

Utilization rhizome of jahe merah (*Zingiber officinale Roscoe*) as a bioreductant in the manufacture of gold nanoparticles with addition of polyvinylpyrrolidone 90 (PVP K90) as a stabilizer

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ABSTRACT

The making of gold nanoparticles (AuNPs) by the biosynthetic method using plants as bioreductors is one of the nanoparticle synthesis methods that is being carried out because it is environmentally friendly and non-toxic to the body. Extract rhizome of Red Ginger (*Zingiber officinale Roscoe*) contains secondary metabolites that function as antioxidants that can be used as bioreductor in the making of gold nanoparticles. The research began with the examination of IC50, the manufacture of gold nanoparticles and the addition of PVP K90 stabilizer. The formation of gold nanoparticles was made in several concentrations, namely 600 g/mL, 900 g/mL and 1200 g/mL. Then the results of the synthesis of gold nanoparticles were characterized by visual observation solution color, UV-Vis spectrophotometry, particle size analyzer and polarization microscope. The results obtained are all variations in concentration can form gold nanoparticles, but 600 g/mL was chosen because it has the smallest particle size compared to other concentrations. However, because gold nanoparticles easily and quickly aggregate to form larger particle sizes, in this study a stabilizer in the form of Polyvinylpyrrolidone 90 (PVP K90) was added. PVP K90 was used with various concentrations of 0.5%, 1%, 2% and 5%. Gold nanoparticles that have been added to PVP K90 are characterized by visual observation the color change of the solution, the maximum wavelength with spectrophotometry UV-Vis and particle size distribution with a particle size analyzer, the results obtained are the addition of 1% concentration of PVP K90 met all standards as a stabilizer of gold nanoparticles with extract rhizome of red ginger.

Keyword: Extract rhizome of Jahe Merah (*Zingiber officinale Roscoe*), Biosynthetic, gold nanoparticle, Polyvinylpyrrolidone90 (PVP K90), characterization.

ABSTRAK

Pembuatan nanopartikel emas (AuNPs) dengan menggunakan metode biosintetik menggunakan tanaman sebagai bioreduktor adalah salah satu metode sintesis nanopartikel yang sedang dilakukan karena ramah lingkungan dan tidak beracun bagi tubuh. Ekstrak rimpang Jahe Merah (*Zingiber officinale Roscoe*) mengandung metabolit sekunder yang berfungsi sebagai antioksidan yang dapat digunakan sebagai bioreduktor dalam pembuatan nanopartikel emas. Penelitian dimulai dengan pemeriksaan IC50, pembuatan nanopartikel emas, dan penambahan PVP K90 sebagai stabilizer. Pembentukan nanopartikel emas dilakukan dalam beberapa konsentrasi, yaitu 600 g/mL, 900 g/mL, dan 1200 g/mL. Hasil sintesis nanopartikel emas dikarakterisasi melalui pengamatan visual warna larutan, spektrofotometri UV-Vis, analisis ukuran partikel, dan mikroskopis polarisasi. Hasil yang diperoleh menunjukkan bahwa semua variasi konsentrasi dapat membentuk nanopartikel emas, namun dipilih konsentrasi 600 g/mL karena memiliki ukuran partikel paling kecil dibandingkan dengan konsentrasi lainnya. Namun demikian, karena nanopartikel emas mudah dan cepat menggumpal membentuk ukuran partikel yang lebih besar, dalam penelitian ini ditambahkan stabilizer berupa Polyvinylpyrrolidone 90 (PVP K90). PVP K90 digunakan dengan variasi konsentrasi 0,5%, 1%, 2%, dan 5%. Nanopartikel emas yang telah ditambahkan PVP K90 dikarakterisasi melalui pengamatan visual perubahan warna larutan, panjang gelombang maksimum dengan spektrofotometri UV-Vis, dan distribusi ukuran partikel dengan analisis ukuran partikel. Hasil yang diperoleh menunjukkan bahwa penambahan PVP K90 dengan konsentrasi 1% memenuhi standar sebagai stabilizer nanopartikel emas dengan ekstrak rimpang jahe merah.

Kata Kunci: Ekstrak rimpang Jahe Merah (*Zingiber officinale Roscoe*), Biosintetik, nanopartikel emas, Polyvinylpyrrolidone 90 (PVP K90), karakterisasi.

