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ORIGINAL RESEARCH PAPER

Green Synthesis of Silver Nanoparticles Using Different Extracts of Spirulina platensis: A Study on Antimicrobial Properties

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ABSTRACT

The green synthesis of nanoparticles is popular due to its efficiency, cost-effectiveness, and environmental sustainability. This study investigated the biosynthesis of silver nanoparticles using Spirulina platensis extracts and their antimicrobial properties. The experiment was a two-factor factorial design with three replications, examining different extracts (supernatant, nano supernatant, water extract, nano water extract, ethanol extract, nano ethanol extract) and bacteria (Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Xanthomonas campestris, Erwinia amylovora). The water extract of Spirulina was prepared by boiling the Spirulina powder in water at 100°C, while the ethanol extract was prepared by soaking the Algae powder in ethanol at room temperature for 24 hours. Nanoparticles were characterized using UV-VIS spectrophotometry, X-ray diffraction (XRD), scanning electron microscopy (FESEM), energy dispersive spectrometry (EDS), and Fourier transform infrared spectroscopy (FTIR). The nanoparticles were crystalline and spherical, with sizes ranging from 22-104 nm (supernatant), 26-58 nm (water extract), and 58-93 nm (ethanol extract). Antimicrobial activity was assessed by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Silver nanoparticles from Spirulina extracts showed lower MIC and MBC than non-nano extracts, with the supernatant having the lowest values. Erwinia amylovora was the most sensitive strain. The study suggests that algae extracts can effectively reduce silver ions to form nanoparticles, highlighting the potential of Spirulina platensis silver nanoparticles as antimicrobial agents.

Keywords: *Biosynthesis, X-ray diffraction, FTIR, MIC, MBC.*

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INTRODUCTION

Nanotechnology, particularly the manipulation of materials at the nanoscale, has garnered significant attention in recent years due to its potential applications across various fields, including medicine, engineering, and environmental science[1]. The prefix "nano," derived from the Greek word for "dwarf," refers to structures that are typically between 0.1 and 100 nanometers in size[2].

One of the most pressing challenges in contemporary healthcare is antibiotic resistance, a phenomenon that enables pathogenic microorganisms to survive and proliferate despite antibiotic treatment[3]. This issue has prompted

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researchers to explore alternative therapeutic agents, including natural compounds and nanoparticles, which have shown promise in overcoming the resistance mechanisms employed by bacteria. [3]. Nanoparticles exhibit antimicrobial activity that can overcome the resistance mechanisms developed by bacteria, such as enzyme inactivation, reduced cell permeability, and overexpression of efflux pumps [4]. Nanoparticles have various properties that make them suitable alternatives to conventional antibiotics. First, the large surface-to-volume ratio of nanoparticles enhances the contact surface with target organisms. Nanoparticles can act as nano-scale molecules that interact with bacterial cells, alter membrane permeability, and disrupt molecular pathways. Second, nanoparticles can augment the inhibitory effects of antibiotics [5]. However, the antibacterial properties of nanoparticles depend on the complexity of physicochemical properties, such as size, shape, chemical modifications, solvent, and environmental factors, during nanoparticle synthesis and interaction with bacteria[6], [7]. Among nanoparticles, silver nanoparticles have antimicrobial, anticancer, and antioxidant properties [8].

Among the various nanoparticles, silver nanoparticles (AgNPs) have emerged as a focal point of research due to their intrinsic antimicrobial properties. The mechanisms by which AgNPs exert their antibacterial effects are multifaceted, involving: a) Reactive Oxygen Species (ROS) Generation: Silver nanoparticles (AgNPs) can induce oxidative stress in bacterial cells by generating reactive oxygen species (ROS). These ROS can damage cellular components, including lipids, proteins, and DNA, ultimately leading to cell death[9]. b) Cell Membrane Disruption: AgNPs can interact with bacterial cell membranes, leading to alterations in membrane permeability. This disruption can result in leakage of intracellular contents, which is detrimental to bacterial survival [10]. C)Release of Silver Ions: As silver nanoparticles dissolve, they release silver ions, which possess intrinsic antibacterial properties. Silver ions can interfere with cellular processes by binding to proteins and nucleic acids, inhibiting bacterial growth and replication [11]. d) Biofilm Inhibition: AgNPs can prevent biofilm formation by inhibiting the adherence of bacteria to surfaces. This ability is crucial in combating infections associated with biofilms, as these structures often confer increased resistance to antibiotics [12]. E) Enhanced Antibiotic Efficacy: Silver nanoparticles have been shown to enhance the efficacy of conventional antibiotics. When used in combination with antibiotics, AgNPs can increase bacterial susceptibility, providing a potential strategy to overcome antibiotic resistance [13]. These mechanisms not only highlight the potential of AgNPs as effective antimicrobial agents but also underscore their capability to enhance the efficacy of conventional antibiotics, providing a strategic avenue to combat antibiotic resistance.

Biological synthesis of nanoparticles is a method that utilizes microorganisms, fungi, and algae to produce nanoparticles by reducing metal ions [14]. This method is low-cost and efficient, and it produces crystalline nanoparticles with various sizes depending on the plant extract concentration, pH, temperature, and incubation time [15]. Algae, such as Spirulina platensis, are of particular interest due to their abundance, non-toxic nature, and rich content of bioactive compounds[16] [11]. Marine algae can also reduce metal ions and transform them into nanoparticles in an extracellular or intracellular manner, owing to the presence of different phytochemical compounds such as proteins, carbohydrates, alkaloids, steroids, phenols, saponins, and flavonoids [17]. These bioactive compounds play a crucial role in the nanoparticle synthesis process. For instance, proteins and carbohydrates can act as reducing and stabilizing agents, facilitating the formation of nanoparticles. Alkaloids, steroids, phenols, saponins, and flavonoids contribute to the reduction of metal ions and enhance the stability and biological activity of the synthesized nanoparticles. The presence of these compounds in Spirulina makes it an excellent candidate for green synthesis of nanoparticles, offering a sustainable and eco-friendly approach to nanoparticle production[18].

Spirulina is a blue-green alga from the Oscillatoriaceae family, multicellular, filamentous, and spiral-shaped. Spirulina is in the form of a single-celled spiral trichome, which is usually regular. Spirulina has beneficial biological activities such as antioxidant, anticancer, antibacterial (against gram-negative and gram-positive bacteria) [19], and probiotic effects. Spirulina has a higher level of vitamin B12 than plant and animal food sources. Spirulina is a rich source of vitamin A (beta-carotene), vitamin C, vitamin E, thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), folic acid (B9), and cobalamin

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(B12). Phenolic compounds are considered the most important natural antioxidants [20]

Various marine algae have been used to synthesize different metal nanoparticles by green methods. For example, silver nanoparticles have been synthesized by algae such as Spirulina [21], Caulerpa racemosa [22], Gracilaria corticata [23], [24], Linum[25], Padina tetrastromatica [26], Sargassum angustifolium [27], Ulva fasciata [28], Desmodesmus [29], Sargassum [30]. Other metal nanoparticles have also been synthesized by algae, such as gold nanoparticles by Cystoseira indica [31] and Spirulina [32], [33]

In addition, several studies have reported the antibacterial properties of nanoparticles biosynthesized from cyanobacteria, algae, and various extracts against different pathogens. For instance, Sharma et al. (2015) found that silver nanoparticles synthesized from Spirulina inhibited Proteus vulgaris[34], while Usharani et al. (2015) reported that methanolic and ethanolic extracts of Spirulina inhibited Staphylococcus aureus and Candida albicans[35]. Similarly, Duda-Chodak (2013) showed that water extract of Spirulina algae inhibited yeasts, bacteria, and molds[36], and Ghasemipour et al. (2017) demonstrated that silver nanoparticles extracted from cyanobacterium Oscillatoria inhibited Staphylococcus aureus, Escherichia coli, and Acinetobacter baumannii[37]. Furthermore, Soltani Nejad et al. (2017) and Guilger-Casagrande et al. (2019) reported that silver nanoparticles inhibited plant pathogens such as Sclerotinia sclerotiorum and Rhizoctonia solani[38], [39].

Despite the growing body of literature on the synthesis of metal nanoparticles via green methods, there remains a notable gap in comparative studies assessing the effectiveness of different algae extracts in synthesizing silver nanoparticles and their subsequent antimicrobial properties. This study aims to address this gap by systematically investigating the biosynthesis of silver nanoparticles using Spirulina platensis extracts and evaluating their antimicrobial potential against a range of pathogenic microorganisms. By elucidating the efficacy of Spirulina-derived silver nanoparticles, this research endeavors to contribute to the development of novel antimicrobial strategies that leverage the unique properties of nanotechnology in the fight against antibiotic-resistant infection

MATERIALS AND METHODS

Experimental design

This study employed a two-factor factorial experiment with a completely randomized design and three replicates. The first factor was the type of extracts (cell-free supernatant, supernatant with silver nanoparticles, water Spirulina extract, water Spirulina extract with silver nanoparticles, ethanol Spirulina extract, ethanol Spirulina extract with silver nanoparticles), and the second factor was the type of bacteria (gram-positive *Staphylococcus aureus*, gram-negative *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Xanthomonas campestris*, *Erwinia amylovora*).

The preliminary cultivation of microalgae in liquid culture medium

Spirulina platensis cyanobacteria obtained from the National Algae Bank of Iran (INA-S-013) were cultured in a standard Zarrouk medium [40] with a pH of 8.5-9. The culture medium was autoclaved at 1 atm pressure and 121°C to eliminate microbial and fungal contamination. Then, 1 mL of Spirulina suspension in the logarithmic growth phase was inoculated into 250 mL Erlenmeyer flasks containing the sterile medium. The flasks were incubated in a shaker at 25 ± 2°C and 2500 lux with a 16:8 light/dark cycle.

Evaluation of the daily growth rate of algae

The growth trend of algae over 22 days was investigated by measuring the light absorption of algae at 750 nm wavelength every two days using a spectrophotometer. Then, the daily growth curve was plotted [41].

