RASĀYAN *J. Chem.*
Vol. 19 | No. 2 | 898-906| April-June | 2026
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

MARINE ALGAE-DRIVEN NANOPARTICLE SYNTHESIS: COMPREHENSIVE CHARACTERIZATION OF AgNPs DERIVED FROM *Sargassum echinocarpum* EXTRACTS

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ABSTRACT

The development of sustainable nanomaterials through eco-friendly approach has become increasing important in alignment with green chemistry principle. This study reports a green synthesis of silver nanoparticles (AgNPs) using marine macroalga *Sargassum echinocarpum* (SE) extract as bio reducing and stabilizing agent. The biosynthesis process was rapid and visually confirmed by color change with UV-Vis spectroscopy revealing a characteristic surface plasmon resonance peak at 425 nm, indicate successful nanoparticles formation. Transmission Electron Microscopy (TEM) analysis demonstrated predominantly spherical and hexagonal morphologies with particle size ranging from 10-65 nm, while Dynamic light scattering (DLS) analysis showed a hydrodynamic diameter of 238 nm and polydispersity index 0,2312, indicated good stability with zeta potential values ranging from -29,7 mV to 16,9 mV. Phytochemical screening and Fourier Transform Infrared (FTIR) spectroscopy confirmed the presence of flavonoid and polyphenol, which played a crucial role as reducing and capping agents. X-ray diffraction (XRD) analysis revealed a face-centered cubic (FCC) crystalline structure, confirming the crystalline nature of the synthesized nanoparticles. The findings demonstrated thar *sargassum echinocarpum* extract is an effective, low-cost, and environmentally benign biomaterial for producing stable AgNPs. This study contributed responsible nanomaterial fabrication aligned with sustainable Development Goal (SDG) 12 on responsible consumption an production and SDG 14 life below water

Keywords: green synthesis, silver nanoparticles, capping agent, marine macroalgae, *Sargassum echinocarpum*. Phytochemical reduction

RASĀYAN *J. Chem.*, Vol. 19, No.2, 2026

INTRODUCTION

Nanotechnology has revolutionized various fields, including biomedicine, catalysis, and environmental science[1]. Among nanomaterials, silver nanoparticles (AgNPs) have gained substantial attention because of their distinctive features. Silver is a stable chemical element and plays a role as an active conductor in catalytic processes in addition to its biomedical properties[2]. Silver, as a noble metal nanoparticle, is currently attracting attention due to its unique optical, electronic, and mechanical properties[3]. Conventional manufacturing techniques AgNPs generally employ energy-intensive processes and hazardous chemicals, posing health and environmental risks. Eco-friendly synthesis, utilizing biological resources, offers a sustainable alternative. Plant-assisted metal synthesis is more environmentally friendly, simpler, less hazardous, and less expensive[4]. Marine environments with extensive biodiversity include many organisms that produce bioactive natural products. Marine plants provide a rich source of compounds with various structural classes and biological activities[5]. In the synthesis of silver metal, biological methods utilizing bioactive materials from marine plants are responsible for replacing the use

of hazardous chemicals to reduce Ag^+ ions to Ag^0.[6] Many potentially beneficial plant bioactive substances, including steroids, flavonoids, fucoxanthins, polysaccharides, and terpenoids, have been found in various *sargassum* species, such as *sargassum muticum*[5], *sargassum lotifolium*[7], and *sargassum denticulatum*[8], and *chaetomorpha* sp.[9] have been successfully used as bioreductants in nanometal synthesis. Green, brown, and red seaweeds are often composed primarily of polysaccharides. Numerous polysaccharides, including alginic acid and alginate, carrageenan and agar, laminarin, fucoidan, ulvan, and their derivatives, make up algal cell walls. Fucoidan and laminarin are the principal water-soluble polysaccharides found in brown algae. Even within the same species, the content of fucoidan differs depending on the species and place of origin.[10]

Sargassum echinocarpum is a species of the s*argassum* genus in the sargassaceae family that lives near the coast of Bintan, Riau Islands, Indonesia. This brown algae species has been utilized for food and traditional medicine and is a reservoir of bioactive chemicals such as flavonoids, polyphenols, tannins, and alkaloids.[11,12] These compounds can act as reducing agents and stabilizers in an environmentally friendly, low-cost, high-production procedure during nanoparticle synthesis. Using *sargassum echinocarpum* algal extract, this study investigates the green synthesis of AgNPs for the first time, emphasizing the extract's dual function as a reducing and capping agent. This study supports the principles of green chemistry by minimizing hazardous chemical usage through a biologically mediated synthesis pathway. The approach aligns with the United Nations Sustainable Development Goal 12 (Responsible Consumption and Production) by promoting environmentally sound chemical processes and sustainable nanomaterial fabrication, and further relates to Sustainable Development Goal 14 (Life Below Water) through the responsible utilization and valorization of marine biomass resources.

