation: TMK, GC, SSO, ME, Formal Analysis: TMK, GC, SSO, ME, Investigation: TMK, GC, Methodology: TMK, GC, SSO, ME, Project Administration: SSO, ME, Resources: SSO, ME, Supervision: ME, Visualization: TMK, GC, SSO, ME, Writing -original draft: TMK, Writing -review and editing: GC, SSO, ME.

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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References

Ahmad, S.A., Das, S.S., Khatoon, A., Ansari, M.T., Afzal, M., Hasnain, M.S., & Nayak, A.K. (2020). Bactericidal activity of silver nanoparticles: A mechanistic review. *Materials Science for Energy Technologies*, *3*, 756–769. https://doi.org/10.1016/j.mset.2020.09.002

Ahmed, S.F., Mofijur, M., Rafa, N., Chowdhury, A.T., Chowdhury, S., Nahrin, M., Saiful Islan, A.B.M. & Ong, H.C. (2022). Green approaches in synthesising nanomaterials for environmental nanobioremediation: Technological advancements, applications, benefits and challenges. *Environmental Research*, *204*, 111967. https://doi.org/10.1016/j.envres.2021.111967

Aigbe, U.O. & Osibote, U.A. (2024). Green synthesis of metal oxide nanoparticles, and their various applications. *Journal of Hazardous Materials Advances, 13*, 100401. https://doi.org/10.1016/j.hazadv.2024.100401

Agarwal, H., Nakara, A., Soumya, M., & Shanmugam, V. (2019). Eco-Friendly Synthesis of Zinc Oxide Nanoparticles Using Cinnamomum tamala Leaf Extract and its Promising Effect Towards the Antibacterial Activity. *Journal of Drug Delivery Science and Technology*, *53*,101-212. https://doi.org/10.1016/j.jddst.2019.101212

Aguilar-Ruiz, R.J., Sánchez-Machado, D.I., Martinez-Macias, M.R., López-Cervantes, J., Nateras-Ramírez, O., Campas-Baypoli, O.N., & Villegas-Peralta, Y. (2022). Effects of light intensity and culture medium on the chemical composition and growth parameters of Nannochloropsis oculate and Porphyridium cruentum. *Aquaculture Research, 53*, 5239–5252. https://doi.org/10.1111/are.16007

Al-Salhi, M.S., Elangovan, K., Ranjitsingh A.J.A., Murali, P. & Devanesan, S. (2019). Synthesis of Silver Nanoparticles Using Plant Derived 4-N-Methyl Benzoic Acid and Evaluation of Antimicrobial, Antioxidant and Antitumor Activity. *Saudi Journal of Biological Sciences*, *26*(5), 970-978. https://doi.org/10.1016/j.sjbs.2019.04.001

Bouafia, A., & Laouini, S.E. (2020). Green Synthesis of Iron Oxide Nanoparticles by Aqueous Leaves Extract of Mentha pulegium L.: Effect of Ferric Chloride Concentration on the Type of Product. *Materials Letters*, *265*, 127364. https://doi.org/10.1016/j.matlet.2020.127364

Caliskan, G., Mutaf, T., Agba, H.C., & Elibol, M. (2022). Green Synthesis and Characterization of Titanium Nanoparticles Using Microalga, Phaeodactylum tricornutum. *Geomicrobiology Journal*, *39*(1), 83–96. https://doi.org/10.1080/01490451.2021.2008549

Cepoi, L., Rudi, L., Zinicovscaia, I., Chiriac, T., Miscu, V., & Rudic, V. (2021). Biochemical changes in microalga Porphyridium cruentum associated with silver nanoparticles biosynthesis. *Archives of Microbiology, 203*, 1547–1554. https://doi.org/10.1007/s00203-020-02143-z

Çalışkan, G., Mutaf, T., Öncel, S.Ş., & Elibol, M. (2020). Green Synthesis of Metal Nanoparticles Using Microalga Galdieria sp. *IFMBE Proceedings*, 73. https://doi.org/10.1007/978-3-030-17971-7_34

