GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *CAULERPA CHEMNITZIA* : CHARACTERIZATION AND APPLICATIONS

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Abstract
Biosynthesis of silver nanoparticles (AgNPs) has emerged as a sustainable and eco- friendly alternative to conventional chemical and physical synthesis methods. In this study, we explored the potential of the green macroalga Caulerpa chemnitzia as a biological agent for the synthesis of silver nanoparticles. The biosynthesis process was carried out by mixing aqueous extracts of C. chemnitzia with silver nitrate solution under controlled conditions. Formation of AgNPs was confirmed through a color change in the reaction mixture and further characterized using UV-Vis spectroscopy, which exhibited a surface plasmon resonance peak typical of AgNPs.Additional characterization techniques, including Fourier- transform infrared spectroscopy (FTIR), X-ray diffraction (XRD),were employed to analyze the functional groups involved in nanoparticle stabilization, crystalline structure, and morphology, respectively. This study underscores the feasibility of using C. chemnitzia as a cost-effective and sustainable platform for silver nanoparticle's biosynthesis and provides a foundation for future investigations into its applications in nanotechnology and medicine.

Keywords: *silver nanoparticles, algae, FTIR, XRD, nanotechnology*

Introduction

Nanotechnology has revolutionized numerous scientific fields, offering innovative solutions in medicine, agriculture, and environmental remediation. Among various nanomaterials, silver nanoparticles (AgNPs) have garnered significant attention due to their unique physicochemical properties, including high surface area, excellent conductivity, and remarkable antimicrobial activity. Traditional methods for synthesizing AgNPs often involve chemical and physical processes that are costly, energy-intensive, and associated with environmental risks due to the use of toxic reagents. Consequently, there is a growing demand for sustainable and eco-friendly approaches to nanoparticle synthesis.

Biological synthesis of AgNPs, also known as green synthesis, has emerged as an environmentally benign alternative that leverages the reducing and stabilizing capabilities of biological entities such as plants, fungi, and algae. Algae, in particular, are attractive candidates due to their rapid growth, abundance, and ability to produce a diverse array of bioactive compounds such as polysaccharides, proteins, and phenolic compounds that facilitate nanoparticle formation.

Algae are widespread organisms that flourish in almost every inhabitable environment on the planet. Although they are most commonly found in stagnant waters, they also thrive in a variety of settings, including hot springs, icy regions, moist soils, rock crevices, tree bark, aged walls, garbage dumps, and waste sites. Large algae that grow in marine, coastal, and brackish water environments are collectively known as seaweeds.

These primitive, chlorophyll-containing organisms can be either prokaryotic or eukaryotic, displaying a wide range of structural diversity from single-celled forms to complex multicellular structures. Their sizes span from tiny microalgae to substantial macroalgae, commonly referred to as seaweeds. Macroalgae predominantly occupy the littoral zone and are classified into green, red, and brown algae. In contrast, microalgae inhabit benthic and littoral environments as well as the open ocean, where they function as phytoplankton (Amitha et al., 2024).

Caulerpa chemnitzia, a green macroalga found in marine ecosystems, has been recognized for its rich biochemical composition, including secondary metabolites with potential reducing and capping properties. Beyond their ecological significance, seaweeds play a vital role across various industries, serving as sources of food, pharmaceutical compounds, and industrial products such as alginates and carrageenans. Rich in nutrients and bioactive compounds, they provide substantial health benefits while also demonstrating remarkable potential in bioremediation and climate regulation. These attributes underscore their critical importance to environmental stability, economic development, and human health. Seaweeds are thus multifaceted resources essential for maintaining ecological balance, supporting livelihoods, and enhancing overall well-being. Despite its known ecological and pharmaceutical significance, its application in nanoparticle biosynthesis remains underexplored(T V et al., 2023). Marine natural products have garnered significant scientific interest due to their valuable and promising compounds with potential applications in combating cancer and other diseases. Over the past decades, numerous bioactive compounds have been extracted from marine resources, demonstrating remarkable pharmacological effects compared to conventional medicines.(Movahhedin et al., 2014)

Further research could focus on exploring algae for novel compounds to develop alternatives for combating diseases. Compared to higher plants, lower forms like seaweeds are recognized for their exceptional biochemical and nutritional properties. As a result, seaweeds hold great potential as alternative food sources for current and future generations.

