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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, CO_2 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 **(Khorshed & 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 continous 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 Ag^0) by the *Cosmarium* sp. extracts **(Enakshi, 2013)**.

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 sscaning eelectron 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 form 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)**.

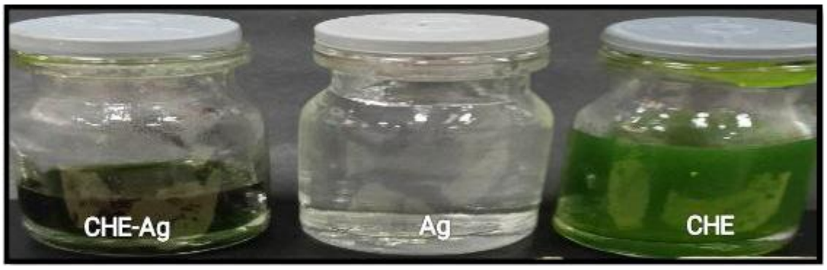


Fig. 1. The change in color using the *Cosmarium* hexane extract. Ag: silver nitrate solution, CHE: *Cosmarium* hexane extract, CHE-Ag (AgNPs): silver nanoparticles synthesized by *Cosmarium* hexane extract.

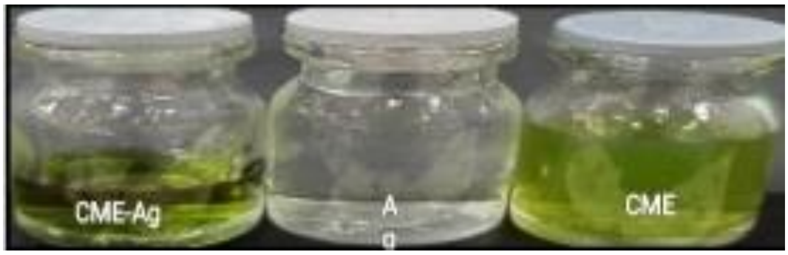
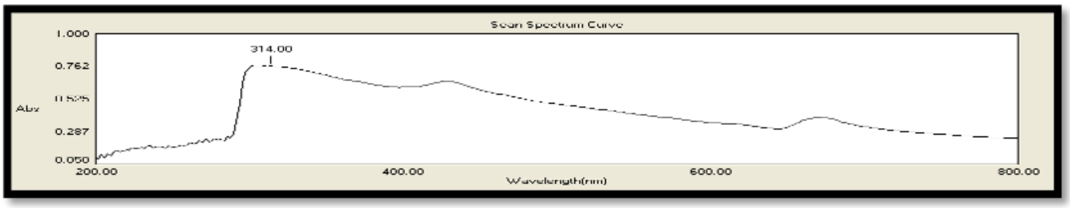


Fig. 2. The change in color using the *Cosmarium* methanolic extract. Ag: silver nitrate solution, CME: *Cosmarium* methanolic extract, CME-Ag (AgNPs): silver nanoparticles synthesized by *Cosmarium* methanolic extract.

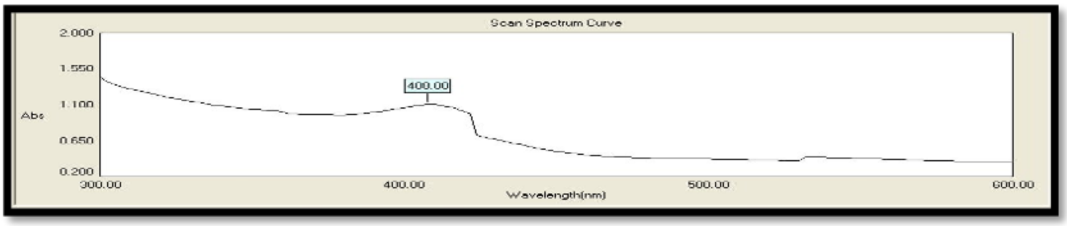
As illustrated in Table (1) and Fig. (3), the absorption peaks of the *Cosmaiurm*-mediated AgNPs were recorded at wavelengths of 408- 414nm. In addition, the data of ultraviolet spectrum at a wavelength of 200- 800nm showed the appearance of an absorption peak at a wavelength of 408-414 nm.

