

Antioxidant activity of silver nanoparticles synthesized using *Holothuria atra* extract

Irwan Irwan^{1,*}, Mutia Ramadhaniaty¹, Eli Nurliza²

¹Department of Marine Sciences, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh, Indonesia.

²Department of Indonesian Language Education, Faculty of Teacher Training and Education, Universitas Serambi Mekkah, Banda Aceh, Indonesia.

ARTICLE INFO

Keywords:

Antioxidant
Seacucumber
Green Synthesis
Silver Nanoparticles
DPPH

ABSTRACT

Sea cucumber (*Holothuria atra*) was used as a reducing and capping agent in the green synthesis process to produce silver nanoparticles (AgNPs). The antioxidant activity of the generated silver nanoparticles was then evaluated. Seacucumber extract was mixed with the AgNO₃ solution and homogenized using a magnetic stirrer to create the silver nanoparticles. It was determined that silver nanoparticle production had occurred using a UV-Vis spectrophotometer. Before being examined for antioxidant activity, the generated nanoparticles were first given a characterizing using Fourier Transform Infra Red (FTIR) spectrometers. The maximum uptake was obtained at wavelengths of 440 cm⁻¹ using a UV-Vis spectrophotometer. Functional groups that play a role in the synthesis of nanoparticles were -OH- C=O, and -C-O groups. The free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) with an absorbance maximum (517 nm) was used for antioxidant properties. The antioxidant assay showed that Silver nanoparticles had higher antioxidant activity than sea cucumber extract alone, with an IC₅₀ value of 4 ppm.

DOI: 10.13170/depik.11.3.28485

Introduction

Free radicals are molecules that have unpaired electrons, are unstable, and highly reactive to bind and damage the electrons of protein, lipid, and DNA cell molecules that have the potential to cause disturbances in the body's balance, cell damage, gene function abnormalities, cardiovascular disease, diabetes mellitus, neurodegenerative and cancer (Young *et al.*, 2001; Abheri, *et al.*, 2010; Phaniendra *et al.*, 2015). Antioxidants are substances that are formed from oxidative metabolism and play a role in preventing, neutralizing, and protecting cells from damage caused by free radical compounds (Maharani, *et al.*, 2022). Sources of antioxidants can be classified into synthetic antioxidants and natural antioxidants. The use of synthetic antioxidants such as BHT (butylated hydroxy Toluene) and BHA (butylated hydroxy aniline) can cause lung and liver function disorders and are carcinogenic (Kikuzaki, *et al.*, 2002). Natural antioxidants can be isolated from plants, fruits, terrestrial and marine organisms

(Avigail *et al.*, 2019). One of the new alternative sources of antioxidants is sea cucumber (*Holothuria atra*).

Sea cucumbers are one of the invertebrate animals that have potential as nutraceuticals and are rich in bioactive compounds resulting from mechanical and chemical self-defence mechanisms (Bordbar *et al.*, 2011; Azwir *et al.*, 2019). Bioactive compounds reported from several types of sea cucumbers include saponins, sterols, polyphenols, flavonoids, terpenoids, steroids, alkaloids, and lectins (Bordbar *et al.*, 2011; Soltani *et al.*, 2014). In addition, the results of research on the activity of bioactive compounds in sea cucumbers show their potential as antitumor (*Holothuria mobile*) (Shimada, 1969), antibacterial (*Holothuria edulis*, *Holothuria leucospilota*) (Sergey, *et al.*, 2009; Arash *et al.*, 2014; Manoppo *et al.*, 2017) antifungal (*H. atra*) (Nimah *et al.*, 2019) and antioxidant (*H. atra*, *S. Horrens*, *Holothuria scarba* and *Stichopus hermanni*) (Rasyid, 2012; Nobsathian *et al.*, 2016; Akerina *et al.*, 2019). The presence of phenolic

* Corresponding author.

Email address: irwan@unsyiah.ac.id

compounds is closely related to antioxidant activity because phenolic compounds have many phenol groups that play an essential role in protecting oxidative stress and preventing free radical initiation reactions (Fitriansyah et al., 2017; Diniyah et al., 2020). Theoretically, the phenolic components contained in bioactive compounds have metal salt reducing properties, stabilizing agents, and capping agents so that they can be used as bioreductors in the synthesis of Ag nanoparticles (Philips, 2010; Masakke et al., 2015; Dewi et al., 2019).