Preparation of cell-free supernatant

The supernatant was prepared by centrifugation. After 22 days of Spirulina growth, the culture medium with Spirulina cyanobacterial cells was centrifuged for 10 minutes at 8000 rpm and 4°C. Then, the supernatant was filtered through a sterile 0.22-micron membrane filter and stored at 4°C for further experiments [42].

Spirulina extraction

A) water extract preparation: The water extract of Spirulina was prepared by the decoction method. In this method, 5 g (dry weight) of Spirulina platensis cyanobacterial biomass was added to 100 mL of sterile double-distilled water and boiled for

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15 minutes at 100°C in a conical Erlenmeyer flask. Then, the mixture was cooled and centrifuged for 15 minutes at 10,000 rpm. The clear supernatant was filtered and stored at 4°C for further analysis.

B) Ethanol extract preparation: The ethanol extract of Spirulina algae was prepared by the maceration method. 5 g of plant powder was mixed with 100 ml of ethanol and left for 24 hours. Then, the extract was filtered using Whatman paper and stored at 4°C for further tests.

Silver nanoparticles synthesis

Silver nanoparticles were synthesized using different concentrations of silver nitrate and Spirulina extracts to find the optimal combination for nanoparticle synthesis. For the supernatant-based synthesis, 92.35 mL of supernatant was mixed with 7.65 mL of 0.1 mM silver nitrate solution[21]. For the water and ethanol extracts of Spirulina algae, 20 mL of water extract and 10 mL of ethanol extract were mixed with 80 mL and 90 mL of 1 mM silver nitrate solution, respectively. The solution was kept in the dark, at room temperature, and at pH 8.2 for 24 hours to reduce the silver ions. The color change from light yellow to dark brown indicated the formation of silver nanoparticles. The silver nanoparticle suspension was washed with distilled water three times. Then, the samples were centrifuged for 20 minutes at 6000 rpm. To obtain pure silver nanoparticles powder, the samples were washed with 90% ethanol and dried at 75°C for 2 hours[43]

Characterization and determination of silver nanoparticle properties

To characterize and verify the production and quality of silver nanoparticles synthesized using Spirulina platensis seaweed extract, a UV-visible spectrometer (model SPECORD 250, Analytik Jena, Germany), an electron microscope, and a Fourier infrared spectrometer were used.

Characterization and confirmation of silver nanoparticle synthesis using UV-visible spectrometer (UV-Vis)

To verify the reduction of silver ions and the synthesis of silver nanoparticles, the color change of the mixture of silver nitrate and Spirulina extracts was observed. Then, 4 microliters of the nanoparticle-containing extract were loaded into the device cell, and its absorption was measured by the UV-visible spectrometer at wavelengths ranging from 300 to 700 nanometers[43]

X-ray diffraction (XRD) analysis

The nanoparticle-containing extracts were centrifuged for 20 minutes at 18000 rpm [44]. Then, the supernatant was discarded, and the pellet was dried in the oven. The crystal structure of the silver nanoparticle powder was analyzed by XRD (model Panalytical X'Pert Pro, Netherlands). The crystal size was calculated according to the Debye-Scherrer equation.

$$D = K\lambda/\beta COS\theta$$

Where k=0.9 and ƛ is the wavelength of the X-ray beam, which is equal to 1.5406 Å, and B is the full width at half maximum of the diffraction peak, and Θ is the angle related to the diffraction peak.

Field emission scanning electron microscope (FESEM) and energy dispersive spectroscopy (EDS) analysis

The size and morphology of the nanoparticles and the composition of the nanodrums were analyzed by field emission scanning electron microscopy (FESEM model ZEISS Sigma, Germany) and energy-dispersive X-ray spectroscopy (EDS model Oxford Instruments system, United Kingdom).

Fourier transform infrared spectroscopy (FTIR) analysis

The aim was to identify the functional groups and potential biomolecules that reduced silver ions and coated the synthesized silver nanoparticles. The dried samples were analyzed by Fourier Transform Infrared Spectroscopy using a Spectrum Two device from PerkinElmer, manufactured in the United States.

Antimicrobial assay

Introduction of bacterial strains

The following bacteria were tested in this study:

- Staphylococcus aureus (ATCC 6538), a gram-positive bacterium
- Escherichia coli (ATCC 8739), a gram-negative bacterium
- Pseudomonas aeruginosa (ATCC 9027), a gram-negative bacterium
- Proteus vulgaris (ATCC 1079), a gram-negative bacterium
- Xanthomonas campestris (ATCC 1473), a gram-negative bacterium

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- Erwinia amylovora, a gram-negative bacterium

Description of the culture media used
Nutrient agar culture medium

The culture medium was prepared according to the instructions by dissolving an appropriate amount of culture medium powder in distilled water in an Erlenmeyer flask. The flask was heated with a heater and a magnetic stirrer until the agar was fully dissolved. The medium was sterilized in an autoclave for 15 minutes at 121°C. Then, the medium was poured into sterile plastic disposable plates, and the lids were carefully placed. This operation was done near the flame and under sterile conditions. The plates were left at room temperature to solidify the medium. Then they were stored inverted in the refrigerator until use. Nutrient broth culture medium: The culture medium was prepared according to the instructions by dissolving an appropriate amount of culture medium powder in distilled water. The liquid medium was distributed into glass tubes and sealed and sterilized in an autoclave for 15 minutes. Then the tubes were stored at 4°C.

Preparation of microbial suspensions

The bacterial strains stored in sterile microcentrifuge tubes with 40% glycerol at -20°C were cultured twice in nutrient broth medium at 37°C for 18 hours and then plated on nutrient agar to obtain single colonies. Several colonies of each bacterium were resuspended in sterile saline using a sterile loop near the flame. A suspension with approximately 10^6 bacterial cells and an absorbance between 0.08 and 0.1 was prepared using a UV-VIS spectrophotometer at 600 nm wavelength. The bacterial cell count in the suspension was verified by serial dilution and plating methods. The suspension was prepared fresh for each experiment.

Determination of minimum inhibitory concentration (MIC)

The MIC was determined using a 96-well microplate. First, each well was filled with 100 microliters of nutrient broth. Then, the extract with a concentration of 10000 mg/L was serially diluted in the wells to obtain final concentrations of 5000, 4000, 2500, 1250, 1000, 500, 250, 125, 62.5, 31.25, and 0 mg/L. The wells were then inoculated with a standard cell suspension with a final dose of 10^4 and incubated at 37°C. After 24 hours, the microbial growth and turbidity in the wells were assessed, and the lowest concentration that inhibited microbial growth and turbidity was defined as the MIC[45].

Determination of minimum bactericidal concentration (MBC)

A sterile loop was used to streak the contents of the wells with no microbial growth onto a nutrient agar plate. The plate was incubated for 24 hours at 37°C. The lowest extract concentration that killed the microorganisms and prevented bacterial colony formation on the agar surface was defined as the MBC[46]

Data analysis

Finally, after examination and classification, the collected data were analyzed using SAS software version 2/9.

RESULTS

Daily growth curve based on light absorption

Figure 1 shows the growth trend of Spirulina platensis in the present study.

The microalgae go through five growth stages during their growth cycle. The first stage is the induction or lag phase, in which the microalgae grow very slowly until they adjust to the new culture conditions. This stage lasted until the fourth day. The second stage is the rapid growth (logarithmic

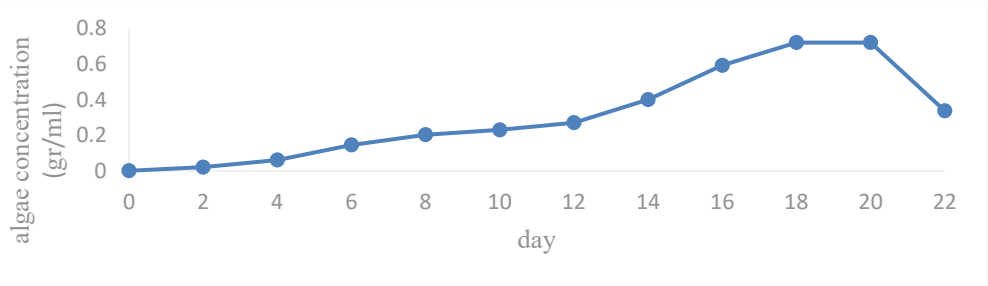

Fig. 1. Growth curve of Spirulina in a shaker incubator.

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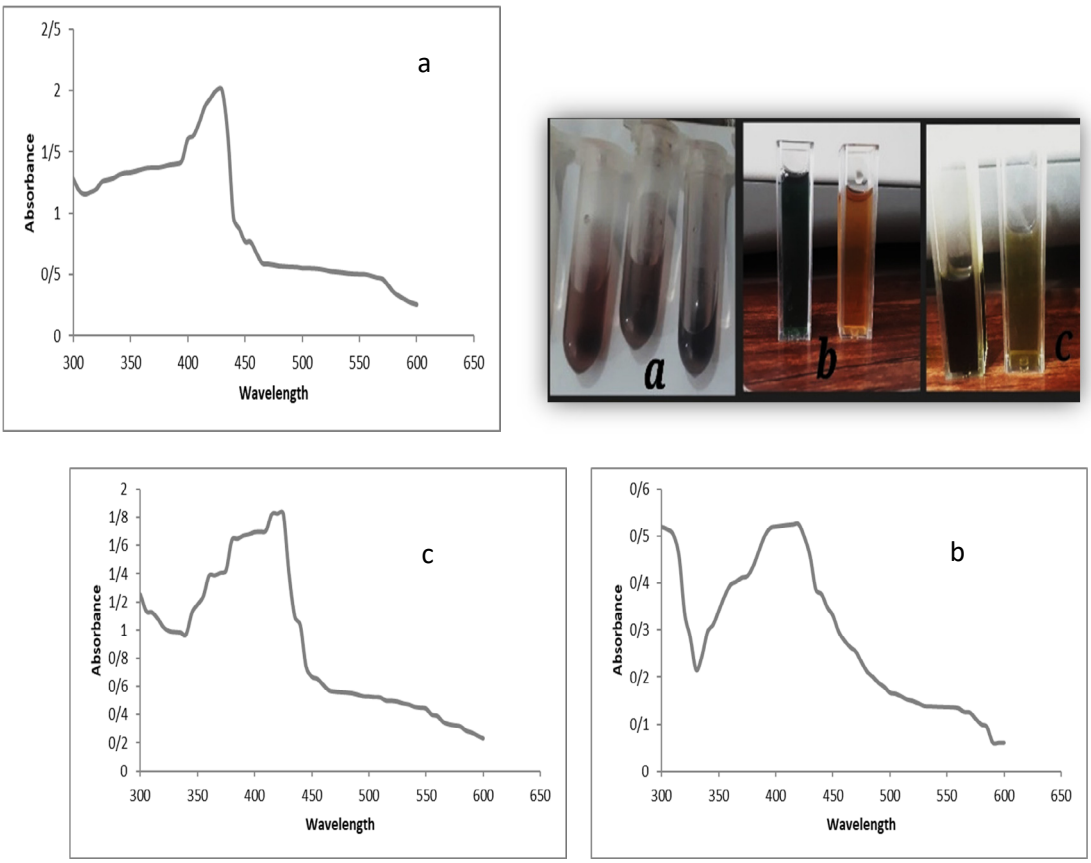

