Research Article

Characterization of the Biosynthesized Silver Nanoparticles from the Sea Cucumber Bohadschia marmorata (Jaeger, 1833)

Wilson L. Laranang1,2* and Paulina A. Bawingan2,3

1Department of Pathology and Laboratories, Ilocos Training and Regional Medical Center, City of San Fernando, La Union, 2500 Philippines
2School of Advanced Studies, Saint Louis University, Baguio City, Benguet 2600 Philippines
3Center for Advanced Maritime Studies, Maritime Academy of Asia and the Pacific, Mariveles, Bataan, 2106 Philippines

ABSTRACT

Silver nanoparticles (AgNPs) from marine invertebrates, particularly sea cucumbers, are relatively few or even lacking. This study aimed to determine the potential of the body wall of Bohadschia marmorata as a reducing agent to synthesize silver nanoparticles and characterize the generated AgNPs using a direct and environment-friendly procedure. Although limited, the generated AgNPs were confirmed with an absorbance peak of 416 nm, indicative of surface plasmon resonance, with strong signals of elemental silver acquired by EDS at 3 keV. The potential functional groups responsible for the reduction of silver ions and capping of the nanoparticles include O–H (alcohol and carboxylic acids), C–C (alkene), N–H, and C–N (amine) groups detected by FT-IR. Scanning electron microscopy showed a nearly spherical morphology with an approximate size of 288 nm which tends to polydisperse and aggregate. Thus, this study showed that the sea cucumber body wall can be used in the biosynthesis of AgNPs. However, refinement of the procedures and operational parameters used is needed to produce more and better-quality nanoparticles.

Introduction

Advances in nanotechnology resulted in innovations in producing nanomaterials from various biological sources, particularly marine invertebrates such as sea cucumbers. The unique characteristics of nanoparticles, including optical, thermal, and strong electrical conductivity, lead to diverse utility in health and medicine (Jeong et al., 2022). Currently, chemically derived AgNPs are incorporated into different commercial products to improve their quality and functionality, but they pose safety issues for health and the environment. However, the

How to cite:
chemical synthesis of nanoparticles has various consequences, including pollution, high energy consumption, and potential health problems (Guan et al., 2022).

Alternatively, biogenic synthesis of AgNPs using plant extracts instead of chemicals to reduce metal ions was more beneficial in reducing the cost, decreasing environmental impact, and enhancing the safety of usage (Dos Santos et al., 2014; Calderón-Jiménez et al., 2017). In the study of Jyoti et al. (2016), AgNPs synthesized from aqueous leaf extracts of Urtica dioica (Linn.) demonstrated antibacterial activity against Gram-positive and Gram-negative bacterial pathogens. Hamouda et al. (2019) also showed that AgNPs synthesized using aqueous extracts of Oscillatoria limnetica as the reducing agent had antibacterial properties against Bacillus subtilis and Pseudomonas aeruginosa.

The reduction of silver ions in the biosynthesis of AgNPs, including their morphology and stability, is influenced by several factors. The presence of sulfur or phosphorus-containing functional groups in the biological extract may stabilize AgNPs by attaching to silver ions. In the studies of Hamouda et al. (2019) and Thiruvengada and Bansod (2021), Fourier Transform-Infrared (FT-IR) spectroscopy detected functional groups of proteins, and free amino and sulfur-containing amino acids, respectively. The presence of C–N, C–C, C–H, and N–H groups detected was responsible for the capping and stability of the formed AgNPs.

Besides plants and algae, extracts of marine invertebrates were used in the green synthesis of AgNPs. Singh et al. (2014) produced polychaete silver nanoparticles at room temperature and demonstrated a typical surface plasmon resonance (SPR) between 418 nm and 420 nm with an average particle size of 40–90 nm analyzed using scanning electron microscopy (SEM). The biosynthesized AgNPs from the marine invertebrate show maximum antibacterial activity against the human pathogen Staphylococcus aureus. In another study, AgNPs from polychaete Diopatra claparedii extract were synthesized for 8 weeks, and the maximum absorption peak was 400–440 nm (Hussain et al., 2018).

Despite the growing importance of biosynthesized silver nanoparticles (bAgNPs), several operational parameters influence their function, including the amount of silver ion precursor, ratio of silver ion solution and extract, contact time and temperature, and pH. UV-Vis spectroscopy confirmation provides an absorption spectrum due to SPR on the shape of bAgNPs. Further, FT-IR characterization reveals the nature, structure, and physiochemical properties of bAgNPs, which affect their activity, behavior, distribution, and safety.

