

## Synthesis of Silver Nanoparticles (NPPs) Using *Xylocarpus Granatum* Fruit Extract and its Application in Heavy Metal Pollution Detection

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### ABSTRACT

Heavy metals are dangerous pollutants because they are toxic, non-biodegradable, and stable. Mercury (Hg) is a type of heavy metal that is often a source of pollution in the aquatic environment. The biosynthesis process of nanoparticles using *Xylocarpus granatum* fruit extract as a bio-reductant has been carried out using the green synthesis method. The research was conducted to synthesize silver nanoparticles (NPP) from *Xylocarpus granatum* fruit extract to detect heavy metals. The optimum condition of NPP was obtained through the comparison of AgNO<sub>3</sub> concentration with extract and reaction time. The optimum NPP produced was at a concentration of AgNO<sub>3</sub> 1 mM 1:1 at a synthesis time of 2 hours. The NPP formed is selective to Hg metal as can be seen from the color change of NPP from dark brown to clear and sensitive to Hg metal at a concentration of 40 ppm. The calibration curve formed obtained a linear regression value of the relationship between the concentration of Hg standard solution and absorbance, namely  $y = -0.01330 + 3.71658x$  with a coefficient of determination ( $R^2$ ) value of 0.99229. Based on the standard curve obtained NPP mangrove fruit extract *Xylocarpus granatum* has an accuracy of 99.229% in predicting the concentration of the sample.

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## INTRODUCTION

Heavy metals are harmful pollutants because they are toxic, non-biodegradable, and stable. One example is mercury (Hg), which has a high level of toxicity.  $\text{HgCl}_2$  is an inorganic mercury compound that is very soluble in water and has a high level of toxicity. The presence of  $\text{Hg}^{2+}$  ions in environments with high concentrations is very dangerous (Azhar, 2019). Mercury waste that contaminates food sources will be very dangerous if it enters the brain through the blood circulation because it can cause mental disorders and even death (Putranto, 2011).

In this regard, nanotechnology or nanoparticle technology continues to develop. With nanotechnology, it has become possible to make materials on the nanoscale (Ristian dkk., 2014). Nanotechnology is a material with a size of 1-100 nm that can function as a detector and also for detecting heavy metals. Nanoparticles with a type of silver nanoparticles have attracted a lot of interest from researchers (Qiao & Qi, 2021). Silver has the ability to carry out several oxidation processes and oxidize other compounds. Generally, silver is preferred because it has low toxicity properties and is more resistant to corrosion. Silver ions are neutral in water, resistant to acids, salts, and weakly alkaline.

Nanoparticles can generally be synthesized by physical methods (top-down) and chemical methods (bottom-up). The physical method involves the decomposition of solid metals into nano-sized particles, while the chemical method produces nanoparticles by chemical reactions (Adriansyah dkk., 2017). However, the process requires high preparation costs and energy requirements, requiring stabilizers that are harmful to the environment (Qiao & Qi 2021). To overcome this problem, an alternative method of silver nanoparticle synthesis known as green syhnthesis was developed. This synthesis method is nanoparticle biosynthesis by utilizing biological sources such as plant extracts, microorganisms, enzymes, as well as biopolymers such as proteins and carbohydrates (Jannah & Amaria, 2020). The use of plant orgasm is preferable because nanoparticles from plant extracts can produce nanoparticles with higher stability and less synthesis time required than microorganisms. This can happen because the biosynthesis process involves biomolecules such as enzymes, proteins, amino acids, vitamins, polysaccharides, and organic acids, which are environmentally friendly but have high chemical complexity (Willian, 2018).

For the detection of mercury metals, nanoparticles must have high selectivity and sensitivity, be stable, and not form aggregations. Therefore, it is necessary to determine the optimal conditions in nanoparticle biosynthesis. Factors influencing nanoparticle biosynthesis include the comparison of  $\text{AgNO}_3$  with plant extracts, the duration of synthesis, and the content of secondary metabolite compounds in plant extracts (Adriansyah et al. 2017). One of the plants that has the potential to be used as a bioreducer for NPP biosynthesis is the fruit *Xylocarpus granatum*. This *Xylocarpus granatum* plant has chemical content in the form of antimicrobial, antifilariasis, CNS-depressant activity, antidiabetic, antidyslipidemic, antimalarial, anticancer, antioxidant, cardiotoxic activity, and antifeedant (Irawan et al., 2023).

