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BIOSYNTHESIS OF SILVER NANOPARTICLES USING *KAPPAPHYCUS ALVAREZII* EXTRACT

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ABSTRACT

Recently many scientists draw interest in nanotechnology using silver nanoparticles. It is because its promising as an antimicrobial agent. In this reseach, silver nanoparticles were synthesized using seaweed extract (*Kappaphycus alvarezii*) extract as reduction agent with $AgNO_3$ as precursor. Extract of seaweed were gained using solvent extraction in stages method from smallest to highest polarity. n-hexane, etyl asetat and ethanol were the solvent that been used. The rendement of the seaweed extract are 0,22 grams. The formation and stability of the reduced AgNPs in the colloidal solution were monitored by UV–vis spectrophotometer analysis. The results showed that the crude extract is a potention bio-reductor agent for synthesizing silver nanoparticle in mild condition.

INTRODUCTION

The development of nanoparticle technology or called nanotechnology in general can be defined as a technology of excitation, the creation of manipulating objects at the nanoscale. This technology is in full swing for research. Interestingly, the amount of research conducted on metal nanoparticles has shown great resistance to bacteria. One of the popular metals is silver which is getting a lot of attention from researchers because it has properties such as chemical stability, good conductivity, catalyst, antibacterial, antiviral and antifungal. Silver nanoparticles effectively kill bacteria by disrupting the structure of the cell membrane and denaturing nucleic acids so that they interfere with their enzymatic activity [1-3].

The function of silver nanoparticles is very broad. The food industry such as packaging, and electronic components. For biomedical applications; added to wound creams, antiseptics, rags and others (Ahmed et al., 2016). The shape and size of silver nanoparticles are very important in determining their optical, electrical, magnetic, catalyst and antimicrobial properties. The smaller the size, the effectiveness of the large meal because the surface area is greater than the volume. Factors that can affect the particle size in the synthesis are solution temperature, salt concentration, reducing agent and reaction time. There are many methods used to make nanoparticles including photo chemistry, sonochemistry, ultrasonic radiation, solvothermal synthesis, and others. however, these methods are considered ineffective because they can cause high toxicity in addition to being expensive and not environmentally friendly.

Indonesia as one of the largest archipelagic countries is known for its biodiversity. This diversity is widely cultivated, one of which is the seaweed *Kappaphycus alvarezii* which is known in the market as Eucheuma cotonii. In 2010, Indonesia was the first exporter of seaweed in the world, amounting to 3.4 million tons and increasing every year. In 2013 Indonesia became the number one exporting country of Eucheuma cotonii in the world. In Indonesia, the potential for untapped planting areas reaches almost 50%. The total potential of seaweed land that is still available is 769.5 thousand hectares. Currently, the land used is only 384.7 thousand hectares [4,5].

Seaweed as a commodity, has the potential to be developed as well because the cultivation technique of seaweed is

relatively easy and cheap with a very low risk of crop failure, high productivity, and harvesting can be done every 45-60 days or about 4 harvests a year. In addition, the nutritional content of seaweed is very high. in *Kappaphycus alvarezii* seaweed contains alkaloids, saponins, tannins, flavonoids, phenols, terpenoids, coumarin, protein, and carbohydrates [6]. Seaweed (*Kappaphycus alvarezii*) has high economic value. However, so far it has only been sold in the dry form which is used for fermentation. Therefore, this research aims to increase the commodity value by making silver nanoparticles from the extract of the seaweed plant, *Kappaphycus alvarezii*. The use of this extract is very environmentally friendly.

MATERIALS AND METHODS

The materials used in this study wereseaweed *Kappaphycus alvarezii* , n-hexane, ethyl acetate, ethanol, aquades, and AgNO3. While the tools used include hot plate (Corning PC-420D), magnetic stirrer, UV-Vis spectrophotometer (Biochrome Libra S12 series), rotary evaporator (IKA HB10 Basic), vortex, centrifugation tool (hermle Z326K series), knives, scales. digital, micro pipette, measuring pipette, measuring cup, erlenmeyer, spatula, vial bottle, aluminum foil, filter paper, and cup glass.