Literature shows that by adding metal ions, silver ions bind to –OH and –COOH groups present in water-soluble and protein compounds (Huang et al., 2015). Parlinska-Wojtan et al. (2016) stated that terpenoids adsorb on the AgNP surface, stabilize, and prevent nanoparticles from agglomerating. Thus, terpenoids reduce Ag+ ions to AgNPs by converting the functional groups of terpenes from the C–O group to the –C–O group (Shankar et al., 2004).

The increasing prospects of nanotechnology using marine sources provide remarkable nanomaterials with various biological activities, such as antiviral activity against SARS-CoV-2, the causative agent of COVID-19 (Asmathunisha & Kathiresan, 2013; Soufi et al., 2020). So far, there have been no reports regarding the biosynthesis of AgNPs from sea cucumbers. The only study the researcher has encountered was the synthesis of sea cucumber Holothuria scabra nano-collagen. The quality of nano-collagen was associated with amide A and B, and amide I, II, and II functional groups present in a standard collagen compound (Sari, 2020).
This study hoped to add to this limited study on sea cucumbers in this aspect. This study determined the potential of the body wall of the sea cucumber, *Bohadschia marmorata* (Jaeger, 1833) as a reducing agent in the synthesis of AgNPs. *Bohadschia marmorata* is a low-value sea cucumber usually collected and sold in the market by local fishermen in the province of La Union, Philippines. The body wall, which is the edible part and largest part of the sea cucumber, is rich in collagen and contains bioactive compounds such as saponin with various biological activities (Sellem *et al.*, 2017; Hossain *et al.*, 2020).

**Materials and Methods**

The study protocol was approved by the Research Ethics Committee of Saint Louis University, Baguio City (Protocol No. SLU-REC 2022-035 on June 15, 2022). A gratuitous permit to use the sea cucumber in this study was secured and issued by the Department of Agriculture-Bureau of Fisheries and Aquatic Resources (Gratuitous Permit No. 0236-022).

**Sample Collection and Preparation**

Sea cucumber *B. marmorata* was bought from a local market in Barangay Paraoir, Balaoan, La Union, and identified based on observed features (Olavides *et al.*, 2010; Kim *et al.*, 2013; Jontila, 2023). The sea cucumber body wall was eviscerated with distilled water, sun-dried, and powdered before synthesis (Singh *et al.*, 2014).

**Preparation of Silver Nitrate Solution, 1 mM**

Silver nitrate, AgNO₃ (Merck, Germany) was provided by the Philippine Science High School-Ilocos Region Campus, and 1 mM AgNO₃ was prepared by adding 0.1699 g of silver nitrate to 1 liter of deionized water (Hamed *et al.*, 2015; Columbano *et al.*, 2021; Osorio-Echavarría *et al.*, 2021).

**Biosynthesis of Silver Nanoparticles**

The formation of Ag⁰ was modified by mixing 1 mL of powder-dried sea cucumber body wall in a 15-mL conical tube with 9 mL of 1 mM AgNO₃ incubated at 40°C for 72 hours (Husseiny *et al.*, 2015; Bhuyar *et al.*, 2020; Rosman *et al.*, 2020). After 72 hours of incubation, the solution was centrifuged at 2, 500 rpm for 5 minutes, and the supernatant was filtered using a 0.22 µm nylon mesh. The color change of AgNO₃ and *B. marmorata* powder dried body wall solution was observed visually. A control set-up of silver nitrate was also maintained during the process (Azizi *et al.*, 2013; Dada *et al.*, 2018; Khalil *et al.*, 2021; Al-Soub *et al.*, 2022).

