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Green Synthesis of Silver Nanoparticles Using *Ulva intestinalis* with Cytotoxic and Antioxidant Activity

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ABSTRACT

The cost-effective and biosafe synthesis of "green" nanoparticles is becoming increasingly popular. Therefore, this study aimed to explore the potential of green algae (*Ulva intestinalis*) extract as a reducing and stabilizing agent for producing silver nanoparticles (AgNPs). *U. intestinalis* contains numerous secondary metabolites that play a crucial role in the formation of AgNPs. Characterization was confirmed using UV-visible spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and a particle size analyzer (PSA). The cytotoxic and antioxidant effects of the product were determined using the Brine Shrimp Lethality Test (BSLT) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The results showed that the AgNPs had a spherical shape, with size variations of 4040 nm (19%), 771 nm (78.6%), and 97.3 nm (2.4%). The obtained AgNPs were found to have higher cytotoxic and antioxidant activity than the *U. intestinalis* extracts with an LC50 value of 128.2 μg/mL (toxic category) and IC50 = 100.4 μg/mL (strong category). This study proved that the AgNPs produced herein have the potential to be used for anticancer and antioxidant purposes.

Keywords: Antioxidant; BSLT; Cytotoxicity; DPPH; Silver nanoparticles; *U. intestinalis*

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1. Introduction

Nanotechnology is renowned for its role in opening up numerous opportunities for discoveries and advancements in the field of medicine and beyond; this field involves the synthesis, characterization, and construction of nanometer-sized materials for which there are vast potential applications [1]. Modern studies use nanotechnology, specifically in health applications including gene therapy, as antibacterial, antifungal, anti-inflammatory, and anticancer agents, as well as biosensors, biomedicine, drug delivery, biotechnology, cosmetics, agriculture, oil and gas, optics, energy, solar cells, environment, catalysts, and electronics [2-7]. Various physical and chemical techniques used in the production of nanoparticles are often disregarded due to high costs and potential hazards. Consequently, the green approach to the synthesis of nanoparticles takes precedence over physical and chemical approaches due to its high yield, stability, reproducibility, lower toxicity, cost-effectiveness, and eco-friendliness [8, 9]. The synthesis of environmentally friendly nanoparticles (NPs) is greatly required to replace toxic substances in a variety of fields. Metals utilized in traditional non-green nanoparticle synthesis and engineering include gold, zinc, palladium, copper, platinum, iron, titanium, and silver [10, 11]. Meanwhile, biosynthesized silver nanoparticles (AgNPs) are cheap and sustainable biocompatible substances with medical and pharmacological applications [12].

Living organisms including plants and microorganisms namely microbes, algae, bacteria, fungal, and actinomycetes can be used as potential material sources in the biosynthesis of AgNPs [13, 14]. Algae are a significant component of marine ecology. Algae are able to synthesize halogenated secondary metabolites to protect them from harsh environmental conditions and predators; these metabolites can be co-opted for use as bioactive compounds in medicine [15]. These microorganisms are rich in proteins and many secondary metabolites including phenolics, glycosides, flavonoids, alkaloids, and steroids that could be used as nutraceuticals and possess anticancer, antibacterial, anti-inflammatory, antioxidant, and antidiabetic properties [16-18]. Green algae species used as bioreductors in the synthesis of AgNPs include *Spirogyra hyalina* [19], Caulerpa sertularioides [20], *Chaetomorpha ligustica* [21], and *Ulva lactuta* [22]. These studies reported that green algae have antibacterial, anticancer, antifungal, and antioxidant properties. However, investigations into the production of silver nanoparticles from the green algae species *U. intestinalis* are not reported.

U. Intestinalis is a species of green algae for which previous studies have only reported the chemical and antibacterial activities from its extract [23, 24]. This study presents preliminary information on the green synthesis of AgNPs using *U. Intestinalis*. The bioactive compounds that are present in the extract of *U. intestinalis* can acts as reducing and chapping agents for AgNP formation. Characterization was carried out using UV spectroscopy, FTIR, and PSA analysis to analyze the physical and chemical features of the particles. This is important to establish more effective products that benefit humans, fisheries, and are environmentally friendly. In this study, the cytotoxic and antioxidant activities of AgNPs made from *U. intestinalis* were investigated.

2. Materials and Methods

2.1 Algae sample collection and preparation

A green algae sample was collected from the Ulee lheue coast, northern Aceh Water, Indonesia and identified [25]. The algae were cleaned completely with sea water and fresh water to eliminate epiphytes, debris, and miscellaneous items, with necrotic sections subsequently excised. Next, algae were shade dried for 7 days and then oven dried at 40 0C until it reached stable mass. This research was carried out from sample preparation, extraction, phytochemical screening, and toxicity tests conducted at the Marine Chemistry and Biotechnology Laboratory of Universitas Syiah Kuala. Meanwhile, the antioxidant test was performed at the THP Laboratory, Universitas Syiah Kuala. The identification results of the algae are presented in Table 1.

