Characterization, Antioxidant, and Antibacterial Activity Silver Nanoparticle of *Gelidium spinosum*

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Abstract. The purpose of this study was to characterize AgNP, and determine antioxidant and antibacterial activities. This research is exploratory descriptive research. The characterization method used PSA (Particle Size Analysis) and FTIR (Fourier Transform Infrared Spectroscopy). The antioxidant activity method uses the DPPH (1.1-diphenyl-2-picrylhydrazyl). The antibacterial activity test used the inhibition zone test with paper disc diffusion (Kirby and Bauer Method), the MIC test used the tube dilution method, and the MBC test used the total plate count method. The results of the research on PSA characterization showed that characterization AgNPs *Gelidium spinosum* has a size of 107.1 nm, while the FTIR results showed that AgNPs *G. spinosum* had 7 spectral peaks at wave numbers 466.37 and 534.83(-(CH2)n), 714.11 and 874.27 (C-H aromatic rings), 1035.43 (C-O alcohols, ethers, carboxylic acids, and esters), 1406.24 (C-H alkanes), 1644.42 (C=C alkenes), and 3323.07 (O-H hydrogen bonds in alcohols and phenols). The results of the antioxidant activity of AgNPs *Gelidium spinosum* an IC50 value of 18.34 ± 5,147 ppm, while the antibacterial inhibition zone test on the sample of AgNPs *G. spinosum* against *E. coli* was included in the strong category with an average value of the diameter of the inhibition zone 10.18 mm different from the others sample in the medium category. The results of the minimum inhibitory concentration (MIC) in *G. spinosum* AgNPs were 18.75 ppm, while the *G. spinosum* extract was 37.5 ppm. The result of the minimum bactericidal concentration (MBC) on AgNPs *G. spinosum* was 18.75 ppm, and the extract of *G. spinosum* is 37.5 ppm. Based on the results of this study AgNPs *Gelidium spinosum* had strong antioxidant and antibacterial activity against gram-positive and gram-negative bacteria.

Keywords: silver nanoparticle · Gelidium spinosum · antioxidant · antibacterial

1 Introduction

Indonesia has good marine potential to be developed, one of which is seaweed. Indonesia contributed nearly 40% of the world's seaweed. Meanwhile, according to trade map data, Indonesia became the first seaweed exporting country with a contribution of 30% of total

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world exports, about 213 thousand tons. Most of the red algae are easily found in shallow ocean waters and public waters. Red algae usually live in warm temperatures in tropical areas such as Indonesia, one of which is the algae *Gelidium spinosum* [1, 2]. *Gelidium spinosum* has the characteristics the fibrous holdfast, and talus cylindri, some talus has branches such as small protrusions. The thallus has a curved, pointed tip and some are rounded, and is purplish-red to deep purple [3, 4].

Algae has secondary metabolites and pigments. One of the secondary metabolites contained is a phenol, terpenoid, and tannin group. Phenol compounds have an efficient antioxidant role [5, 6].

The natural antioxidants in plants are obtained from phenols and their derivatives, such as flavonoids, coumarins, derivatives of hydroxamic compounds, tocopherols, and organic acids [7]. These metabolites have also been reported to have antibacterial, cytotoxic, antitumor, antiviral, and antifungal activities [8] Steroids and tannins can inhibit bacterial growth by changing the permeability of the bacterial cell membrane, microsomes, and lysosomes so that bacteria will lysis or be destroyed [9, 10].

Based on the potential of these macroalgae, many technologies are being developed that can utilize natural resources through technology are nanoparticles. Nanoparticle technology can be used widely in various fields such as biomedical, ecosystem, electronics, and cosmetics [11] Nanoparticles have the advantage of increasing the affinity of the system, which is caused by increasing the contact surface area [12]. The a nanosize of $\pm$ 1–100 nm. This nanomaterial has a slightly different character than the pure material [13].

Nanoparticles are a good delivery system, can ward off excess moisture, good absorption, and can increase the penetration of active substances. One type of nanomaterial that is often used in the research is silver nanoparticles [14]. Green synthesis is one of a method for synthesizing nanoparticles using metabolite compounds in plants as bioreductants. Extraction of plant will obtain enzymes, proteins, flavonoids, and terpenoids as bioreducing and stabilizer agents for nanoparticles [15] Silver nanoparticles also have more effective antibacterial agents than zinc, copper, and titanium. This is due to the highly reactive nature of silver ionization so that it can bind to proteins on cell walls and bacterial cell membranes so cells will be damaged and die. The research also produced AgNPs that were synthesized from the *Gelidium amansii* which can effectively inhibit the growth of various gram-negative bacteria (*P. aeruginosa, V. parahamolyticus, E. coli and A. hydrophila*) and gram-negative bacteria (*B. pumilus and S. aureus*) [16].

FTIR (Fourier Transform Infrared Spectroscopy) analysis is used to identify the functional groups present in a compound. The wavelength of the absorbed light is an important feature of chemical bonds which can be seen by annotating the spectrum. The spectrum formed from the absorption of infrared waves to determine chemical bonds in a compound under study [17]. The infrared spectrum is generated from the transmission of light that passes through the sample, measuring the light intensity with a detector and compared with the intensity without the sample as a function of wavelength. The infrared spectrum obtained is then plotted as a function of energy intensity, wavelength (μm) or wave number (cm^{-1}). FT-IR has advantages such as high sensitivity and speed, increased optical output, activating all frequencies measuring metabolites simultaneously, and efficient data interpretation (Selamat *et al.*, 2021) [18, 19].