Table 1. UV-visible spectroscopy data

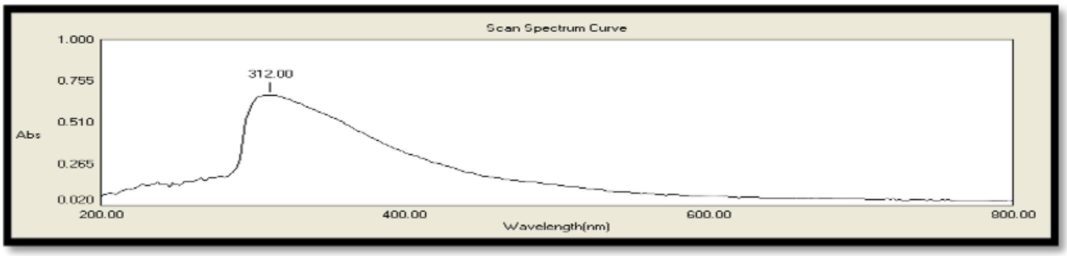
***Cosmarium* methanol extract= CME**		***Cosmarium* hexane extract= CHE**	
CME-Ag (AgNPs)	CME	CHE-Ag (AgNPs)	CHE
414 nm	312 nm	408 nm	314 nm



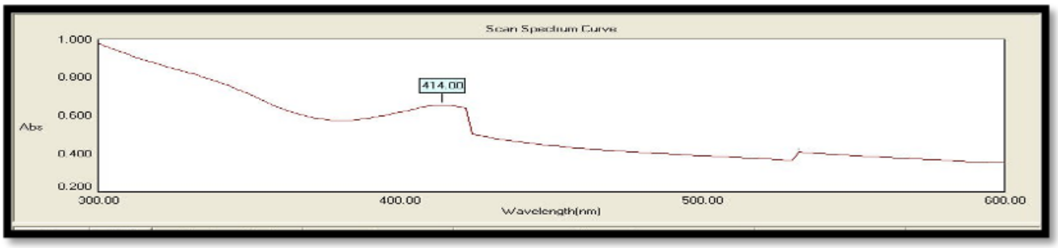
Cosmarium hexane extract = CHE



CHE-Ag (AgNPs)



Cosmarium methanol extract = CME



CME-Ag (AgNPs)

Fig. 3. UV-Vis spectra of AgNPs synthesized by the *Cosmarium* methanolic and hexane extracts

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.

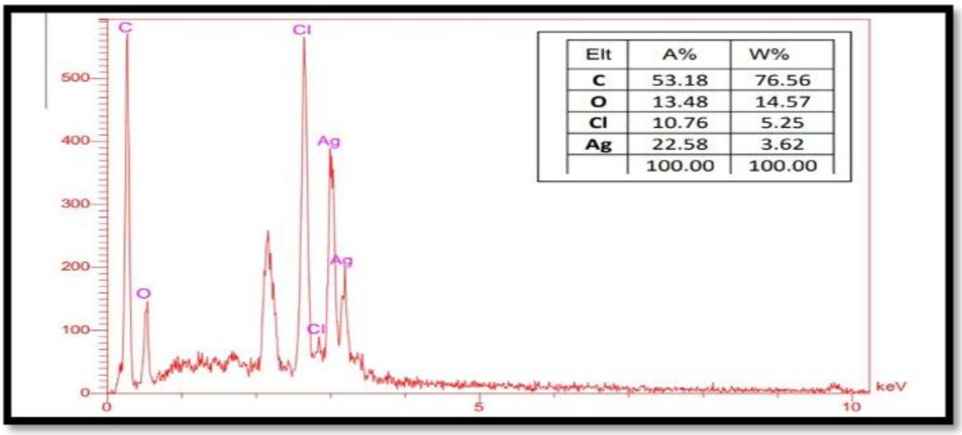


Fig. 4. Energy dispersive X-ray diffraction (EDX) pattern of *Cosmarium* -mediated synthesized AgNPs