Dağlıoğlu, Y., & Yılmaz Öztürk, B. (2019). A novel intracellular synthesis of silver nanoparticles using Desmodesmus sp. (Scenedesmaceae): different methods of pigment change. *Rendiconti Lincei. Scienze Fisiche e Naturali*, 30, 611–621. https://doi.org/10.1007/s12210-019-00822-8

Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., Khorasani, S., & Mozafari, M.R. (2018). Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems. *Pharmaceutics*, *10*(2), 57. https://doi.org/10.3390/pharmaceutics10020057

Dahoumane, S.A., Mechouet, M., Alvarez, F.J., Agathos, S.N., & Jeffryes, C. (2016). Microalgae: An Outstanding Tool in Nanotechnology. *Bionatura*, *1*(4), 196-201. https://doi.org/10.21931/RB/2016.01.04.7

Deb, A. & Sutradhar, A. (2024). Microalgal nanobiotechnology for biosynthesis of metallic nanoparticles: In-depth into the strategies, mechanism and nanofluidic hydrodynamics. *Biocatalysis and Agricultural Biotechnology, 56*, 103046. https://doi.org/10.1016/j.bcab.2024.103046

Dhandapani, K.V., Anbumani, D.i Gandhi, A.D., Annamalai, P., Muthuvenkatachalam, B.S., Kavitha, P., & Ranganathan, B. (2020). Green Route for the synthesis of Zinc Oxide Nanoparticles from Melia azedarach Leaf Extract and Evaluation of Their Antioxidant and Antibacterial Activities. *Biocatalysis and Agricultural Biotechnology*, *24*,101517. https://doi.org/10.1016/j.bcab.2020.101517

Domingos, R.F., Rafiesi, Z., Monteiro, C.E., Khan, M.A.K. & Wilkinson, K.J. (2013). Agglomeration and dissolution of zinc oxide nanoparticles: role of pH, ionic strength and fulvic acid. *Environmental Chemistry, 10*, 306-312. https://dx.doi.org/10.1071/EN12202

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., & Smith F. (1956). Colorimetric Method for Determination of Sugars and Related Substances. *Division of Biochemistry*, *28*(3), 350-356.

Fakhari, S., Jamzad, M., & Fard, H.K. (2019). Green synthesis of zinc oxide nanoparticles: a comparison. *Green Chemistry Letters and Reviews*, *12*(1), 19-24. https://doi.org/10.1080/17518253.2018.1547925

Fernandes, C., Jathar, M., Sawant, B.K.S. & Warde, T. (2023). Scale-Up of Nanoparticle Manufacturing Process. In: Jindal, A.B. (eds) Pharmaceutical Process Engineering and Scale-up Principles. *AAPS Introductions in the Pharmaceutical Sciences*, 13. Springer, Cham. https://doi.org/10.1007/978-3-031-31380-6_12.

Fuentes, M.M.R., FernaÂndez, G.G.A., PeÂrez, J.A.S., & Guerrero, J.L.G. (2000). Biomass nutrient pro®les of the microalga Porphyridium cruentum. *Food Chemistry, 70*, 345-353. https://doi.org/10.1016/S0308-8146(00)00101-1

Gallego, R., Martínez, M., Cifuentes, A., Ibáñez, E., & Herrero, M. (2019). Development of a Green Downstream Process for the Valorization of Porphyridium cruentum Biomass. *Molecules*, *24*(8), 1564. https://doi.org/10.3390/molecules24081564

Gallón, S.M.N., Alpaslan, E., Wang, M., Larese-Casanova,P., Londono, M.E., Atehortua, L., Pavon, J.J., & Webster, T.J. (2019). Characterization and Study of the Antibacterial Mechanisms of Silver Nanoparticles Prepared with Microalgal Exopolysaccharides. *Materials Science&Engineering*: C, *99*(2019), 685-695. https://doi.org/10.1016/j.msec.2019.01.134

Gupta, D., Boora, A., Thakur, A. & Gupta, T.K. (2023). Green and sustainable synthesis of nanomaterials: Recent advancements and limitations. *Environmental Research*, *231*(3), 116316. https://doi.org/10.1016/j.envres.2023.116316