The research about the characteristics of antioxidant and antibacterial tests on silver nanoparticles (AgNPs) using the *Gelidium spinosum* red algae has never been encountered before, so this research needs to be carried out with the purpose of testing the physical and phytochemical characterization using PSA (Particle Size Analyzer) and FT-IR (Fourier Transform Infrared Spectroscopy) to determine the functional groups of algae nanoparticles (AgNPs) and for determining the antioxidant and antibacterial activity of silver nanoparticles synthesized using the *Gelidium spinosum* red algae.

2 Material and Method

The materials used in this study were dry algae of *Gelidium spinosum* (from algae farmers in Pangasan Beach, Pacitan, East Java), Silver nitrate ($AgNO_3$) (Merck), distilled water, DPPH solution, ascorbic acid, isolates of *Staphylococcus aureus* and *Escherichia coli* bacteria. The results of sub-cultures from the Microbiology Laboratory of UIN Malang, NB (Nutrient Broth) media, NA (Nutrient Agar) media, $BaCl_2.2H_2O$ 1.175%, H_2SO_4 0.36 N, plastic wrap, aluminum foil, cotton, gauze, 70% alcohol, absolute ethanol, and chloramphenicol.

2.1 Extraction of Gelidium Spinosum

The algae extraction used a research reference by Rajivghandi *et al.,* (2020) with a modification, the algae *Gelidium spinosum* is dried, then cut into small pieces, put in a blender, and mashed. Then filtered through a 0.01 mm Whatmann. Samples were weighed as needed (10 g). After that, the sample was dissolved with 100 ml of distilled water (1:10 g/ml ratio). Then homogenized with a stirrer at a temperature of 30 °C for ± 15 min. It was put into a 15 ml tube and centrifuged at 4000 rpm at 4 °C for 15 min. After that, the supernatant was taken from the extraction of *Gelidium spinosum.*

2.2 Synthesis of Silver Nanoparticles (AgNPs) Gelidium spinosum

The synthesis of silver nanoparticles with a modification, taking 100 ml of 1 mM silver nitrate ($AgNO_3$) and 100 ml of *Gelidium spinosum* algae extract (1:1 ratio). The color change was observed after 1 h when the solution turned yellow-brown at room temperature, indicating that nanoparticles had been formed. Then the solution was centrifuged for 30 min at 20 °C. Then the centrifuged pellets were dried in an oven at 45 °C for 24 h and then the pellets were pulverized, the AgNPs *Gelidium spinosum* ready for characterization, antioxidant and antibacterial tests [9].

2.3 Physical Characterization Using PSA (Particle Size Analysis)

The procedure in the particle size characterization used the PSA (Particle Size Analysis), the first PSA tool is heated for about 20 min. After that, turn on the computer device that is connected to the tool. Then start to make settings on the tool. The standard solution of 20 dilutions was shaken using a vortex mixer for ±1 min. Then put it in a clean cuvette until it is filled 2/3 of the cuvette. After that, the cuvette containing the standard solution

is inserted into the tool and closed with a sensor. Before being measured, the temperature is conditioned first at 25 °C by pressing the "Temp.Panel" menu. The standard starts to be measured by pressing the "Auto1" menu. Then the tool will automatically measure the size of the particle as much as six times the measurement. The same procedure was carried out for standard solutions of 200 dilutions and 2000 dilutions. The next procedure uses an automatic method with a sharp distribution form. The procedure is the same as in the first automatic method, but the distribution graph settings are replaced with sharp shapes [10].

2.4 Characterization of Function Groups Using FT-IR (Fourier Transform Infrared Spectroscopy)

The characterization of the functional groups of silver nanoparticles using FT-IR. The silver nanoparticle was centrifuged at 3500 rpm for 15 min, after which the pellet was re-dispersed in deionized water. The processes of centrifugation and redispersion with deionized water were repeated three times to ensure a better separation of the free entities of metal nanoparticles. The purified pellets were then dried and powdered with potassium bromide for FT-IR spectroscopic measurements. The spectrum is recorded using FT-IR and the transmittance mode operates at a resolution of 4/cm [20, 21].

2.5 Antioxidant Activity

Testing of antioxidant activity used DPPH method with modifications [22]. First, the concentration series was determined, then 500 μl of sample solution (50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm) and 500 μl of DPPH solution were taken into the tube and made with 3 replications. Then the solution was incubated at room temperature for 30 min in the dark. Then read the absorbance of the solution using a UV-Vis spectrophotometer at a wavelength of 517 nm and the reading was repeated 3 times [23].

Preparation of a serial solution for ascorbic acid (positive control) was carried out as a standard by making a stock solution of ascorbic acid by dissolving 0.5 mg of ascorbate in 100 ml of distilled water. The concentrations tested were 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm, so the volume of ascorbate taken was 40 μl, 80 μl, 160 μl, and 200 μl and dissolved in distilled water up to 10 ml. This antioxidant test using the DPPH method is then calculated using the following formula:

$$\% \text{ inhibition} = \frac{A\ blanko - A\ sampel}{A\ blanko} \times 100\% \quad (1)$$

2.6 Antibacterial Activity

The test of the inhibition zone of *S. aureus* and *E. coli* with several modifications, using the Kirby and Bauer method [24]. Then the disc paper was inserted into 200 μl of the sample with several levels of concentration (25 ppm, 50 ppm, 75 ppm, and 100 ppm) and soaked for about 30 min. After that, the paper discs were placed on the surface of solid agar (NA) media which had been given a bacterial suspension solution and incubated at