Synthesis of silver nanoparticles can be carried out through various methods, such as physical, chemical, and biological methods. Chemical and physical methods can produce pure particles but are inefficient because they are expensive and harmful to the environment (Willian and Pardi, 2021). While the green synthesis method is more prevalent in synthesizing nanoparticles because it has the advantages of being eco-friendly (environmentally friendly), more reproducible, non-toxic, low cost, fast reaction, does not require high energy, and good morphology (Mailu et al., 2010, Mittal et al., 2012 and Kim et al., 2016).

Silver nanoparticle sources for biological synthesis (green synthesis) use microorganisms and plants. Many studies have been carried out on the use of plants as bioreductors in the biosynthesis of silver nanoparticles, such as *Syngonium podophyllum* leaves, *Carthamus tinctorius* L leaves, and *Acacia cyanophylla* leaves which have antibacterial activity (Romana et al., 2021; Felix et al., 2021; Jalab et al., 2012). In contrast, the use of marine organisms such as deepsea cucumbers as a bioreductant of silver nanoparticles has never been reported. Therefore, this research is important to do to increase the potential of black sea cucumber (*Holothuria atra*) as a new bioreductant in the synthesis of silver nanoparticles and its wide use in the biomedical field.

Materials and Methods

Location and time of research

This research was conducted from April to July 2022 at the Laboratory of Marine Chemistry and Fisheries Biotechnology, Faculty of Marine Affairs and Fisheries, Universitas Syiahkuala. A Sampling of sea cucumbers at Inong Balee Fort, Aceh Besar regency. Sampling locations can be seen on the following map.

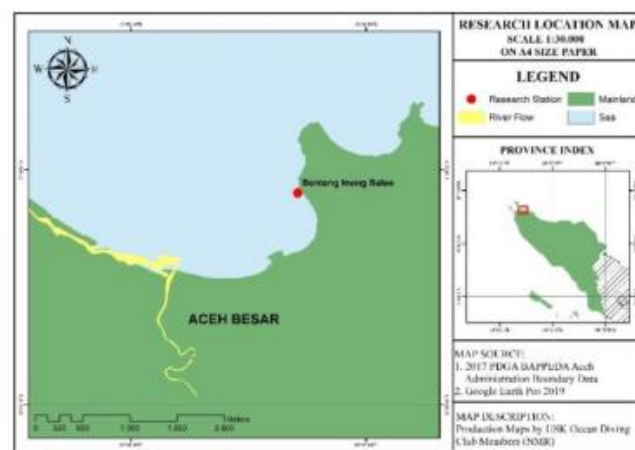


Figure 1. Map of research area.

Material

Wet seacucumber (*Holothuria atra*) were collected from Benteng Inong Balee, Aceh Besar regency. The chemicals such as silver nitrate (Merck) and Methanol (Merck).

Experimental

Extraction of *Holothuria atra*

Wet seacucumber (*Holothuria atra*) were macerated for 3 x 24 hours using methanol solvent with the ratio of solvent and sample is 1:2. The extraction results were filtered to separate between filtrate and residue (maserate). The maserate was concentrated in a rotary evaporator and the filtrate obtained was stored in a closed container to be used as a bioreductant in the synthesis of silver nanoparticles.

Synthesis of silver nanoparticles (AgNPs)

10 mL of seacucumber extract was mixed into 90 mL of 0.1 mM AgNO₃ solution. The mixture was stirred with a magnetic stirrer for 1 x 24 hours at room temperature until a precipitate was formed. Then centrifuged at 4000 rpm for 20 minutes to form silver nanoparticles. The filtrate and residue obtained were filtered. The filtrate result was stored and analyzed using a UV-Vis spectrophotometer to identify the formation of silver nanoparticles. Meanwhile, the precipitate obtained after centrifuge process was dried in the oven at 80 °C for 2 hours. The dried precipitate was characterized using Fourier transform infrared (FTIR)

Antioxidant test

Preparation of stock solution (1000 ppm) by weighing 25 mg of sea cucumber extract and then dissolving in 25 mL of ethanol. Each stock solution was diluted with distilled water to a concentration of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm. For every 1 mL of concentration, 2 mL of methanol and 1 mL of 0.1 mM DPPH were added to ethanol. The next step was vortexing and restricting for 30 minutes at room temperature in dark conditions. The

absorbance of the solution was measured at a wavelength of 517 nm (Niraimathi *et al.*, 2013 and Thilagavathi *et al.*, 2016). The blank solution was distilled water, while the control used a mixture of 1 mL DPPH 0.1 mM and 2 mL ethanol.