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INTRODUCTION

Gold is one of the metal elements with the symbol Au which has an atomic number of 79. Gold is characterized by a shiny yellow color, solid, stable in air and water without being oxidized, does not rust and has high electrical conductivity (Nengsih, 2020). Gold ions have attenuation activity against free radicals that cause damage to some parts of cells that cause aging (as anti-aging) (Nengsih, 2020). With the advancement of science, cosmetic manufacturers produce beauty products with gold-based ingredients. This product acts as an anti-aging agent and keeps the skin moist. To maximize the work of gold is made in the form of nanoparticles. Nanoparticles are particles with a size of 1 to 100 nanometers. Material properties can change when the size approaches nano, the effect will increase further (Priyo, 2017). Gold nanoparticles themselves have advantages, namely unique optical properties, biocompatible, easy to reach target cells due to their smaller size and large surface area, not cytotoxic to normal cells and easy to synthesize (Khan et al., 2018). Gold nanoparticles are *anti-aging* because they are able to reduce free radicals (Taufikurohmah et al., 2012), the smaller the particle size, the greater the antioxidant effect (GM et al., 2021).

In general, there are two approaches to the synthesis of nanoparticles namely “*top to bottom*” and “*bottom to top*”. In a *bottom-to-top approach*, nanoparticles are synthesized by chemical (reduction) and biological (using plants, microorganisms) methods by assembling atoms into new nuclei that grow on a nanoscale. While the *top to bottom approach* the material breaks down into fine particles, for example: *grinding, milling, sputtering laser/thermal abrasion* (Tran et al., 2013). However, the physical and chemical methods used are dangerous because of the use of chemicals (reductants) and the price is quite expensive (Ahmed, 2016).

Biosynthesis was chosen because it has the principle of utilizing plants and microorganisms as reducing agents, besides that it is the safest, easiest and relatively inexpensive production cost (Mulfinger et al., 2007). Bioreductants are found in plant extracts that contain secondary metabolites in the form of flavonoids, phenolics and tannins (Priya et al., 2016). Jahe Merah contains secondary metabolites in the form of flavonoids, phenols, terpenoids, and essential oils (Rachmin, 2018), ketones as well as aldehydes and sesquiterpenes as bioreductors (Haryani et al., 2016).

Based on previous research, the best results were found at a concentration of 600 ppm because nanoparticles with a wavelength of 500-600 nm were formed. The characterization of gold nanoparticles with PSA resulted in a size of 369.6 nm with a PI (*Polydispersity Index*) value of 0.298 which indicated the heterogeneity of the particle size (Sari, 2021). Therefore, it is continued with new research with the addition of a stabilizer in the form of PVP K90 in the hope that gold nanoparticles will be more stable. *Polyvinylpyrrolidone* (PVP) is a water and citrate soluble polymer, and is commonly used as a stabilizer for the synthesis of gold and silver metals. Besides functioning as a stabilizer, PVP also functions as a reducing agent as well as a complexing agent (Amir. D., Ricca. RN, Nurul. SEI, Sarina. S., 2021).

This study purpose to utilize natural ingredients, namely rhizome extract of Jahe Merah as a bioreductant in the manufacture of gold nanoparticles by biosynthetic methods, and to determine the effectiveness of adding *polyvinylpyrrolidone* (PVP K90) as a stabilizer in gold nanoparticles.

RESEARCH METHOD

1. Macroscopic Test

Macroscopic testing is done by looking at *simplicia* and *simplicia* powder directly with the naked eye, paying attention to the shape, color, smell and taste (Yuliani et al., 2016).

2. Microscopic Test

Microscopic testing by taking a small amount of *simplicia* powder, placing it on an object glass. Warm over a spirit lamp, taking care not to boil. Cover with a cover glass. Observe *simplicia* with a magnification according to the characteristics of *simplicia* (Yuliani et al., 2016).