This study aims to determine the synthesis process of silver nanoparticles in *Xylocarpus granatum* fruit extract and its use as an indicator in mercury metal analysis using the spectrophotometry method.

## **THEORETICAL REVIEW**

### ***Nanoparticles***

Nanoparticles are a technology that involves the design and utilization of material structures with dimensions at the nanometer scale. The material in question has a size on the nanoscale (1-100 nm). Nanoparticles can be made of several metals such as gold (Au), Silver (Ag), palladium (Pd), and Platinum (Pt). Silver nanoparticles are preferred because of their uniqueness that makes them easy to use and have a high affinity for binding biological molecules. This property allows it to control the growth of bacteria without toxic effects on humans, animals, and plants (Kurniawati et al., 2022).

Silver can undergo some oxidation process and oxidize other substances and is used because of its low toxicity and resistance to corrosion. Silver ions are neutral in water, resistant to acids, salts, and weak bases (Dwistika, 2018).

Silver nanoparticles are stable and can be modified in various fields such as detectors, catalysts, antimicrobial agents, optical sensors. Its small size results in a larger surface area (Dwistika, 2018). The ability of nanoparticles to detect heavy metals is due to the presence of Surface Plasmon Resonance (SPR), which gives silver nanoparticles a high level of sensitivity and selectivity to heavy metal ions (Adriansyah et al., 2017).

### ***Biosynthesis of Silver Nanoparticles***

Nanoparticle synthesis methods are generally divided into two types: physical methods (top-down) and chemical methods (bottom-up). The physics method involves the decomposition of solid metals into nano-sized particles, while the chemical method produces nanoparticles by chemical reactions (Adriansyah et al., 2017). Chemical reduction is carried out by reducing Ag<sup>+</sup> metal ions using reducing agents so that silver nanoparticles are formed. To maintain the stability of silver nanoparticles, stabilizers are often added that prevent aggregation. However, stabilizing agents such as polyvinylalcohols (PVA), polyvinylporolidine (PVP), carbon tetrachloride (CCl<sub>4</sub>), and benzene tend to be difficult to degrade and harmful to the environment.

Therefore, the methods required in the synthesis of nanoparticles must be environmentally friendly and use reductors from biological sources such as plant extracts, microorganisms, enzymes, as well as biopolymers such as proteins and carbohydrates (Jannah & Amaria, 2020). The synthesis of nanoparticles with plants is preferred over microorganisms because the process is simpler and more biologically suitable, making it more suitable in various fields. Most components in plants play an important role in reducing metal salts, as well as acting as stabilizing and capping agents in nanoparticle synthesis (Fitriany et al., 2023).

### ***Xylocarpus granatum***

Mangrove forests are a type of forest ecosystem that grows in tidal areas, especially along protected beaches and river estuaries. The condition of mangrove forests is waterlogged during high tide conditions and there is no inundation at low tide, where the constituent plant communities can tolerate salinity. Mangrove forests are a very important link in maintaining the balance of the biological cycle of aquatic ecosystems (Irawan et al., 2023).

One of the plant species that make up the mangrove ecosystem is *Xylocarpus granatum* J.Koenig which is known by the local names nyirih, niri, nilih, nyireh, nyuru, jombok ivory, buli, white feather, black buli, inggili, siri, nyireh flower, nyiri shrimp, nyiri forest, tree approximately, jomba, banang-banang, nipa, niuniri-kara, kabau, mokmof (Rusila Noor, Y., M. Khazali, 2006). Nyirih is included in the category of true mangroves. Etymologically, the scientific name nyirih, *Xylocarpus granatum* comes from the Greek language, *xylon* means wood refers to its hard fruit like wood, while *carpos* means fruit, then the granatum species designation refers to pomegranate or pomegranate because the shape of the nyirih fruit is similar to a pomegranate (Irawan et al., 2023)