Inhibition of DPPH absorption in percentage is calculated using the formula below:

$$\% \text{ inhibition} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100 \%$$

Note: Control absorbance = Absorbance of DPPH;
sample absorbance = Absorbance in DPPH

Results

Formation of silver nanoparticles

The success synthesising silver nanoparticles (AgNPs) from seacucumber extract can be indicated by a change in the colour of the solution. Based on the study's result, it is known that there has been a change in the color of the seacucumber extract from orange to blackish brown after the addition of AgNO₃ solution. This indicates that the formation of silver nanoparticle compounds has occurred. The colour change of the solution can be seen in Figure 2.

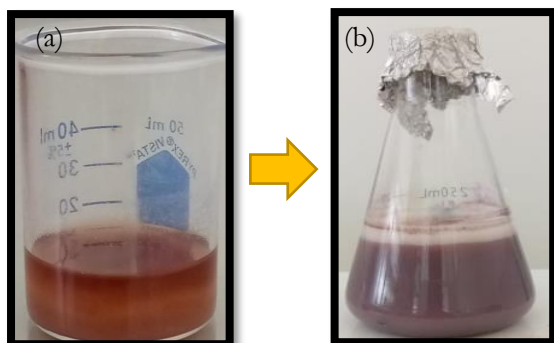


Figure 2. Solution color of (a) Sea cucumber extract and (b) AgNPs.

UV-visible spectroscopy analysis

UV-Vis spectrophotometer analysis was carried out to identify the formation of silver nanoparticles. The UV-Vis spectrophotometer analysis showed the maximum absorption peak of silver nanoparticles at 440 nm, as shown in Figure 3.

Analysis FT-IR

FT-IR analysis in this study aims to determine functional groups extracts' interaction with silver nanoparticles. The results of the FT-IR test on sea cucumber extracts and silver nanoparticles (AgNPs) can be seen in Figure 4 and 5.

Antioxidant activity data

In this research, the antioxidant activity of silver nanoparticles and sea cucumber extract will be tested using the DPPH method, which has the advantage of being simple and fast. The test results of antioxidant

activity between sea cucumber extract and AgNPs can be seen in the Table 1 below.

Tabel 1. Antioxidant activity results between seacucumber extract and silver nanoparticle (AgNPs).

Sample	Concentration (ppm)	% Inhibition	IC ₅₀
Extract	10	46.19	27.59
	20	47.1	
	30	49.64	
	40	53.2	
	50	57.27	
AgNPs	10	51.1	4.0
	20	52.1	
	30	53.8	
	40	55.5	
	50	56.8	

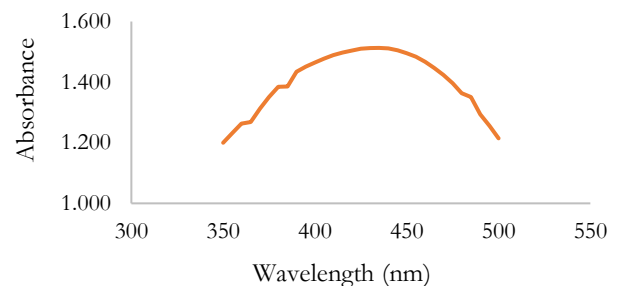


Figure 3. UV Vis Spectrophotometer of AgNPs.

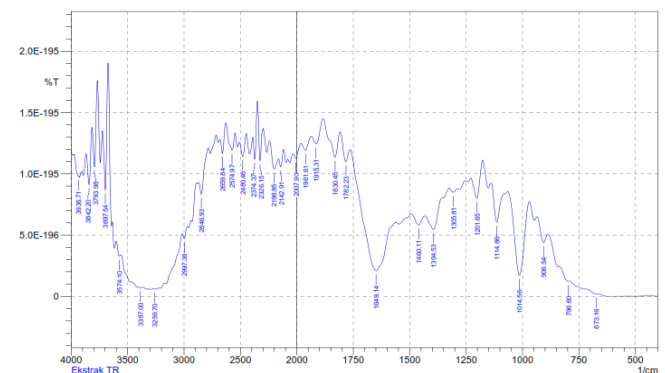


Figure 4. FT-IR sea cucumber extract.