Jena, J., Pradhan, N., Dash, B.P., Panda, P.K., & Mishra, B.K. (2015). Pigment Mediated Biogenic Synthesis of Silver Nanoparticles Using Diatom Amphora sp. and its Antimicrobial Activity. *Journal of Saudi Chemical Society*, *19*(6), 661-666. https://doi.org/10.1016/j.jscs.2014.06.005

Jeon, M.S., Han, S., Park, Y.H., Kim, H.S., & Choi, Y. (2021). Rapid green synthesis of silver nanoparticles using sulfated polysaccharides originating from Porphyridium cruentum UTEX 161: evaluation of antibacterial and catalytic activities. *Journal of Applied Phycology, 33*, 3091–3101. https://doi.org/10.1007/s10811-021-02540-x

Kandav, G & Sharma, T. (2024). Green synthesis: an ecofriendly approach for metallic nanoparticles synthesis. *Particulate Science and Technology, 42*(5), 874–894. https://doi.org/10.1080/02726351.2023.2281452

Karpagavinayagam, P., & Vedhi, C. (2019). Green Synthesis of Iron Oxide Nanoparticles Using Avicennia marina Flower Extract. *Vacuum*, *160*, 286-292. https://doi.org/10.1016/j.vacuum.2018.11.043

Kathiraven, T., Sundaramanickam, A., Shanmugam, N., & Balasubramanian, T. (2015). Green Synthesis of Silver Nanoparticles Using Marine Algae Caulerpa racemosa and Their Antibacterial Activity Against Some Human Pathogens. *Appl. Nanosci., 5*, 499-504. https://doi.org/10.1007/s13204-014-0341-2

Krishnamoorthy, K., Jayaraman, S., Krishnamoorthy, R., Manoharadas, S., Alshuniaber, M.A., Vikas, B., & Veeraraghavan, V.P. (2023). Green synthesis and evaluation of antimicrobial, antioxidant, anti-inflammatory, and anti-diabetic activities of silver nanoparticles from Argyreia nervosa leaf extract: An in vitro study. *Journal of King Saud University – Science, 35*(10), 102955. https://doi.org/10.1016/j.jksus.2023.102955

Lowry, O.H., Rosebrough, N.J., Farr, A.L., & Randall, R.J. (1951). Protein Measurement with Folin-phenol Reagent. *Journal of Biochemical Engineering*, 193, 265-275.

Lu, J., Ali, H., Hurh, J., Han, Y., Batjikh, I., Rupa, E.J., Anandapadmanaban, G., Park, J.K., & Yang, D.C. (2019). The Assessment of Photocatalytic Activity of Zinc Oxide Nanoparticles from the Roots of Codonopsis Lanceolata Synthesized by One-Pot Green Synthesis Method. *Optik-International Journal for Light and Electron Optics*, *184*, 82-89. https://doi.org/10.1016/j.ijleo.2019.03.050

McNamara, K., & Tofail, S.A.M. (2017). Nanoparticles in biomedical applications. *Advances in Physics: X*, *2*(1), 54–88. https://doi.org/10.1080/23746149.2016.1254570

Merin, D.D., Prakash, S., & Bhimba, B.V. (2010). Antibacterial Screening of Silver Nanoparticles Synthesized by Marine Micro Algae. *Asian Pacific Journal of Tropical Medicine*, *3*(10), 797-799. https://doi.org/10.1016/S1995-7645(10)60191-5

Mhatre, A., Navale, M., Trivedi, N., Pandit, R., & Lali A.M. (2018). Pilot Scale Flat Panel Photobioreactor System for Mass Production of Ulva lactuca (Chlorophyta). *Bioresource Technology*, *249*, 582-291. https://doi.org/10.1016/j.biortech.2017.10.058

Mubarak Ali, D., Sasikala, M., Gunasekaran, M., & Thajuddin, N. (2011). Biosynthesis and Characterization of Silver Nanoparticles Using Marine Cyanobacterium, Oscıllatorıa willei Ntdm01. *Digest Journal of Nanomaterials and Biostructures*, *6*(2), 385-390.

