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Biosynthesis And Characterization Of Silver Nanoparticles Using Cacao (Theobroma cacao L) Skin Extract

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Abstract. Synthesis of silver nanoparticles has been successfully carried out using extracts of cocoa pod (*Theobroma cacao* L). Silver nanoparticles were synthesized using the green synthesis method. Biosynthesis was carried out by mixing 1 mM 40 ml AgNO3 solution with 0.5 ml of cocoa pod husk extract, stirring for 1 minute, after a few minutes the solution will change color from yellowish to dark colored, the color change that occurs shows the ion reduction process so that silver nanoparticles are formed. When silver nanoparticles are formed, the UV-Vis absorption spectrum is at a wavelength between 400-500 nm. Characterization and stability using a UV-Vis spectrophotometer with a time variation of 4 hours, 24 hours, 3 days and 7 days of reduction time. With increasing time the resulting long wave gets higher and bigger the size of the aggregation. Sample (1) shows 446, 446, 446, 447.5 nm, obtained a maximum wavelength of 447.5 nm, repeated repetition is labeled sample (2) shows 447, 446.5, 450, 449.5 nm with a maximum wavelength of 495 , 5 nm seen from the wavelength value tends to be stable so there is no need to add a stabilizing agent. The absorbance value increases with time, the greater the absorbance value, the more nanoparticles formed.

Introduction

In the last few decades, the synthesis of nano-sized particles and characterization has developed due to its wide application especially in the fields of catalysis, biomedicine, optics, and energy (Rasheed, 2017). Making nanoparticles is easier to produce on a large scale so that many are made by chemical methods, but there are some deficiencies in their use that damage the environment such as high energy consumption and hazardous waste, so it is necessary to develop methods for making nanoparticles that are more environmentally friendly (Masakke, 2015).

Nanotechnology is a modern field of science deals with synthesis and application of nanoparticles (NPs), it have a size of 1 – 100 nm. NPs have been studied extensively because of their unique physicochemical characteristics including antibacterial properties, catalytic activity, optical properties, electronic properties, and magnetic properties (Murugan and Shanmugasundaram, 2014).

Due to the completely new or more refined properties of nanoparticles, their applications are growing rapidly in areas such as biomedicine, pharmaceuticals, catalysis, drug delivery, and antimicrobials, etc. Due to miniaturization, changes in physicochemical properties offer the development of new functional attributes of nanomaterials. Nanoparticles are found to be used in various fields such as health care, biomedicine, tissue engineering, gene delivery, drug delivery, optics, mechanics, the food industry, the environment, the aerospace industry, optical devices, and even in every field they have been widely relied on (Ahmed *et al*, 2016).

Research efforts on the use of nanoparticles have increased in this century because they defined chemical, optical, and mechanical properties. As microbes resistant to

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metal ions, antibiotics, and the development of strain resistance growing, metal nanoparticles show the most promise due to their good antimicrobial properties and large surface area to volume ratio, which are proving to be the focus for many researchers (Eun Yun & Gun Lee, 2017)

Chemical reduction still is the most common method used to obtain AgNPs, despite it involves the use of toxic compounds. The integration of green chemistry principles into nanotechnology is essential to promote both, efficiency and safety in the production process (Lopez *et al*, 2017). Moreover, the synthesis of nanoparticles using the plant extract as a reducing and capping agent was more beneficial than microbial synthesis (Arumugam *et al*, 2017)

Metal nanoparticles have been used to increase the non-linearity of molecular research for use in selective imaging of the structure and physiology of nanometric regions in cellular systems, potential applications of waste radioactive bioremediation, sensor technology, opto-electronic recording media and optics (Singh *et al*, 2012)

One of the requirements for advances in nanotechnology is the development of reliable experimental protocols in the synthesis of nanomaterials over a range of high biological composition, size and monodispersity (Vahabi, 2011).