3. Phytochemical Screening

a. Polyphenol Identification

A total of 1 g of the sample was heated with water on a water bath, filtered. The filtrate was added with 3 drops of 1% iron (III) chloride solution. Positive results containing polyphenols will form a blue-black green color in the solution (Sulasmı et al., 2018).

b. Identification of Flavonoids

3 mL extract into a test tube, added with 2 mL of H₂SO₄ p.a solution. Positive results in the sample will experience a striking color change in the solution, namely yellow, red or brown (Rachmin, 2018).

c. Identification of Tannins

2 mL of the extract was pipetted into a test tube, 1 mL of 1% FeCl₃ solution was added. Positive results in samples containing tannins will experience a color change to blackish green in the sample solution (Rachmin, 2018).

d. Identification of Alkaloids

The extract was dissolved with a few drops of 2N sulfuric acid and then tested with 2 alkaloid reagents, namely Dragendorph's reagent and Mayer's reagent. The test result is positive if a red to orange precipitate (dragendorf) and a yeowish white precipitate (Mayer) are formed(Rachmin, 2018).

e. Quinone Identification

Simplicia powder as much as 1 g is heated with water on a water bath, then filtered. The filtrate is added 2-3 drops of KOH solution. A positive result in the sample will indicate the formation of a yeow to red color in the solution (Farnsworth, 1966)

f. Identification of Saponins

The test was carried out using the Forth method by inserting 2 mL of the extract into a test tube then adding 10 mL of warm water and shaking it for 30 seconds, observing the changes that occurred. If a 1 cm high foam is formed that does not disappear for 30 seconds, it can be ascertained that the positive sample contains saponins (Rachmin, 2018).

g. Identification of Steroids and Triterpenoids

1 gram of simplicia powder was ground with 20 mL of ether, then filtered. The filtrate was evaporated in a steamer dish to dryness. On the dry residue, the Liebermann-Burchard reagent was dripped. A positive result in the sample will form a purple color (triterpenoid positive) and a green-blue color (steroid positive) (Farnsworth, 1966).

h. Identification of Monoterpenes and Sesquiterpenes

The simplicia powder was ground with ether, then the solution was filtered. The filtrate is evaporated in a vaporizer cup, until it dries. The drying residue was added with vanillin SO₄ reagent. If the sample is positive for monoterpenes and sesquiterpenes, it will produce colors on the dry residue on the evaporating dish (Harborne, 1987).

4. Making Rhizome Extract of Jahe Merah

The rhizomes of Jahe Merah were cleaned of dirt, washed with running water, cut into small pieces, then dried in an oven at 50°C. Furthermore, it was mashed using a blender and sieved with a 60 mesh. Extraction was carried out with 400 grams of rhizome Jahe Merah samples macerated with 1.5 L of methanol for 72 hours, then filtered. The maceration and filtering processes were carried out many times until a clear filtrate was obtained. The filtrate was evaporated with a rotary evaporator at a temperature of 40°C to form a thick extract (Rachmin, 2018).

5. Antioxidant Activity Testing

a. Preparation of 50 ppm DPPH Solution

A total of 5 mg of DPPH was dissolved with 100 mL of pro-analytical methanol in a volumetric flask that had been covered with aluminum foil to avoid light, shaken until dissolved. The test was carried out by pipetting 4 mL of DPPH solution, incubating for 30 minutes at 37°C in a dark room (donein triples). The solution was measured at an absorption waveength of 400-700 nm and the absorbance value of the DPPH solution.

b. Preparation and Measurement of Red Ginger Rhizome Extract Test Solution

Weighed 50 mg of red ginger extract and dissolved with 50 mL of methanol pro-analysis, homogenized. Made 6 different concentrations. In the vial, 2 mL of the test solution was reacted with 2 mL of the obtained DPPH solution, then the vial was wrapped in aluminum foil and incubated for 30 minutes, its absorbance was measured using a spectrophotometer UV-Vis with a maximum waveength of DPPH solution (donein triple).

Calculation of free radical attenuation IC₅₀ (Khan et al., 2018):

$$\%I = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

A calibration curve is made from the calculated % inhibition results so that the equation $y = bx + a$ is obtained with the value of y being replaced by a value of 50 so that the IC₅₀ value of is obtained from the value of x.

6. Preparation of 0.5 mM. HAuCl₄ solution

Weighed 0.05 grams of gold, then dissolved it with a mixture of HCl pa and HNO₃ in a ratio of 3:1 (HCl:HNO₃) / aquaregia solution. Mixing was carried out with the hep of heating until there was no metal left and there was no smoke or evaporation, then the solution was diluted with demineralized aqua in a 500 mL volumetric flask to the mark and homogenized.

7. Gold Nanoparticle Manufacturing

10,000 ppm rhizome extract of Jahe Merah was prepared as the main liquid, then diluted concentrations of 600 ppm, 900 ppm and 1200 ppm were prepared. Each concentration with a volume of 5000 µL into a vial that has been coated with aluminum foil to prevent exposure to light. Then 5000 µL of 0.5 mM HAuCl₄ was added.