**Ultraviolet-Visible (UV-Vis) Spectroscopy Analysis**

The reduction of Ag⁺ ions in the formation of AgNPs was confirmed by UV-Vis spectroscopy. Briefly, the absorbance of the bAgNPs was determined using an aliquot of approximately 3 mL in a cuvette and scanned using UV-Vis i1900 (Shimadzu Corporation, Japan) in the wavelength range of 300–700 nm with a scanning speed of about 29, 000 nm/min (Zia *et al.*, 2017; Abdel-Raouf *et al.*, 2019; Columbano *et al.*, 2021).
Fourier Transform-Infrared (FT-IR) Spectroscopy

The AgNPs were centrifuged at 2,500 rpm for 5 minutes to concentrate silver nanoparticles. The pellet containing bAgNPs was re-dispersed three times with sterile deionized water, and the samples were dried overnight at 60°C in an oven. Dried bAgNPs ground with KBr pellets were analyzed using Spectrum Two FT-IR Spectrometer (PerkinElmer Spectrum IR Version 10.6.0; PerkinElmer Inc., USA), in the diffuse reflectance mode with a resolution of 4 cm⁻¹. Both spectra of the powder-dried sea cucumber body wall and bAgNPs were compared to determine the attached functional groups (Abdel-Raouf et al., 2019; Bawazeer et al., 2021).

Scanning Electron Microscopy

Scanning electron microscopy (Hitachi TM4000Plus, Japan) coupled with EDS (energy dispersive spectroscopy) was used to observe the surface morphology and identify the elemental composition of bAgNPs, respectively (Scimeca et al., 2018; Hamida et al., 2020; Columbano et al., 2021). The SEM samples were prepared in a thin film on a carbon-coated copper grid by sprinkling a tiny amount of bAgNPs. Excess samples were removed using blotting paper, then the samples were dried on the SEM grid under a mercury lamp for 5 minutes. The SEM was conducted with a 15 kV (10–15 kV) accelerator voltage at 50 µm x 1000 magnification (Hussain et al., 2018). Subsequently, qualitative spectrum acquisition of elements present was performed remotely on AZtecLive software, https://nano.oxinst.com/products/azteclive (Vivek et al., 2011; Singh et al., 2014; Zia et al., 2017; Scimeca et al., 2018). The SEM images taken were processed and enhanced using Image J software (https://imagej.net) and Artguru (https://www.artguru.ai/photo-enhancer/), and particle analysis was performed on Mountains10® Surface Analysis Software Free Trial Version (https://www.digitalsurf.com).

Results and Discussion

Biosynthesized Silver Nanoparticles

The treatment of the powder-dried body wall of B. marmorata with 1 mM AgNO₃ developed from yellow to dark brown indicating silver nanoparticle formation (Fig. 1, upper right inset). Abdel-Raouf et al. (2019) showed a similar color reaction change in the biosynthesis of silver nanoparticles from the marine brown alga Padina pavonia. The color change was due to surface plasmon excitation, its vibration, and the reduction of Ag⁺ to Ag⁰ (Thirumurugan et al., 2010; Jalab et al., 2021). This is the first report on surface plasmon resonance (SPR) vibrations using the sea cucumber B. marmorata.

The bAgNPs were confirmed using UV-Vis spectroscopy, showing a maximum absorption peak at 416 nm (Fig. 1). This observed absorption peak of the bAgNPs from the sea cucumber body wall was also displayed by the biosynthesized silver nanoparticles from the fungus Aspergillus niger, Paracoccus sp. bacteria and fruit extracts from Nothapodytes nimmoniana (Graham) Mabb (Muniyan et al., 2017; Ninganagouda et al., 2014; Zhang et al., 2019). On the other hand, Yurtluk et al. (2018) measured a maximum absorbance peak at 416 nm at pH 8 in Bacillus species.
Several factors affect the biosynthesis of AgNPs, including reaction time, pH, temperature (Hashemi et al., 2015), and the concentration of silver metal salt (Dhaka et al., 2023). According to Bhuyar et al. (2020), the frequency and width of the surface plasmon absorption depend on the morphology of metal nanoparticles, the dielectric constant of the metal used, and the surrounding medium.

**Fourier Transform-Infrared (FT-IR) Measurements**

The FT-IR spectra of bAgNPs from the body wall of *B. marmorata* shown in Figure 2 show various absorption bands in the range of 4000 to 600 cm\(^{-1}\). The signals were observed at 3339.82, 2608.71, 1635.01, and 1183.03 cm\(^{-1}\) (Table 1). The absorption bands in the 3200-3550 cm\(^{-1}\) and 2500-3300 cm\(^{-1}\) were assigned as hydroxyl group O–H stretching for alcohols and carboxylic acids, with strong and broad peaks, respectively. Moreover, the absorption bands of 1635.01 cm\(^{-1}\) and 1186.03 cm\(^{-1}\) were assigned as alkene (C=C) and amine (C–N) stretching with medium peaks (Segneanu et al., 2012; Majeed et al., 2018; Rosman et al., 2020; Aatab et al., 2023; FTIR Functional Group Database Table with Search – InstaNANO, 2024). The result was comparable to the attached functional groups of nano-collagen detected at 3418.97 cm\(^{-1}\) showing N–H stretching vibration, C–N stretching at 1227.74 cm\(^{-1}\), and 1236.43 cm\(^{-1}\) (Sari, 2020).

