Green Bio-Manufacturing of Silver Nanoparticles Using the Green Microalga Cosmarium sp. (Desmidiaceae) and Assessment of Their Microbial Efficacy

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ABSTRACT
This study aimed to prepare silver nanoparticles (AgNPs) using the green microalga Cosmarium sp. by mixing its crude methanolic and hexane extracts with silver nitrate solution (1:1, v/v), as well as assessing their antimicrobial characterization. Alga-mediated AgNPs were characterized by color change. UV-Vis spectroscopy, field emission scanning electron microscopy (FE-SEM), Fourier transform infrared spectroscopy (FTIR), and energy dispersive X-ray diffraction (EDX) analysis were used in this study. This green bio-manufacturing approach led to the formation of AgNPs based on the color changing evidence after 10-15 min of the mixing process. The UV absorption peaks at 408 nm and 414 nm, for the hexane and methanolic extracts, respectively, confirmed AgNPs formation. FE-SEM and EDX approaches showed that AgNPs particles were rod-shaped and ranged between 50-77 nm in size. For their antibacterial activity, the methanolic nano-extract of Cosmarium sp. was more effective against Klebsiella pneumonia, where average diameter of the inhibition zone was 20 mm, while the hexane nano-extract exhibited the most antibacterial effect against Salmonella typhi with a diameter range of 22 mm. In addition, the synthesized AgNPs exhibited a prominent antifungal activity, particularly against Mucor racemosus, where the inhibition zones were 23 mm and 30 mm for the algal nano-methanolic and nano-hexane extracts, respectively.

INTRODUCTION
Microalgae are living microorganisms characterized by their ability to form a wide range of high-valued, bioactive compounds using sunlight, CO₂ and water (Al-Hayali & Al-Katib, 2020). Among these microorganisms, green microalgae constitute the most heterogeneous group of phototrophic primitives, which is considered one of the main sources of chemical compounds including nutrients, fats and vitamins (Khorsheed & Al-Katib, 2021; Yaqub et al., 2022). In general, microalgae are among the richest biological sources of bioactive compounds produced from secondary metabolism and they are key sources of several phenolics, alkaloids and carotenoids (Al-Taie & Al-Katib, 2020). Nowadays, a great attention has been drawn to exploit the ecofriendly algae-mediated nanoparticles as powerful antimicrobial agents to manage and control human and plant diseases.
Several previous investigations pinpointed that silver nanoparticles (AgNPs) manufactured by microalgae have a wide spectrum of biotechnological applications such as antimicrobial, anticancer, biosensing characteristics (Al-Hasso et al., 2022). Moreover, they have been applied in catalysis (Atwan & Hayder, 2020; Abd & Mohammed, 2021). AgNPs are high-valued nanoparticles due to their feasible utilization in different fields. Indeed, AgNPs are characterized by their low economic cost as well as being non-toxic and interfacially safe, and thus it is possible to use them in the field of secondary medicine (Wu et al., 2017). There are three basic methods for the manufacture of nanoparticles, including chemical, physical and biological. The latter is also named “green manufacturing”, which is the most preferred approach (Abbasi et al., 2016). The green manufacturing technology using microalgae is currently considered one of the best methods since it is environmentally friendly, safe, low cost, easily handled, speedy in forming nanoparticles (Al-Katib et al., 2015), and chemically more stable compared to other biological manufacturing methods using bacteria, fungi, yeasts and viruses (Vanlalveni et al., 2021). Therefore, algae-based nanotechnology opened horizons in the field of nanomedicine to prepare potent nanoparticles, which can be used as alternative antibiotics to combat the problem of multiple drug resistance (MDR), which is shown by several plant- and human- infecting pathogens (Morell & Balkin, 2010).

To the best of our knowledge, utilization of the green desmid Cosmarium sp. in the preparation of nanoparticles is still an underestimated topic. The present work was sought to prepare AgNPs, using the methanolic and hexane extracts of the microalga Cosmarium sp. in addition to assessing their antimicrobial characterization.

MATERIALS AND METHODS

Biosynthesis of silver nanoparticles (AgNPs)

The green macroalga Cosmarium sp. was isolated from a water tank at house in Bartella sub-district, Shaquli village, eastern Mosul City, Nineveh Governorate, Iraq. The isolation and cultivation processes were previously described in the study of Khorshed and Al-Katib (2021). Silver nanoparticles were produced from the green microalga Cosmarium sp. by adding a solution of silver nitrate (0.0421g) to 100ml of deionized water to obtain a concentration of 1mM (Nagati et al., 2013). Then, the hexane and methanolic extracts were prepared from Cosmarium sp. (Verawaty et al., 2017) by using 10g of dried algal powder, which was inserted inside the thimble of the Soxhlet device, followed by the addition of about 150ml of the desirable solvent.