EXPERIMENTAL

Chemical

Analytical-grade chemicals were employed in this investigation. All of the chemical reagents were analytical grade and were utilized without additional purification. Silver nitrate ($AgNO_3$) and distilled water were used to make everything function.

Collection and Preparation of *Sargassum echinocarpum* (SE)

The macroalgae *sargassum echinocarpum* was collected along the coast of Pengudang Village, Bintan, Riau Islands, Indonesia. The steps for making the algal extract were simple: First, healthy algal samples were washed several times with distilled water to remove undesirable objects such as epiphytes, foreign materials, and necrotic matter. They were air-dried, cut into little pieces, and then ground into a fine powder. The SE extract preparation process was carried out by modifying the procedure by R. Vanayagam, et al. 2024 [6]. Ten grams of dried *sargassum* powder and one hundred milliliters of distilled water were boiled for an hour at 80 degrees Celsius before being filtered using Whatman filter paper number 1. Until it was ready for use, the filtered material was gathered and kept at 4 degrees Celsius.

Preliminary Phytochemical Screening (PPS)

Sargassum echinocarpum (SE), a brown algae, belong to the *sargassum* genus and the *Sargassacea*e family. This algae is abundantly distributed along the Indonesian coast in Bintan Regency and has long been utilized as a traditional medicine and food.This species was identified in Raja Ali Haji Maritime University's marine laboratory. To achieve a stock concentration of 1% (w/v), the freeze-dried extract was dissolved in 100 milliliters of distilled water. Standard techniques were then applied for phytochemical analysis.The testing process was carried out according to references Waterman, et.al (1993)[11] and Satpathy, S et.al. (2020)[12]. Alkaloids (Mayer, Hager, and Wagner tests), saponins (foam test), terpenoids (Libermann-Burchard test), tannins and polyphenols (ferric chloride test), flavonoids (Shinoda test), carbohydrates (Molisch test), and protein and amino acid tests (Ninhydrin and Biuret tests).

Synthesis of colloidal silver nanoparticle (SE-AgNPs)

AgNPs were formed according to Balaraman, P. *et a, (2020)*[13] with modifications. In a 250 mL Erlenmeyer flask, combine 5 mL of SE extract with 45 mL of 1 mM and 0.5 mM $AgNO_3$ solutions. Bioreduction was achieved by changing the pH solution (8-9) and stirring continuously for three hours at

room temperature in the presence of light. It was believed that the color change demonstrated that Ag^{+} had communicated with nano-silver Ag^{0}.

Characterization of silver nanoparticles Characterization

UV-Vis Spectroscopy was used to monitor surface plasmon resonance (SPR) in the produced nanoparticles. UV-Vis absorbance of SE-AgNPs colloidal solution generated during synthesis was measured using a Genesys 10S dual beam UV-Visible Spectrophotometer (Thermo Scientific) with VISION Lite software. The morphology and size of synthesized SE-AgNPs were visualized employing High- Resolution Transmission Electron Spectroscopy (HRTEM H9500). X-Ray Diffraction (XRD) An X-ray diffractometer (XRD, Philips Xpert MPD to analyze crystallinity. Shimadzu's FTIR 400S was used to determine the groups of functions implicated in the capping step. Dispersion, homogeneity, along with the polydispersity index (PDI) were all decided using dynamic light scattering (DLS) measurements (Malvern pro zetasizer).

RESULTS AND DISCUSSION

Preliminary Phytochemical Screeining (PPS)

Based on the procedures that have been followed, the phytochemical content of *Sargassum echipocarpum* (SE) was produced as shown in Table-1. Most of the secondary metabolites in seaweed are phenolic and play an important role in various fields such as biomedicine.[14] Photochemical analysis of S. *echinocarpum* water extract shows the existence of alkaloids, flavonoids, steroids, terpenoids, proteins, amino acids, phenol carbohydrates and tannins. Similar research was also found in *sargassum polycystum*[15], *sargassum tenerrium* [16], *sargassum polycystum.*[15] *Sargassum* sp.'s main bioactive molecule is fucoidans, which contain fucose as its main monomer. The chemical structure of fucoidans is determined by a variety of parameters, including species, geographical location of recovery, climatic circumstances, etc.[17]