This study investigates the potential of *C. chemnitzia* for the biosynthesis of AgNPs. By utilizing its natural bioactive compounds, the study aims to develop a simple, sustainable, and efficient method for producing AgNPs. The synthesized nanoparticles were characterized using advanced analytical techniques to confirm their formation, structure, and morphology. This work contributes to the growing body of knowledge on algal-based nanoparticle synthesis and sets the stage for further exploration of *C. chemnitzia* in nanotechnology.

Materials and Methods Biosynthesis of Silver Nanoparticles

The selected dried green seaweed, after a 30-day drying period, was pounded into a fine powder with a mortar and pestle. After dissolving the resultant powder in 1000 millilitres of Millipore water, the mixture was boiled for 20 minutes at 80 degrees Celsius. The solution was then filtered using Whatman No. 1 filter paper. After being extracted with methanol using a Soxhlet device, the filtrate from the crude plant material was macerated at 37 °C. Following the methanol extraction, the extract was dried for one day at 45 °C under low pressure in a rotary evaporator. In a 250 ml sterile conical flask, 10 ml of the extract and 100 ml of AgNO3 aqueous solution were mixed sequentially. After being blended, the samples were placed in a water bath at 90 °C for an hour. The pH of the solution was adjusted using 1N NaOH and 1N H3PO4. The color of the reaction mix gradually changes from yellow to reddish-brown after incubation, indicating the synthesis of Ag NPs in the mixture.

Characterization of Silver Nanoparticles UV Vis Spectroscopy

The Ultraviolet (UV) spectrophotometer (Merck, India) was initially employed to verify the synthesis of Ag NPs. Ag-NPs solution was scanned at a modest scanning rate between 300 and 700 nm utilizing the UV-vis spectrophotometer. UV-Vis spectroscopy is widely used for the evaluation of plasmonic resonance in nanoparticles and the confirmation of the creation of silver nanoparticles. Determining the absorbance data from UV-Vis spectroscopy is a dependable method to verify the existence of silver nanoparticles and explore their plasmonic characteristics. A preliminary estimation of the nanoparticle size can also be made using this analytical method based on the observed absorbance characteristics (Alim-Al-Razy et al., 2020).

X- Ray Diffraction Analysis

The XRD pattern was initiated concurrently and monitored at a 2θ angle from 10 to 90°.(Wan Mat Khalir et al., 2020) The sample was then adequately dissolved with KBr to form a fine pellet and XRD analysis was carried out on the sample (Zhang et al., 2020).

Scherrer's relationship, which can be stated as follows, was used to determine the sample's particle size:

$$D = 0.9\lambda/(B\cos\theta)$$

The x-ray wavelength is denoted by λ, the Bragg's diffraction angle is θ, and the broadening of the diffraction line is B, which is represented as half of the maximum intensity in radians. The width of the XRD signal is used to infer the sample's particle size. This technique provides important new information on the phase nature, crystalline grain size, lattice parameters and crystalline structure. The Scherrer equation is specifically used to quantify the crystalline grain size. This includes measuring the broadening of the most intense peak in an XRD measurement for a given sample(Kumar, 2022) .

Fourier Transform Infrared Spectroscopy

The FTIR analysis was used to determine which possible biomolecules were capping the Ag ions and reducing the Ag ions. The potential involvement of the functional groups has been demonstrated by analyzing and detecting them using FTIR spectroscopy on the freeze-dried nanoparticles(Hieu et al., 2022)Fourier-transform infrared (FTIR) spectroscopy is employed to get the infrared absorption or emission spectra of different states of matter, such as solids, liquids and gases. The capacity to concurrently record high-resolution images over a wide spectral range, typically between 4000 and 400 cm^{-1} is what distinguishes this method (Fadlelmoula et al., 2022).

Result and Discussions

The production of silver nanoparticles (AgNPs) was successfully carried out using algae samples from the Caulerpa species. The biogenesis of AgNPs was initially identified through visual inspection, where color changes indicated the reduction of silver nitrate ($AgNO_3$). Upon adding the aqueous $AgNO_3$ solution to the marine algal extract, the solution became opaque, signaling the commencement of the reaction. This was followed by a transformation in color to orange-brown and greenish-brown, corresponding to the biogenesis of AgNPs in *Caulerpa chemnitzia*. Similarly, in *Chaetomorpha antennina*, the biogenesis of AgNPs was marked by a transformation of the initially colorless solution into a pale reddish-brown color, indicating the onset of AgNP production (Ramalingam et al., 2024).