The preparation of nanoparticles using this biosynthetic method involves antioxidant compounds such as flavonoids and vitamin C, which can reduce Ag^+ to Ag0 in the form of Silver Nanoparticles. Flavonoids have a reduction potential of +0.33, this is smaller than the reduction potential of Ag^+ which is +0.80, therefore flavonoids are able to reduce Ag^+ ions to Ag0 in the form of Silver Nanoparticles (Maryani *et al*., 2017)

Studies have shown that biomolecules such as proteins, phenols, and flavonoids not only play a role in reducing the size of the ion to nanomedicine, but also play an important role in the limitation of nanoparticles. In the process of reducing Ag + ions, carried out by a combination of biomolecules such as vitamins, enzymes / proteins, organic acids such as citrate, amino acids, and environmentally friendly polysaccharides, many have started, but have not yet been chemically complex (Rupiasih et al., 2013).

(Baghkheirati, 2016) states that in nanoparticle biosynthesis, plant extracts can be implemented as a source of electrons and stabilizing agents in the chemical reduction reaction process of metal ions in the formation of nanoparticles. The research procedure has a very important role in the synthesis of nanoparticle products.

Biomolecules present in plants such as terpenoids, flavones, ketones, aldehydes, proteins, amino acids, vitamins, alkaloids, tannins, phenolics, saponins, and polysaccharides play an important role in reducing metals. A systematic comparison of the literature, based on the bioreduction capacity of various plant biomass / extracts against various metals under different experimental conditions, has also been carried out. Various instrumental techniques used to characterize nanoparticles are also discussed (Vijayaraghavan, 2017).

The potential of natural resources, especially cocoa in Indonesia, really depends on the production of cocoa produced by farmers and the availability of plantation land. However, the results of cocoa processing are still not optimal for increasing cocoa production. This is due to disruption of pests and diseases and the large number of cocoa farmers who have changed land use for fast-growing plants. The results of cocoa processing produce cocoa pod husk waste (Wijaya et al, 2017).

Cocoa pod husks have not been used optimally. Most of them are still cocoa plantation waste because they are only collected in holes and then dumped or thrown around the cocoa plants. Therefore, it is necessary to find a way to use cocoa pod husks that are more efficient and have higher economic value. Cocoa shell consists mostly of polysaccharides (cellulose and hemicellulose) and lignin, and a small portion consists of phenolic compounds, tannins, purine alkaloids, and cocoa butter. (Jusmiaty et al, 2015)

The development of AgNPs biosynthesis is carried out using cocoa pod skin as a bio-reducing agent in the manufacture of silver nanoparticles to determine the capability and stability of the cocoa pod extract in the biosynthesis process.

Experimental

Material and Methods

Analytical Scales, Magnetic Stirrer, Hot Plate Stirrer, UV-VIS Spectrophotometer, a set of glass tools, scissors, tweezers, electric heating, 10 ml scale pipette, Erlenmeyer, measuring flask, pH / pH indicator. meter, petri dish, and stirring rod

Preparation of AgNO3 Solution

Make a solution of AgNO3 by weighing 0.0425 grams of AgNO3 powder then dissolve and stir slowly. After dissolving, then dilute to a volume of 250 ml into a measuring flask with a size of 250 m. then the AgNO3 solution is shaken until evenly distributed and the solution can be used immediately, when the silver nitrate solution is not used the solution is stored in the refrigerator.

Preparation of Cocoa Pod Skin Extract

Plants used for the biosynthesis process are the waste from the skin of Theobroma cacao (Cocoa). These plants were obtained from Toraja, South Sulawesi. The part of the plant used is the skin of the cocoa pod that has been removed. The skin of the cocoa pods is washed using distilled water then dried until the washing water drains. After the skin of the cocoa pods is drained, it is followed by chopping the skin of the cocoa pods with the same size to weigh 50 grams, after weighing the pods, boil them with 250 ml of aquabides into a 500 ml beaker until boiling, the boiling water is allowed to boil for 5 minutes. The cooking water will be filtered using filter paper when the temperature has reached room temperature. The filtered boiled water can be used directly for the biosynthesis process, if it is not used directly, save the boiled water in the refrigerator.