8. Gold Nanoparticles with Addition of Polyvinylpyrrolidone90 (PVP K90)

Rhizome extract of Jahe Merah solution was added with PVP K90 then heated at 60°C for 1 minute. Furthermore, HAuCl₄ Solution was added with a ratio of rhizome extract of Jahe Merah solution : 0.5 mM HAuCl₄ Solution (1:1).

Table 1. Formula for Adding Gold Nanoparticles Solution with Polyvinylpyrrolidone 90 (PVP

Formula	Gold Nanoparticles with Elxtract of Jahel Melrah	PVP concelntration K90
F0	600 ppm	-
F1	600 ppm	0.5%
F2	600 ppm	1%
F3	600 ppm	2%
F4	600 ppm	5%

9. Gold Nanoparticle Characterization

a. Visual Observation

Visual observations seen from the color changes that occur in the gold nanoparticle solution were carried out at 5', 10', 15', 30', 60', 90', 3 hours, 5 hours and 24 hours. If there is a change in size to nano, the color of the gold solution will change from pale yeow to purple.

b. UV-Visible Spectrophotometry

A sample of 1000 µL was taken into a disposable cuvette. The blanko used is aqua demineralization. Furthermore, the extract of Jahe Merah solution that had been mixed with 0.5 mM HAuCl₄, was observed for the waveength of the gold nanoparticles in the range of 400–600 nm using a spectrophotometer UV-Vis. The waveength of gold nanoparticles is in the range of 500-600 nm (Taufikurohmah et al., 2012).

c. Polarizing Microscope

The use of a polarizing microscope aims to see the color and size of the particles formed in the gold nanoparticle solution. Samples were taken 1-2 drops on a glass slide, evaporated to dryness, then observed the habit and morphology of the particles with a polarizing microscope. A good result is that no particles can be seen on the results of the polarization microscope because the particle size is already nano-sized.

d. Particle Size Analyzer (PSA)

Samples were taken as much as 2-3 mL, then the sample was inserted into the cuvette, then inserted into the cuvette holder for particle measurement on the PSA tool.

e. Stability Test

Visual observations, observations with UV-Vis spectrophotometry, observations with a polarizing microscope and size observations with a Particle size analyzer at 5', 10', 30', 60', 90', 3 hours, 5 hours and 24 hours in a solution of gold nanoparticles of rhizome of Jahe Merah which has added stabilizer polyvinylpyrrolidone90 (PVP K90).

RESULTS AND DISCUSSION

In this study, the rhizome of Jahe Merah used was obtained from the city of Bogor, West Java. Before being used for research, a determination was made at the Plant Taxonomy Laboratory, Department of Biology, FMIPA, Padjadjaran University. This determination is carried out to ensure the correctness of the types of plants used as needed so that there are no errors in the use of plants. The results of the determination showed that the plant used was a Jahe Merah plant species *Zingiber officinale* Roscoe.

The rhizome of Jahe Merah plant that will be used is examined for characteristics first as a standardization of the material to be used in the study. Among them, characteristics were carried out in the form of macroscopic examination, microscopic examination, phytochemical screening, determination of total ash content, determination of water soluble ash content, determination of acid insoluble ash content and determination of water content.

Macroscopic examination was carried out to visually identify the plants to be used including the color, sme, taste and shape of the rhizome Jahe Merah as we as free fiber and free of fractures. The results of the macroscopic examination of the rhizome of Jahe Merah are listed in table 2.

Microscopic examination was carried out to see the fragments contained in the plant. The results of microscopic observations showed the presence of starch grains, carrier bundles and fibers. The results of microscopic examination of Jahe Merah rhizomes are listed in Table 3.

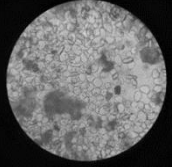
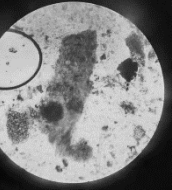

Another characterization examination is the determination of water content and ash content. The water content was checked, namely to determine the water content contained in the simplicia using azeotropic distillation method. The water content that has been obtained is 3.1% ± 2.35, these results are in accordance with the provisions of the water content

contained in the Indonesian Herbal Pharmacopoeia which is less than 10% to avoid the growth of microorganisms during the storage period.