Solvent Extraction in Stages

50 grams of small pieces of kappaphycus were dissolved in 150 ml of n-hexane (1: 3 w / v) in erlenmeyer, wrapped in aluminum foil and left in the room for three days. Furthermore, it is filtered so that the extract and pulp will be obtained. The waste was extracted again with ethyl acetate and filtered. In the same way the pulp is extracted again with ethanol. The resulting extract was separated from the solvent using a rotary evaporator and a centrifuge.

Nanoparticle Synthesis

The solutions prepared in this study were: first, a solution of silver nitrate (AgNO3), 4Mm in 100mL with 0.068 grams of Ag. Second, a 100mM solution of silver nitrate (AgNO3) in 100 mL with 1.7 grams of Ag. 22 ml of crude seaweed extract solution with one-time dilution of ethanol (0.22: 22, w / v). In this experiment, there are two samples to analyze the synthesis of nanoparticles. The first sample, nanoparticle synthesis was obtained by making a solution of 20 ml silver nitrate, 4mM with a solution of seaweed extract with a ratio (18: 2, v / v). The second sample, nanoparticle synthesis was obtained by making a solution of 50 ml silver nitrate, 100 mM with a solution of seaweed extract with a ratio (45: 5, v / v). Next, ainserted into each solution magnetic stirrer was and placed on a hot plate of 200 rpm, 0ºC. The nanoparticles formed were then characterized using UV-Vis by taking samples at the specified times.

RESULTS AND DISCUSSION

In the Extraction of seaweed, the samples were wrapped in aluminum foil and stored in the room so that the solution was not exposed directly to sunlight which could cause evaporation of the solvent or extract. The factor affecting the solubility of a substance is its polarity with the solvent. A substance in principle will dissolve more easily in a solvent of the same polarity [7].

Table 1. Chemical Constituent and Its Polarity of *Kappaphycus*

Substance	Non polar	Semipolar	polar
alkaloids		✓	
saponins			✓
tannin			✓
flavonoid			✓
phenols		✓	
terpenoids	✓		
coumarin	✓		
protein			✓
carbohydrate			✓

Solvents used are n-hexane, ethyl acetate and ethanol. n-hexane as a non-polar solvent can dissolve terpenoids and coumarin. While alkaloids and phenols are effectively dissolved in semi-polar ethyl acetate. The substances dissolved in ethanol polar solvents are saponins, tannins, flavoloid, protein and carbohydrates [8]. From the data, the con-

tent ofcontains kappapyhcus more polar substances, this can also be seen from the number and color of the seaweed filtrate [9]. In table 2, the most seaweed filtrate was obtained with 142 ml of ethanol as a solvent. In Figure 1, after being left for three days there is a significant color change in the extracts with their respective solvents. The color of the filtrate in ethanol is darker than n-hexane and ethyl acetate. Extract separation and dissolution used a rotary evaporator. The working system of this tool is the same as simple condensation. The solution will be heated according to the boiling point of the solvent, the solvent vapor will enter the cold tube and will condense and come out in the form of a liquid.

Figure 1. Color of the extract solution with the solvent after 3 days (n-hexane, ethyl acetate, ethanol, from left to right respectively.)

Table 2. Results of *filtration* after 3 days

Dry Weight	Solvent	volume	filtrate	Yield
50 grams	n-hexane	150ml	116ml	
50 grams	Ethyl acetate	150ml	132ml	
50 grams	Ethanol	150ml	142ml	0.22 grams