Ecologically, *Xylocarpus granatum* grows in areas affected by tides on riverbanks. In addition, *Xylocarpus granatum* commonly grows on the edges of mangrove ecosystems. This species grows in large groups in tidal areas. *Xylocarpus granatum* generally prefers sandy soils, not silt or clay soils (Prinasti et al., 2020). *Xylocarpus granatum* is usually found in the back mangrove forests adjacent to the mainland (Irawan et al., 2021). *Xylocarpus granatum* can grow up to 10-20 m. This tree has plank roots that spread sideways and the trunk is often hollow, especially old trees. The bark is light brown to yellowish, thin, and peels easily. The leaves are rather thick, arranged in pairs or alone with a size of 4.5-17 cm x 2.5-9 cm. The flowers have two sexes or only females. Flower bunches emerge from the base of the petioles, usually consisting of 8-20 flowers with irregular formations. The fruit resembles a ball (coconut), is brownish-green in color, and can reach a weight of 1-2 kg. The fruit of *Xylocarpus granatum* hangs close to the surface of the hidden ground. Inside this fruit are 6 to 16 large, woody, and tetrahedral in shape. The arrangement of the seeds inside the 10 fruit resembles a puzzle, which makes it called the "puzzle fruit" in English (Rusila Noor, Y., M. Khazali, 2006).

## **METHODOLOGY**

### ***Chemicals, Equipment, and Instrumentals***

The ingredients used are fruit *Xylocarpus granatum*, AgNO<sub>3</sub>, HgCl<sub>2</sub>, and aquades.

The tools used are UV-Vis spectrophotometer, cuvette, analytical balance, flask, hotplate magnetic stirrer, mouthpiece, Whatman filter paper, filter cloth, droppipette, measuring pipette, suction rubber, micropipette, beaker glass, vial bottle, spray bottle, spatula, aluminium foil.

## **Research Procedure**

### *Sample Preparation*

The fruit part used is the flesh that has been cleaned, cut into small pieces and then in the oven at a temperature of 65°C for 5-7 days. Next, it is mashed using a blender. The extract is made by weighing 10 grams of powder and adding 100 mL of aquades then put into a 250 ml beaker in a stirrer with a temperature of 65°C for 1 hour. Then the extract is filtered with a filter cloth and then filtered again using *Whatman paper*. The extract is stored in the refrigerator if not in use. AgNO<sub>3</sub> artens are made by weighing 0.017 grams and 0.034 grams of AgNO<sub>3</sub> crystals, dissolved in 100 mL of aquades to the cut-off mark. The solution is stirred until homogeneously mixed. The solution can be used immediately.

### *Biosynthesis of Silver Nanoparticles*

Biosynthesis was carried out by mixing the extract with 1 mM and 2 mM AgNO<sub>3</sub> solutions in a 1:1 ratio, then heated in sunlight for 5, 15, 30, 45, and 60 minute time variations, as well as for 2 hours and 3 hours. Then, observations were made on color changes. The nanoparticles formed were then measured for absorption using a UV-Vis spectrophotometer in the wave range of 280-700 nm. The maximum detectable absorbance is located in the wave range of 400-500 nm.

### *Silver Nanoparticle Selectivity Test*

The selectivity test was processed by mixing 2 ml of optimum condition silver nanoparticles and 1 ml of standard metal solution HgCl<sub>2</sub> at a concentration of 100 ppm. After that, observations were made on the color change and its absorption was measured. The selectivity of nanoparticles is assessed based on the decrease in absorbance value to Hg metal.

### *Silver Nanoparticle Sensitivity Test*

The sensitivity test was carried out by mixing 2 ml of nanoparticles and 1 ml of Hg metal solution which varied in concentration, namely; 0,2; 0,6; 1; 10; 20; 40; 60; 80; 100; 150; 250 ppm. Then the color change was observed and the absorption was measured using a UV-Vis spectrophotometer. The sensitivity of the nanoparticles was tested by comparing the color difference between nanoparticles that have Hg metal added to them and nanoparticles that do not have Hg metal added.