Fig. 2. Color change of extracts after adding silver nitrate and UV-Vis spectrum of silver nanoparticles produced after 24 hours: nano supernatant(a), nano water extract(b), and nano ethanolextract (c)of Spirulina platensis algae.

or exponential) phase, in which the microalgae have adapted to the new culture conditions and multiply logarithmically for several days, and their density increases. This stage lasted from the fourth to the twelfth day. The third stage, from the twelfth to the eighteenth day, was the algae growth period, a linear phase in which the microalgae keep increasing their density. The fourth stage is the stationary, quiescent, or stable phase, in which the cell density of the microalgae stays almost constant. The fifth stage is the death or decline phase, in which the cyanobacterial cells start to die due to the severe depletion of nutrients in the culture vessels, and as a result, the cell density of the microalgae is drastically reduced. Other researchers have reported a similar trend [47].

Synthesis of silver nanoparticles from Spirulina extracts:

The addition of silver nitrate solution to the Spirulina extracts resulted in a color change from light yellow to dark brown or date, indicating the formation of a colloidal suspension of silver nanoparticles (fig. 2).

Spectrophotometric analysis:

The UV-visible spectrum was measured in the wavelength range of 300-600 nm to verify the synthesis of silver nanoparticles. The optimal peak was seen after 24 hours of reaction time in the range of 420-425 nm (Fig. 2).

XRD analysis:

The formation of silver nanocrystals was verified by XRD analysis (database code AMCSD: 0013118)[48]. As shown in Fig. (3), the XRD pattern displays Miller indices on planes 111, 200, 220, and 311 that match the angles 38.2°, 44.4°, 64.6° and 77.1° in ethanol and water nano extracts and the angles 38.3°, 46.4°, 64.6° and 77° in nano supernatant, confirming the presence of silver nanocrystals in various Spirulina extracts and

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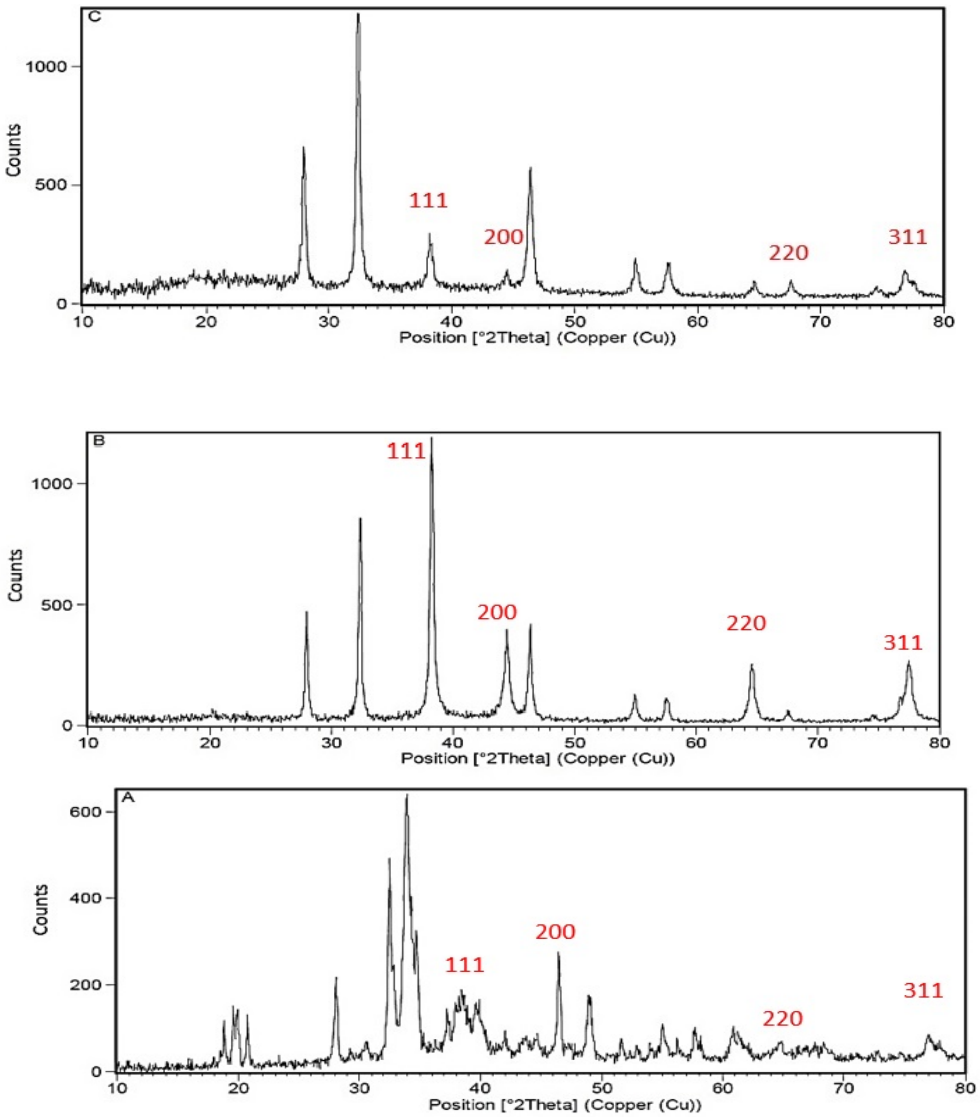

Fig. 3. XRD pattern of silver nanoparticles: nano supernatant(A), nano ethanol extract (B), and nano water extract (C) Spirulina platensis algae.

supernatant. The current results agree with those of Salari et al. (2014).

FTIR analysis of the synthesized nanoparticles (FTIR)

The following figures (4) show the FTIR results for the silver nanoparticles synthesized from three different forms of Spirulina platensis microalgae (supernatant, ethanol extract, and water extract). The FTIR spectrum reveals the existence of various compounds based on the peaks. The water extract of Spirulina platensis microalgae produced silver nanoparticles with peaks at 3438, 2927, 2345, 1620, 1382, 1025 and 612 (cm-1) (Fig. 4). The ethanol extract had peaks at 3445, 2922, 1615, 1383, 1008, 595 and the supernatant had peaks at 3422, 1625, 1414, 1115, 1015, 881, and 617. In general, FTIR analysis shows that different groups, such as carboxyl (-C=O), hydroxyl (-OH), and amine (N-H) in Spirulina platensis microalgae, play a role in reducing Ag^{+} ions to Ag0 nanoparticles (Fig. 4).

FESEM analysis

The size and morphology of the silver nanoparticles were assessed by FE-SEM scanning electron microscopy. Figure (5) displays the