Table-1: The phytochemical content of *Sargassum echinocarpum* (SE)

Chemical Class	Present	Method
Flavonoid	+++	NaOH test H_2SO_4
Alkaloid	++	Mayer's test Wagner's test,hager test
Steroid	+	Liebermann-Burchard Analysis
Tannin	+	Tannins Analysis
phenolics	+++	Ferric chloride check
Protein and amino acid	+	Ninhydrin test Biuret check
Carbohydrate	++	Molisch Analysis
Triterpenoid	++	Liebermann-Burchard check

+++: Highly present; ++: Present; +: Sparingly present.

Most of the secondary metabolites in seaweed are phenolic and play an important role in various fields such as biomedicine.[14] Similar research was also found in *sargassum polycystum*[15], *sargassum tenerrium*[16], and *sargassum swartszii.*[18] *Sargassum* species include bioactive chemicals high in phenolic and flavonoid content, which are expected to have a function in the reduction process and as a capping agent to avoid agglomeration.

Characterization of the nanoparticles

UV–Vis Spectrophotometer analysis

When *sargassum* extract SE comes into contact with metal salts (Ag^{+}), the first thing expected is a color change. Therefore, the first technique to validate the creation of nanoparticles is UV-Vis spectroscopy.[19] A visible color shift verifies the creation of silver nanoparticles. After 30 minutes, the reaction blend's color transformed from yellow-brown to dark brown, signifying the creation of silver nanoparticles.[20] Figure-1 shows the spectra of Ag^{+}, extract, SE-AgNPs, and the color change of the solution. The surface plasmon resonance (SPR) action and the reduction about $AgNO_3$ are responsible for the dark brown.[21] When metal nanoparticles are exposed to light, the Coulomb attraction between the electrons and the nucleus generates the restoring force, which ultimately causes the electron cloud to oscillate in relation to the nuclear framework. Meanwhile, the electrical field on the light enables the electron plume to move in relation to the nucleus.[22]

The phenomenon of coherent oscillation of metal electrons resonating with an electromagnetic field is referred to as the surface plasmon resonance (SPR) band.[22, 23] The study demonstrated how temperature, reaction time, and plant extract content impact the production of silver nanoparticles, one of it generates a distinct surface plasmon resonance (SPR) band. In addition, the flavonoid, polyphenol, and terpenoid content found in *sargassum* has a crucial role in the synthesis of silver nanoparticles, aiding the biological reduction of metal nanoparticles in plant extracts.[24]

UV-vis spectral examination was used to confirm the synthesis of SE-AgNPs. A stable color shift to dark brown that occurred upon heating the mixture of algae extract and silver nitrate solution acted as an initial confirmation for the formation of SE-AgNPs. This color change occurred through localized surface plasmon resonance (LSPR).[25] The spectra of SE extract and SE-AgNPs between 200 and 800 nm in Figure 1b-e. The spectrum of the extract (279 nm) and the spectrum of SE-AgNPs (425 nm) are caused by the electronic transitions (n to π*) or (π to π*) of the phenolic groups attached to the extract.[26] The SE-AgNPs spectrum illustrates the realization of NPs, which is confirmed by the surface plasmon resonance (SPR) peak. This strengthens the idea that the reducing agent from the algal extract is involved in the formation of SE-AgNPs. Similar investigations were discovered during the Manufacturer of biogenic AgNPs by extracts of the marine brown algae *padina pavonia* at 410 nm[14], 420 nm using *sargassum myriocystum*[13], 424 nm using *callistemon lanceolatus*[6], and 422 nm using extracts of *Sargassum serratifolium.*[25]