Murillo-Rábago, E.I., Vilchis-Nestor, A.R., Juarez-Moreno, K., Garcia-Marin, L.E., Quester, K., & Castro-Longoria, E. (2022). Optimized Synthesis of Small and Stable Silver Nanoparticles Using Intracellular and Extracellular Components of Fungi: An Alternative for Bacterial Inhibition. *Antibiotics*, *11*(6), 800. https://doi.org/10.3390/antibiotics11060800

Mutaf, T., Çalışkan, G., Meydan, C., Öncel, S.Ş. & Elibol, M. (2020). Biogenic Nanoparticle Synthesis Using Marine Alga Schizochytrium sp. *IFMBE Proceedings*, 73. https://doi.org/10.1007/978-3-030-17971-7_33.

Mutaf, T., Caliskan, G., Ozel, H., Akagac, G., Oncel, S.Ş., & Elibol, M. (2023). Green synthesis of titanium nanoparticles using a sustainable microalgal metabolite solution for potential biotechnological activities. *Asia-Pac J Chem Eng*., *18*(5), e2954. https://doi.org/10.1002/apj.2954

Patel, V., Berthold, D., Puranik, P., & Gantar, M. (2015). Screening of Cyanobacteria and Microalgae for Their Ability to Synthesize Silver Nanoparticles with Antibacterial Activity. *Biotechnology Reports*, *5*, 112-119. https://doi.org/10.1016/j.btre.2014.12.001

Prabha, S., Arya, G., Chandra, R., Ahmed, B., & Nimesh, S. (2016). Effect of size on biological properties of nanoparticles employed in gene delivery. *Artificial Cells,*

Nanomedicine, and Biotechnology, *44*, 83–91. https://doi.org/10.3109/21691401.2014.913054

Rani, N., Rani, S., Patel, H., Bhavna, Yadav, S., Saini, M., Rewat, S. & Saini, K. (2023). Characterization and investigation of antioxidant and antimicrobial activity of zinc oxide nanoparticles prepared using leaves extract of Nyctanthesarbor-tristis. *Inorganic Chemistry Communications, 150*, 110516. https://doi.org/10.1016/j.inoche.2023.110516

Restrepo, C.V., & Villa, C.C. (2021). Synthesis of silver nanoparticles, influence of capping agents, and dependence on size and shape: A review. *Environmental Nanotechnology, Monitoring & Management*, *15*, 100428. https://doi.org/10.1016/j.enmm.2021.100428

Rinawati, M., Sari, L.A., & Pursetyo, K.T. (2020). Chlorophyll and carotenoids analysis spectrophotometer using method on microalgae. *IOP Conf. Series: Earth and Environmental Science, 441*, 012056. https://doi.org/10.1088/1755-1315/441/1/012056

Ruiz-Ruiz, F., Benavides, J., & Rito-Palomares, M. (2013). Scaling-up of a B-phycoerythrin production and purification bioprocess involving aqueous two-phase systems: Practical experiences. *Process Biochemistry, 48*(4), 738–745. https://doi.org/10.1016/j.procbio.2013.02.010

Salem, D.M.S.A., Ismail, M.M., & Aly-Eldeen, A. (2019). Biogenic Synthesis and Antimicrobial Potency of Iron Oxide (Fe_3O_4) Nanoparticles Using Algae Harvested from the Mediterranean Sea, Egypt. *Egyptian Journal of Aquatic Research*, *45*(3), 197-204. https://doi.org/10.1016/j.ejar.2019.07.002

Saif, S., Tahir, A., & Chen, Y. (2016). Green Synthesis of Iron Nanoparticles and Their Environmental Applications and Implications. *Nanomaterials*, *6*(11), 209. https://doi.org/10.3390/nano6110209

Sánchez-Saavedra, M.P., Castro-Ochoa, F.Y., Nava-Ruiz, V.M., Ruiz-Güereca, D.A., Villagómez-Aranda, A.L., Siqueiros-Vargas, F., & Molina-Cárdenas, C.A. (2018). Effects of nitrogen source and irradiance on Porphyridium cruentum. *J Appl Phycol*, *30*, 783–792. https://doi.org/10.1007/s10811-017-1284-2