### *Hg Metal Calibration Curve Making*

Variations in solution concentration 10; 20; 30; 40; 50; 60 ppm have been made, each 1 ml is taken and then mixed with 2 ml of nanoparticle solution. The absorption value was measured with a UV-Vis spectrophotometer at a wavelength of 430 nm to obtain the linear regression line equation and the determination coefficient (R<sup>2</sup>) value.

## RESULTS

### *Results of Biosynthesis of Extracts *Xylocarpus granatum* and $\text{AgNO}_3$*

The principle of green nanoparticle synthesis is to utilize biological materials such as plants as bioreactors. Based on the results of this study, the fruit of the *Xylocarpus granatum* was successfully used as an NPP bio reducer which was characterized by a change in color after the *Xylocarpus granatum* extract was added with  $\text{AgNO}_3$ , a color change from clear yellow, light brown to dark brown as the irradiation time with sunlight increased at 5, 15, 30, 45, 2 hours and 3 hours variations. The color change of the synthesis results can be observed in the following figure:



**Figure 1** Color before synthesis of extract and  $\text{AgNO}_3$  1 mM



**Figure 2** Color after synthesis of extract and  $\text{AgNO}_3$  1 mM

Figure 1 shows that the color before synthesis is light brown, after the addition of  $\text{AgNO}_3$  1 mM in Figure 2, it can be seen that the color changes to dark brown at the time of synthesis 5 minutes using sunlight and the color becomes more intense as the synthesis time increases.



**Figure 3** Color before synthesis of the extract and  $\text{AgNO}_3$  2 mM



**Figure 4** Color after synthesis of extract and  $\text{AgNO}_3$  2 mM

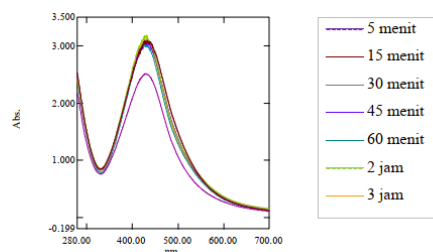
Figure 3 shows that the color before synthesis is clear brown, after the addition of  $\text{AgNO}_3$  2 mM then in Figure 4 it can be seen that the color changes to light brown at the time of synthesis for 5 minutes using sunlight and the color gets darker as the synthesis time increases.

In producing silver nanoparticles with the principle of green synthesis, the main principle is to reduce silver ions ( $\text{Ag}^+$ ) to silver nanoparticles ( $\text{Ag}^0$ ) (Willian, 2023). The more intense the color produced, the more organic compounds are released and the more abundant  $\text{Ag}^+$  is reduced to  $\text{Ag}^0$ . Irradiation with sunlight can help accelerate the reaction rate of silver particle formation. Exposure to ultraviolet (UV) rays from the sun causes the formation of free radicals due to the instability of secondary metabolite compounds. These free radicals then convert  $\text{Ag}^+$  ions into  $\text{Ag}^0$  ions (Notriawan et al., 2023).

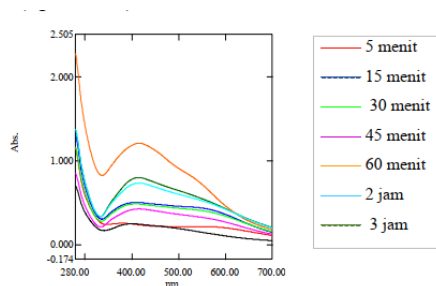
This discoloration process is caused by the reaction of  $\text{AgNO}_3$  solution and *Xylocarpus granatum* fruit extract, which changes color from clear yellow to dark brown after exposure to sunlight. This phenomenon occurs due to *Surface Plasmon Resonance* (SPR) produced by the oscillation of surface plasmon in silver nanoparticles (Willian, 2023). *Surface Plasmon Resonance* (SPR) is a series of vibrations from the transfer of electrons. *Surface* is the polarization of surface charges arising from the oscillation of electrons, while *Plasmon* analogues are caused by the vibration of electrons in plasma gas. When a material interacts with light (electromagnetic waves), oscillations occur on the surface of the material. The oscillating charge can be amplified on the surface of the material, known as *local surface Plasmon resonance* (LSPR) (Irwan R et al., 2016).