Table 1. Classification of algae.

Classification	Plant
Kingdom	Plantae
Division	Chlorophyta
Class	Ulvophyceae
Order	Ulvales
Familia	Ulvaceae
Genus	Ulva
Species	*Ulva intestinalisLinnaeus, 1753*

2.2 Materials

The green algae investigated in this study was *U. intestinalis*. The chemicals used for analysis were AgNO3 (Merck), ethanol (Merck), and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH; Sigma-Aldrich). Equipment included Fourier Transform Infrared (FTIR) (Bruker Alpha), Aquamate UV-8100 VIS spectrophotometer, and Pore Size Analyzer (PSA) (Microtrac).

2.3 Sample extraction

U. intestinalis was extracted using the maceration process, where 100 grams of dry *U. intestinalis* were immersed in 300 mL of ethanol with a dry sample-to-solvent ratio of 1:3. The sample was left for three days and then filtered to separate the residue and filtrate. The filtrate was collected and concentrated using a rotary evaporator set to 40°C. Finally, the crude extract was sealed in a container and preserved as a reductant in the synthesis of AgNPs.

2.4 Green synthesis of AgNPs

The Biosynthesis of AgNPs followed a modified method described previously [19, 22]. In summary, 90 mL of a 1 mM AgNO3 solution was combined with 10 mL of green algae extract in a flask at 60°C, using a magnetic stirrer for 45 minutes. Light-induced oxidation was prevented by covering the flask with aluminum foil. Finally, the solution was incubated for 24 hours, indicating the production of silver nanoparticles by the change in its color.

2.5 Characterization of AgNPs

Characterization of AgNPs was carried out using Fourier transform infrared spectroscopy (FTIR), and UV-visible spectroscopy, while the Particle Size Analyzer (PSA) was used to assess physicochemical properties.

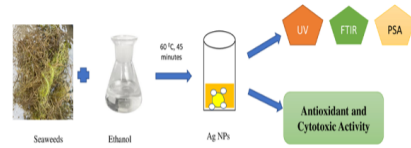

Fig. 1. Synthesis and Characterization of AgNPs.

2.6 Antioxidant activity

Antioxidant activity was determined by the DPPH method [26, 27]. The free radical scavenging ability of green algae extracts, and AgNPs was evaluated at different concentrations of 25, 50, 100, 150, and 200 ppm. About 2 ml for each concentration extract and AgNPs was mixed with 0.1 mM DPPH in ethanol. The mixture was quickly mixed and left at room temperature for 30 minutes in a dark location. Finally, the absorbance of the sample was determined at 517 nm using a spectrophotometer. All measurements were carried out in triplicate. The scavenging ability percentage was determined using the following Eq. **??**.

$$\%\text{DPPH activity} = \frac{A_0 - A_S}{A_S} \times 100\%, \tag{2.1}$$

where A_S is the sample absorbance and A_0 is the blank absorbance. Following the charting of DPPH scavenging, the IC50 was established. The straight line equation was verified, and the IC50 was regarded as "x" in the equation formula $y = mx + b$ where y=50. Furthermore, the values of slope (m) and intercept (b) were given in the graphical equation of scavenging percentages versus concentration.

2.7 Cytotoxic activity

The brine shrimp lethality test (BSLT) was used to determine the toxicity of algae extract and AgNPs to V*rtemia salina* larvae. This method has the advantage of being easy, fast, accurate, and correlated with the toxicity level of an anticancer compound.

A toxicity assessment was conducted following the procedure outlined previously [28, 29]. Larval *A. salina* were subjected to different concentrations of extract and AgNP solutions, including 100, 250, 500, 1000, and 2000 ppm. A total of 10 *A. salina* larvae were introduced into a vial containing the respective concentration of the stock extract solution. The experiment was repeated thrice over 24 hours, and the mortality of *A. salina* larvae was observed. The toxicity level towards *A. salina* was examined using percent mortality. The LC_50 value was determined through probit analysis.

3. Results and Discussion

3.1 Green synthesis of AgNPs

The first check was conducted visually by analyzing the formation of silver nanoparticles. The presence of discoloration after *U. Intestinalis* extract was added into the AgNO3 solution signified the formation of AgNPs. The change in color from green to yellow showed the reduction of Ag+ in the solution [30, 31], as described in Fig. 2.

Fig. 2. Green synthesis of Ag NPs using green algae. (a) Green algae *U. intestinalis*, (b) Extract (c) Silver nanoparticles.