Table 2. Macroscopic Examination of Rhizomes Jahe Merah

Parameter	Results
Color	The outside is brownish red and the inside is yeowish white
Flavor	Spicy
Sme	The sting of ginger
Slice shape	Egg round shape
Free fiber	Sometimes there is free fiber
Break free	There are prominent fibers

Table 3. Test Microscopic on Rhizome of Jahe Merah

Parameter	Results
	Starch Grains
	Carrier File
	fibers

Determination of ash content which includes total ash content, water soluble ash content and acid insoluble ash content was carried out by means of simplicia ashing in a crucible into a furnace at a temperature of 500°C. At this temperature, it can destroy and evaporate organic matter and its derivatives, leaving only minerals and inorganic elements. The results of determining the ash content in Jahe Merah rhizomes are listed in table 4.

Table 4. Results of Determination of Ash Content of Jahe Merah Rhizome Simplicia

Characterization	Results of Determination of Ash Content
Total Ash Content	4.9% ± 0.12
Water soluble ash content	4.6% ± 0.32
Acid Insoluble Ash Content	1.81% ± 0.35

Table 5. Phytochemical Screening of Rhizome Jahe Merah

Secondary Metabolites	Results
Alkaloids	+
Polyphenol	+
Tannins	-
Flavonoids	+
Quinone	+
Saponins	-
Monoterpenes	-
Triterpenoids	+

Description :
 (+) = Detected
 (-) = Not Detected

Phytochemical screening of Jahe Merah rhizome simplicia was carried out to qualitatively determine the content of secondary metabolites contained in rhizome of Jahe Merah. Based on the results of the phytochemical screening, the rhizome of Jahe Merah contains metabolites in the form of alkaloids, quinones, triterpenoids, polyphenols and flavonoids. Flavonoids themselves can be used as bioreductants in the manufacture of gold nanoparticles. The results of phytochemical screening are listed in table 5.

Rhizome of Jahe Merah extract was obtained from the extraction process by maceration using 96% ethanol as solvent. Maceration was chosen because it uses a simple tool and does not use heating, so the simplicia does not decompose and allows a lot of compounds to be extracted. The maceration method was carried out for 3 days with 800 grams of simplicia in 9 liters of 96% ethanol. After the maceration process, the extract was evaporated using a rotary evaporator and a water bath to obtain a thick extract. The results of the thick extract obtained were 201 g with a results percentage of 22.33%, the results were in accordance with the provisions in the Indonesian herbal pharmacopoeia, which was not less than 17.0%.

The ethanol extract rhizome of Jahe Merah was tested for antioxidant activity using the DPPH method (2,2-diphenyl-1-picrylhydrazil). The DPPH method is a simple method with stable free radicals but sensitive to light, pH, oxygen and the type of solvent used. The mechanism for reducing free radicals with this method is that the antioxidant will donate oneelectron / hydrogen atom to the DPPH free radical so that all electrons in the DPPH will pair up to form a stable molecule. The happening of antioxidant activity causes the DPPH solution which was originally purple to turn into a pale yeow color, which indicates that the free radicals have been reduced.

The parameter used to determine the antioxidant activity is based on the IC50 value which is the concentration of antioxidant substances that provide a 50% inhibition percent. Rhizome of Jahe Merah which contains secondary metabolites in the form of flavonoids will donate its hydrogen atom (H+) to DPPH free radicals so that DPPH will become stable. DPPH measured the maximum waveength on a UV-Vis spectrophotometer, which is 517 nm (Khan et al., 2018). The measurement of the maximum waveength of DPPH (2,2-diphenyl-1-picrylhydrazyl) that has been carried out produces a maximum waveength of 516 nm, the results of these observations are shown in Figure 1.

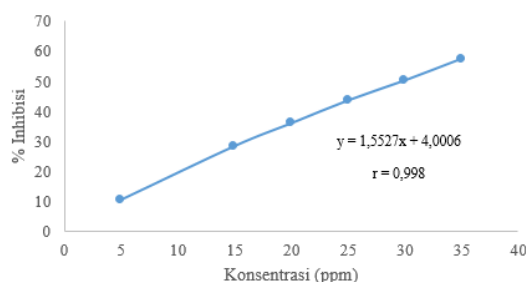


Figure 1. The curve between the concentration of rhizome Jahe Merah and the percentage of inhibition

Based on the picture above, the antioxidant test using the DPPH method obtained a linear regression equation, namely $y = 1.5527x + 4,0006$ with $r = 0.998$ so that the IC50 value of rhizome Jahe Merah was 29.63 g/mL.