### Table 1. FT-IR Peaks and Corresponding Functional Groups of Biosynthesized Silver Nanoparticles from *B. marmorata* Body Wall

<table>
<thead>
<tr>
<th>FT-IR peak cm(^{-1})</th>
<th>Functional Group</th>
<th>Class</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>3339.82</td>
<td>O–H</td>
<td>Alcohol</td>
<td>Andas &amp; Idris (2021) Singh et al. (2022)</td>
</tr>
<tr>
<td>2608.71</td>
<td>O–H</td>
<td>Carboxylic Acids</td>
<td>Muzamil et al. (2014) Singh et al. (2022)</td>
</tr>
<tr>
<td>1635.01</td>
<td>C=C</td>
<td>Alkene</td>
<td>Majeed et al. (2018) Singh et al. (2022)</td>
</tr>
<tr>
<td>1186.03</td>
<td>C–N</td>
<td>Amine</td>
<td>Singh et al. (2022)</td>
</tr>
</tbody>
</table>

Mahendran et al. (2016) studied AgNPs derived from *N. nimmoniana* fruit extracts. They identified the possible biological components linked to the capping agent on the nanoparticle, such as the C–O functional group with an FT-IR spectrum band shift from 1061.62 to 1120.44
cm\(^{-1}\). Bar et al. (2009), cited by Abdel-Raouf et al. (2019), supported that biomolecules’ functional groups interact with metal salts and mediate their reduction to nanoparticles. Singh et al. (2014) identified analogous infrared signatures of synthesized silver nanoparticles from marine invertebrates (polychaete). They found the presence of prominent peaks at 3345, 2922, 1670, 1384, 1088, and 1037 cm\(^{-1}\), corresponding to different functional groups such as \(-\text{NH}, \text{O–H}, \text{C≡N}, \text{N–O}, \text{C=C}, \text{C–N}, \text{and C=CH}_2\).

Based on the FT-IR analysis, the bAgNPs in this present study display capping ligands for stabilization such as O–H groups, carboxylic acids and their derivatives, alkene, and amine groups associated with amino acids and proteins, and saponins (Bhuyar et al., 2020). Further, the functional groups of the metabolites interact with metal ions (Ag\(^+\)), which is responsible for their reduction to nanoparticles (Marslin et al., 2018).

![FT-IR Spectra](image)

**Figure 2.** FT-IR Spectra (Percent Transmittance, %T) of Biosynthesized Silver Nanoparticles from *B. marmorata* Body Wall

According to Zhao et al. (2018) and Nguyen et al. (2020), the sea cucumber body wall contains a high proportion of saponin, which serves as a chemical defense and affects the size of AgNPs produced. Muniyan et al. (2017) supported the presence of C–O, C=C, C=O, and O–H functional groups involved in the reduction and stability of saponin-conjugated AgNPs. Thus, amine groups’ interaction with the silver surface stabilizes the colloidal particles (Roldán et al., 2012). Also, carboxylic acids encapsulate AgNPs and provide a barrier to particle size growth, as Muzamil et al. (2014) suggested. Moreover, free amino acids in the solution prevent the agglomeration of nanoparticles adhering to the surface (de Matos et al., 2017).

In the study, SEM images of bAgNPs from *B. marmorata* body wall could not be resolved under lower magnification (Fig. 3). The surface morphology predominantly revealed a relatively spherical morphology of biosynthesized silver nanoparticles. The findings were similar to the morphology observed by Rajesgkumar et al. (2012), Mahendran et al. (2016) and Ohiduzzaman et al. (2024). Spherical-shaped nanoparticles with a size of 20.3 nm were also produced from the red alga *Gracilaria birdiae* by de Aragao et al (2019). Spherical-shaped AgNPs were also observed from *Crescentia cujete*, *Antidesma bunius* L. Spreng, and *Sargassum siliquosum* J. G. Agardh (Fabregas et al., 2021; Legaspi & Fundador, 2020;
Vazquez et al., 2016). However, the study of Singh et al. (2014) showed spherical and triangular shapes.