The dried sample was left immersed in solvent for 24h, after which the heater was turned on, and its temperature was fixed at 60°C. Later, using a magnetic stirrer, a continuous stirring was maintained to mix the extract and silver nitrate solution with a ratio of 1:1 (v/v) at a temperature of 80°C for 15min until the color of the extracts was changed. This preliminary evidence indicates the completion of bioreduction of Ag ions (from Ag⁺ to Ag⁰) by the Cosmarium sp. extracts (Enakshi, 2013).
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**Characterization of alga-synthesized nanoparticles**

*Changing color of the solution*

After adding the silver nitrate solution to the raw *Cosmarium* sp. extracts, we noticed a change in color, which was also observed upon the addition of the silver nitrate solution to the phenolic extract of the algae (*Ranganath et al., 2012*).

*UV-visible spectroscopy*

This test was conducted using Unicum UV-300 UV/Vis spectrophotometer (Spectronic Unicam, Rochester, USA) to diagnose metallic nanoparticles and determine the concentration of the biosynthetic solution, where a beam with a wavelength ranging between 180- 1100nm was passed through the biosynthesized nanoparticles (*Islam et al., 2017*).

*Field emission scanning electron microscopy (FE-SEM)*

Scanning emission electron microscopy was used to diagnose the shape and size of bio-manufactured AgNPs. This test was carried out at the CAC laboratories, University of Tabriz, Iran using Inspect F50 FE-SEM, FEI Company, Germany. Briefly, AgNPs were dried at room temperature for 24h on a glass slide. The adhesive carbon tape was fixed on the aluminum stubs, and then the sample was fixed on the surface of the stubs, and the sample was placed in a special chamber inside the vacuum sputter coater for 10min (*Echlin, 2009*).

*Fourier-transform infrared spectrum (FTIR)*

This screening was conducted using FTIR spectroscopy (SHIMADZU Spectroscopy, Japan) to determine the functional groups of biomolecules in the *Cosmarium* extracts, which are responsible for synthesis and capping of alga-synthesized nanoparticles.

*Energy dispersive X-ray spectroscopy (EDX)*

Energy dispersive X-ray spectrometry (EDX) was applied to determine the type of chemical elements present in the sample to be examined. It is a type of X-ray spectrometer symbolized as EDX or EDS.

**Antimicrobial activity of *Cosmarium*-synthesized AgNPs**

The well-diffusion method described by *Magaldi et al. (2004)* was used. Seven pathogenic bacterial strains were used, viz., *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi, Bacillus cereus* and *Bacillus coagulanse*. These strains were obtained from the postgraduate laboratories of the College of Education for Pure Sciences, and the laboratories of the College of Pharmacy, University of Mosul, Iraq. Additionally, five fungal strains were assessed: *Trichoderma asperallum, Mucor racemosus, Candida albicans, Aspergillus niger* and *Trichoderma harzianum*. These strains were obtained from the laboratories
of the College of Environmental Sciences and Technologies, University of Mosul, Iraq.

RESULTS AND DISCUSSION

After adding the *Cosmarium* extracts to a silver nitrate solution and placing it in a dark place, a change in the color of the mixture was observed (Figs. 1, 2). This color change is a visual evidence of the formation of biological AgNPs. The change in color is due to changes in the oxidation process of metals and the transformation of silver from normal size to nanoscale by some bioparticles in the *Cosmarium* extracts (Ibraheem et al., 2016).

**Fig. 1.** The change in color using the *Cosmarium* hexane extract. Ag: silver nitrate solution, CHE: *Cosmarium* hexane extract, CHE-Ag (AgNPs): silver nanoparticles synthesized by *Cosmarium* hexane extract.

**Fig. 2.** The change in color using the *Cosmarium* methanolic extract. Ag: silver nitrate solution, CME: *Cosmarium* methanolic extract, CME-Ag (AgNPs): silver nanoparticles synthesized by *Cosmarium* methanolic extract.

As illustrated in Table (1) and Fig. (3), the absorption peaks of the *Cosmarium*-mediated AgNPs were recorded at wavelengths of 408-414 nm. In addition, the data of ultraviolet spectrum at a wavelength of 200-800 nm showed the appearance of an absorption peak at a wavelength of 408-414 nm.

**Table 1.** UV-visible spectroscopy data

<table>
<thead>
<tr>
<th><em>Cosmarium</em> methanol extract= CME</th>
<th><em>Cosmarium</em> hexane extract= CHE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CME-Ag (AgNPs)</td>
<td>CME</td>
</tr>
<tr>
<td>414 nm</td>
<td>312 nm</td>
</tr>
<tr>
<td></td>
<td>CHE-Ag (AgNPs)</td>
</tr>
<tr>
<td></td>
<td>CHE</td>
</tr>
<tr>
<td></td>
<td>408 nm</td>
</tr>
<tr>
<td></td>
<td>314 nm</td>
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</tbody>
</table>
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The results of the UV-Vis spectra highly coincide with the findings of Muthukrishnan et al. (2015) who confirmed that the absorbance region of AgNPs is at a wavelength of 400-450 nm. UV–Vis spectrophotometer is an important analytical tool for the detection of AgNPs formed in the matrix as a result of the optical properties shown by the nanoparticles resulting from (SPPR) surface plasmon polarization resonance, with absorbance at specific wavelengths strongly related to the size and relative dimensions of the nanoparticles (Xin et al., 2018).