UV-visible spectroscopy was employed to analyze the absorption spectra of the nanosized silver samples produced. *Caulerpa chemnitzia* has been identified as a significant biological agent for the extracellular synthesis of stable silver nanoparticles (AgNPs). It is well-established that AgNPs exhibit a pale yellow to brown coloration. The UV-Vis spectra of silver nitrate solutions incubated with the marine green alga over time confirmed the synthesis of AgNPs, displaying surface plasmon resonance (SPR) peaks within the 430–450 nm range.

In this study, *Caulerpa chemnitzia* exhibited a distinct SPR peak at 404 nm, consistent with previously reported SPR peaks for various metal nanoparticles with sizes ranging from 2 to 100 nm. Similarly, F. Abdelbaset et al. (2021) investigated the UV absorption spectra of silver nanoparticles synthesized using four different algal extracts as a function of reaction time. Their results revealed characteristic SPR peaks, confirming successful nanoparticle formation: 416 nm for silver nanoparticles synthesized with *Chlorella vulgaris* extract, 420 nm for *Sargassum denticulatum* extract, 430 nm for *Ulva lactuca* extract, and 379 nm for nanoparticles synthesized with *Spirulina platensis* extract.

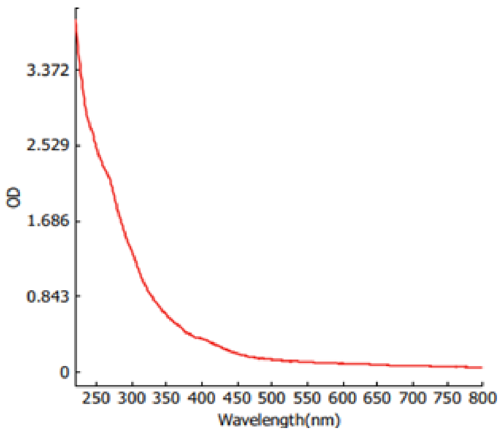
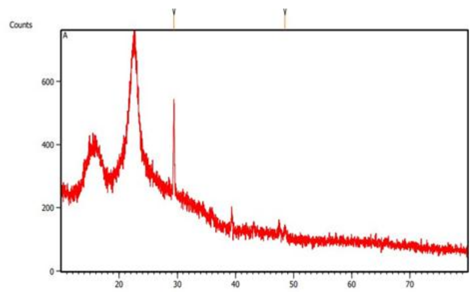


Chart 1 UV–Visible spectra of silver nanoparticles Biosynthesized *Caulerpa chemnitzia*

X-ray diffraction (XRD) analysis was conducted to examine the appearance and crystalline structure of the biosynthesized nanoparticles. In *Caulerpa chemnitzia*, distinct diffraction peaks associated with silver nanoparticles (AgNPs) were observed at 12.24, 29.72, 37.44, and 46.40, corresponding to the Bragg reflection planes of AgNPs. The absence of reflection peaks related to nitrate ions or other contaminants indicated that the nanoparticles were highly pure. The sharp reflections with high intensities further confirmed the crystalline nature of the nanoparticles. The XRD pattern revealed an average particle size of 7.13 nm for the AgNPs produced by *C. chemnitzia*, confirming their crystalline structure and purity, with no peaks for Ag_2O or other contaminants. Similarly, Thiurunavukkarau et al. (2022) studied the crystallinity of silver nanoparticles synthesized from *Sargassum polycystum* using XRD. The pattern displayed peak values at 13.75, 27.45, 33.33, and 38.44, supporting the crystalline structure of the silver nanoparticles produced from *Sargassum polycystum*. These findings underscore the consistent crystalline nature of silver nanoparticles across different algal species.