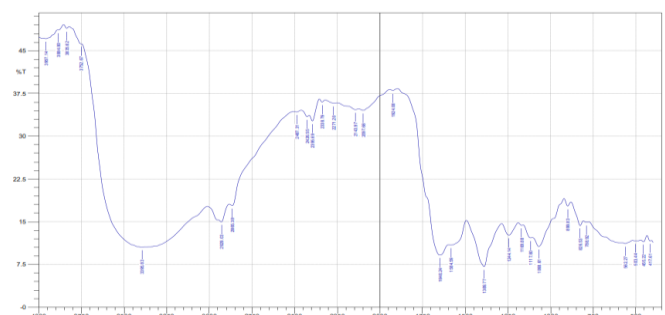


Figure 5. FT-IR silver nanoparticles.

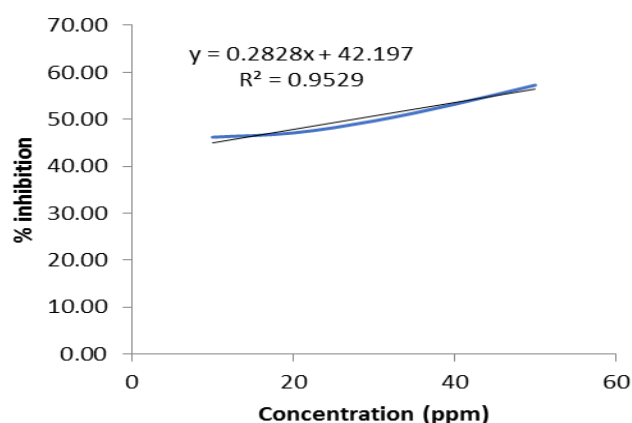


Figure 6. % Inhibition Vs seacucumber extract.

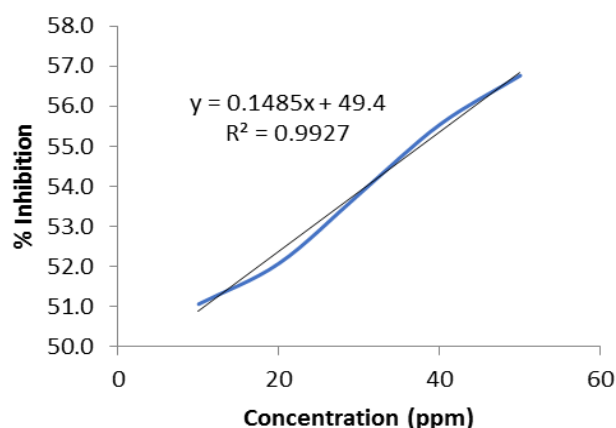


Figure 7. % Inhibition Vs silver nanoparticles.

Based on the data (IC_{50}) in the table 1 above, the antioxidant activity of AgNPs is higher than sea cucumber extract. As for the IC_{50} value, it is obtained based on the regression line equation in Figures 6 and 7 for each substance.

Discussion

Synthesis of silver nanoparticles (AgNPs)

The colour change indicates that there has been a reduction process of silver ions (Ag^+) to (Ag^0) in secondary metabolites contained in the extract (Elumalai et al., 2011 and Ibrahim, 2015). According to Thirumurugan et al. (2010), the color change of the extract from yellow to brown indicates the formation of silver nanoparticles.

UV-Visible spectroscopy analysis

The absorption at maximum wavelength indicates the formation of silver nanoparticles. UV light absorbed by nanoparticle compounds will cause the excitation of electrons on the metal surface, which has unique optical properties that will bring up variations in the shape and size of the particles (Devi et al., 2016). According to Srikar et al (2016), the range of the formation of silver nanoparticles is a wavelength of 400-450 nm. According to Solomon et al. (2007), silver nanoparticles with a wavelength of 420-440 nm have a

particle size of 35-80 nm. This difference in particle size is controlled by the amount of reducing agent used to reducing silver ions (Agnihotri et al., 2014).