### **Best Nanoparticle Biosynthesis**

The formation of silver nanoparticles can be recognized from the color change and increased peak absorbance at 400-450 nm, a characteristic of silver nanoparticles (Prasetiowati et al., 2018). In this study, the reaction contact time and the variation of  $\text{AgNO}_3$  concentration were observed using a uv-vis-spectrophotometer at a wavelength of 280 nm-700 nm to determine the most optimal NPP. The time variations used in this study were 5, 15, 30, 45, 60 minutes, 2 hours and 3 hours with  $\text{AgNO}_3$  concentrations of 1 mM and 2 mM in a 1:1 ratio.



**Figure 5** NPP wavelength curve with  $\text{AgNO}_3$  1mM



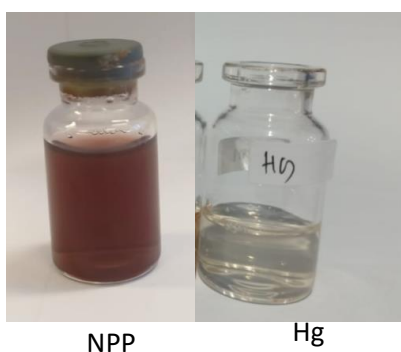
**Figure 6** NPP wavelength curve with  $\text{AgNO}_3$  2 mM

From the NPP absorbance curve, it can be seen that at the 1 mM  $\text{AgNO}_3$  variation, a single peak is formed that is conical and quite high than the 2 mM  $\text{AgNO}_3$  variation. Based on the above data, it can be concluded that the  $\text{AgNO}_3$  variation of 1 mM is the most optimal because it produces a conical absorbance peak with the largest absorption, which is 3,192 at the synthesis time of 2 hours with a wavelength of 430 nm. Ristian et al., (2014) stated that low concentrations of  $\text{AgNO}_3$  can produce stable nanoparticles and have a small nanoparticle size distribution.

This result is supported by research (Willian et al., 2023) where synthesis using *Rhizophora stylosa* fruit extract produces a peak absorption at a maximum wavelength of 430 nm after a synthesis time of 2 hours. Research (Maryani et al., 2017) synthesis using passion fruit extract produced NPP at a wavelength of 461 nm at a synthesis time of 2 hours. These optimum conditions will be used for the manufacture of NPP which will be used for the analysis of mercury metal.

### ***Hg Metal Selectivity Test***

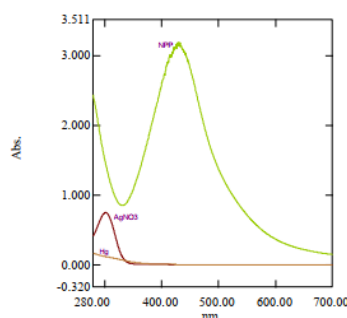
The synthesized nanoparticles were tested for selectivity against  $\text{Hg}_2$  standard metal solutions with a concentration of 100 ppm as much as 1 mL. The selectivity test was carried out to see the ability of nanoparticles to absorb or bind mercury metal (Hg) which is an indicator in the analysis of colorimetry (Adriansyah et al., 2017).



**Figure 7** Results of NPP selectivity test data

Based on figure 7 above, it can be seen that the NPP of *fruit Xylocarpus granatum* is selective for mercury metal. This is evident from the occurrence of a color change after adding 1 ml of mercury solution to the mixture, a very significant color change occurs. The results of this study are supported by the research of Azhar (2019), which shows that nanoparticles added with 1 ml of Hg

metal solution undergo a change in color to become clear. The discoloration of Hg metal occurs because  $\text{Hg}^{2+}$  can oxidize brown  $\text{Ag}^0$  into clear  $\text{Ag}^+$  ions.  $\text{Hg}^{2+}$  (+0.92) metal has a higher standard reduction potential than  $\text{Ag}^+$  (+0.80) so Hg metal tends to act as an oxidizer. So when nanoparticles are reacted with Hg ions, a redox reaction will occur spontaneously (Jannah & Amaria, 2020).