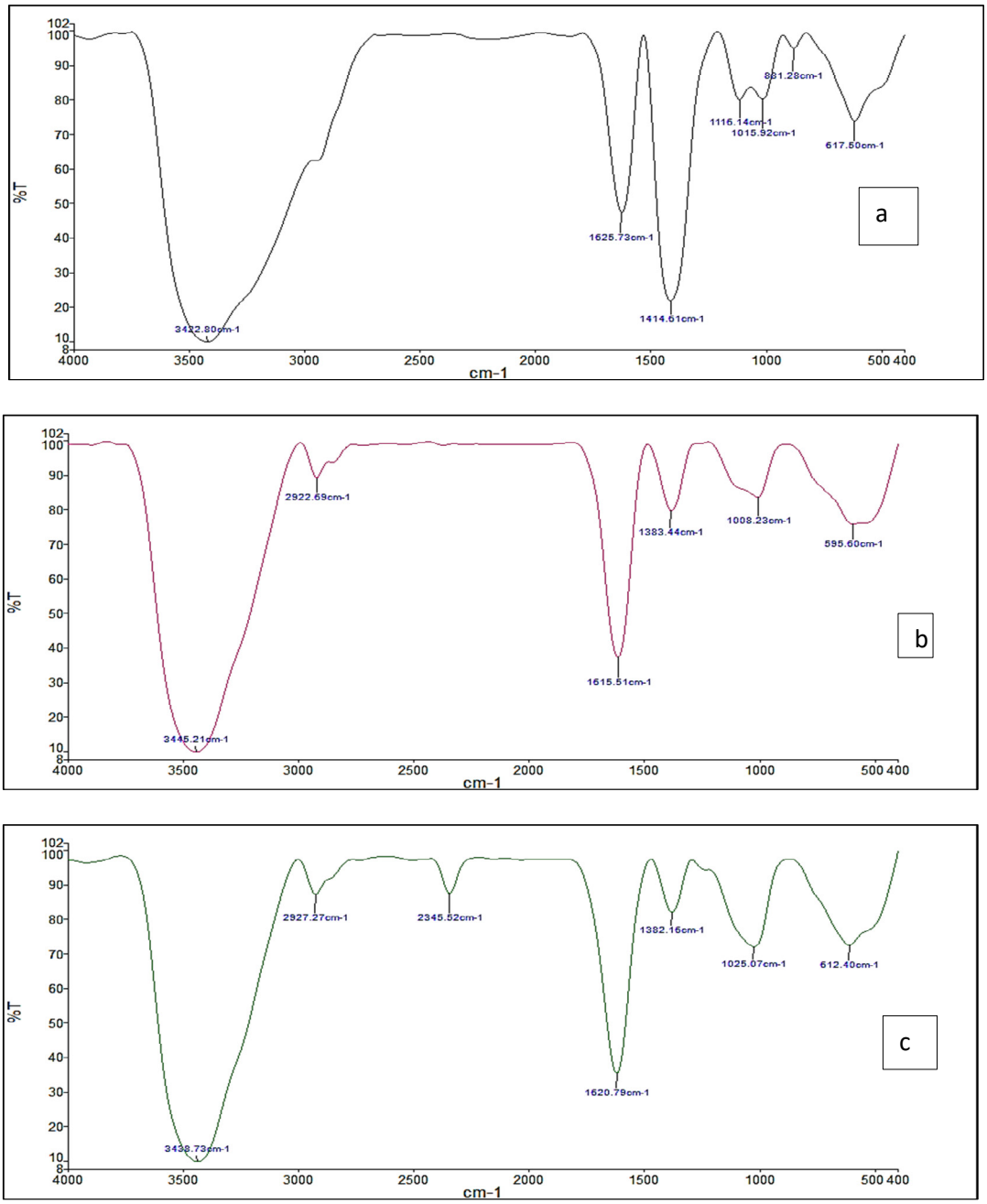

Fig 4. FT-IR spectrum of nanoparticles: nano supernatant(a), nano ethanol extract(b), and nano water extract (c) of Spirulina platensis algae.

surface structure of silver nanoparticles produced from three different types of Spirulina platensis microalgae extract (supernatant, ethanol extract, and water extract). The images confirm the existence of silver nanoparticles and show their size.

The FE-SEM results verified the existence of stable silver nanoparticles. The supernatant, ethanol extract, and water extract produced silver nanoparticles with sizes ranging from 22 to 104 nanometers, 58 to 93 nanometers, and 26 to 58 nanometers, respectively. The water extract yielded smaller nanoparticles than the other two extracts. The silver nanoparticles had a spherical shape.

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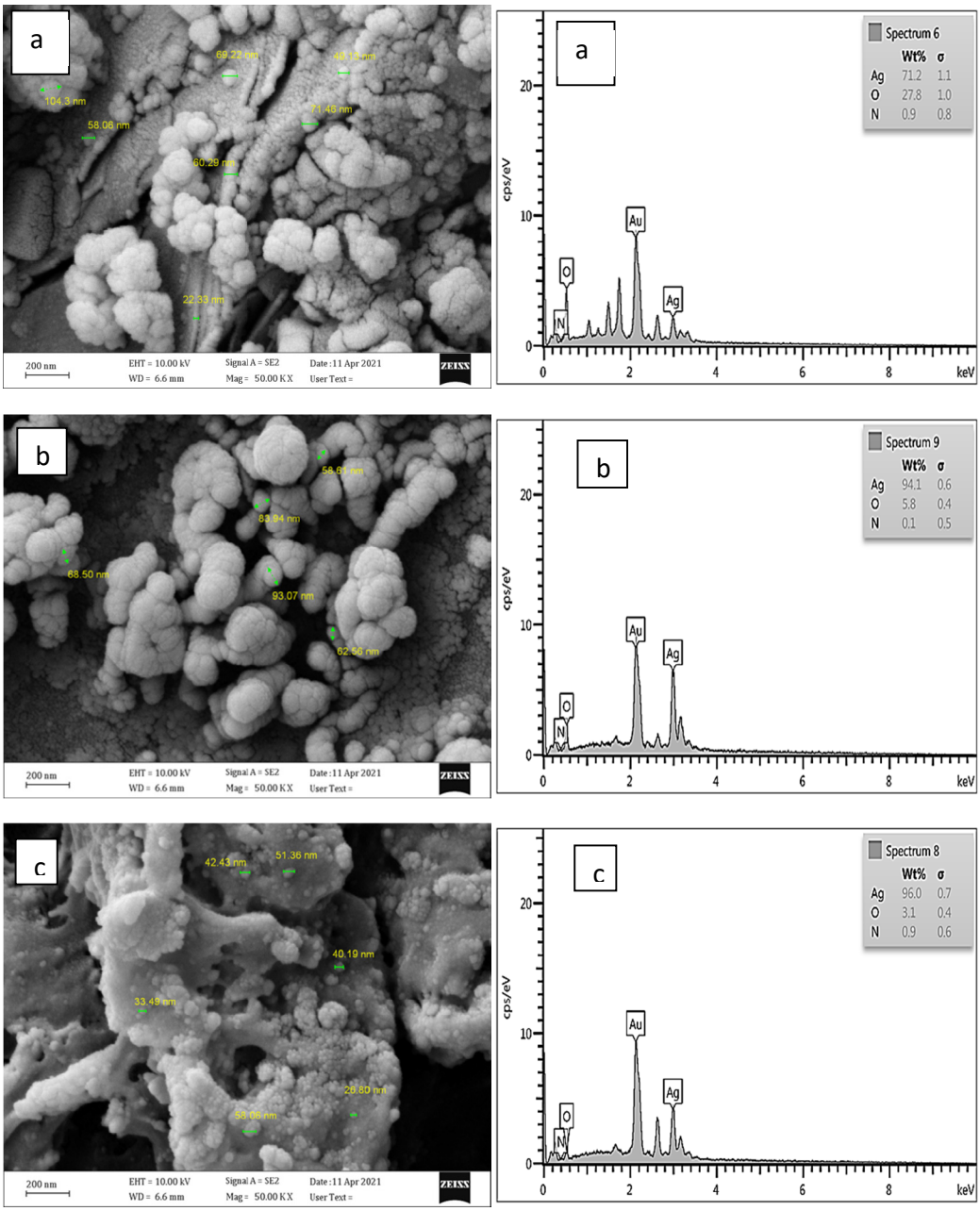

Fig. 5. FE-SEM image and EDS spectrum of silver nanoparticles synthesized from the nano-supernatant(a), nano-ethanol extract(b), and nano-water extract (c) of Spirulina algae.

X-ray energy dispersive spectroscopy analysis (EDS)

The EDS spectrum revealed the existence of elements such as silver metal, oxygen, and nitrogen in the nanoparticles. Silver metal was the most abundant element in all three types of nanoparticles. The supernatant, ethanol extract, and water extract contained 71.2%, 94.1%, and 96% of silver, respectively (Fig. 5)

The results of the antimicrobial activity of silver nanoparticles synthesized from Spirulina microalgae

The antimicrobial activity of silver nanoparticles derived from Spirulina microalgae (water extract, ethanol extract, and supernatant) and of non-nano Spirulina microalgae (water extract, ethanol extract, and supernatant) was tested against Escherichia coli, Proteus vulgaris, Xanthomonas campestris,

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Table1. Analysis of variance of MIC and MBC

S.O. V	df	MIC (mg/L)	MBC (mg/L)
Bacteria	5	2535267.6**	12754783.3**
extracts	5	27822043.2**	90927960.4**
Bacteria*extracts	25	1183918.2**	2525095.8**
Error	70	2459.5	144.7
C.V		3.44	0.42

*, **, ns, significant in 95%, 99% and non-significant.

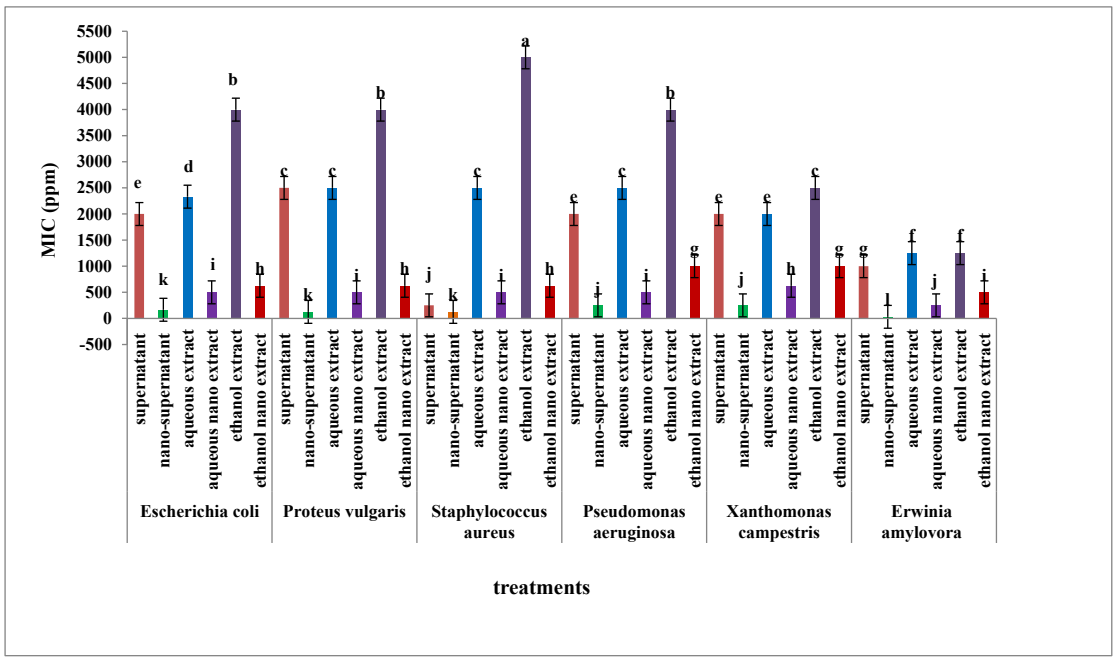

Fig. 6. Comparison graph of the average interactions between bacteria and extract (MIC).