Peak List: (Bookmark 3)

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
30.4180	291.25	0.1171	3.03626	100.00
48.5077	24.08	0.4015	1.87676	8.27

Chart 2 The XRD Graph of Silver Nanoparticles Biosynthesized C*. Cheminitzia*

Fourier-transform infrared spectroscopy (FTIR) was utilized to determine the functional or biomolecules involved in the green synthesis of silver nanoparticles with Sargassum polycystum. Peaks were found at 3888.62 cm−1, indicating the presence of alcohol (O-H), 3395.99 cm−1, corresponding to the stretching of Amide II (N-NH), 2921.93 cm−1, indicating alkanes (-CH2-), and 2129.99 cm−1, signifying alkynes (-C-C). A band at 1582.96 cm−1 indicates the presence of an aromatic ring (C=C). Marine seaweeds include high levels of phenolic chemicals and flavonoids, which are also found in Caulerpa taxifolia silver nanoparticles (Thiurunavukkarau et al., 2022).

The FT-IR analysis revealed significant functional groups in both *Caulerpa chemnitzia* and *Caulerpa sertularioides* extracts, which are involved in the reduction of $AgNO_3$ to silver nanoparticles (AgNPs). A large peak between 3200-3500 cm^{-1} indicated the presence of OH functional groups in both extracts, possibly due to primary NH_2 groups. In *C. chemnitzia*, a distinct peak within the broad band at 3379.29 cm^{-1} highlighted the presence of primary and secondary amines or amides, attributed to N-H stretching. A signal around 1620 cm^{-1} suggested the presence of double bonds, likely from secondary metabolites, while a faint signal near 700 cm^{-1} indicated thioester groups. The fingerprint region displayed additional smaller peaks and bands.

The FT-IR spectra of silver nanoparticles synthesized from *C. chemnitzia* showed prominent peaks at 3332.99, 1635.64, 686.66, 601.79, 555.50, and 486.06 cm^{-1}, while the spectra of silver nanoparticles from *C. sertularioides* exhibited similar peaks at 3278.99, 1635.64, 686.66, 601.79, 555.50, and 447.49 cm^{-1}. These spectral similarities suggest that similar biomolecules are involved in the reduction process across both algae species, contributing to the formation of silver nanoparticles.

Table 1 FT-IR Spectra of Selected Sample *Caulerpa Chemnitzia*

Peak Values (cm-1)	Functional groups
3379.29	N-H Stretching
1635.54	C=C Stretching
1550.77	C=C Bending
1234.44	Aryl O Stretch
1026.13	C-N Stretching
671.23	N-H Wagging

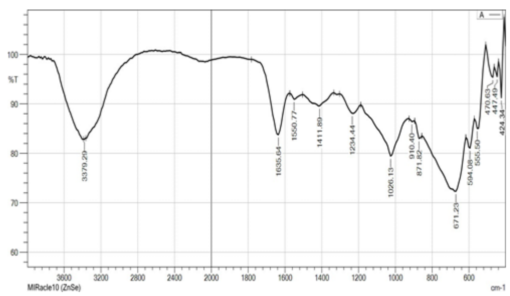


Chart 3 FT-IR Spectra of Selected Sample *CaulerpaChemnitzia*

Conclusion

Green seaweeds are significantly underrepresented compared to red and brown macroalgae. The biosynthesis of silver nanoparticles (AgNPs) in *Caulerpa chemnitzia* was successfully achieved, with clear indications of nanoparticle formation through visual and spectroscopic analyses. The color change in the algal extract upon the addition of silver nitrate ($AgNO_3$) confirmed the reduction of $AgNO_3$ to AgNPs. X-ray diffraction (XRD) analysis revealed that the synthesized nanoparticles were highly crystalline with an average particle size of 7.13 nm, and no impurities or Ag_2O peaks were observed, indicating the high purity of the AgNPs. Additionally, Fourier-transform infrared (FT-IR) spectroscopy identified functional groups, such as primary and secondary amines, double bonds, and thioester groups, which are likely involved in the reduction and stabilization of the nanoparticles. These findings suggest that *C. chemnitzia* is an effective biosource for the green synthesis of silver nanoparticles, which may have potential applications in various fields, including medicine, environmental science, and nanotechnology.Greater research efforts are required to unravel the intricate relationships between environmental factors—varying across regions and time—and the biochemical composition of these species. Such insights could pave the way for enhancing the production of specific target compounds.

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