Biosynthesis of Silver Nanoparticles

Biosynthesis was carried out by mixing 50 ml of pipette AgNO3 solution and 0.5 ml of pipetting cocoa pod skin extract. The AgNO3 solution sample and the cocoa pod skin extract will be mixed and then stirred using a stirrer for 1 minute until the color changes to yellow, the color change that occurs indicates that nanoparticles have been formed.

Characterization of Silver Nanoparticles

After mixing AgNO3 and cocoa pod husk extract and forming a solution of nanoparticles, analysis will be carried out using a UV-Vis spectrophotometer after 4 hours, 24 hours, 3 days, and 7 days. Includes Reproducibility Testing and Aging Effect Testing. Reproducibility Testing is a test of the ability of Silver Nanoparticles to be reproduced with the same characteristics. This test was carried out by making Silver Nanoparticles twice repetitions with the same composition, material conditions and environmental influences. Then perform the analysis using a UV-VIS Spectrophotometer. While the Aging effect test describes the effect of time on the performance and characteristics of Silver Nanoparticles. This test was carried out by looking at the changes that occurred in Silver Nanoparticles, both visually and optically (characterization by UV-VIS spectroscopy) during a certain period.

Result and Discussion

Biosynthesis of Silver Nanoparticles

Biosynthesis was carried out by utilizing cocoa pod husk extract, where the pod husk extract was mixed with 1 mM $AgNO_3$ solution. 50 ml of cocoa pod husk extract then added 0.5 ml of $AgNO_3$ solution. After being mixed, stir it using a magnetic stirer for 1 minute until the color changes occur at 4 hours, 24 hours, 3 days and 7 days. $AgNO_3$ acts as an oxidizer which will undergo a reduction reaction. This is due to the addition of electrons from phenolic compounds to $AgNO_3$. $AgNO_3$ compounds are reduced to Ag + and NO3 - This reaction forms unstable charged silver. The presence of energy in the form of electrons will donate electrons to unstable silver to convert Ag^{+}, Ag^{2+} or Ag^{3+} to Ag (0)

Figure 1. Solution of silver nanoparticles with different times (a). 4 hours; (b). 24 hours; (c). 3 days; (d). 7 days.

The $AgNO_3$ solution mixed with the cocoa pod husk extract solution was initially clear. After the reaction runs for 1 minute with stirring the solution will change color to yellowish and over time the color of the solution will become more and more concentrated, according to (Shankar et al. 2004) the increasing reaction time makes the color of the solution change from clear to yellowish to brown and continues to increase. over time the color change that occurs shows the ion reduction process so that the formation of silver nanoparticles.

When silver nanoparticles are formed, the UV-Vis absorption spectrum is at a wavelength between 400-500 nm. This is because the colloidal characteristics of silver nanoparticles will absorb visible light at 400-500 nm, so that the color seen by the eye is yellow and the absorbance value increases with increasing contact time.

Characterization and Stability of Silver Nanoparticles

After it was known that the color change was an indication of the formation of nanoparticles, then tested using a UV-Vis spectrophotometer to determine the absorbance peak value at the wavelength absorption indicating silver nanoparticles had formed, which ranged from 400-500 nm.

Table 1. Measurement results from samples (1) of biosynthetic silver nanoparticles using cocoa pod extract.

No.	$AgNO_3$: Pod Extract (mL)	Time (h)	Measurement Result	
			Wavelength (nm)	Absorbance (a.u)
1.	50 : 0,5	4	446	0.423
2.	50 : 0,5	24	446	0.482
3.	50 : 0,5	3	446	0.312
4.	50 : 0,5	7	447,5	0.415

From the results of the analysis on the UV-Vis spectrophotometer, it can be seen that the measurement results of the nanoparticles formed over time. The maximum wavelength and absorbance from the results of research and observations can be seen the relationship of absorbance and maximum wavelength to time. The increasing contact time, the greater λmax obtained, meaning that the larger the size of the nanoparticles produced.