Based on these data, the IC50 value of the rhizome Jahe Merah ethanol extract obtained was less than 50 g/mL. So it can be classified as a very strong antioxidant (Tristantini D, et al, 2016). The IC50 value test can be used as a reference in determining the concentration of rhizome Jahe Merah which will be used as a bioreductant in the manufacture of gold nanoparticles.

The manufacture of gold nanoparticles (AuNPs) in this study was carried out by means of biosynthesis using ethanol extract of rhizome Jahe Merah as a bioreductant. The gold nanoparticle synthesis process was carried out by reducing 0.5 mM HAuCl₄ solution with a natural bioreductant, namely ethanol extract of rhizome Jahe Merah in a ratio of 1: 1.

In the process of forming gold nanoparticles, HAuCl₄ which is a weak acid in solution forms an equilibrium system, so that the number of AuCl₄⁻ ions produced is not much so that it is possible to reduce it to Au⁰. When in the form of ions, they will repe each other due to the influence of similar charges, but when it is reduced to Au⁰ then the charge will be neutral and allow Au atoms to interact with each other through bonds between metals to form nano-sized clusters (Amiruddin, 2013).

Preparation of 0.5 mM HAuCl₄ solution using gold precious metal dissolved in aquaregia. Aquaregia which has strong acid properties is able to dissolve metal compounds including gold metal. In its manufacture, a redox reaction occurs where the uncharged Au ion (Au⁰) is oxidized to trivalent Au (Au³⁺) and tetrachlorate anion (III) is formed, with the following reaction equation (Amiruddin and (Amiruddin, 2013) :






The reaction produces NO₂ and H₂, so it is necessary to heat to remove the gas. When heated on the hotplate, visible explosions are NO₂ and H₂ gases. The dissolution was carried out in a fume hood until all the solid gold dissolved into a clear yeow color. Another purpose of heating is so that the residual acid in the aquaregia that may still exist in the solution can be completely evaporated, marked by no more gas being formed. Furthermore, the HAuCl₄ solution was diluted with 500 mL aquademineralization to obtain a 0.5 mM HAuCl₄ solution. The use of aquademineralization is to reduce the content of other metals that may be dissolved. Then observations were made with UV-Vis spectrophotometry to see the maximum waveength entering the range of 200-400 nm because the color of the solution was not yeowish so it did not fall into the visible color range. The maximum waveength obtained is 316.4 nm. This waveength is the initial waveength before the addition of a bioreductant.

Synthesis of gold nanoparticles has the aim of making gold into nanometer size with the hep of a bioreductant in the form of rhizome Jahe Merah. The active compound in rhizome Jahe Merah will reduce gold (Au³⁺) to gold (Au⁰). The manufacture of gold nanoparticles using concentration variations, namely 600 ppm, 900 ppm and 1200 ppm. In the manufacturing process, incubation is protected from light because it avoids the oxidation reaction of the HAuCl₄ solution by light which will affect the results of the formation of gold nanoparticles.

The formation of gold nanoparticles is characterized by a visual (qualitative) color change. The color change that occurred was from a colorless solution to a burgundy to purple solution, and was observed for 24 hours. The formation of color can be seen in table 6.

Table 6. Visual Results of Red Ginger Rhizome Gold Nanoparticles Color after 60 Minutes

Picture	Concentration
	600 ppm
	900 ppm
	1200 ppm

At the beginning of mixing the colorless solution, then incubated at room temperature and in a dark place and protected from light. This protection is doneso that the results are not disturbed by light which can oxidize HAuCl₄. From all concentration variations, gold nanoparticles can be formed because within 60 minutes there has been a change in color from colorless to purple. However, for further research, a concentration of 600 ppm was chosen because at 900 ppm and 1200 ppm it seemed to have an excessive purple color. The formation of the purple color indicates that there has been a reduction process in the gold nanoparticle solution from Au³⁺ to Au⁰.

Visual observation can not be used as the main result, there must be support for other tests. The next test is to use UV-Vis spectrophotometry to see the maximum waveength in the gold nanoparticle solution. In general, gold nanoparticles when observed on UV-Vis spectrophotometry will give a maximum waveength in the range of 500-600 nm (Sekarsari & Taufikurrohmah, 2012).