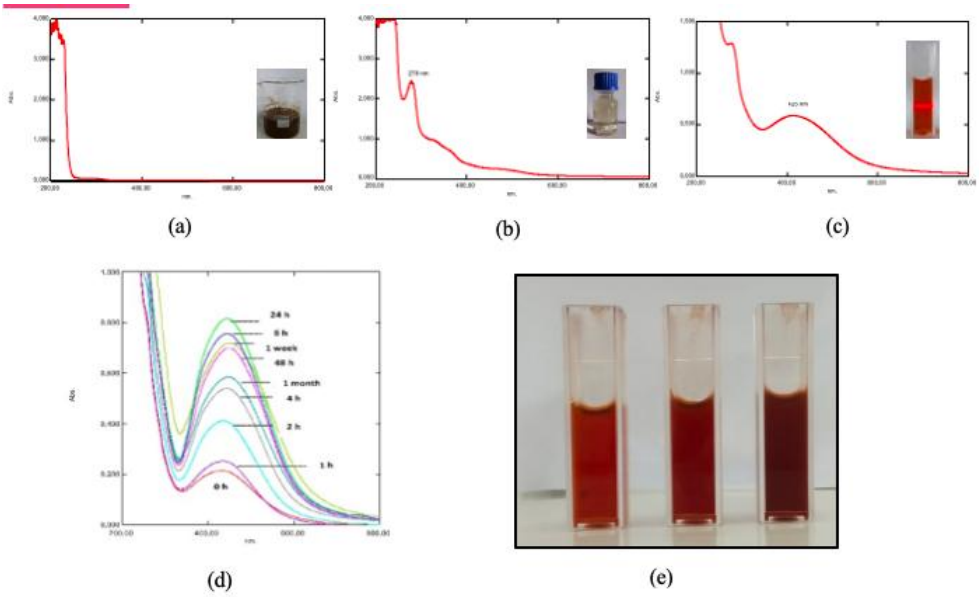


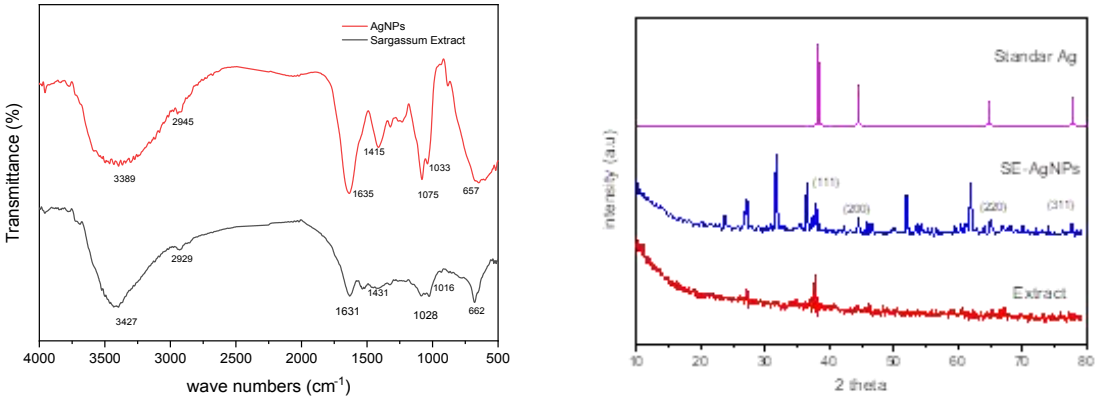
Fig.-1: The UV-visible absorption spectrum of (a) Ag^+ solutions, (b) *Sargassum echinocarpum*(SE), (c). SE-AgNPS, (d) Stability absorption during 1 month (e) Simultaneous color change of SE-AgNPs during 2 hours of synthesis from light brown to dark brown

Fourier transform infrared spectroscopy analysis (FTIR)

Potential functional biomolecules that reduce Ag^+ ions and capping agents from *Sargassum echinocarpum* macroagla to generate E-AgNPs were identified using the FTIR spectrum (Fig.-2).

The FTIR spectrum of SE extract (black line) shows prominent peaks at ~3427 cm^{-1}(–OH stretching), ~1631 cm^{-1}(C=C aroma), and ~1028 cm^{-1}(C–O stretching), indicating the presence of flavonoids, polyphenols, and glycosides, reflecting the complex nature of the algae powder. The AgNP sample's shifting peaks (red line) indicate that it interacted with silver ions during reduction and capping. The peaks 3427–3389 and 1631–1635 cm^{-1} are shifted. The production of N-H from strong hydrogen bonds between two molecules is responsible for the peak at about 3420 ccm^{-1}. It is important to note that amine groups N-H and hydroxyl groups (O-H) are present in the U-shaped peak located between 3200 and 3500 cm^{-1}. Therefore, compared to its control, this peak was more noticeable in silver nanoparticles made from algae powder. Therefore, the peak's shift from 3427 to 3389 cm^{-1} suggests that this group may be

involved in the creation of nanoparticles.[14] This outcome is consistent with studies that plant systems rich in polyphenols serve as decrease and stabilize agents during the synthesis of silver nanoparticles.[27,28]