Table 2. Measurement results from samples (2) of biosynthetic silver nanoparticles using cocoa Pod extract.

No.	$AgNO_3$: Pod Extract (mL)	Time (h)	Measurement Result	
			Wavelength (nm)	Absorbance (a.u)
1.	50 : 0,5	4	447	0.452
2.	50 : 0,5	24	446.5	0.463
3.	50 : 0,5	3	450	0.380
4.	50 : 0,5	7	449.5	0.491

Due to the reproducibility testing of silver nanoparticles, the biosynthesis was carried out twice or repeated with the same composition and conditions in the first manufacture and it can be seen that time affects wavelength and absorbance value.

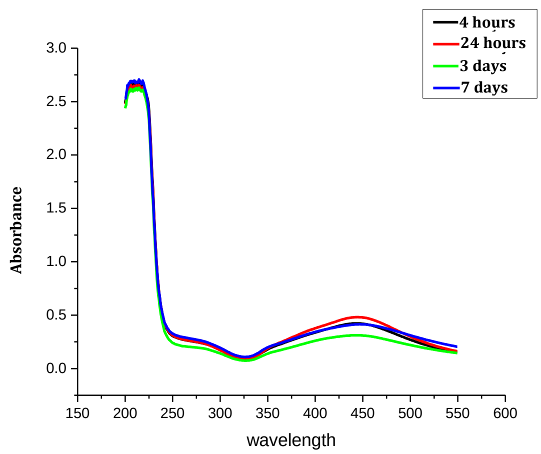

Figure 2. UV-Vis spectrum of silver nanoparticles of biosynthesis (Sample 1).

Figure 2 and 3 shows the UV-Vis absorption spectrum of biosynthetic silver nanoparticles at various times of synthesis in sample (1) and sample (2). Seen in the spectrum image shows the peaks of each absorbance band at wavelengths. The most optimal nanoparticles produced were characterized by the greatest absorbance at a wavelength of 400-500 nm with a single, conical peak. It can be seen that at the peak of the spectrum wavelength (a) it is known that the highest wavelength is at 24 hours followed by 7 days, while in sample (b) it is known that the highest wavelength is at 7 days.

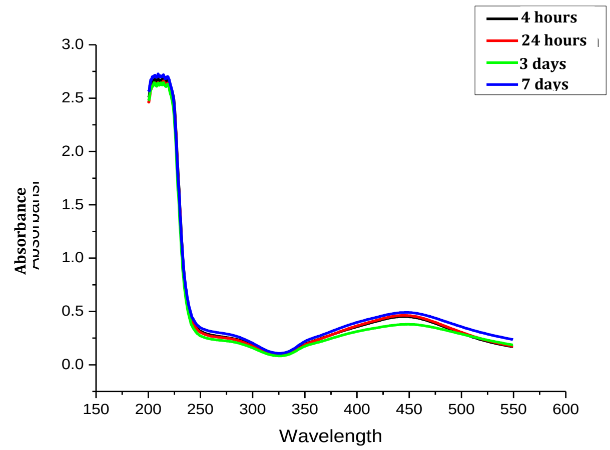

Figure 3. UV-Vis spectrum of silver nanoparticles of biosynthesis (Sample 2).

In Figure 3 the graph of the relationship between time and wavelength in the sample (1) it is known that the wavelengths of the nanoparticles that are formed over time tend to be stable, seen at 4 hours, 24 hours and 76 hours (3 days) the wavelength value of 446 nm and at 168 hours (7 days) the wavelength value became 447.5. The larger the wavelength produced, the greater the aggregation size.