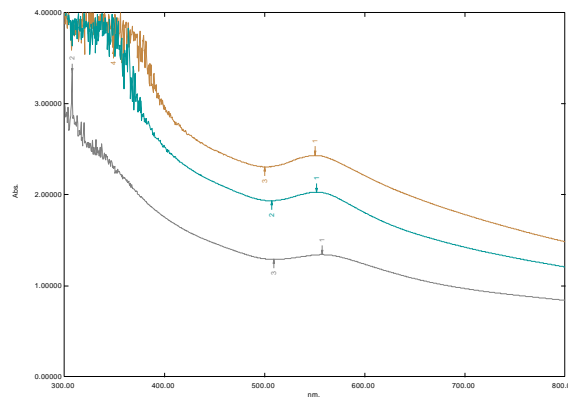


Figure 2. Results of the Absorption Spectrum of Gold Nanoparticles

Figure 2 shows the results of the absorption spectrum of gold nanoparticles with the addition of rhizome Jahe Merah extract. In all concentration variations, namely 600 ppm, 900 ppm and 1200 ppm, the maximum wavelengths were 557.5 nm, 552 nm and 550 nm. This indicates that the formation of gold nanoparticles is true at this concentration variation, because the results obtained fall into the wavelength range of gold nanoparticles, namely 500-600 nm. The difference in the maximum wavelength produced is caused by the gold nanoparticles having different size distributions, so that the size reading at the wavelength will have different results.

To further prove the results of the formation of nanoparticles, it was checked using a polarizing microscope at 100x magnification. By evaporating the solution on top of the object glass, you can see spots that indicate the solution is not water but nanoparticles formed. The results obtained are in the form of nothing else that can be seen, meaning that these particles have become nano-sized, so they cannot be read by a polarizing microscope. Polarizing microscope itself aims to see micro-sized particles.

Gold nanoparticles were subjected to another characterization test using Particle Size Analyzer (PSA) to determine the value of the particle size distribution and the polydispersity index of the particles. PSA itself has a principle that is based on the scattering of laser light by particles experiencing Brownian motion and measuring the diversity of particle sizes. In the results listed in Table 7, the results obtained at all concentration variations have entered the nanoparticle size range, namely the range of 1 -1000 nm (Tiyaboonchai, 2003) and are included in the range of Polydispersity Index values, which are in the range of 0.2-0.7.

Table 7. Results of Particle Size and Polydispersity Index Formation of Gold Nanoparticles

No	Formula	Concentration (ppm)	24 Hours Time Results		
			Max (nm)	Particel Sizel (nm)	Max (nm)
1	F1	600	560	185.9	0.265
				201.8	0.319
				195.6	0.365
2	F2	900	546.5	311.8	0.266
				257.7	0.442
				218.2	0.426
3	F3	1200	547.5	242.2	0.280
				252	0.352
				254.3	0.378

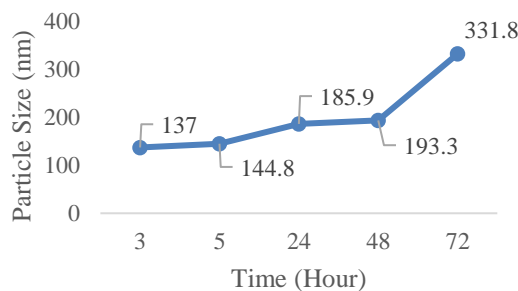


Figure 3. Growth of Gold Nanoparticle Size on Storage Time

Gold nanoparticles tend to aggregate due to strong interparticle forces so that the particles will approach and gather to form clusters (GM et al., 2021). In this study, the concentration of 600 ppm was chosen, because at a concentration of 600 ppm it has a smaller particle size value compared to other concentrations. The growth of gold nanoparticles was observed at a concentration of 600 ppm, to prove the occurrence of aggregation or changes in particle size during the storage period. The results of observations of the growth of gold nanoparticles at a concentration of 600 ppm are shown in Figure 3.

Due to the growth of particle size / aggregation occurred during the storage period, a polymer, namely polyvinylpyrrolidone (PVP) was added as a stabilizer for gold nanoparticles. PVP itself is a stabilizer that is able to prevent aggregation of gold nanoparticles and acts as a typical surfactant in the growing gold metal core (Zhao et al., 2015).