Senapati, S., Syed, A., Moeez, S., Kumar, A., & Ahmad, A. (2012). Intracellular Synthesis of Gold Nanoparticles Using Alga Tetraselmis kochinensis. *Materials Letters*, *79*, 116-118. https://doi.org/10.1016/j.matlet.2012.04.009

Sharifiaghdam, M., Shaabani, E., Asghari, F. & Faridi-Majidi, R. (2021). Chitosan coated metallic nanoparticles with stability, antioxidant, and antibacterial properties: Potential for wound healing application. *J Appl Polym Sci*., *139*(10), e51766. https://doi.org/10.1002/app.51766

Sharma, B., Purkayastha, D.D., Hazra, S., Thajamanbi, M., Bhattacharjee, C.R., Ghosh, N.N., & Rout, J. (2014). Biosynthesis of Fluorescent Gold Nanoparticles Using an Edible Freshwater Red Alga, Lemanea fluviatilis (L.) C.Ag. and Antioxidant Activity of Biomatrix Loaded Nanoparticles. *Bioprocess Biosyst Eng*., *37*, 2559–2565. https://doi.org/10.1007/s00449-014-1233-2

Shumi, G., Demissie, T.B., Eswaramoorthy, R., Bogale, R.F., Kenasa, G. & Desalegn, T. (2023). Biosynthesis of Silver Nanoparticles Functionalized with Histidine and Phenylalanine Amino Acids for Potential Antioxidant and Antibacterial Activities. *ACS Omega, 8,* 24371-24386. https://doi.org/10.1021/acsomega.3c01910

Singh, P., Kim, Y.J., Zhang, D., & Yang, D.C. (2016). Biological Synthesis of Nanoparticles from Plants and Microorganisms. *Trends in Biotechnology*, *34*(7), 588-599. https://doi.org/10.1016/j.tibtech.2016.02.006

Soleimani, M., & Habibi-Pirkoohi, M. (2017). Biosynthesis of Silver Nanoparticles using Chlorella vulgaris and Evaluation of the Antibacterial Efficacy Against Staphylococcus aureus. *Avicenna Journal of Medical, Biotechnology*, *9*(3), 120-125.

Stanier, R.Y., Kunisawa, R., Mandel, M., & Cohen-Bazire, G. (1971). Purification and Properties of Unicellular Blue-Green Algae (Order Chroococcales). *Bacteriological Reviews*, *35*(2), 171-205.

Subhapriya, S., & Gomathipriya, P. (2018). Green Synthesis of Titanium Dioxide (TiO_2) Nanoparticles by Trigonella foenum-graecum Extract and its Antimicrobial Properties. *Microbial Pathogenesis*, *116,* 215-220. https://doi.org/10.1016/j.micpath.2018.01.027

Tounsi, L., Ben Hlima, H., Fendri, I., Abdelkafi, S. & Michaud, P. (2024). Photoautotrophic growth and accumulation of macromolecules by Porphyridium cruentum UTEX 161 depending on culture media. *Biomass Conv. Bioref*., *14*, 26323–26340. https://doi.org/10.1007/s13399-023-04703-x

Vijayaram, S., Razafndralambo, H., Sun, Y.Z., Vasantharaj, S., Ghafarifarsani, H., Hoseinifar, S.H. & Raeeszadeh, M. (2024). Applications of Green Synthesized Metal Nanoparticles — a Review. *Biological Trace Element Research, 202*, 360–386. https://doi.org/10.1007/s12011-023-03645-9

Wishkerman, A., Arad, M., & Shoshana, A. (2017). Production of Silver Nanoparticles by the Diatom Phaeodactylum tricornutum. *Nanotechnology VIII*, 10248. https://doi.org/10.1117/12.2264706

Yedoti, V. & Supraja, N. (2024). A Review on Algal Mediated Synthesized Metallic Nanoparticles: An Eco-Friendly Approach for Sustainable Nanotechnology. *Current Journal of Applied Science and Technology, 43*(6), 1-10. https://doi.org/10.9734/cjast/2024/v43i64381