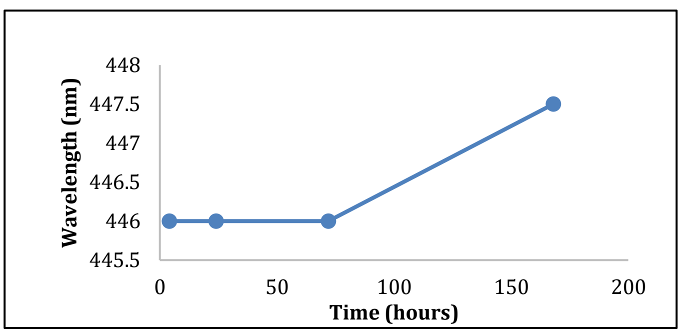

Figure 4. Correlation between time and sample wavelength (1).

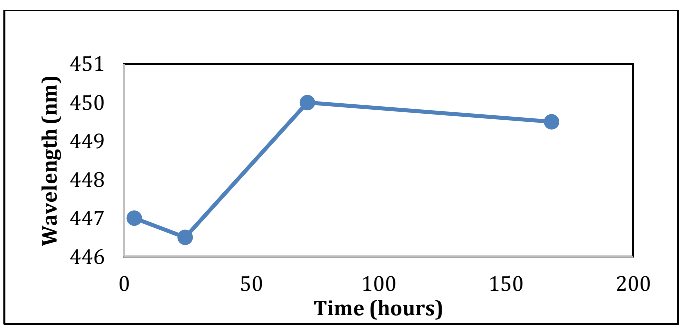

Figure 5. Correlation between time and sample wavelength (2).

In this graph it is known that the wavelengths of the nanoparticles that are formed over time decrease and increase where from 4 hours to 24 hours it begins to decrease but there is no drastic decrease then the wavelength value increases at 76 hours (3 days) and towards the time of 168 days (7 days) there was a decrease but the decline was not drastic. In biosynthesis using plants, it is known that the aggregation occurs fast or there is a drastic increase in wavelength. Therefore, the nanoparticle wavelength tends to be stable.

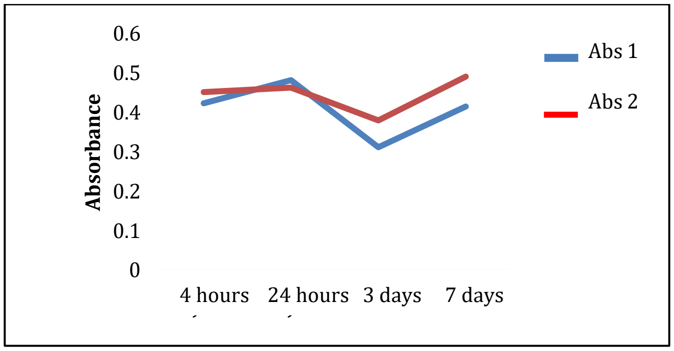

Figure 5. Comparison of the absorbance values of samples (1) and (2).

Figure 5 is a comparison of the absorbance values in samples (1) and (2). It is known that the absorbance value is the number of nanoparticles formed. The reaction time greatly affects the silver nanoparticles formed. It is known that the longer the reaction time, the higher the absorbance value or tends to be stable or the more nanoparticles are formed.

Conclusion

Based on the results and discussion, it can be concluded that silver nanoparticles can be synthesized by a reduction method using an extract of the skin of cocoa pods (Theobroma cacao L). The stability of silver nanoparticles using cocoa bark extract (Theobroma cacao L) tends to be stable by testing the reproducibility of the nanoparticles by making repetitions with the same composition. 447.5 nm day. While the sample (2) 4 hours 447 nm, 24 hours 446.5, 3 days 450 nm and 7 days 449.5. Characterization of silver nanoparticles from AgNO3 biosynthesis with cocoa pod husk extract (Theobroma cacao L) was carried out by aging effect testing where the more time the nanoparticles were formed, and the higher the wavelength value where the wavelength was between 400-500 nm.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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