3.2 UV-Visible spectroscopy analysis

UV-Vis spectrophotometer characterization is a well-established way to measure AgNP production. The UV-Vis spectrum showed significant absorbance peaks of the AgNPs at 360, 395, 415, and 445 nm (Fig. 3). These absorption bands were identified as surface plasmon resonance. The existence of chromophores in organic substances influences visible light, while UV-Vis ray attenuation of silver metal is asso-

ciated with the formation of localized surface plasmon resonance. The characteristic wavelength of AgNPs is at 451 nm. In addition, other absorption peaks were found, which could be attributed to the presence of the bioactive molecule associated with reducing and capping AgNPs [19]. The distinctive absorbance peak confirmed successful synthesis.

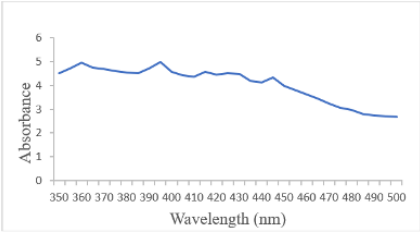

Fig. 3. UV-visible spectra of AgNPs.

3.3 FTIR Analysis

FT-IR spectroscopy showed the attachment of functional groups to silver nanoparticles and identified those responsible for capping and reducing Ag^+ ions. The spectra of *U.intestinalis* extracts and silver nanoparticles were analyzed in the 4000-400 cm^{-1} wavenumber range. The spectrum of *U. intestinalis* extract had prominent signals at wave numbers 3361 cm^{-1}, 2976 cm^{-1}, and 1651 cm^{-1}, indicating the stretching vibration of -OH groups, -CH, and C=C aromatic, respectively (Fig. 4). Meanwhile, -OH and -C=C groups in the AgNPs were at wave numbers 3375 cm^{-1} and 1641 cm^{-1}, respectively (Fig. 3). The presence of carbohydrates or proteins in the sample causes a weakening of the stretching bond present at 2976 cm^{-1} [16]. The broad absorption peak of this functional group shows that bioactive compounds in the extract and silver solution interacted to produce AgNPs. The stretching vibration of C=O has a prominent spectral peak at 1641 cm^{-1}, and its change in AgNPs shows reactions between the phenolic compounds and the nanoparticles [32]. Moreover, the interaction between the functional groups of the extract and the AgNO3 solution was considerably process-capped, resulting in a reduction of Ag(I) to Ag(0) [33].

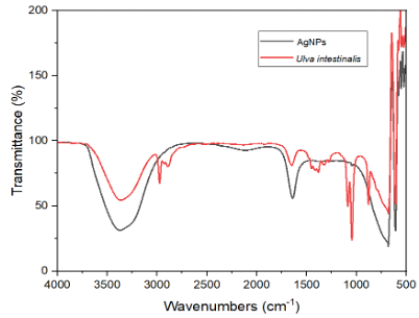

Fig. 4. FTIR of *U. intestinalis* AgNPs.

3.4 PSA analysis

A PSA was used to ascertain the average size of the AgNPs. The variant mean diameter of *U. intestinalis* AgNPs (UI-AgNPs) was 4040 nm (19%), 771 nm (78,6%), and 97.3 nm (2.4%), respectively (Fig. 5), predominantly spherical in shape. PSAs are essential instruments for measuring the distribution of nanoparticle shape and size [34, 35]. Also important is the fact that temperature of synthesis affects the variation in nanoparticle size [36].

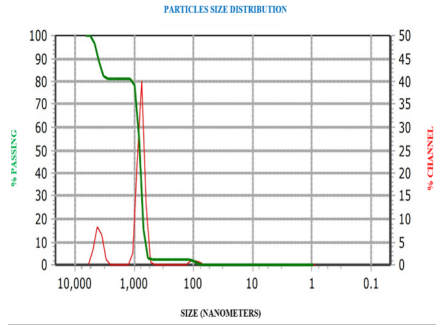

Fig. 5. PSA image of *U. intestinalis* AgNPs.

3.5 Antioxidant activity

Antioxidants are compounds that protect cells from oxidative damage caused by free radicals. The assessment of antioxidant activity for AgNPs was conducted using the DPPH assay. A direct relationship was observed; the higher the concentration of both the extract and AgNPs, the greater the percentage of scavenging activity (Table 1). This implied that the polyphenolic substance in the sample experienced a DPPH reduction process by gaining hydrogen atoms in antioxidant molecules [37]. The mechanism of DPPH interaction with the antioxidant is depicted in Fig. 6.

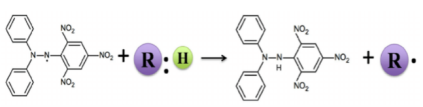

Fig. 6. Reaction mechanism between DPPH and antioxidant.

The antioxidant activity test results with IC50 as a parameter showed the sample concentration needed to inhibit the activity of DPPH by 50% (Table 2).