![Figure 3](image3.jpg)

**Figure 3.** Scanning Electron Micrograph of Biosynthesized Silver Nanoparticles from *B. marmorata* Body Wall

The particle analysis showed an approximate size of 288 nm, which tends to polydisperse, and the size was slightly comparable to the nano-collagen in the study of Sari (2020), with a particle size of 285 nm. These findings differed from those of Rosman et al. (2020), with a smaller particle size with an average of 40.19 nm, and size ranges from 20 nm to 480 nm by Vazquez et al. (2016). These differences in morphology of silver nanoparticles were likely affected by the dispersant, reducing agent present in the sea cucumber body wall, and the presence of amine, including its concentration, pH, reaction time, and temperature during biosynthesis (Natsuki et al., 2015; Rosman et al., 2020; Malik et al., 2022), and post-mechanical manipulation to concentrate bAgNPs.

![Figure 4](image4.jpg)

**Figure 4.** Energy Dispersive Spectroscopy of Biosynthesized Silver Nanoparticles from *B. marmorata* Body Wall

The spectral analysis (Fig. 4) demonstrated a strong elemental silver signal at 3 keV (2.5-3.5 keV) showing AgNP formation typical of nanocrystallites from SPR vibrations (Mahendran et al., 2016). The presence of minor peaks of carbon (C) and oxygen (O) was consistent with the
findings of Yu et al. (2019) associated with the capping of AgNPs by the bioactive compounds (Arunachalam et al., 2015). By contrast, Muthukrishnan et al. (2015) detected sodium and chloride in their plant-mediated silver nanoparticle synthesis using Ceropogia thwaitesii. The strong chloride (Cl) and minor sodium (Na) peaks were due to residual salt. Weaker signals (Al, Si, Zr) were likely contaminants from the plastic container, and aluminum foil used during the biosynthesis and drying of silver nanoparticles.

The size and distribution of the biosynthesized AgNPs exhibit optical and electrical features that can be used for microbiology and environmental applications. Functional groups of compounds attached to the surface of nanoparticles, like amine, carboxylic, and hydroxyl groups, keep the particles stable and improve their ability to spread and interact with biological systems in ways that are antiviral and antibacterial. Additionally, the approximate size (288 nm) and roughly spherical form indicate reactivity and penetration capabilities, suggesting that these nanoparticles could be used for drug delivery and biosensing. These results demonstrate the wide therapeutic potential of these biosynthesized AgNPs and are consistent with other research on the bioactive chemicals found in sea cucumbers.

**Conclusion and Recommendations**

This study was the first to explore the synthesis of AgNPs using the sea cucumber B. marmorata body wall as a reducing agent. The successful biosynthesis of AgNPs from the sea cucumber B. marmorata body wall used simple, direct, and eco-friendly procedures. The results provide useful information for optimization and standardization of the procedures for future research. Further assessment of the operational parameters used includes concentration of sample and metal precursor, reaction time, temperature, and pH during biosynthesis, surface morphology evaluation by transmission electron microscopy, and particle size analysis. The properties of the nanoparticles obtained in this study support their possible use as antibacterial or antiviral. Hence, it is suggested to test its antiviral properties using a surrogate viral agent in vitro and its antibacterial properties against WHO critical pathogens.

**Acknowledgment**

The authors are grateful to Dr. Ronnaleen N. Orteza and Ms. Michelle B. Ducusin of the Philippine Science High School-Ilocos Region Campus, San Ildefonso, Ilocos Sur for the use of facility equipment and assisting in the analysis of the nanoparticles, Professor Victoria N. Malaya of the College of Fisheries, Don Mariano Marcos Memorial State University-South La Union Campus, Santo Tomas, La Union, and Ms. Dianne A. Peralta of the University Research and Extension Office, Don Mariano Marcos Memorial State University-North La Union Campus, Bacnotan, La Union for the identification of the target sea cucumber.

**Statement of Conflict of Interest**

There is no conflict of interest regarding the publication of this research article in any journal between the authors and host institutes.
References


Laranang and Bawingan – Characterization of the Biosynthesized Silver Nanoparticles


Laranang and Bawingan – *Characterization of the Biosynthesized Silver Nanoparticles*