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

Size of AgNPs	Shape of AgNPs
50.43-77.37 nm	rod-shaped

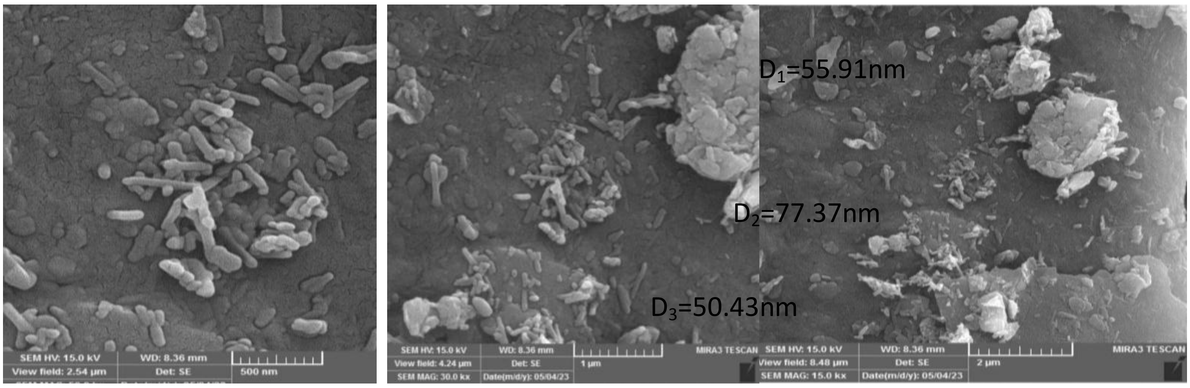
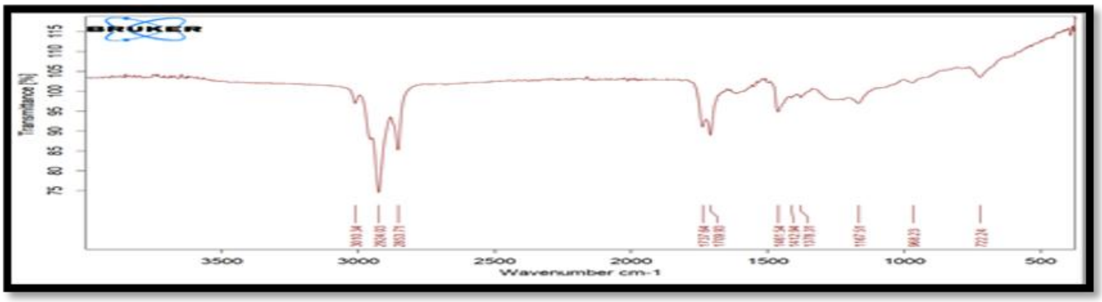


Fig. 5. Shape and size of silver nanoparticles (AgNPs) synthesized by the *Cosmarium* methanolic and hexane extracts

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 & 1200cm^{-1}, which referred to the O-CH_3 group **(Mumtaz *et al.*, 2012)**.



Cosmarium methanolic extract (CME)