Fig. (4) shows the energy dispersive X-ray diffraction (EDX) spectra analysis of AgNPs in the Cosmarium methanolic extract to which silver nitrate was added. The element (C) appeared and its energy peak was at (0.3 keV), while the element Ag had its energy peak at 3.2 keV, and two elements appeared, O and Cl, and their energy peak was (0.5, 3) keV.
As shown in Table (2) and Fig. (5), the shape of AgNPs was rod-shaped, and the size ranged between 50 & 77nm.

Table 2. The shape and size of silver nanoparticles (AgNPs) synthesized by the Cosmarium methanolic and hexane extracts

<table>
<thead>
<tr>
<th>Size of AgNPs</th>
<th>Shape of AgNPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>50.43-77.37 nm</td>
<td>rod-shaped</td>
</tr>
</tbody>
</table>

The results of diagnosing nanoparticles using FTIR technique (Fig. 6) showed bands associated with the functional groups, as previously documented in the study of Laskar et al. (2018). Thus, the Cosmarium extracts indicating the emergence of bands is located between 1188 & 1200 cm\(^{-1}\), which referred to the O-CH\(_3\) group (Mumtaz et al., 2012).
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The results of Table (3) show the activities and effect of the algae extract added to the silver nitrate solution, as the most sensitive bacteria to the methanol nano-extract are *Salmonella typhi*, with an inhibition diameter of 22mm, and the most sensitive bacteria to the hexane nanoextract are also *S. typhi*, with a diameter of 20 mm. Table (3) reveals that, the most sensitive fungus to the methanolic nano-extract is *Mucor racemosus*, with an inhibition diameter of 23mm. While, for the nano-hexane extract, it was more effective on *M. racemosus*, with a diameter of 30mm. The algae either cyanobacterial or green alga extract succeeded as an antibacterial agent. It was reported that, two types of Iraqi fresh water alga, *Scenedesmus* sp. and *Oscillatoria* sp., were recorded for some of their active compounds that play an important role as antioxidants and antimicrobial against some bacteria. Three types of bacteria *Salmonella* sp., *Escherichia coli*, and *Staphylococcus* sp. that cause spoilage for food were tested (Karm, 2019). The results show that biosynthesized silver nanoparticles are more effective than bacterial supernatant on human pathogenic microbes, as stated in the study of Kadhum and Hussein (2020). Thus, the production of silver nanoparticles and addressing their antimicrobial activity is a new important, beneficial field in scientific research.

**Table 3.** Inhibition zones (mm) of antibacterial activity of AgNPs synthesized by the Cosmarium methanolic and hexane extracts

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>AgNPs synthesized by the Cosmarium methanolic extract</th>
<th>AgNPs synthesized by the Cosmarium hexane extract</th>
<th>AgNO₃ solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella typhi</em></td>
<td>20</td>
<td>20</td>
<td>–</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>10</td>
<td>10</td>
<td>–</td>
</tr>
</tbody>
</table>
**Fig. 7.** Antibacterial assay of *Cosmarium*-synthesized AgNPs, as shown in the present study

**Table 4.** Inhibition zones (mm) of antifungal activity of AgNPs synthesized by the *Cosmarium* methanolic and hexane extracts

<table>
<thead>
<tr>
<th>Fungal strains</th>
<th>AgNPs synthesized by the <em>Cosmarium</em> methanolic extract</th>
<th>AgNPs synthesized by the <em>Cosmarium</em> hexane extract</th>
<th>AgNO₃ solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>11</td>
<td>22</td>
<td>–</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>20</td>
<td>13</td>
<td>–</td>
</tr>
<tr>
<td><em>Trichoderma asperellum</em></td>
<td>18</td>
<td>18</td>
<td>–</td>
</tr>
<tr>
<td><em>Trichoderma harzianum</em></td>
<td>22</td>
<td>15</td>
<td>–</td>
</tr>
<tr>
<td><em>Mucor racemosus</em></td>
<td>23</td>
<td>30</td>
<td>–</td>
</tr>
</tbody>
</table>
Fig. 8. Antifungal assay of Cosmarium-synthesized AgNPs, as shown in the present study

CONCLUSION

In this study, the green synthesis of AgNPs using the methanolic and hexane extracts of the green microalga Cosmarium sp. was investigated. The synthesized AgNPs were rod-shaped, and their sizes ranged between 50-77 nm. UV absorption peaks, FTIR and EDX analyses confirmed the formation of AgNPs. Based on our preliminary findings, the Cosmarium-synthesized AgNPs showed good antimicrobial activity against a wide group of pathogenic bacteria and fungi. Therefore, it is recommended to utilize the Cosmarium-synthesized AgNPs as an alternative to commercial antibiotics, but this needs further in-depth investigations.

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REFERENCES