The categorization of antioxidant strength was determined using IC_{50} values, with category boundaries set as follows: highly potent (IC_{50} < 50 ppm), potent (50 <IC_{50} <100 ppm), moderate (100 <IC_{50} <150 ppm), weak (50 <IC_{50}< 200 ppm), and very weak (IC_{50} >200 ppm) [38]. Analysis of the IC_{50} values showed that AgNPs had higher antioxidant activity (strong) compared to *U. intestinalis* extract (very weak). The strong antioxidant efficacy of AgNPs was attributed to the existence of diverse reducing agents in the extract, mainly phenolic compounds [39, 40]. Phenolic complexes play a crucial role in antioxidant mechanisms, providing hydrogen ions or electrons for stability and neutralizing free radicals [41]. Additionally, previous studies have established a direct relationship between antioxidant capacity and polyphenol content [42-44]. The high activity of AgNPs might be linked to the existence of bioactive compounds and various types of functional groups, as confirmed by FTIR, which were relevant for the bio-reduction of silver ions. This shows the potential use of AgNPs as an alternative antioxidant in the therapy of illnesses induced by free radicals. The bioactive compound may donate H+ from its hydroxyl group (-OH) to free radicals, forming stable phenoxyl radicals [45, 46].

3.6 Cytotoxic activity

The cytotoxic activity of extract and AgNPs was analyzed with the BSLT. The toxicity of the materials was evaluated by determining the 24-hour LC_{50} value, representing the concentration at which half of the examined animals died after 24 hours [47]. The LC_{50} values in the BSLT analysis were categorized as non-toxic (>1000 ppm), toxic (30-1000 ppm), and very toxic (< 30 ppm) [28]. The results showed that the extract and AgNPs were in the toxic category, with the toxicity of AgNPs exceeding that of the extract (Table 3).

The BSLT results showed that at higher UI-AgNP concentrations, the number of dead A. salina larva was higher than it was at lower concentrations. As was found in a previous study, the size and shape of AgNPs caused increased toxicity [48]. Furthermore, the higher the concentration of AgNPs, the greater the cytotoxicity, resulting in a decline in the quantity of shrimp larvae alive after 24 hours. Furthermore, UI-AgNPs have the potential to be used as an anticancer agent. According to certain studies, the cytotoxicity of AgNPs in mammalian cells is highly dependent on the nanoparticle size, shape, sur-

Table 2. Antioxidant activities of *U. intestinalis* extracts and AgNPs.

Sample	Concentration (ppm)	% Scavenging activity	IC_{50} (ppm)
Extract of *U. intestinalis*	200	14.81 ± 2.08	953.08
	150	13.76 ± 1.81	
	100	12.17 ± 1.16	
	50	9.52 ± 0.92	
	25	6.35 ± 0.79	
AgNPs	200	72.12 ± 2.67	100.40
	150	54.90 ± 1.89	
	100	47.21 ± 1.14	
	50	41.92 ± 1.03	
	25	38.32 ± 0.86	

Table 3. The value of 24-hours LC50 *U. intestinalis* extract and AgNPs.

Sample	Concentration (ppm)	% Mortality	LC_{50} (ppm)
Extract of *U. intestinalis*	2000	76.33 ± 2.52	563.24
	1000	66.67 ± 2.51	
	500	49.32 ± 2.16	
	250	25.31 ± 2.05	
	100	18.23 ± 1.24	
AgNPs	2000	98.23 ± 3.40	128.20
	1000	91.43 ± 2.62	
	500	84.07 ± 1.70	
	250	65.52 ± 1.65	
	100	46.24 ± 0.94	

face charge, dosage, oxidation state, aggregation, and cell type [49]. The anticancer activity of smaller AgNPs is stronger because they have a higher surface-area-to-volume ratio as well as a larger total surface area, all else being equal (50). The wide-spectrum bioactivity of AgNPs can be utilized to diagnose and treat a variety of cancers [51]. When aquatic fish are exposed to AgNPs, it can lower acetylcholine esterase activity, disrupt T-lymphocyte selection, change the balance of chloride and potassium in the blood plasma, raise cortisol levels, and change the histology of the fish's skin, gills, liver, and kidneys [52, 53].

4. Conclusion

This study has demonstrated the successful synthesis of AgNPs using the green algae *U. intestinalis* as both a capping and reducing agent. The bioactive components in *U. intestinalis* stabilized Ag+ during the reduction process, as confirmed by UV, FTIR, and PSA characterizations. These AgNPs have strong anticancer and antioxidant properties, suggesting their potential as therapeutic agents. Further cytotoxicity testing for in-vivo applications is needed to ensure the biosafety of silver. To develop safe and effective anticancer and antioxidant medicinal products, additional methods for the application of AgNPs must be investigated.

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