37 °C for 24 h. The test was repeated 3 times. Then the clear zone that appears around the paper disc is observed and its diameter is measured with a caliper and recorded and then entered into the table and the average inhibition zone is calculated with the following formula [21]:

$$\text{Inhibitory zone diameter} = \text{Clear zone diameter} - \text{Paper disc diameter} \quad (2)$$

The MIC test method was carried out by tube dilution method using sterile test tubes containing NB (Nutrient Broth) media with several modifications [16, 25], 200 µl of *Gelidium spinosum* AgNPs solution was included in the first well. Then, gradual dilutions were carried out (75 ppm; 37.5 ppm; 18.75 ppm; 9.375 ppm; 4.6875 ppm, and 2.34375 ppm) by taking 100 l of the test solution from the first well and adding it to the next well which was filled. 100 µl of NB medium successively up to the last tube. Next, the microbial suspension was adjusted with Mac solution. Farland 0.5 was added to all wells as much as 100 µl and incubated at 37 °C for 24 h. The lowest concentration of antibacterial in clear wells indicated that there was no bacterial growth and was reported as a MIC result. Furthermore, the MBC test was carried out by inoculating the suspension from each well as much as 100 µl with the pour plate method on NA media and carried out three times. Then, it was incubated at 37 °C for 24 h. The minimum concentration of wells that did not contain bacterial growth was reported as the result of MBC. Furthermore, the number of bacterial colonies was calculated using a digital colony counter and entered into the Total Plate Count with the following formula:

$$\Sigma cell = \Sigma colony \times \frac{1}{fp} \quad (3)$$

3 Result and Discussion

3.1 Physical Characterization Using PSA (Particle Size Analyzer)

The particle size distribution of silver nanoparticles (AgNPs) formed in this study had an average size of 107.1 nm after analysis using the Particle Size Analyzer (PSA), which can be seen in Fig. 1. This shows that the red algae extract Gelidium spinosum can produce silver nanoparticles. By following the opinion of Ahdyani *et al.*, (2020) that particles measuring 10–1000 nm can be called nanoparticles. The treatment carried out during the manufacture of nanoparticles can affect the size and shape, such as temperature, $AgNO_3$ concentration, pH, and the method used. Mustari, *et al.,* (2019) added that plant extraction will obtain enzymes, proteins, flavonoids, and terpenoids as bioreducing agents and stabilizer agents in the synthesis of nanoparticles [26].

3.2 Characterization of Function Group Using FTIR (Fourier Transform Infrared Spectroscopy)

The results of the functional group characterization using FT-IR on all samples on *Gelidium spinosum* nanoparticles, *Gelidium spinosum* extract and $AgNO_3$ based on

the wave absorption values are presented in the form of a spectrum graph as shown in Fig. 2.

Based on the functional groups in the FITR results on AgNPs and *Gelidium spinosum* extract (Fig. 2) it is known that there are several functional groups whose spectra shifted but not too widely, namely (529.12 to 534.83), (713.11 to 714.11), (1032.58 to 1035.43), (1404.82 to 1406.24), (1641.57 to 1644.42) and (3311.66 to 3323.07). In addition, there are functional groups that are not found in nanoparticles but are found in *Gelidium spinosum* extract, namely in spectrum 1143.82 which shows the C-O-C ether functional group, and spectrum 2926.59 which shows the C-H alkane functional group. All functional groups contained in AgNPs *Gelidium spinosum* are also found in *Gelidium spinosum* extract. Examples are the C-H aromatic functional groups and the O-H alcohols and phenols. These functional groups are the constituents of flavonoid compounds and their derivatives. According to Harborne (2006) functional groups of polar compounds such as functional groups C-H aromatic and O-H alcohol will be easily dissolved by polar solvents [17]. By following the opinion of Alfaridz (2018) that two aromatic groups combined by a carbon bridge (C_6-C_3-C_6) become the basic structure

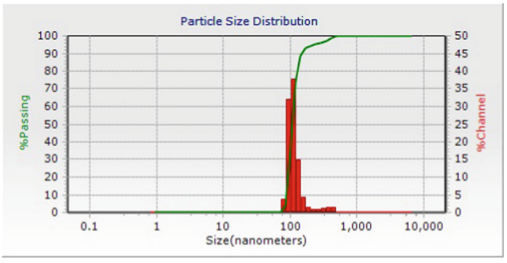

Fig. 1. Particle Size Distribution of AgNPs *Gelidium spinosum*

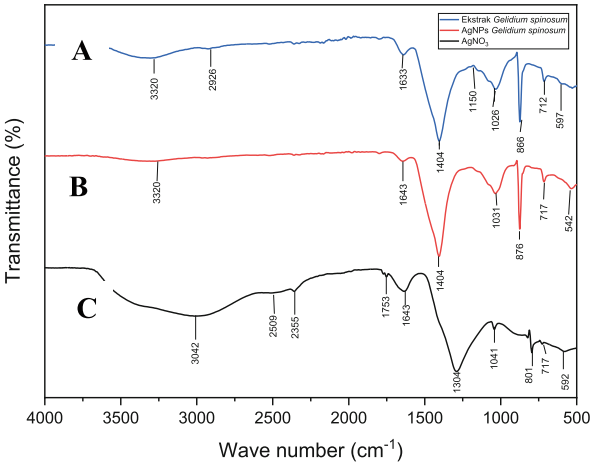

Fig. 2. Spectrum peak values of FTIR A) *Gelidium spinosum* extract; B) AgNPs *Gelidium spinosum*; C) AgNO3

of flavonoid compounds [27]. Flavonoid derivatives are produced by a combination or interaction between the structure of the aromatic functional group with other groups. The flavonoids have antibacterial, anti-inflammatory, antioxidant, and cytotoxic activity [28].