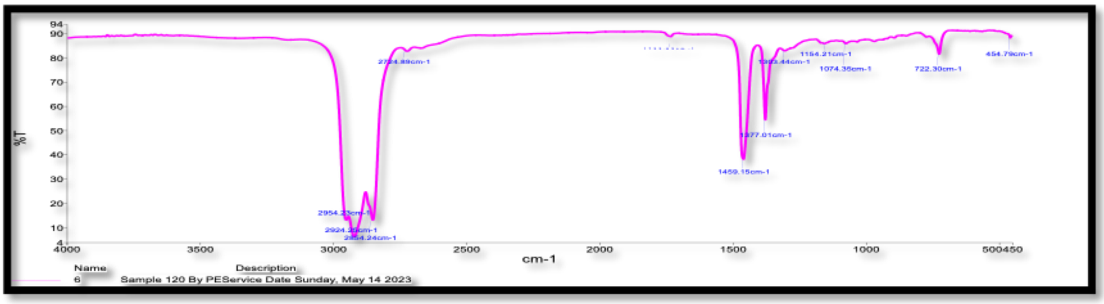


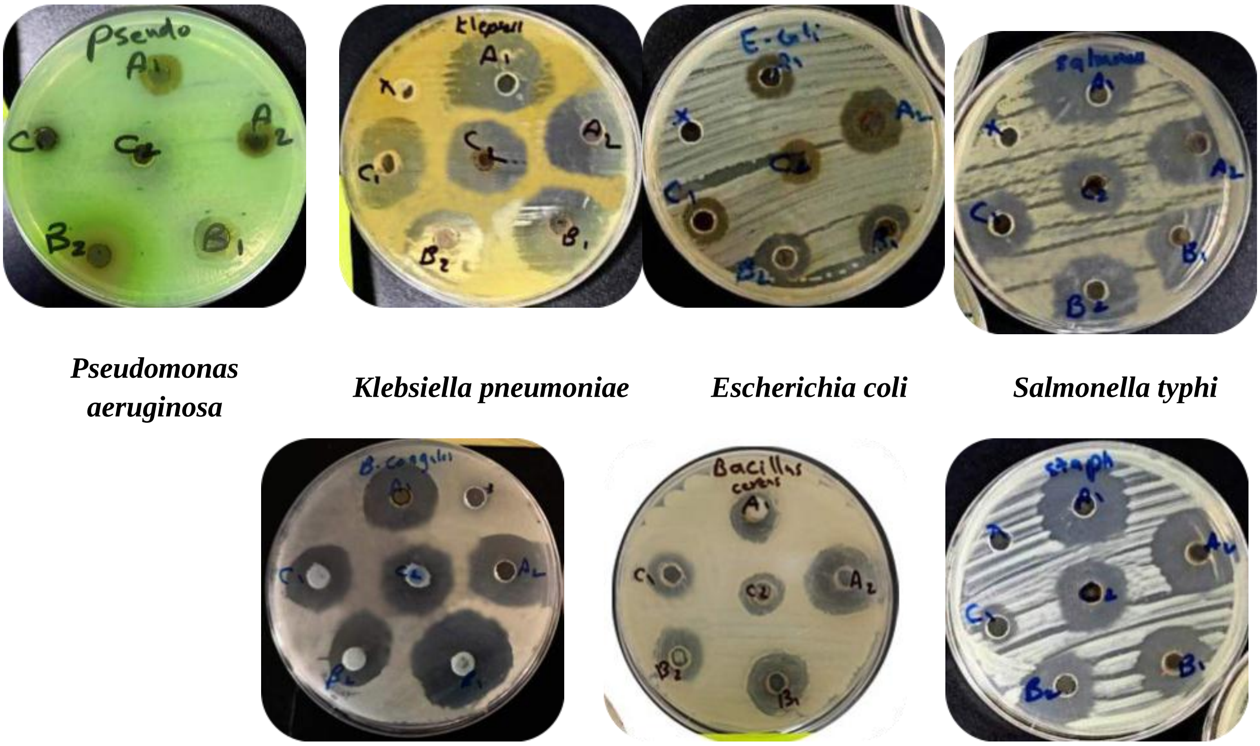
Fig. 6. FTIR spectra of *Cosmarium*-synthesized AgNPs

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 algae 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

Bacterial strains	AgNPs synthesized by the *Cosmarium* methanolic extract	AgNPs synthesized by the *Cosmarium* hexane extract	$AgNO_3$ solution
Salmonella typhi	20	20	–
Escherichia coli	10	10	–

Klebsiella pneumoniae	20	19	–
Pseudomonas aeruginosa	–	–	–
Staphylococcus aureus	12	19	–
Bacillus cereus	10	8	–
Bacillus coagulans	10	15	–



Pseudomonas aeruginosa ***Klebsiella pneumoniae*** ***Escherichia coli*** ***Salmonella typhi***

Bacillus coagulanse ***Bacillus cereus*** ***Staphylococcus aureus***

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

Fungal strains	**AgNPs synthesized by the *Cosmarium* methanolic extract**	**AgNPs synthesized by the *Cosmarium* hexane extract**	**$AgNO_3$ solution**
Candida albicans	11	22	–
Aspergillus niger	20	13	–
Trichoderma asperellum	18	18	–
Trichoderma harzianum	22	15	–
Mucor racemosus	23	30	–