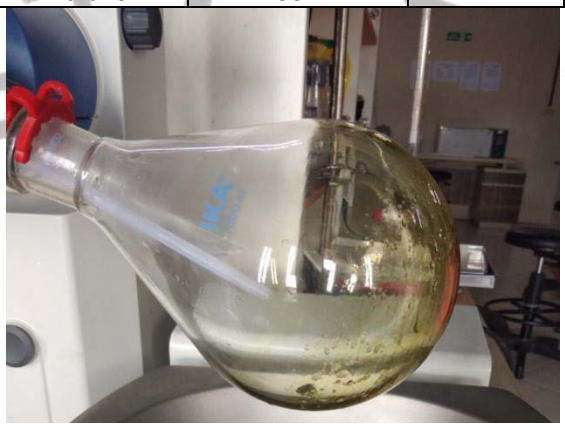

Figure 2. Crude Extract results after being separated from n-hexane solvent

Theextract in Figure 2. Extracted using a spatula. However, the extract obtained is very small because it sticks to the tube which has the same polarity and the seaweed sample is used little. To take the extract, ethanol is added to each tube of the extract. The use of a rotary evaporator was not optimal, so the process of separating the extract and the solvent was carried out using a 5000 rpm centrifuge with a temperature of 24ºC in 15 minutes. This tool serves to separate solutions of different densities [10]. After 15 minutes the precipitated seaweed extract was taken. The weight of the crude seaweed extract obtained was 0.22 grams. The extract obtained was too thick, so it was diluted once with 22 ml of ethanol (1: 100, w / v).

Characterization of UV-Vis spectrophotometry.

The synthesis solution of nanoparticles did not experience a significant color change during 24 hours of data collection. The UV-Vis spectrum measurements performed were used to see the stability of the silver nanoparticles that had been synthesized based on the time function. The stability of silver nanoparticles can be seen from the absorbance

value. Ideally, if the absorbance value shifts to a larger wavelength, the higher it indicates that agglomeration of silver nanoparticles has occurred. The occurrence of agglomeration causes the absorption value to increase and the color of the nanoparticle synthesis changes over time [11].

The wavelength used is from 300-600 with an interval of 50. In Figure 4, the absorbance in each wave tends to decrease and is more unstable. In Figure 4, the first 22 hours show no peaking. After observing it, it turns out that there is still silver aggregate in the synthesis solution. This causes the absorbed UV rays that are not as concentrated as it can also be seen on the graph.

Synthesized silver nanoparticles tend to aggregate to form large sizes. The stability of silver nanoparticles plays a very important role when it is characterized and applied to a product. Nanoparticles tend to experience aggregation (large size). Efforts to prevent aggregates between nanoparticles can be done by adding particle coating materials or molecules. The compounds commonly used to stabilize the size of the nanoparticles are polymers. The addition of polyvinyl alcohol (PVA) to stabilize the size of the successfully performed in previous studies [12-14]

Table 3. Spectrometer data (18: 2, v / v)

Waktu/λ	300	350	400	450	500	550	600
1 hour	0.264	0.114	0.071	0.049	0.038	0.03	0.026
2 hours	0,232	0,113	0,110	0,050	0,030	0.020	0.018
3 hours	0,252	0,129	0,116	0,058	0,038	0,028	0,023
4 hours	0,240	0,122	0,113	0,056	0,038	0,028	0,028
5 hours	0,230	0,104	0,096	0,060	0,035	0,022	0,018
6 hours	0,241	0,114	0,112	0,071	0,047	0,032	0,026
7 hours	0,229	0,105	0,103	0,064	0,040	0,026	0,020
8 hours	0,726	0,322	0,151	0,104	0,079	0,062	0,054

Currently, many types of seaweed have been used as a reducing agent for metal nanoparticles [15-20]. In table 5, we can see several lists of seaweed species that have been researched and their extracts can be used for nanoparticle biosynthesis.

Table 5. A list of some types of seaweed can produceAgNPs

	seaweed species	Nanoparticles
1	*Turbinaria conoides*	Silver
2	*gracilaria birdiae*	Silver
3	*Gracilaria corticata*	Silver
4	*Chaetomorpha antennina*	Silver
5	*Acanthophora specifera*	Silver
6	*Codium capitatum*	Silver
7	*Sargassum cinereum*	Silver
8	*Dictyota bartayresiana*	Silver
9	*Padina Boeregeseni*	Silver
10	*Sargassum longifolium*	Silver

Conclusion

Indonesia is rich in seaweed diversity. With a fast, cheap and environmentally friendly production process, seaweed can be developed as a commodity and its selling value is not only in the form of raw seaweed and food. In this research and previouslyextract *Kappaphycus alvarezii* can be used as a reducing agent for nanoparticles, especially silver. To obtain stable AgNPs, a stabilizer is needed.

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