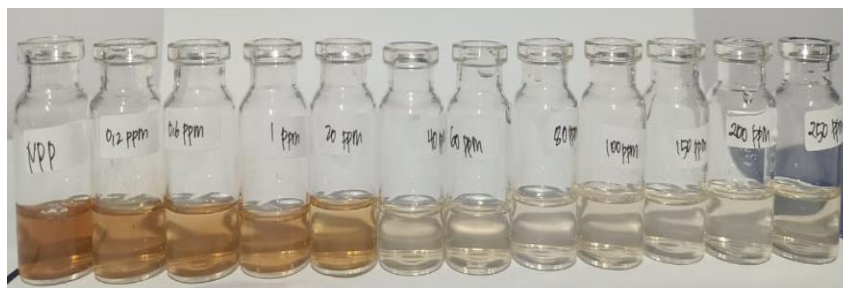
**Figure 8** UV-Vis Hg absorbance curve 100 ppm

From Figure 8, it can be seen that there is a decrease in the peak of absorbance in Hg metal where there is no longer a peak of absorbance and produces a decreasing curve. The loss of absorbance peaks occurs due to the oxidation reaction process between Hg metal and NPP which results in  $\text{Ag}^0$  in NPP changing to  $\text{Ag}^+$ .

Based on the results of the study, it can be concluded that the NPP of *Xylocarpus granatum* fruit is formed selectively to Hg metal. Furthermore, it is followed by an NPP sensitivity test to Hg metal to determine the limit of NPP's ability to detect Hg metal ions spectrophotometrically.

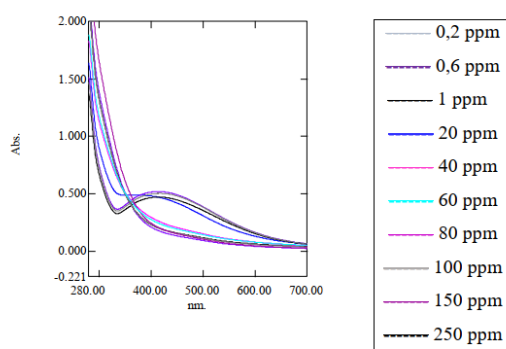
### **Hg Metal Sensitivity Test**

Nanoparticle sensitivity tests were performed to determine the detection limit of Hg metal ions using spectrophotometry. The concentration of  $\text{Hg}^{2+}$  metal ions varied from 0.2 ppm to 250 ppm. Adriansyah et al. (2017) stated that the sensitivity of silver nanoparticles (NPP) was measured by observing the change in color of NPP to clear and the change in absorbance after adding a standard solution of Hg metal, compared to a blank solution (without the addition of Hg metal).



**Figure 1** NPP sensitivity test to Hg metal of various concentrations

Based on Figure 9, it is known that the sensitivity of NPP to mercury metal can be directly seen from the physical reaction of silver ions through the discoloration that occurs from a concentration of 40 ppm. The greater the concentration of  $\text{Hg}^{2+}$  added to the NPP solution, the more the solution will fade and even become clear, because the greater the number of  $\text{Hg}^{2+}$  ions added, the more  $\text{Hg}^{2+}$  ions will oxidize the NPP, this oxidation process is caused by the reduction potential value of  $\text{Hg}^{2+}$  which is greater than the reduction potential of  $\text{Ag}^+$  ions. Therefore, a UV-Vis test was carried out to determine the difference in the absorbance spectrum at each addition of  $\text{Hg}^{2+}$  concentration to NPP.



**Figure 10** Absorbance curve of uv-vis sensitivity test results

From Figure 10, it can be seen that the decrease in absorbance value is greater when Hg is added with a larger concentration so that the color of NPP will be clearer. The absorbance of NPP in  $\text{Hg}^{2+}$  solution tends to decrease with increasing concentration of mercury ions, accompanied by a slight shift in the value of the maximum wavelength. The decrease in absorbance and the not too large shift in the maximum wavelength indicate that the size and amount of NPP in the solution do not change, so the color of the solution is the same as the color of the NPP before the reaction. When the solution becomes clear, the absorbance of NPP is significantly expanded and evenly distributed, indicating that the NPP in the solution is no longer present, because  $\text{Ag}^0$  has been oxidized to  $\text{Ag}^+$ .