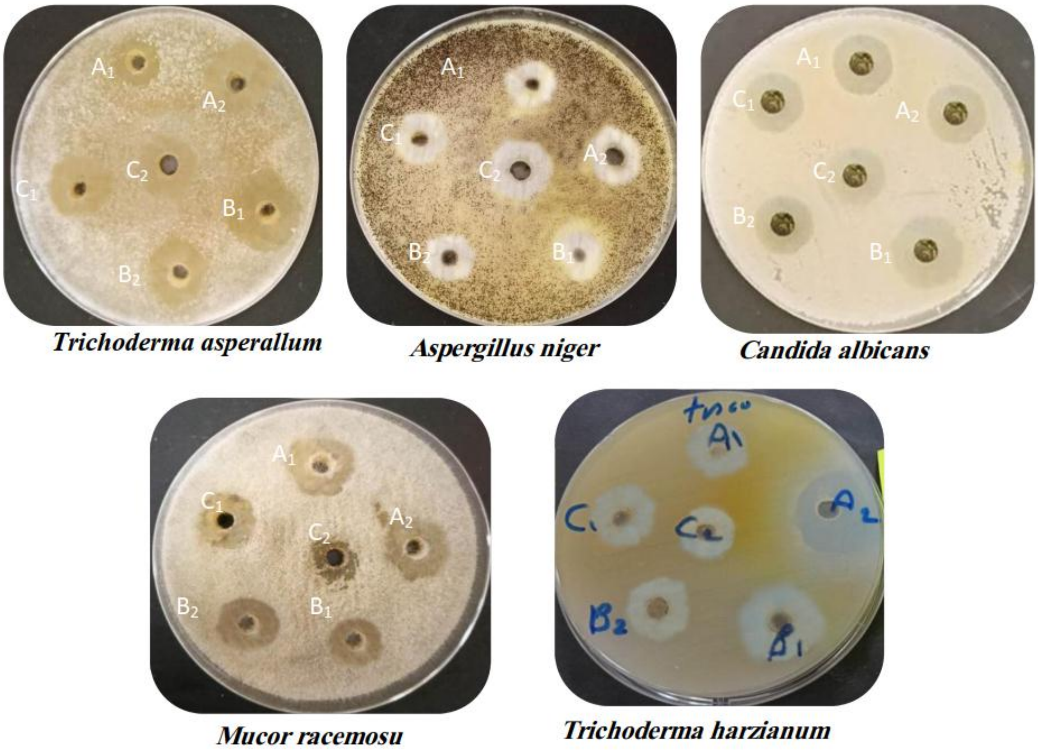


Fig. 8. Antifungal assay of *Cosmarium*-synthesized AgNPs, as shown in the present study

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- 77nm. 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

Abbasi, A.; Khojasteh, H. ; Hamadanian, M. and Salavati-Niasari, M. (2016). Synthesis of $CoFe_2O_4$ nanoparticles and investigation of the temperature, surfactant, capping agent and time effects on the size and magnetic properties. Journal of Materials Science: Materials in Electronics, 27: 4972-4980.

Abd, I. N. and Mohammed-Ridha, M. J. (2021). Simultaneous adsorption of tetracycline and amoxicillin by *Cladophora* and *Spirulina* algae biomass. Iraqi Journal of Agricultural Sciences, 52(5): 1290-1303.

Al-Hayali, A. Z. and Al-Katib, A. M. (2020). Isolation and diagnosis of phenolic compounds of *Gleocapsa* sp. PCC7428 and their antimicrobial activity. Plant Archives, 20(2): 8773-8783.

Al-Katib, M; Al-Shahery Y. and Al-Niemi, A. (2015). Biosynthesis of silver nanoparticles by cyanobacterium *Gloeocapsa* sp. Inter. J. Enhance Res. Sci., 4: 60-73.

Al-Hasso, Z. Y. K.; Al-Katib, M.A. and Alrahman, G.Y.A. (2022). Biosynthesis of gold nanoparticles by *Lyngbya* sp. clone Zen/Mira 16S ribosomal RNA gene and its antibacterial activity. International Journal of Health Sciences, 6(S6): 10810-10819.

Al-Taie, M. F. and Al- Katib, M. A. (2020). Beta-carotene extraction from some microalgae, cynaobacetria and chlorophyta with its antibacterial and antifungal activity. Plant Archives**,** 20(2), 8085-8097.

Atwan, Q. S. and Hayder, N. H. (2020). Eco-friendly synthesis of silver nanoparticles by using green method: Improved interaction and application *in vitro* and *in vivo*. The Iraqi Journal of Agricultural Science, 51: 201-216.

Echlin, P. (2009). Handbook of sample preparation for scanning electron microscopy and X-ray microanalysis. Springer Science & Business Media.

Enakshi, D. (2013). Development of nano structure plasmon gold by green synthesis for fabrication of bio/chemical sensor. Research Journal of Recent Sciences, 2277, 2502.

Khorshed, B. H. and Al-Katib, M. A. (2021). Screening of some algal oils to select the best algal biodiesel resource. Egyptian Journal of Aquatic Biology & Fisheries, 25(3): 571-588.