Columns that have at least one letter in common do not have a significant difference based on the LSD test at the 5% probability level

Pseudomonas aeruginosa, and Erwinia amylovora as gram-negative bacteria, and Staphylococcus aureus as a gram-positive bacterium. The analysis of variance revealed that the simple effect of bacteria, the simple effect of extract, and the interaction of bacteria and extract on (MIC) and (MBC) were significant at the 1% probability level (Table 1).

The results indicated that the Spirulina extracts had varying effects on the growth inhibition of the bacteria (Fig. 6). The nano-supernatant extract had the highest inhibitory effect on *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Xanthomonas campestris*, and *Erwinia amylovora* at concentrations of 166.67, 125, 250, 250, 250, and 31.25 micrograms per milliliter, respectively. The ethanol extract of algae had the lowest inhibitory effect on the bacteria, and it was not significantly different from the water extract of algae for *Proteus vulgaris*, *Xanthomonas campestris*, and *Erwinia amylovora*. The MIC values for the ethanol extract of algae for *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Xanthomonas campestris*, and *Erwinia amylovora* were 4000, 4000, 5000, 4000, 2500, and 1250 micrograms per milliliter, respectively. A similar pattern was observed for the minimum bactericidal concentration (MBC) factor for the different extracts. That is, the nano-supernatant had the lowest MBC, and the water and ethanol extracts of algae had the highest MBC. However, the minimum bactericidal concentration for the bacteria varied. The MBC value for the nano-supernatant for *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Xanthomonas campestris*, and *Erwinia amylovora* was 208.3, 250, 250, 500, 500, and 31.5 micrograms per milliliter, respectively. The MBC value for the water and ethanol extracts of algae

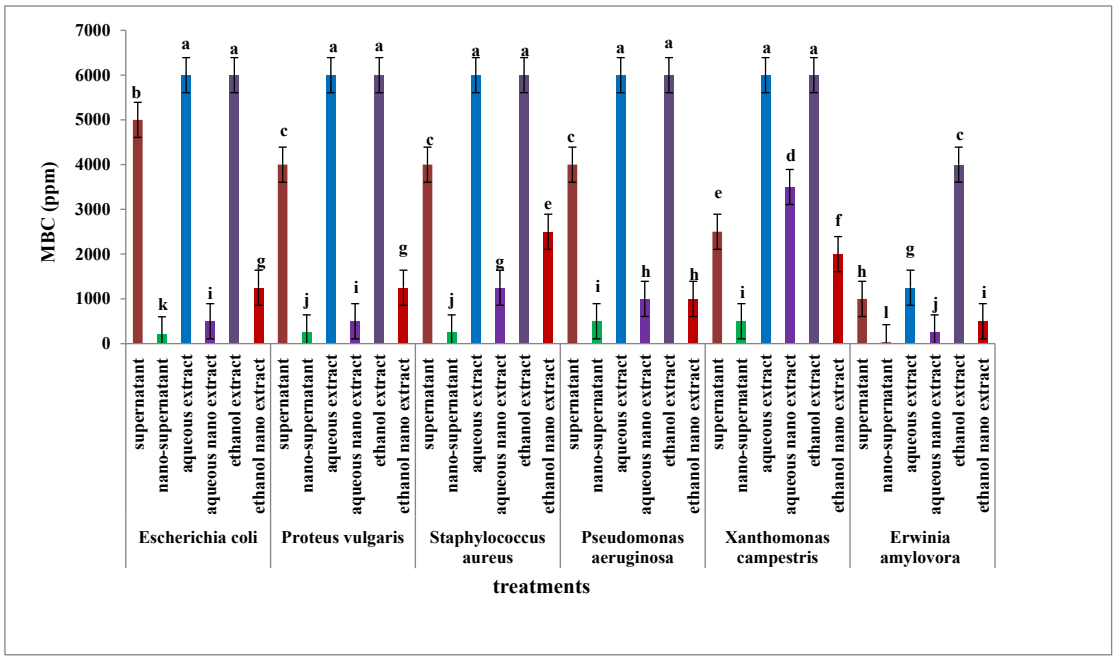

Fig. 7. Comparison chart of the average interactions between bacteria and extract (MBC).

Columns that have at least one letter in common do not have a significant difference based on the LSD test at the 5% probability level

for all the bacteria (except *Erwinia amylovora*) was 6000 micrograms per milliliter.

Extracts

The extracts had different effects on the growth inhibition and killing of the bacteria (Fig. 7). The results indicated that the nano extracts had significantly higher growth inhibition and killing effects on the bacteria than the other extracts. The nano supernatant, water nano extract, and ethanol nano extract required concentration ranges of 250-31.25, 625-250, and 1000-500 micrograms per milliliter, respectively, to inhibit the growth of different bacteria. In contrast, the supernatant, water, and ethanol extracts required concentration ranges of 2500-1000, 2500-1250, and 5000-1250 micrograms per milliliter, respectively, to achieve the same effect. A similar pattern was observed for the killing effects of the different extracts. That is, the nano extracts had much higher killing effects than the other extracts. The nano supernatant, water nano extract, and ethanol nano extract had concentration ranges of 500-31.25, 3500-250, and 2500-500 micrograms per milliliter, respectively, for the minimum concentration required to kill the bacteria. However, the water and ethanol extracts needed concentrations higher than 6000 micrograms per milliliter to kill all the bacteria (except Erwinia).

The bacteria also reacted differently to the different extracts. The Spirulina algae extracts had higher antibacterial activity against *Erwinia amylovora* than the other bacteria, and this bacterium had the lowest bactericidal concentration for all the extracts. The bactericidal concentration for Erwinia ranged from low to high as follows: nano supernatant (31.25), water nano extract (250), ethanol nano extract (500), supernatant (1000), water extract (1250), and ethanol extract (4000). The effect of the different extracts on the minimum inhibitory concentration of Erwinia growth followed a similar trend to the bactericidal concentration of the extracts. The minimum inhibitory concentration for Erwinia was as follows: nano supernatant (31.25), water nano extract (250), ethanol nano extract (500), supernatant (1000), water extract (1250), and ethanol extract (1250).

DISCUSSION

The results demonstrated that the Spirulina algae extracts changed color from light yellow to dark brown, suggesting the creation of a colloidal suspension of silver nanoparticles. This color alteration was consistent with other studies that produced silver nanoparticles using *Gracilaria*

gracilis, *Spirulina platensis*, and *Nostoc linckia* algae [49], [50], [51]. Various mechanisms have been proposed for the color change in the green synthesis of nanoparticles. Algae extract contains biologically active compounds that reduce silver to a nanoform that is compatible with living organisms [52]. Mainly, secondary metabolites such as terpenoids, flavonoids, phenols, alkaloids, proteins, and carbohydrates act as reducing agents [53] and they can help in stabilizing and synthesizing silver nanoparticles and enhancing their antibacterial properties [10]. Mulvaney (1996) and Kumar et al. (2013) attributed it to the surface plasmon resonance in metal nanoparticles, which indicates the reduction of silver nitrate to silver nanoparticles[49], [54]. Medina-Ramirez et al. (2009) assigned the silver nanoparticle formation to the hydrophilic-hydrophobic interaction of silver ions[55]. Sharma et al. (2015) also associated the brown color change with the reduction of silver ions to silver nanoparticles in the reaction of Spirulina algae extract with silver nitrate salt solution[34].

Metal nanoparticles exhibit various physicochemical properties, among which the optical properties are particularly intriguing. The optical properties of metal nanoparticles are determined by surface plasmon resonance, which is influenced by factors such as nanoparticle size, shape, spacing, and refractive index of the surrounding medium [56]. This study showed that silver nanoparticles absorbed light in the range of 400 to 500 nm, and the surface plasmon resonance absorption band appeared at 420 nm for silver nanoparticles, as shown in Fig. (3). This peak was consistent with the findings of other studies that reported the optimal peak between 420 and 450 nm for silver nanoparticles [34], [51], [57]. The peak position, intensity, and color were affected by nanoparticle size. In other words, the optical properties of silver nanoparticles were strongly dependent on the nanoparticle diameter. In this study, the light absorption of water, ethanol, and supernatant extracts was 0.5, 1.8, and 2 nm, respectively. The light absorption increased as the nanoparticle size decreased. Therefore, it was expected that the supernatant nanoparticles would have the smallest size.

XRD analysis was used to verify the crystal structure of silver nanoparticles. The XRD pattern revealed the face-centered cubic structure of silver nanocrystals, with Miller indices on planes 111, 200, 220, and 311 matching the angles 38.2°, 44.4°, 64.6°, and 77.1° in ethanol and water nano extracts and the angles 38.3°, 46.4°, 64.6°, and 77° in nano supernatant. Miller indices indicate the orientation of crystal planes. The peak intensity represents the atomic arrangement in the crystal planes. The higher the order of the atoms, the higher the peak intensity. The crystal size of silver nanoparticles produced from water and ethanol extracts was 33.3 nm, and the crystal size of silver nanoparticles produced from supernatant was 55.5 nm. The crystal size of nanoparticles denotes the average diameter of the crystals that form the nanoparticles. The crystal size of nanoparticles affects their physical and chemical properties. Generally, the smaller the crystal size of nanoparticles, the more varied and intriguing their properties become. This is because the decrease in crystal size increases the surface-to-volume ratio of nanoparticles and consequently the number of surface atoms and structural anisotropy. These factors increase the chemical and physical activity of nanoparticles. The difference between the outcomes of other studies and the current study is related to the type of species, the type of extraction, and the size of silver nanoparticles generated. Some specific compounds, such as phenols, flavonoids, terpenes, and other compounds, have more reducing, antioxidant, and antimicrobial properties and affect the size of nanoparticles. Nanoparticles with a size below 50 nm have more penetration and impact power [57].