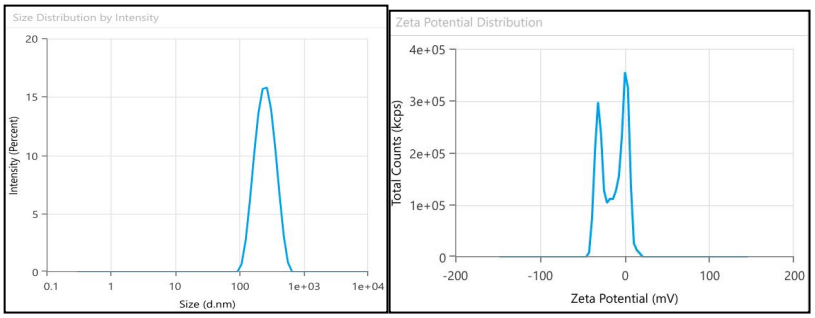


(a). (b).

Fig.-2: (a), shows the FTIR spectra of AgNPs (Red line) and extract of *sargassum echinocarpum* (black line) showing bioactive functional groups., (b). XRD pattern of extract SE, SE-AgNPs and Ag standard base data of JCPDS NO. 04-0783

Dynamic Light Scattering (DLS) and Zeta Potential

The DLS data consolidate the size variation patterns derived from UV-vis spectra and FTIR investigations. The differences in SE-AgNPs size measured by DLS and TEM may be due to differences in their working principles. DLS diameter results are typically greater than TEM diameter values for the same suspension.[29] It is well-known that DLS measures the thickness of bio compounds present on the nanoparticle surface, including the original size of metal nanoparticles. Figure-3 displays the DLS analysis used to identify the distribution of SE-AgNPs. hydrodynamic size of SE-AgNPs. The average size of SE-AgNPs measured using this method was 238 nm at 25°C with a Polydispersity Index (PI) value of 0.2312. This value indicates that agglomeration has not occurred in the synthesis of SE-AgNPs. This reveals that the extract can reduce silver ions and prevent most of the quasi-spherical nanoparticles from clumping. In this study, we demonstrated that the surface charge plays an important role in forecasting agglomeration.[30]



(a) (b)

Fig.-3: (a).DLS reveals the colloidal size and, (b). Zeta potential of SE-AgNPs. Previous studies on the production of AgNPs using *sargassum*

Zeta Potential colloidal SE-AgNPs

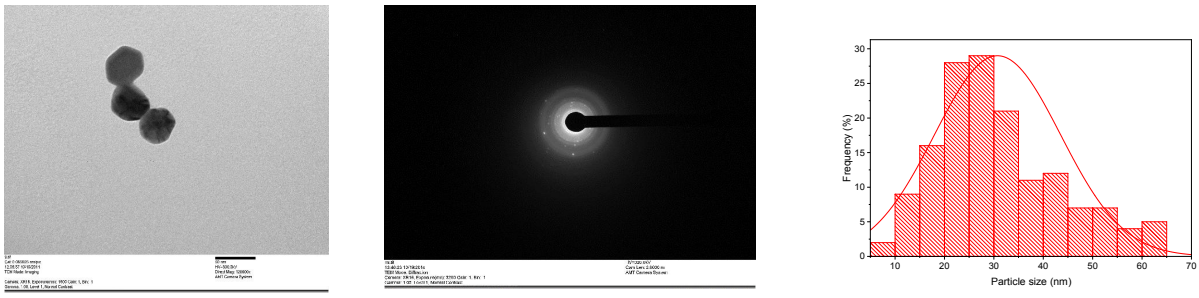
Determination of the zeta potential allows for the estimation of surface charge, which can be used to assess the physical stability of metal nanoparticles.[31] Particles having large positive or negative charges are thought to reject one another, resulting in stable particles with little tendency to cluster together. For stable suspensions, the zeta potential usually falls between 20 and 30 mV. AgNPs may collect in dispersions with lower zeta potentials because of van der Waals forces.[32] The zeta potential of SE-AgNPs in water as a dispersant was evaluated over a period of 1 month, and Fig.-3 displays the outcomes. Zeta potential values ranged from −29.7 mV to −16.9 mV, indicating the presence of a negative charge on the

nanoparticle surface, indicating that SE-AgNPs are quite stable. These values indicate that AgNPs have a negatively charged surface, implying strong repulsive forces between particles, leading to the prevention of aggregation and stabilization of AgNPs in the medium. Previous investigations found that NPs with charges between ±30 mV and −30 mV were highly stable, whereas those with values between ±15 mV and −25 mV were quite stable[33]. The polyphenol compounds that have been confirmed in FTIR in the aqueous SE extract could be the reason for the negative zeta potential shown by SE-AgNPs.