Ibraheem, I. B. M.; Abd-Elaziz, B. E. E.; Saad, W. F. and Fathy, W. A. (2016). Green biosynthesis of silver nanoparticles using marine red algae *Acanthophora specifera* and its antimicrobial activity. J. Nanomed Nanotechnol, 7(409): 1-4.

Islam, Z. A.; Mondal, S. and Islam, M. (2017). Applications, synthesis and characterization of gold nano particles. PhD dissertation, BRAC Univeristy.

Kadhum, M. M. and Hussein, N. N. (2020). Detection of the antimicrobial activity of silver nanoparticules biosynthesized by *Streptococcus pyogenes* bacteria. Iraqi Journal of Agricultural Sciences, 51(2): 500–507.

Karm, I. F. A. (2019). Investigation of active compound in clove (*Syzygium aromaticum*) extract and compared with inhibitors of growth of some types of bacteria causing food poisoning. Iraqi Journal of Agricultural Sciences, 50(6).:1645-1651.

Laskar, I. B.; Rajkumari, K.; Gupta, R.; Chatterjee, S.; Paul, B. and Rokhum, S. L. (2018). Waste snail shell derived heterogeneous catalyst for biodiesel production by the transesterification of soybean oil. RSC advances, 8(36): 20131-20142.

Magaldi, S.; Mata-Essayag, S.; De Capriles, C. H.; Perez, C.; Colella, M. T.; Morell, E. A. and Balkin, D. M. (2010). Methicillin-resistant *Staphylococcus aureus*: a pervasive pathogen highlights the need for new antimicrobial development. The Yale Journal of Biology and Medicine, 83(4): 223-233.

Mumtaz, M. W.; Adnan, A.; Mukhtar, H.; Rashid, U. and Danish, M. (2012). Biodiesel production through chemical and biochemical transesterification: Trends, technicalities, and future perspectives. In: *Clean Energy for Sustainable Development*, pp. 465-485, Academic Press.

Muthukrishnan, S.; Kumar, T. S. and Rao, M. V. (2015). Anticancer activity of biogenic nanosilver and its toxicity assessment on *Artemia salina*-evaluation of mortality, accumulation and elimination: An experimental report. Journal of environmental chemical engineering, 5(2): 1685-1695.

Nagati, V. B.; Alwala, J.; Koyyati, R.; Donda, M. R.;Banala, R. and Padigya, P. R. M. (2013). Green synthesis of plant-mediated silver nanoparticles using *Withania somnifera* leaf extract and evaluation of their antimicrobial activity. *Asian Pac J Trop Biomed*, 2: 1-5.

Ranganath, E.; Vandana, R. and Afreen, B. (2012). Biosynthesis of silver nanoparticles by *Lactobacillus* sp. and its activity against *Pseudomonas auerogenosa*. Asian Journal of Biochemical and Pharmaceutical Research, 3 (2): 49-55.

Vanlalveni, C.; Lallianrawna, S.; Biswas, A.; Selvaraj, M.; Changmai, B. and Rokhum, S. L. (2021). Green synthesis of silver nanoparticles using plant extracts and their antimicrobial activities: A review of recent literature. RSC advances, 11(5): 2804-2837.

Verawaty, M.; Melwita, E.; Apsari, D. and Wiyahsari, M. (2017). Cultivation strategy for freshwater macro- and micro- algae as Biomass stock for lipid production. J. Eng. Technol. Sci., 48(2): 261-274.

Wu, Y. S.; Ngai, S. C.; Goh, B. H.; Chan, K. G.; Lee, L. H. and Chuah, L. H. (2017). Anticancer activities of surfactin and potential application of nanotechnology assisted surfactin delivery. Frontiers in Pharmacology, 8, 761.

Xin, H.; Namgung, B. and Lee, L. P. (2018). Nanoplasmonic optical antennas for life sciences and medicine. Nature Reviews Materials, 3(8): 228-243.

Yaqub, H. M.; Saeed, J. A. and Al-Katib, M. A. (2022). Allelopathic activity of the aqueous extracts of *Cladophora* and *Spirogyra* on the germination and growth of three cultivars of bread wheat *Triticum aestivum* L. . International Journal of Health Sciences, 6(S8): 3666-3675.