FTIR spectroscopy is a useful tool for identifying functional groups and interactions between metals and biological molecules [58]. Silver nanoparticles produced from various Spirulina extracts exhibited functional groups such as O-H, C-H, C=O, C-O, and N-H. The FTIR results showed peaks at 3438, 3445, and 3422 cm^{-1}, indicating the presence of the O-H group. This group is characterized by a peak in the range of 3200 to 3600 cm^{-1} in the FTIR spectrum and can be associated with absorbed water or alcoholic or phenolic groups.

Additionally, peaks at 2927, 2922, and 2920 cm^{-1} were observed, indicating the presence of the C-H group, which is marked by peaks in the range of 2800 to 3000 cm^{-1} in the FTIR spectrum and can be associated with aliphatic or aromatic groups. The C=O group, related to carbonyl compounds such as esters, ketones, aldehydes, acids, or amides, was indicated by peaks between 1600 and 1800 cm^{-1}. Specifically, peaks at 1620, 1615, and 1625 cm^{-1} were observed.

The C-O group in the FTIR spectrum is marked by peaks in the range of 1000 to 1300 cm^{-1} and can be associated with alcohol, ester, ether, or carboxyl groups. Peaks at 1025, 1008, 1015, and 1115 cm^{-1} indicated the presence of the C-O group. The N-H group, related to amino or amide compounds, was indicated by a peak at 3450 cm^{-1} in all three extracts (water, ethanol, and supernatant). The N-H group in the FTIR spectrum is marked by peaks in the range of 3300 to 3500 cm^{-1}.

The FTIR results of other studies on silver nanoparticles produced from Spirulina extract reported similar findings, confirming the presence of hydroxyl, carboxyl, carbonyl, amide, and amine groups, as well as proteins in the extracts [34], [51], [58]. Secondary metabolites such as terpenoids, flavonoids, phenols, alkaloids, proteins, and carbohydrates in the extract act as reducing agents[53].. Moreover, the stability and proper dispersion of nanoparticles are attributed to their interaction with proteins. During the production process of nanoparticles, proteins likely form a coating on nanoparticles and prevent their aggregation and densification by stabilizing them[59]. Among the functional groups, proteins and hydroxyl groups play a more significant role in the stability of silver nanoparticles. Proteins form a protective layer around the nanoparticles, while hydroxyl groups contribute to the reduction and stabilization processes[60]. Therefore, biological molecules can likely perform the reduction and stabilization of silver nanoparticles in an aqueous environment[34].

The FE-SEM results verified the existence of stable silver nanoparticles. The nanoparticles had different sizes depending on the extracts. The supernatant, ethanol extract, and water extract produced silver nanoparticles with sizes of 22 to 104 nanometers, 58 to 93 nanometers, and 26 to 58 nanometers, respectively. The water extract yielded the smallest nanoparticles among the three extracts. The silver nanoparticles had a spherical shape. Previous studies have reported different sizes for silver nanoparticles derived from algae. For example, Palanisamy et al. (2017) reported that most of the nanoparticles synthesized from the water extract of Spirulina were spherical and oval with sizes from 2 to 18 nanometers[30]. Also, sizes of 21.211 nanometers [51], 30-50 nanometers [34] in Spirulina algae, and 55.06 ± 9.67 nanometers in *Chlorella vulgaris* algae [61] have been reported. The variation in nanoparticle size in this study and other studies is related to changes in temperature, pH, duration, and different concentrations of interaction between the salt solution and extract, which leads to inconsistency in nanoparticle size in various research [62].

The EDS spectrum revealed the existence of elements such as silver metal, oxygen, and nitrogen in the nanoparticles. Silver metal was the most abundant element in all three types of nanoparticles. The oxygen and nitrogen elements in this spectrum might be due to the X-ray scattering from proteins and sugars in algae[23], [63]. Carbon, potassium, nitrogen, oxygen, and sulfur were also detected in the nanoparticles derived from the red algae Gracilaria [50]. The absorption peak seen at around 3 keV was typical of metallic silver nanocrystals and was related to surface plasmon resonance [64].

The analysis of variance for the antibacterial activity of silver nanoparticles showed that the simple effect of bacteria, the simple effect of extract, and the interaction of bacteria and extract on (MIC) and (MBC) were significant at the 1% probability level. The results indicated that the bacteria responded differently to the extracts. The Spirulina algae extracts had higher antibacterial activity against *Erwinia amylovora* than the other bacteria, and this bacterium had the lowest bactericidal concentration for all the extracts. The bactericidal and growth inhibitory concentrations (31.25) for Erwinia were observed in the nano supernatant. The antibacterial potential of Spirulina algae extracts and silver nanoparticles (AgNPs) has been investigated by various studies. AgNPs from different extracts of *Spirulina platensis* showed inhibitory effects on several bacterial strains, such as *Escherichia coli*, *Proteus vulgaris* [34], *Staphylococcus aureus* [34], [65], [66], and *Pseudomonas aeruginosa* [65], [66] at different concentrations. Spirulina phycocyanin also exhibited antibacterial activity against *Escherichia coli* [51], [67] and *Staphylococcus aureus* [67] and *Proteus vulgaris* [51] with specific values of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Moreover, AgNPs from other algae species, such as green algae[68], red marine algae [69], and *Chlorella vulgaris* [70], demonstrated antibacterial effects on *Xanthomonas campestris* and *Erwinia amylovora*. The difference in the minimum inhibitory concentration and the minimum bactericidal concentration in different types of nanoparticles can be explained by the difference

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in size. Also, the difference in these results may be related to the shape of the nano, structural, and genetic differences of the strains. Physically, the size and shape of the particles are important factors for the antibacterial properties of silver nanoparticles. Reducing the size of nanoparticles enables better interaction with bacteria and increases the release of silver ions, which enhances the antibacterial property [71]. Guzman et al. (2012) demonstrated that the antibacterial properties of nanoparticles increase as their size decreases[72]. According to reports, irregular-shaped, spherical, and rod-shaped nanoparticles are, respectively, the best shapes of silver nanoparticles to prevent bacterial growth [71], [72]. In the present study, the FE-SEM analysis showed that the nanoparticles had a spherical shape.

Algae extract contains biologically active compounds that reduce silver to a nanoform compatible with living organisms [52]. Mainly, secondary metabolites such as terpenoids, flavonoids, phenols, alkaloids, proteins, and carbohydrates act as reducing agents [53] and they can help in stabilizing and synthesizing silver nanoparticles and enhancing their antibacterial properties [10]. Generally, metal nanoparticles have four antibacterial mechanisms, which are as follows: 1- Small size and high surface-to-volume ratio of nanoparticles, which increase their contact area with bacterial cells [73]. 2. Release of metal ions and electrostatic interaction between metal ions and the negative charge of the cell wall, well-dispersed nanoparticles have more antibacterial properties than aggregated particles [74]. 3- They generate reactive oxygen species, O2- and OH damage the bacterial cell walls, causing membrane rupture and disruption of cell function and preventing DNA replication of bacteria, consequently leading to leakage of internal components and destruction of bacteria [75]. Strong reaction of metal nanoparticles with functional groups in the bacterial cell wall: silver nanoparticles bind to sulfur-containing proteins on the bacterial membrane surface, alter the bacterial electron transfer chain, and ultimately affect cellular respiration and cause bacterial death[76].

The antibacterial property of silver nanoparticles is related to the release of silver ions, although the exact mechanism by which silver ions create antibacterial effects is still unclear. Some of the proposed

mechanisms include damage to the cell membrane, production of free radicals, and inhibition of DNA replication [71], [77], [68], delay in DNA replication due to the interaction of silver nanoparticles and thiol groups of bacterial proteins [78].

CONCLUSION

In this study, silver nanoparticles were synthesized using Spirulina microalgae extracts (water, ethanol, and supernatant), and their antibacterial properties were evaluated. The results demonstrated that the Spirulina algae extracts changed color from light yellow to dark brown, indicating the formation of a colloidal suspension of silver nanoparticles. This color change was consistent with other studies that produced silver nanoparticles using various algae species.

The synthesized silver nanoparticles exhibited significant antibacterial activity, particularly against *Erwinia amylovora*, with the lowest bactericidal concentration observed in the nano supernatant extract. The nanoparticles from the supernatant extract had the smallest size, which contributed to their higher antibacterial efficacy. The XRD analysis confirmed the face-centered cubic structure of the silver nanoparticles, with crystal sizes of 33.3 nm for water and ethanol extracts and 55.5 nm for the supernatant extract.

The FTIR spectroscopy results identified functional groups such as O-H, C-H, C=O, C-O, and N-H, which played a crucial role in the reduction and stabilization of the silver nanoparticles. The FE-SEM analysis revealed that the nanoparticles had a spherical shape, with sizes ranging from 22 to 104 nm, depending on the extract used.

The significance of these findings lies in the potential application of silver nanoparticles synthesized from Spirulina microalgae as effective antimicrobial agents. The smaller size and higher surface-to-volume ratio of the nanoparticles enhance their interaction with bacterial cells, leading to increased antibacterial activity. Compared to previous studies, the nanoparticles synthesized in this study demonstrated comparable or superior antibacterial properties, highlighting the effectiveness of Spirulina microalgae extracts in producing stable and potent silver nanoparticles.