Analysis FT-IR

Based on the spectrum of sea cucumber extract in Figure 4, it can be seen that there is a clearly absorption signal at wave numbers 3387 cm^{-1} , 2997 cm^{-1} , 1649 cm^{-1} that indicates the interaction of $-OH$ groups, $-CH$ stretching, and $C=C$ aromatic stretching at polyphenolic compounds contained in extract. Meanwhile, in Figure 5 shows the presence of $-OH$ and $-CH$ groups at wave numbers 3395 cm^{-1} and 2929 cm^{-1} in silver nanoparticles. The change in the absorption intensity of this functional group indicates that there has been an interaction between bioactive compounds in the extract and silver solution to form silver nanoparticles. The research result by Agustina et al. (2021) showed that the polyphenolic compounds contained in seacucumber extract (*Holothuria atra*) were flavonoids, saponins, and triterpenoids. According to Gurunathan et al. (2009), hydroxide ions (OH^-) play a vital role in accelerating the reduction reaction of silver ions in the formation of nanoparticles. According to Felix et al. (2021), The reaction mechanism between bioactive compounds and silver solution ($AgNO_3$) begins through the reduction of silver ions by the functional groups contained in the extract, then the nucleation stage until the formation of silver nanoparticles. The green synthesis pathway of silver nanoparticles can be seen in Figure 8 (Felix et al. 2021):

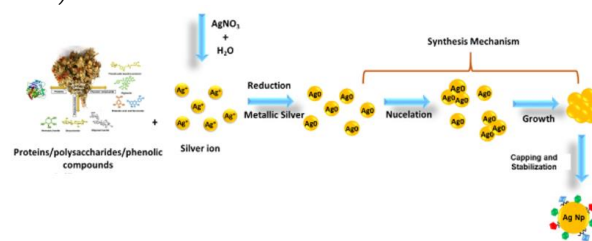


Figure 8. Synthesis of silver nanoparticles mechanism.

Antioxidant activity

Antioxidants are substances that prevent cell damage from oxidation reactions caused by free radicals. Based on Table 1, it can be seen that the higher the concentration of the extract and AgNPs, the higher the % inhibition. This could be due to the occurrence of a DPPH reduction reaction by the polyphenolic compounds in the sample. This reduction reaction occurs because DPPH gaining hydrogen atoms in antioxidant compounds. The mechanism of this reduction reaction can be seen in

Figure 9 below (Szabo et al, 2006 and Liang and Kitts, 2014):

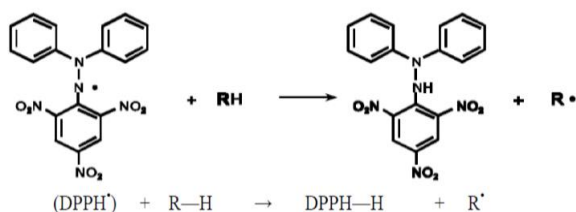


Figure 9. Reduction mechanism.

The activity test results in Table 1 show that silver nanoparticles and sea cucumber extract showed very strong antioxidant activity. According to Molyneux, (2004) material can be classified as having very strong antioxidant activity if the IC₅₀ value is <50 ppm. The IC₅₀ value indicates the ability of inhibition (50%) of free radicals in a substance. In addition, based on the IC₅₀ value, it is also known that silver nanoparticles have higher antioxidant activity than sea cucumber extract. According to Bedlovicova et al. (2020) and Elemike et al. (2017), the high antioxidant activity of silver nanoparticles can be caused by a large number of reducing agents contained in an extract (phenolic compounds, flavonoids, and terpenoids).

Based on the graphs in Figures 6 and 7, it is known that the regression equation (R²) of silver nanoparticles is higher than that of sea cucumber extract. It shows that the higher concentration of silver nanoparticles, the higher the antioxidant capacity.

Conclusion

Based on results research, it can be concluded that the bioactive components of sea cucumber (*holothuria atra*) have an important role in synthesizing silver nanoparticles. The success of the synthesis of silver nanoparticles in this study was indicated by the presence of blackish brown silver nanoparticles at a wavelength of 440 nm and the interaction of -OH, -CH stretching, and C=C aromatic stretching groups on polyphenolic compounds with silver ions. The antioxidant activity of silver nanoparticles was higher than sea cucumber extract, with an IC₅₀ value of 4 ppm.

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How to cite this paper:

Irwan, I., M. Ramadhaniaty, E. Nurliza. 2022. Antioxidant activity of silver nanoparticles synthesized using *Holothuria atra* extract. *Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan*, 11(3): 341-346.