The results of the phytochemical characterization of $AgNO_3$ using FTIR are presented in Fig. 2 in the form of an IR spectrum graph with different peak values. Based on the results of these peak values, it shows that there are C=O acyl halide and S=H sulfur groups in $AgNO_3$ compounds that are not present in the peak values of *Gelidium spinosum* nanoparticles. This indicates that both groups were reduced by bioactive of *Gelidium spinosum* during the synthesis of silver nanoparticles. The FTIR results on $AgNO_3$ showed that there were missing peaks, are the amine and thiocarbonyl groups because they were reduced by a capping agent from the bioactive plant compound *Reinwardtia indica* [29].

3.3 Antioxidant Activity

Based on the results of the tests carried out, the results obtained are different IC50 values between nanoparticles and *Gelidium spinosum* extract shown on Table 1.

Table 1 shows that the IC50 value of *Gelidium spinosum* silver nanoparticles (AgNPs) is 18.34 ± 5.147 ppm, which is smaller than the IC50 value of *Gelidium spinosum* extract of 19.57 ± 6.051 ppm, but larger than the positive control in the form of ascorbic acid, which is 1.808 ± 0.0067 ppm. The results of *Gelidium spinosum* silver nanoparticles (AgNPs) are included in the very strong category, so it can be said that the formation of *Gelidium spinosum* AgNPs can increase antioxidant activity in degrading free radicals. The IC50 value of *Gelidium spinosum* AgNPs has a lower value than the extract, this is due to the effectiveness of nanoparticles in degrading DPPH molecules so that their antioxidant activity is stronger than the extract form. The principal of the DPPH method is that the lower the IC50 value, the greater role of antioxidants. The radical in DPPH is organic nitrogen, which is dark purple in color, when it is reduced it will turn yellow [30].

That bioactive compounds in plants or algae can be capping agents and catalysts to produce high antioxidant activity so that silver nanoparticles have better activity when compared to non-nano particles or extracts [31]. That certain functional groups contained in bioactive compounds can be attached to the AgNP surface, thus AgNP becomes stable [32]. The synthesized silver nanoparticles (AgNPs) showed potential antioxidant activity through the DPPH test [33]. These AgNPs also show potential antibacterial activity

Table 1. Antioxidant activity of AgNPs, Extract, and Ascorbic Acid

SAMPLE	**IC_{50} (ppm)**	**CATEGORY (Febrianti, *et al.*, 2017)**
AgNPs	18.34 ± 5.147	Very Strong
Extract	19.57 ± 6.051	Very Strong
Ascorbic acid	1.808 ± 0.0067	Very Strong

against pathogenic bacteria, so it clearly shows that the AgNPs produced by green synthesis can produce effective antioxidants as well as antibacterial agents. The results showed that the antioxidant potential of AgNPs increased from 5–15% concentration.

3.4 Antibacterial Test

The antibacterial tests in this research included the zone of inhibition test, the minimum inhibitory concentration test (MIC), and the minimum bactericidal concentration test (MBC). The results of the inhibition zone test are presented in Table 2.

Based the results of the inhibition zone test in Table 2 and Fig. 3, it shows that the highest average diameter of the inhibition zone is found in *Gelidium spinosum* nanoparticles (AgNPs) inhibit *E.coli* bacteria with a value of 10.18 mm which belongs to the strong category compared to the other samples. The diameter between 10–20 mm is included in the strong category. Samples of *Gelidium spinosum* nanoparticles inhibit *S.aureus*, *Gelidium spinosum* extract inhibit *E.coli* and *Gelidium spinosum* extract inhibit *S.aureus* had an inhibition zone diameter of a medium category with a successive value of 6.50 mm 8.34 mm, and 8.28 mm. The diameter of the barriers ranging from 5–10 mm is included in the medium category [34].

Ibrahemm *et al.,* (2016) in their research also stated that AgNPs synthesized using the algae *Acantophora specifera* could inhibit *E.coli* bacteria indicated by an inhibition zone value of 19.5 mm with a strong category [35]. This is because AgNPs compounds can degrade bacterial cell membranes by absorption of silver ions by bacteria, which is followed by disruption of ATP formation in the process of metabolism and DNA replication and direct damage to bacterial cell membranes. In addition, another factor that allows for increasing the antibacterial activity of nanoparticles is the role of silver. The silver nanoparticles have been used as a detector, catalyst, surface coating agent, and antibacterial. It also has stable properties and potential applications in antibacterial agents, catalysts, and optical sensor detectors [36].

Table 2. Inhibition zone diameter of extract *Gelidium spinosum*, AgNPs *Gelidium spinosum*, chloramphenicol dan aquadest

No	Sample	Bacteria	Inhibition zone (mm)	Category [34]
1.	Extract *Gelidium spinosum*	*E.coli*	8,34	Moderate
2.	Extract *Gelidium spinosum*	*S. aureus*	8,28	Moderate
3.	AgNPs *Gelidium spinosum*	*E.coli*	10,18	Strong
4.	AgNPs *Gelidium spinosum*	*S. aureus*	6,50	Moderate
5.	chloramphenicol	*E.coli*	13,52	Strong
6.	chloramphenicol	*S. aureus*	13,23	Strong
7.	Aquadest	*E.coli*	0,00	None
8.	Aquadest	*S. aureus*	0,00	None