Future research should focus on optimizing the synthesis process to achieve even smaller nanoparticle sizes and exploring the potential applications of these nanoparticles in various fields, including medicine, agriculture, and environmental

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protection. Additionally, further studies are needed to understand the exact mechanisms of antibacterial action and to evaluate the safety and efficacy of these nanoparticles in living organisms.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Allafchian AR, Jalali SAH, Aghaei F, Farhang HR. Green synthesis of silver nanoparticles using Glaucium corniculatum (L.) Curtis extract and evaluation of its antibacterial activity. IET Nanobiotechnol. 2018;12(5):574-8. https://doi.org/10.1049/iet-nbt.2017.0265
2. Lugani Y, Kaur G, Oberoi S, Sooch BS. Nanotechnology: current applications and future prospects [Internet]. 2018 [cited 2025 Aug 24]. Available from: www.wjahr.com
3. Singh P, Kim YJ, Zhang D, Yang DC. Biological synthesis of nanoparticles from plants and microorganisms. Trends Biotechnol. 2016;34(7):588-99. https://doi.org/10.1016/j.tibtech.2016.02.006
4. Dos Santos Ramos MA, Silva PB, Spagnol C, Pereira FG, Borges CP, de Oliveira MA, et al. Nanotechnology-based drug delivery systems for control of microbial biofilms: a review. Int J Nanomedicine. 2018;13:1179-213. https://doi.org/10.2147/IJN.S146195
5. Eleraky NE, Allam A, Hassan SB, Omar MM. Nanomedicine fight against antibacterial resistance: an overview of the recent pharmaceutical innovations. Pharmaceutics. 2020;12(2):142. https://doi.org/10.3390/pharmaceutics12020142
6. Saka R, Chella N. Nanotechnology for delivery of natural therapeutic substances: a review. Environ Chem Lett. 2021;19:1097-106. https://doi.org/10.1007/s10311-020-01103-9
7. Arif ZN, Al-Daamy AAH, Alhidary AQ. Efficacy evaluation of meropenem against resistant Staphylococcus hominis in heart failure patients. 2022.
8. Elemike EE, Onwudiwe DC, Ekennia AC, Jordaan A. Synthesis and characterisation of silver nanoparticles using leaf extract of Artemisia afra and their in vitro antimicrobial and antioxidant activities. IET Nanobiotechnol. 2018;12(6):722-6. https://doi.org/10.1049/iet-nbt.2017.0297
9. Sardar M, Sadeghi M, Rezaei A, Ghasemi F, Karimi Z, Hosseini H. Mechanisms of bacterial inactivation by silver nanoparticles: a comprehensive review. Colloids Surf B Biointerfaces. 2020;194:111328.
10. Parveen KS, Lakshmi D. Biosynthesis of silver nanoparticles using red algae, Amphiroa fragilissima and its antibacterial potential against gram positive and gram negative bacteria. Int J Curr Sci. 2016;19(3):93-100.
11. Jiang W, Yang Y, Wang C, et al. Silver nanoparticles: a promising antibacterial agent. J Nanobiotechnol. 2021;19:42.
12. Y A, AA KMA, KS, Khan KR. The anticancer and antibacterial properties of silver nanoparticles. Front Microbiol. 2019;10:1120.
13. A AS, AR KM, Musa PP. Synergistic effects of silver nanoparticles with antibiotics. Mater Today Commun. 2021;27:102302.
14. Jagtap UB, Bapat VA. Green synthesis of silver nanoparticles using Artocarpus heterophyllus Lam. seed extract and its antibacterial activity. Ind Crops Prod. 2013;46:132-7. https://doi.org/10.1016/j.indcrop.2013.01.019
15. Ajitha B, Reddy YAK, Reddy PS. Green synthesis and characterization of silver nanoparticles using Lantana camara leaf extract. Mater Sci Eng C Mater Biol Appl. 2015;49:373-81. https://doi.org/10.1016/j.msec.2015.01.035
16. Das CGA, Kumar A, Singh R, et al. Antibacterial activity of silver nanoparticles (biosynthesis): a short review on recent advances. Biocatal Agric Biotechnol. 2020;27:101593. https://doi.org/10.1016/j.bcab.2020.101593
17. Rajeshkumar S, Malarkodi C, Gnanajobitha G, et al. Seaweed-mediated synthesis of gold nanoparticles using Turbinaria conoides and its characterization. J Nanostruct Chem. 2013;3:1-7. https://doi.org/10.1186/2193-8865-3-44
18. Gul A, Baig MN, Ahmed D, Najam Z, Aslam T, Ali S. Green synthesis of silver nanoparticles from Spirulina platensis extract: antibacterial and antioxidant potential. Bionanoscience. 2024;14(3):2327-36. https://doi.org/10.1007/s12668-024-01473-2
19. Elshouny WAEF, El-Sheekh MM, Sabae SZ, Khalil MA, Badr HM. Antimicrobial activity of Spirulina platensis against aquatic bacterial isolates. J Microbiol Biotechnol Food Sci. 2017;6(5):1203. https://doi.org/10.15414/jmbfs.2017.6.5.1203-1208
20. Guerreiro-Tanomaru JM, Tanomaru-Filho M, da Silva GF, et al. Effect of zirconium oxide and zinc oxide nanoparticles on physicochemical properties and antibiofilm activity of a calcium silicate-based material. Sci World J. 2014;2014:1-8. https://doi.org/10.1155/2014/975213
21. Mahdieh M, Zolanvari A, Azimee AS, Mahdieh M. Green biosynthesis of silver nanoparticles by Spirulina platensis. Sci Iran. 2012;19(3):926-9. https://doi.org/10.1016/j.scient.2012.01.010
22. Kathiraven T, Sundaramanickam A, Shanmugam N, Balasubramanian T. Green synthesis of silver nanoparticles using marine algae Caulerpa racemosa and their antibacterial activity against some human pathogens. Appl Nanosci. 2015;5:499-504. https://doi.org/10.1007/s13204-014-0341-2
23. de Aragao AP, Farias WRL, de Barros AL, Feitosa APA, de Morais LC, de Medeiros SRB, et al. Green synthesis of silver nanoparticles using the seaweed Gracilaria birdiae and their antibacterial activity. Arab J Chem. 2019;12(8):4182-8. https://doi.org/10.1016/j.arabjc.2016.04.014
24. Ibraheem IBM, Abd-Elaziz BEE, Saad WF, Fathy WA. Green biosynthesis of silver nanoparticles using marine red algae Acanthophora specifera and its antimicrobial activity. J Nanomed Nanotechnol. 2016;7:409.
25. Kannan RRR, Arumugam R, Ramya D, Manivannan K, Anantharaman P. Green synthesis of silver nanoparticles using marine macroalga Chaetomorpha linum. Appl Nanosci. 2013;3:229-33. https://doi.org/10.1007/s13204-012-0125-5
26. Jegadeeswaran P, Shivaraj R, Venckatesh R. Green synthesis of silver nanoparticles from extract of Padina tetrastromatica leaf. Dig J Nanomater Bios. 2012;7(3):991-8.
27. Bhuyar P, Rahim MHA, Sundararaju S, Ramaraj R, Maniam GP, Govindan N. Synthesis of silver nanoparticles using marine macroalgae Padina sp. and its antibacterial activity towards pathogenic bacteria. Beni Suef Univ J Basic Appl Sci. 2020;9:1-15. https://doi.org/10.1186/s43088-019-0031-y
28. Negm MA, Ibrahim HA, Shaltout NA, Shawky HA, Abdel-Mottaleb MS, Hamdona SK. Green synthesis of silver nanoparticles using marine algae extract and their antibac-