### *Hg Standard Metal Calibration Curve*

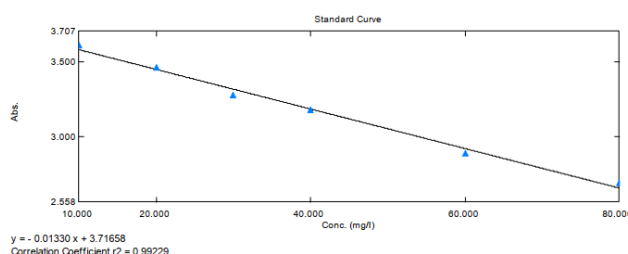
The calibration curve is the main component in performing an element's level test. From the absorbance data obtained, a calibration curve is created to show the relationship between absorbance and concentration. Lambert-Beer's law states that absorbance is directly proportional to concentration, where the higher the concentration, the higher the absorbance.

The standard solution (Hg) is prepared by weighing Hg solids and is made at a concentration of 1000 ppm. The 1000 ppm stock solution is diluted into 250 ppm, 150 ppm, and 100 ppm. Furthermore, a cascade of dilution is carried out to produce a row of standard solutions with concentrations of 0.2 ppm, 0.6 ppm, 1 ppm, 20 ppm, 40 ppm, 60 ppm, and 80 ppm. The absorbance data on the various variations of the concentration of the Hg standard solution measured are listed in the following table:

**Table 1** Peak absorbance of *Xylocarpus granatum* extract

No	Konsentrasi (mg/L)	Absorbansi
1	2.517	3.611
2	3.103	3.463
3	3.098	3.281
4	3.069	3.180
5	3.047	2.884
6	3.192	2.687

Based on Table 1, a standard calibration curve of Hg can be made to determine the linear line equation and the value of the determination coefficient ( $R_2$ ) as follows:



**Figure 11** Standard solution calibration curve Hg

Figure 11 shows the results of the equation of the standard metal calibration curve Hg having a linear tangent of Hg. The curve obtained in accordance with the Lambert-Beer law is that with an increase in concentration, it will produce high absorbance (Nisah & Nadhifa, 2020). From the curve above, the linear regression value of the relationship between the concentration of the standard solution Hg and absorbance is obtained, namely  $y = -0.01330x + 3.71658$  where  $y$  is the absorbance value and  $x$  is the standard Hg. The value of the determination coefficient ( $R_2$ ) is 0.99229. Based on the results above, it means that the standard Hg curve has an accuracy of 99.229% in calculating the concentration of a sample.

## CONCLUSION

Based on the results that have been presented in chapter four, conclusions can be drawn.

1. The bioreductor of *Xylocarpus granatum* fruit extract can be synthesized using the green synthesis method by using silver as a precursor. The results of the UV-Vis  $\text{AgNO}_3$  analysis obtained are the optimum concentration of  $\text{AgNO}_3$  of 1 mM to obtain the best nanoparticles. The resulting nanoparticles are nano colloids with a dark brown color and

absorb *visible radiation* at a wavelength of 430 nm at a reaction time of 2 hours.

2. The selectivity test showed that the nanoparticles were selective to the mercury metal.
3. The sensitivity test showed that the nanoparticles were sensitive to mercury metal at a concentration of 40 ppm with a color change from dark brown to clear.

## RECOMMENDATION

It is necessary to carry out nanoparticle characterization tests to determine the size and size distribution of the silver nanoparticles produced, for example using the Transmission Electron Microscope (TEM) or the Particle Size Analyzer (PSA) test.

## FURTHER STUDY

It is necessary to conduct environmental sample tests to prove the accuracy of the nanoparticles produced and to develop the potential use of *Xylocarpus granatum* in various fields.

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