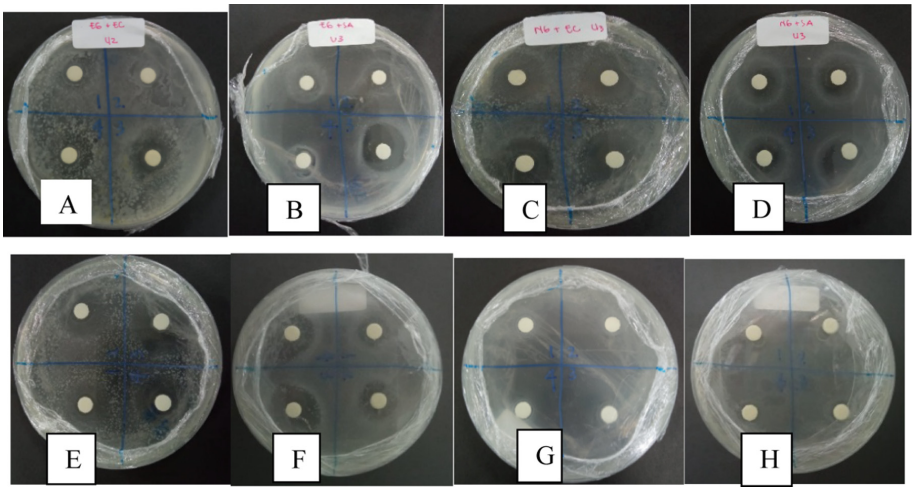

Fig. 3. Inhibition zone diameters a) Extract *Gelidium spinosum* against *E.coli*; b) Extract *Gelidium spinosum* against *S.aureus;* c) AgNPs *Gelidium spinosum* against *E.coli*; d) AgNPs *Gelidium spinosum* against *S.aureus;* e) Chloramphenicol against *E.coli;* f) Chloramphenicol against *S.aureus*; g) Aquades against *E.coli*; and h) Aquadest against *S.aureus*

Samples of *E.coli* and *S.aureus* bacteria had different average diameters of inhibition zones, both in the extract, *Gelidium spinosum* nanoparticles, and chloramphenicol. The samples tested inhibit *E.coli* bacteria had a larger diameter of inhibition zone than the samples with *S.aureus*. This is due to differences in the structure of the bacterial cell walls between gram-positive and gram-negative bacteria. The cell walls of gram-positive bacteria are composed of peptidoglycan which is simpler than gram-negative bacteria, so that antibacterial substances are easy to penetrate the cell wall and interfere with bacterial metabolism which will cause the bacteria to lyse and die [37].

The minimum inhibitory concentration test is used to identify the concentration that is effective in inhibiting the growth of microorganisms. The way to observe the MIC test is to observe the number of bacterial colonies that grow [38]. The results of the minimum inhibitory concentration test are presented in Table 3.

Based on the data from the MIC test results presented in Table 3 it is known that *Gelidium spinosum* nanoparticles inhibit *E.coli* and *S.aureus* bacteria have a minimum inhibitory concentration of 18.75 ppm, while samples of *Gelidium spinosum* extract have a minimum inhibitory concentration of 37.5 ppm. This is known from the observation of the clarity of the test solution. The turbidity will be seen in the tube that does not contain antibacterial substances so that the bacteria are still alive, while the black line visible on the negative control tube is still visible (clear) because the tube does not contain antibacterial substances [39]. *Gelidium spinosum* has alkaloids, saponins, phenolics, and flavonoids that have the potential as antibacterial [40]. Then according to the silver nanoparticles are antibacterial against gram-positive and gram-negative bacteria, because silver ions (Ag^{+}) easily penetrate bacterial cell walls so that even in small concentrations they can inhibit bacterial growth [41].

Table 3. Minimum inhibitory concentration of samples

Sample	Bacteria	Concentration (ppm)					
		75	37,5	18,75	9,375	4,375	2,34375
AgNPs *G.spinosum*	*E. coli*	-	-	-	+	++	+++
AgNP *G.spinosum*	*S. aureus*	-	-	-	++	++	++
Extract *G.spinosum*	*E. coli*	-	-	+	+	++	++
Extract *G.spinosum*	*S. aureus*	-	-	++	++	++	+++
Chloramphenicol	*E. coli*	-	-	-	-	-	-
Chloramphenicol	*S. aureus*	-	-	-	-	-	-
Aquadest	*E. coli*	++	++	++	+++	+++	+++
Aquadest	*S. aureus*	++	++	++	++	+++	+++

(-) trasnparent; (+) low roily; (++) roily; (+++) very roily

This minimum bactericidal concentration test was carried out to determine the smallest concentration of antibacterial substances that could kill 99.9% of the total colonies in the final inoculum that appeared after incubation for 24 h [39]. The results of the MBC test are presented in Table 4.

Based on the data from the MBC test results in Table 4, it shows that the minimum bactericidal concentration of *E.coli* and *S.aureus* bacteria in the *Gelidium spinosum* nanoparticle (AgNPs) sample is 18.75 ppm, while for the *Gelidium spinosum* extract sample is 37.5 ppm. Samples of nanoparticles and also extracts of Gelidium spinosum have antibacterial activity when viewed from the results of MBC even though at different minimum concentrations, this is because there are antibacterial substances present in the sample, causing bacteria to be inhibited or even die.