HR-TEM with SAED

While characterization established the size and shape of the particles, TEM-SAED pictures confirmed the production of SE-AgNPs. Plant extracts reveal that SE-AgNPs are spherical and slightly hexagonal, while TEM studies in Fig.-4 show that AuNPs has a spherical shape having a 10–65 nm diameter. Furthermore, SAED investigation shows ring diffraction, indicating the crystalline nature of the particles. The synthesis results with mangrove plants also revealed differences in the form of the silver nanoparticles.[34,35] Silver nanoparticles synthesized using marine plants rich in phenolic compounds are also affected by the type of marine plant, the amount of extract, and the reaction process conditions. To control particle size, agglomeration, and material morphology, organic capping agents (also known as stabilizers or protecting agents) are typically used in the bottom-up technique during the synthesis of Ag nanopowders.[19]

The creation of cubic and hexagonal AgNPs with sizes ranging from 25 to 44 nm was revealed by SEM and DLS (dynamic light scattering) data in another work that examined the production of AgNPs by the freshwater green alga pithophora oedogonia[36]. The FTIR spectrum revealed distinct peaks that suggested the existence of terpenoids, long-chain fatty acids, and secondary amide derivatives, which coat and stabilize the AgNPs.[36]



(a) (b) (c)

Fig.-4: (a)TEM image, (b) SEAD pattern and (c)a particles size distribution of AgNPs synthesised from *Sargassum echinocarpum*

X-ray diffraction (XRD) Analysis

XRD was used for phase identification and crystal structure characterization of gold nanoparticles.[37,38] Fig.-2b shows a typical XRD pattern of AgNPs coated with SE extract, demonstrating the crystalline character of the produced AgNPs. The XRD pattern shows four specific diffraction peaks at 38.24°, 44.84°, 66.18°, and 77.81°, indexed as the (111), (200), (220), and (311) planes of FCC metallic silver, when compared with the standard base data of JCPDS NO. 04-0783. The presence of additional peaks in the spectrum is due to the organic crystallization of algae extract on the silver surface, which acts as a capping agent, as found in several previous studies.

Based on Fig.-2b, the undefined peak (*) may be due to the organic crystallization of algae extract on the silver surface[6] which acts as a capping agent.[35] The crystallite properties were calculated by the Bragg equation, and the average crystallite size was 32,44 nm, which is close to the average crystallite size from the TEM study. Importance of Capping Agents Capping agents derived from *sargassum* extracts, primarily polyphenols and flavonoids, were crucial in stabilizing AgNPs and preventing agglomeration. These bioactive compounds formed a protective layer around the nanoparticles, enhancing their colloidal stability.

Overall, the comprehensive physicochemical characterization confirms that the biosynthesized SE-AgNPs exhibit well-defined morphology, crystalline structure, and satisfactory colloidal stability, demonstrating

the effectiveness of *Sargassum echinocarpum* extract as both a reducing and stabilizing agent. The presence of bioactive phytochemicals, particularly flavonoids and polyphenols, plays a decisive role in facilitating nanoparticle formation while preserving structural integrity and dispersion stability. Notably, this biosynthetic approach eliminates the need for hazardous chemical reductants and synthetic capping agents that are typically employed in conventional nanoparticle synthesis, thereby significantly reducing chemical waste and environmental risks. The integration of marine-derived biomaterials into nanoparticle fabrication highlights the dual advantage of functional performance and environmental compatibility. These findings not only validate the technical feasibility of algae-mediated AgNP synthesis but also provide a strong foundation for the broader implementation of sustainable nanomaterial production strategies, as further elaborated in the concluding section.

CONCLUSION

The present work demonstrates that *Sargassum echinocarpum*-mediated synthesis of AgNPs provides a stable, efficient, and environmentally benign route for nanoparticle production. The adoption of marine biomass as a bio-reducing and capping agent reduces reliance on hazardous chemical substances and supports sustainable material innovation.

ACKNOWLEDGMENTS

This research was funded by the Ministry of Higher Education, Science, and Technology through the Directorate of Research and Community Service through a Regular Fundamental Research Grant with contract number 002C/UN53.0004/Kontrak. The authors are very grateful for this research grant.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-14: Life below Water*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the author can be verified from their ORCID ids, given below:

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Cite this article as: N. Willian *et al.*, *Rasayan J. Chem.*, 19(2), 898-906(2026), https://doi.org/10.31788/RJC.2026.1929668.

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[RJC-9668/2025]