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terial activity. Sci. 2018;8(3):957-70.
29. Öztürk BY. Intracellular and extracellular green synthesis of silver nanoparticles using Desmodesmus sp.: their antibacterial and antifungal effects. Caryologia. 2019;72(1):29-43.
30. Palanisamy S, Anjali R, Rajasekar P, Kannapiran E, Vaseeharan B, Prabhu NM. Synthesis and distribution of bio-inspired silver nanoparticles using Spirulina extract for control of Vibrio parahaemolyticus infection in aquaculture. Asian J Chem. 2017;29(4). https://doi.org/10.14233/ajchem.2017.20335
31. González-Ballesteros N, Prado-López S, Rodríguez-González JB, Lastra M, Rodríguez-Argüelles M. Green synthesis of gold nanoparticles using brown algae Cystoseira baccata: its activity in colon cancer cells. Colloids Surf B Biointerfaces. 2017;153:190-8. https://doi.org/10.1016/j.colsurfb.2017.02.020
32. Kalabegishvili T, Kirkesali E, Rcheulishvili A. Synthesis of gold nanoparticles by blue-green algae Spirulina platensis. Frank Lab Neutron Phys. 2012.
33. Hamza AA, Chevidenkandy AR, Hassan AH. Antioxidant activity, lipoxygenase inhibitory effect and polyphenolic compounds from Calotropis procera (Ait.) R. Br. Res J Phytochem. 2011;5(2):80-8. https://doi.org/10.3923/rjphyto.2011.80.88
34. Sharma G, Jasuja ND, Kumar M, Ali MI. Biological synthesis of silver nanoparticles by cell-free extract of Spirulina platensis. J Nanotechnol. 2015;2015:1-6. https://doi.org/10.1155/2015/132675
35. Usharani G, Srinivasan G, Sivasakthi S, Saranraj P. Antimicrobial activity of Spirulina platensis solvent extracts against pathogenic bacteria and fungi. Adv Biol Res (Rennes). 2015;9(5):292-8.
36. Duda-Chodak A. Impact of water extracts of Spirulina (WES) on bacteria, yeasts and molds. Acta Sci Pol Technol Aliment. 2013;12(1):33-40.
37. Ghasemipour T, Salehzadeh A, Sa SAS. Biosynthesis of silver nanoparticles using Oscillatoria extract: evaluation of anticancer and antibacterial activities. Armaghane Danesh. 2017;22(4):459-71. Available from: https://www.magiran.com/paper/1770018
38. Soltani Nejad M, Bonjar GHS, Khatami M, Amini A, Aghighi S. In vitro and in vivo antifungal properties of silver nanoparticles against Rhizoctonia solani, a common agent of rice sheath blight disease. IET Nanobiotechnol. 2017;11(3):236-40. https://doi.org/10.1049/iet-nbt.2015.0121
39. Guilger-Casagrande M, Germano-Costa T, Pasquoto-Stigliani T, Fraceto LF, de Lima R. Biosynthesis of silver nanoparticles employing Trichoderma harzianum with enzymatic stimulation for the control of Sclerotinia sclerotiorum. Sci Rep. 2019;9(1):14351. https://doi.org/10.1038/s41598-019-50871-0
40. Zarrouk C. Contribution to the cyanophyceae study: influence of various physical and chemical factors on growth and photosynthesis of Spirulina maxima (Setch et Gardner) Geitler extract. Paris: Univ Paris; 1996.
41. Moheimani NR, Borowitzka MA, Isdepsky A, Sing SF. Standard methods for measuring growth of algae and their composition. In: Borowitzka MA, Moheimani NR, editors. Algae for Biofuels and Energy. Dordrecht: Springer Netherlands; 2013. p. 265-84. https://doi.org/10.1007/978-94-007-5479-9_16
42. Hossain MAA, Rahman MH, Hossain MS, Habib MAB, Uddin MA, Sarker F. Smart production of Spirulina (Spirulina platensis) using supernatant of digested rotten potato (Solanum tuberosum). J Agric Food Environ. 2021;2(1):62-9. https://doi.org/10.47440/JAFE.2021.2111
43. Hanisha R, Kumar S, Ahmed D, et al. Biogenic synthesis of silver nanoparticles using Spirulina maxima extract and their bactericidal activity. J Umm Al-Qura Univ Appl Sci. 2024. doi: 10.1007/s43994-024-00203-4. https://doi.org/10.1007/s43994-024-00203-4
44. Majeed Khan MA, Kumar S, Ahamed M, Alrokayan SA, AlSalhi MS. Structural and thermal studies of silver nanoparticles and electrical transport study of their thin films. Nanoscale Res Lett. 2011;6(1):434. https://doi.org/10.1186/1556-276X-6-434
45. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother. 2001;48(Suppl 1):5-16. https://doi.org/10.1093/jac/48.suppl_1.5
46. Chikezie IO. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method. Afr J Microbiol Res. 2017;11(23):977-80. https://doi.org/10.5897/AJMR2017.8545
47. Córdova O, Ruiz-Filippi G, Fermoso FG, Chamy R. Influence of growth kinetics of microalgal cultures on biogas production. Renew Energy. 2018;122:455-9. https://doi.org/10.1016/j.renene.2018.01.125
48. Suh IK, Ohta H, Waseda Y. High-temperature thermal expansion of six metallic elements measured by dilatation method and X-ray diffraction. J Mater Sci. 1988;23(2):757-60. https://doi.org/10.1007/BF01174717
49. Kumar P, Senthamil Selvi S, Govindaraju M. Seaweed-mediated biosynthesis of silver nanoparticles using Gracilaria corticata for its antifungal activity against Candida spp. Appl Nanosci. 2013;3:495-500. https://doi.org/10.1007/s13204-012-0151-3
50. Ataei-E Jaliseh S, Zamani Kochesfehni M, Zamani Kochesfehani MH. Antibacterial effect of silver nanoparticles synthesized from the red algae Gracilaria gracilis. J Microbial World. 2020;13(4):369-78.
51. Ismail GA, El-Sheekh MM, Samy RM, Gheda SF. Antimicrobial, antioxidant, and antiviral activities of biosynthesized silver nanoparticles by phycobiliprotein crude extract of the cyanobacteria Spirulina platensis and Nostoc linckia. Bionanoscience. 2021;11:355-70. https://doi.org/10.1007/s12668-021-00828-3
52. Mittal AK, Chisti Y, Banerjee UC. Synthesis of metallic nanoparticles using plant extracts. Biotechnol Adv. 2013;31(2):346-56. https://doi.org/10.1016/j.biotechadv.2013.01.003
53. Alshehri MA, Alzahrani AA, Alzahrani A, et al. One-step synthesis of Ag nanoparticles using aqueous extracts from Sundarbans mangroves revealed high toxicity on major mosquito vectors and microbial pathogens. J Clust Sci. 2020;31:177-84. https://doi.org/10.1007/s10876-019-01631-7
54. Mulvaney P. Surface plasmon spectroscopy of nanosized metal particles. Langmuir. 1996;12(3):788-800. https://doi.org/10.1021/la9502711
55. Medina-Ramirez I, Bashir S, Luo Z, Liu JL. Green synthesis and characterization of polymer-stabilized silver nanoparticles. Colloids Surf B Biointerfaces. 2009;73(2):185-91. https://doi.org/10.1016/j.colsurfb.2009.05.015
56. Shams S, Pourseyedi SH. Green synthesis of silver nanopar-

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ticles in Melia azedarach fruit extract and screening its antimicrobial activity. Plant Prod. 2015;38(3):55-63.
57. Heydarzadeh S, Yaghoubi H. Green synthesis and antibacterial effect of silver nanoparticles by using the extract of Citrus aurantium. Razi J Med Sci. 2019;24(157):15-24.
58. Muthusamy G, Thangasamy S, Raja M, Chinnappan S, Kandasamy S. Biosynthesis of silver nanoparticles from Spirulina microalgae and its antibacterial activity. Environ Sci Pollut Res Int. 2017;24(23):19459-64. https://doi.org/10.1007/s11356-017-9772-0
59. Mansuya P, Aruna P, Sridhar S, Kumar JS, Babu S. Antibacterial activity and qualitative phytochemical analysis of selected seaweeds from Gulf of Mannar region. J Exp Sci. 2010;1(8).
60. Suh IK, Ohta H, Waseda Y. High-temperature thermal expansion of six metallic elements measured by dilatation method and X-ray diffraction. J Mater Sci. 1988;23(2):757-60. https://doi.org/10.1007/BF01174717
61. Rajkumar R, Ezhumalai G, Gnanadesigan M. A green approach for the synthesis of silver nanoparticles by Chlorella vulgaris and its application in photocatalytic dye degradation activity. Environ Technol Innov. 2021;21:101282. https://doi.org/10.1016/j.eti.2020.101282
62. Sankar R, Karthik A, Prabu A, Karthik S, Shivashangari KS, Ravikumar V. Origanum vulgare mediated biosynthesis of silver nanoparticles for its antibacterial and anticancer activity. Colloids Surf B Biointerfaces. 2013;108:80-4. https://doi.org/10.1016/j.colsurfb.2013.02.033
63. Bhimba BV, Kumari PR. Phytosynthesis of silver nanoparticles from the extracts of seaweed Ulva lactuca and its antimicrobial activity. Int J Pharma Bio Sci. 2014;5(4).
64. Magudapathy P, Gangopadhyay P, Panigrahi BK, Nair KGM, Dhara S. Electrical transport studies of Ag nanoclusters embedded in glass matrix. Phys B Condens Matter. 2001;299(1-2):142-6. https://doi.org/10.1016/S0921-4526(00)00580-9
65. Ameen F, Abdullah MMS, Al-Homaidan AA, Al-Lohedan HA, Al-Ghanayem AA, Almansob A. Fabrication of silver nanoparticles employing the cyanobacterium Spirulina platensis and its bactericidal effect against opportunistic nosocomial pathogens of the respiratory tract. J Mol Struct. 2020;1217:128392. https://doi.org/10.1016/j.molstruc.2020.128392
66. Al Rashed S, Al Shehri S, Moubayed NMS. Extracellular biosynthesis of silver nanoparticles from cyanobacteria. Biomed Res (India). 2018;29(13):2859-62. https://doi.org/10.4066/biomedicalresearch.29-17-3209
67. Safari R, Reyhani Poul S. Cell culture of Spirulina microalgae (Spirulina platensis) and comparison the efficiency of enzymatic, ultrasound, freeze-defrosting and mineral solvent methods in extraction of phycocyanin pigment. Iran Food Sci Technol Res J. 2023;19(5):649-61.
68. Hassaan MA, Hosny S. Green synthesis of Ag and Au nanoparticles from micro and macro algae: review. Int J Atmos Ocean Sci. 2018;2(1):10-22. https://doi.org/10.11648/j.ijaos.20180201.12
69. Roseline TA, Murugan M, Sudhakar MP, Arunkumar K. Nanopesticidal potential of silver nanocomposites synthesized from the aqueous extracts of red seaweeds. Environ Technol Innov. 2019;13:82-93. https://doi.org/10.1016/j.eti.2018.10.005
70. Hafez EE, Kabeil SS. Antimicrobial activity of nano-silver particles produced by micro algae. J Pure Appl Microbiol. 2013;7:35-42.
71. Emrani S, Zhiani R, Jafari MD. The biosynthesis of silver nanoparticles using plants of Glycyrrhiza glabra and Mentha piperata and its antimicrobial effect on some bacterias that cause tooth decay. J Rafsanjan Univ Med Sci. 2018;16(10).
72. Guzman M, Dille J, Godet S. Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. Nanomedicine. 2012;8(1):37-45. https://doi.org/10.1016/j.nano.2011.05.007
73. Barani H, Montazer M, Samadi N, Toliyat T. In situ synthesis of nano silver/lecithin on wool: enhancing nanoparticles diffusion. Colloids Surf B Biointerfaces. 2012;92:9-15. https://doi.org/10.1016/j.colsurfb.2011.10.062
74. Lee SM, Song KC, Lee BS. Antibacterial activity of silver nanoparticles prepared by a chemical reduction method. Korean J Chem Eng. 2010;27:688-92. https://doi.org/10.1007/s11814-010-0067-0
75. Yadav HM, Kulkarni DK, Gole AN, et al. Preparation and characterization of copper-doped anatase TiO_2 nanoparticles with visible light photocatalytic antibacterial activity. J Photochem Photobiol A Chem. 2014;280:32-8. https://doi.org/10.1016/j.jphotochem.2014.02.006
76. Moritz M, Geszke-Moritz M. The newest achievements in synthesis, immobilization and practical applications of antibacterial nanoparticles. Chem Eng J. 2013;228:596-613. https://doi.org/10.1016/j.cej.2013.05.046
77. Moadi T, Ghahramanzadeh R, Yosofi M, Mohammadi F. Synthesis of silver nanoparticles using four species plant and investigation of their antimicrobial activity. Iran J Chem Eng. 2014;4:1-9.
78. Martínez-Castañon GA, Nino-Martinez N, Martinez-Gutierrez F, Martínez-Mendoza JR, Ruiz F. Synthesis and antibacterial activity of silver nanoparticles with different sizes. J Nanopart Res. 2008;10:1343-8. https://doi.org/10.1007/s11051-008-9428-6