The presence of phenol in the algae *Gelidium spinosum* causes the microbial cell membrane to be damaged, so that antibacterial substances will enter the bacteria and interfere the activity of peptidoglycan transpeptidase, and ultimately cause the bacteria to lyse [1, 42]. In addition, silver nanoparticles also play a role in killing bacteria. The silver nanoparticles will stick to the cell membrane and penetrate bacterial cells so that they will interfere with bacterial metabolism. In addition, the antibacterial mechanism of silver nanoparticles is molecular, namely the interaction between silver ions and sulfhydryl thiol groups in proteins. Silver ions will replace hydrogen cations (H^+) from sulfhydryl thiol groups to produce more stable S-Ag groups on the surface of bacterial cells. This can inactivate proteins, decrease membrane permeability, and lead the cellular death [36].

Table 4. Minimum Bactericidal Concentration (MBC)

Sample	Bacteria	Concentration (ppm)					
		75	37,5	18,75	9,375	4,375	2,343
AgNPs *G.spinosum*	*E. coli*	0	0	0	X	X	X
AgNPs *G.spinosum*	*S. aureus*	0	0	0	X	X	X
Extract *G.spinosum*	*E. coli*	0	0	X	X	X	X
Extract *G.spinosum*	*S. aureus*	0	0	X	X	X	X
Chloramphenicol	*E. coli*	0	0	0	0	0	0
Chloramphenicol	*S. aureus*	0	0	0	0	0	0
Aquadest	*E. coli*	1,36 x 10^4	1,7 x 10^4	X	X	X	X
Aquadest	*S. aureus*	X	X	X	X	X	X

X = too much to count (> 250 colonies)

4 Conclusion

Red algae extract *Gelidium spinosum* can be a bioreductant in the synthesis of silver nanoparticles (AgNPs) which produces nanoparticles with an average size of 107.1 nm based on the results of PSA (Particle Size Analyzer) testing. This is because in the *Gelidium spinosum* red algae there are chemical compounds or secondary metabolites such as terpenoids and flavonoids that can be used as bioreductants.

Functional group characterization using FTIR resulted in 8 peak values on AgNPs *Gelidium spinosum*, namely –(CH2)n functional group, C-H aromatic ring, C-O alcohol, ether, carboxylic acid and ester, C-H alkane, C=C alkene, and O-H hydrogen bonds in alcohol and phenol. There are two functional groups are reduced by Ag^+ ions in the synthesis of AgNPs *Gelidium spinosum*, namely the C-O-C ether and C-H alkane functional groups. As for the extract, there are aromatic O-H, C-H, and C=C groups which indicate that there are flavonoid compounds as bioreductants of AgNPs.

Antioxidant activity of AgNPs *Gelidium spinosum* has inhibitory activity with IC50 value with a value of 18.34 ± 5.147 ppm classified as very strong category. The antioxidant activity of silver nanoparticles (AgNPs) of *Gelidium spinosum* algae was stronger than that of the extract although it was still in the same category, which was very strong. This is because silver nanoparticles have a high surface expansion yield to provide maximum contact with the environment.

AgNPs *Gelidium spinosum* can inhibit *E. coli* bacteria are included in the strong category with an average inhibition zone diameter of 10.18 mm, compared to the extract samples. The results of MIC and MBC on AgNPs *Gelidium spinosum* were 18.75 ppm, while that of *G. spinosum* extract was 37.5 ppm. This indicates that AgNPs *Gelidium spinosum* has a stronger antibacterial activity than *G. spinosum* extract.

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References

1. Pugazhendhi, D. Prabakar, J. M. Jacob, I. Karuppusamy, and R. G. Saratale, "Synthesis and characterization of silver nanoparticles using Gelidium amansii and its antimicrobial property against various pathogenic bacteria," *Microbial Pathogenesis*, vol. 114, pp. 41–45, Jan. 2018, doi: https://doi.org/10.1016/j.micpath.2017.11.013.
2. M. Ghazali, H. Husna dan, P. Studi Biologi, and F. Matematika dan Ilmu Pengetahuan Alam, "Diversitas dan Karakteristik Alga Merah (Rhodophyta) pada Akar Mangrove di Teluk Serewe Kabupaten Lombok Timur," vol. 18, no. 1, 2018.
3. H. M. D. Wang, X. C. Li, D. J. Lee, and J. S. Chang, "Potential biomedical applications of marine algae," *Bioresource Technology*, vol. 244. Elsevier Ltd, pp. 1407–1415, 2017. doi: https://doi.org/10.1016/j.biortech.2017.05.198.
4. D. J. Mchugh, "Worldwide distribution of commercial resources of seaweeds including Gelidium," 1991.
5. H. Zhang and R. Tsao, "Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects," *Current Opinion in Food Science*, vol. 8. Elsevier Ltd, pp. 33–42, Apr. 01, 2016. doi: https://doi.org/10.1016/j.cofs.2016.02.002.
6. S. Agustina, N. N. Aidha, and E. Oktarina, "Ekstraksi Antioksidan Spirulina sp. Dengan Menggunakan Metode Ultrasonikasi Dan Aplikasinya Untuk Krim Kosmetik," *Jurnal Kimia dan Kemasan*, vol. 40, no. 2, p. 105, Oct. 2018, doi: https://doi.org/10.24817/jkk.v40i2.4100.
7. H. Masaki, "Role of antioxidants in the skin: Anti-aging effects," *Journal of Dermatological Science*, vol. 58, no. 2, pp. 85–90, 2010, doi: https://doi.org/10.1016/j.jdermsci.2010.03.003.
8. K. Kemer, D. S. J. Paransa, A. P. Rumengan, D. Desy, and M. H. Mantiri, "ANTIBAKTERI DARI BEBERAPA EKSTRAK PADA ALGA COKLAT," 2015.
9. G. N. Rajivgandhi *et al.*, "Anti-oxidant, anti-bacterial and anti-biofilm activity of biosynthesized silver nanoparticles using Gracilaria corticata against biofilm producing K. pneumoniae," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 600, Sep. 2020, doi: https://doi.org/10.1016/j.colsurfa.2020.124830.
10. W. Nuraeni, I. Daruwati, E. M. W, and M. E. Sriyani, "Verifikasi Kinerja Alat Particle Size Analyzer (PSA) Horiba Lb-550 Untuk Penentuan Distribusi Ukuran Nanopartikel," *Prosiding Seminar Nasional Sains dan Teknologi Nuklir*, pp. 268–269, 2013.
11. L. Prasetiowati *et al.*, "Indonesian Journal of Chemical Science Sintesis Nanopartikel Perak dengan Bioreduktor Ekstrak Daun Belimbing Wuluh (Averrhoa Bilimbi L.) sebagai Antibakteri," 2018. [Online]. Available: http://journal.unnes.ac.id/sju/index.php/ijcs
12. V. J. Mohanraj and Y. Chen, "Nanoparticles-A Review," 2006. [Online]. Available: http://www.tjpr.freehosting.net
13. M. M. Modena, B. Rühle, T. P. Burg, and S. Wuttke, "Nanoparticle Characterization: What to Measure?," *Advanced Materials*, vol. 31, no. 32. Wiley-VCH Verlag, 2019. doi: https://doi.org/10.1002/adma.201901556.
14. D. Saryanti *et al.*, "OPTIMASI KARBOPOL DAN HPMC DALAM FORMULASI GEL ANTIJERAWAT NANOPARTIKEL EKSTRAK DAUN SIRIH (Piper betle Linn)," 2019.
15. M. R. Shaik *et al.*, "Plant-Extract-Assisted green synthesis of silver nanoparticles using Origanum vulgare L. Extract and their microbicidal activities," *Sustainability (Switzerland)*, vol. 10, no. 4, pp. 1–14, 2018, doi: https://doi.org/10.3390/su10040913.
16. N. Nino and J. R. Martinez, "Synthesis and antibacterial activity of silver nanoparticles with different sizes Synthesis and antibacterial activity of silver nanoparticles with different sizes," *J Nanopart Res (2008)*, vol. 10:1343–13, no. January 2015, pp. 1343–1348, 2008, doi: https://doi.org/10.1007/s11051-008-9428-6.

17. M. Asrofi, H. Abral, A. Kasim, and A. Pratoto, "XRD and FTIR Studies of Nanocrystalline Cellulose from Water Hyacinth (*Eichornia crassipes*) Fiber," *Journal of Metastable and Nanocrystalline Materials*, vol. 29, no. August, pp. 9–16, 2017, doi: https://doi.org/10.4028/www.scientific.net/jmnm.29.9.
18. F. Boccuzzi, A. Chiorino, and M. Manzoli, "FTIR study of the electronic e ff ects of CO adsorbed on gold nanoparticles supported on titania," *Surface Science*, vol. 456, pp. 942–946, 2000.
19. X. Liu, S. M. Colman, T. Brown, and E. C. Minor, "Estimation of carbonate, total organic carbon, and biogenic silica content by FTIR and XRF techniques in lacustrine sediments," *Journal of Paleolimnology*, vol. 50, no. 1, 2013, doi: https://doi.org/10.1007/s10933-013-9733-7.
20. N. Nagar, S. Jain, P. Kachhawah, and V. Devra, "Synthesis and characterization of silver nanoparticles via green route," *Korean Journal of Chemical Engineering*, vol. 33, no. 10, pp. 2990–2997, Oct. 2016, doi: https://doi.org/10.1007/s11814-016-0156-9.
21. Muchtaromah, E. S. Safitri, P. D. Fitriasari, and J. Istiwandhani, "Antibacterial activities of curcuma mangga val. extract in some solvents to staphylococcus aureus and Escherichia coli," in *AIP Conference Proceedings*, Apr. 2020, vol. 2231. doi: https://doi.org/10.1063/5.0002490.
22. P. Molyneux, "The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity."
23. Wungkana, E. Suryanto, and L. Momuat, "AKTIVITAS ANTIOKSIDAN DAN TABIR SURYA FRAKSI FENOLIK DARI LIMBAH TONGKOL JAGUNG (Zea mays L.)," 2013.
24. K. Murugan, B. Senthilkumar, D. Senbagam, and S. Al-Sohaibani, "Biosynthesis of silver nanoparticles using Acacia leucophloea extract and their antibacterial activity," *International Journal of Nanomedicine*, vol. 9, no. 1, pp. 2431–2438, Mar. 2014, doi: https://doi.org/10.2147/IJN.S61779.
25. H. Zare, "Biosynthesis of Silver Nanoparticles Using Common Sage Extract and Evaluation of Anticancer Activity," *Biomedical Journal of Scientific & Technical Research*, vol. 28, no. 1, May 2020, doi: https://doi.org/10.26717/bjstr.2020.28.004581.
26. Khodashenas and H. R. Ghorbani, "Synthesis of silver nanoparticles with different shapes," *Arabian Journal of Chemistry*, vol. 12, no. 8. Elsevier B.V., pp. 1823–1838, Dec. 01, 2019. doi: https://doi.org/10.1016/j.arabjc.2014.12.014.
27. F. Alfaridz, R. Amalia, K. Kunci,: Flavonoid, and A.-O. Klasifikasi, "REVIEW JURNAL : KLASIFIKASI DAN AKTIVITAS FARMAKOLOGI DARI SENYAWA AKTIF FLAVONOID."
28. N. Panche, A. D. Diwan, and S. R. Chandra, "Flavonoids: An overview," *Journal of Nutritional Science*, vol. 5. Cambridge University Press, Jan. 08, 2016. doi: https://doi.org/10.1017/jns.2016.41.
29. P. Upadhyay, S. K. Mishra, S. Purohit, G. P. Dubey, B. Singh Chauhan, and S. Srikrishna, "Antioxidant, antimicrobial and cytotoxic potential of silver nanoparticles synthesized using flavonoid rich alcoholic leaves extract of Reinwardtia indica," *Drug and Chemical Toxicology*, vol. 42, no. 1, pp. 65–75, Jan. 2019, doi: https://doi.org/10.1080/01480545.2018.1488859.
30. P. Febrianti, W. C. Prabowo, L. Rijai, L. Penelitian, D. Pengembangan, and F. Tropis, "Proceeding of the 5 th Mulawarman Pharmaceuticals Conferences, AKTIVITAS ANTIOKSIDAN DAN TABIR SURYA EKSTRAK DAUN AFRIKA (Vernonia amygdalina Del.)," 2017.
31. P. Taba, N. Y. Parmitha, and S. Kasim, "SINTESIS NANOPARTIKEL PERAK MENGGUNAKAN EKSTRAK DAUN SALAM (Syzygium polyanthum) SEBAGAI BIOREDUKTOR DAN UJI AKTIVITASNYA SEBAGAI ANTIOKSIDAN Synthesis of Silver Nanoparticles Using Syzygium polyanthum Extract as Bioreductor and the Application as Antioxidant," 2019.

32. S. K. Srikar, D. D. Giri, D. B. Pal, P. K. Mishra, and S. N. Upadhyay, "Green Synthesis of Silver Nanoparticles: A Review," *Green and Sustainable Chemistry*, vol. 06, no. 01, pp. 34–56, 2016, doi: https://doi.org/10.4236/gsc.2016.61004.
33. R. Singh, C. Hano, G. Nath, and B. Sharma, "Green biosynthesis of silver nanoparticles using leaf extract of carissa carandas l. And their antioxidant and antimicrobial activity against human pathogenic bacteria," *Biomolecules*, vol. 11, no. 2, pp. 1–11, Feb. 2021, doi: https://doi.org/10.3390/biom11020299.
34. S. B. Oroh, F. E. Kandou, J. Pelealu, D. Pandiangan, and P. Biologi FMIPA Universitas Sam Ratulangi Manado, "UJI DAYA HAMBAT EKSTRAK METANOL Selaginella delicatula DAN Diplazium dilatatum TERHADAP BAKTERI Staphylococcus aureus DAN Escherichia coli INHIBITION TEST OF METHANOL EXTRACT OF Selaginella delicatula AND Diplazium dilatatum AGAINST BACTERIA Staphylococcus aureus AND Escherichiacoli."
35. K. Elumalai, T. N. V. K. v Prasad, and E. David, "Extracellular synthesis of silver nanoparticles using leaves of Euphorbia hirta and their antibacterial activities Extracellular synthesis of silver nanoparticles using leaves of Euphorbia hirta and their antibacterial activities," *J. Pharm. Sci. & Res*, vol. 2, no. September, p. 594554, 2010.
36. Sirajudin and S. Rahmanisa, "Ahmad Sirajudin dan Soraya Rahmanisa | Nanopartikel Perak sebagai Penatalaksanaan Penyakit Infeksi Saluran Kemih MAJORITY I Volume 5 I Nomor 4 I Oktober 2016 I 1."
37. W. Susanah Rita, N. Putu Eka Vinapriliani, and I. Wayan Gede Gunawan, "FORMULASI SEDIAAN SABUN PADAT MINYAK ATSIRI SERAI DAPUR (Cymbopogon citratus DC.) SEBAGAI ANTIBAKTERI TERHADAP Escherichia coli DAN Staphylococcus aureus," 2018.
38. F. WA, "Green Biosynthesis of Silver Nanoparticles Using Marine Red Algae Acanthophora specifera and its Antibacterial Activity," *Journal of Nanomedicine & Nanotechnology*, vol. 07, no. 06, 2016, doi: https://doi.org/10.4172/2157-7439.1000409.
39. S. Purnamasari,; Isnindar, and Ita Armyanti, "UJI AKTIVITAS ANTIBAKTERI INFUSA BIJI BUAH LANGSAT (Lansium domesticum Cor.) TERHADAP Streptococcus pneumoniae."
40. R. Lutfiyanti, "AKTIVITAS ANTIJAMUR SENYAWA BIOAKTIF EKSTRAK Gelidium latifoliumTERHADAP Candida albicans," 2012. [Online]. Available: http://www.ejournal-s1.undip.ac.id/index.php/jpbhp
41. V. K. Sharma, R. A. Yngard, and Y. Lin, "Silver nanoparticles: Green synthesis and their antimicrobial activities," *Advances in Colloid and Interface Science*, vol. 145, no. 1–2. pp. 83–96, Jan. 30, 2009. doi: https://doi.org/10.1016/j.cis.2008.09.002.
42. Nil, A. M. S, Z. A, and M. E. A. A. A. S, "Effects of season on the yield and quality of agar from Gelidium sesquipedale (Rhodophyta) from Mostaganem, Algeria," *African Journal of Biotechnology*, vol. 15, no. 10, pp. 350–355, Mar. 2016, doi: https://doi.org/10.5897/ajb2015.15004.