Gold nanoparticles with a concentration of 600 ppm were added as stabilizer in the form of PVP K90 with various concentrations of 0.5%, 1%, 2% and 5%. After adding the stabilizer, it was incubated in a closed place and protected from light. Furthermore, it is characterized by using a Particle Size Analyzer (PSA) to determine the distribution of particle size, polydispersity index and zeta potential.

Characterization with PSA was carried out for 28 days with routine checks on day 0 (60 minutes), day 1, day 7, day 14, day 21 and day 28.

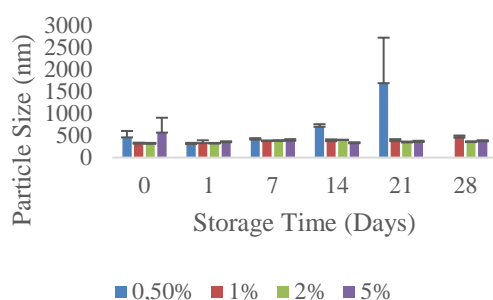


Figure 4 Graph of the relationship between the concentration of the addition of PVP K90 and storage time on the formation of gold nanoparticles on particle size

In figure 4, it can be seen in the nanoparticles with the addition of 0.5% PVP K90 there was a significant increase in particle size due to the aggregation of gold nanoparticles. So that on the 21st day the particle size reached 1689.73 nm because the particle size had formed large clusters and exceeded the size of the nanoparticles. This occurred allegedly due to the addition of PVP K90 concentration which was too small so that it was not sufficient to stabilize the red ginger rhizome gold nanoparticle solution. However, at concentrations of 1%, 2% and 5%, although there was an increase in particle size, it was not significant and was still in the nanoparticle size range of 1-1000 nm.

The value of the polydispersity index (PI) shows a broad or narrow picture of the particle size distribution. The smaller the PI value, the more stable the nanoparticle formula will be (Ambarwati & Rustiani, 2022). Judging from the results of the measurement of the PI value listed in appendix 7, for the concentration of 0.5% addition of PVP K90 there was a significant increase on the 21st day, namely 1.41 which showed the nanoparticles were unstable and aggregation occurred because the PI value was already high and did not enter in the range of PI values, namely 0.2 - 0.7. Meanwhile, the concentrations of 1%, 2% and 5% remained within the range of PI values for nanoparticles.

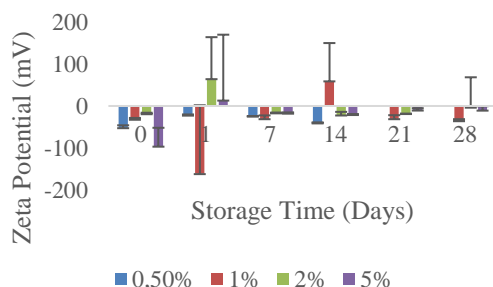


Figure 5. Graph of the relationship between the concentration of addition of PVP K90 and storage time on the formation of gold nanoparticles on the zeta potential

Zeta potential is a parameter of electric charge between colloidal particles. The higher the zeta potential value (negative or positive), the more stable it will be and prevent flocculation, namely the incorporation of colloids from small to large ones. Values higher than ± 30 will have good stability, and values lower than ± 30 will be unstable until aggregation occurs (Ambarwati & Rustiani, 2022). After measuring the zeta, the results obtained are listed in Appendix 7 and Figure

5. The addition of PVP K90 with a concentration of 0.5%, 2% and 5% has a zeta potential that tends to be small, which is smaller than ± 30 mV so that the gold nanoparticles are not stable enough and do not fall into a good range of zeta potential values. While the addition of PVP K90 concentration of 1% tends to be stable because it has a zeta potential value that is more negative than -30 mV and more positive than +30 mV.

CONCLUSIONS AND SUGGESTIONS

Based on the research that has been done, it can be concluded that the rhizome of Jahe Merah (*Zingiber officinale* Roscoe) can be used as a bioreductant to synthesize gold nanoparticles. The IC₅₀ Value of rhizome Jahe Merah obtained is 29.64 g/mL which means it has very strong antioxidant activity. At a concentration of 600 ppm, gold nanoparticles can already be formed after it has been confirmed by conducting nanoparticle characterization. To prevent aggregation of nanoparticles, a stabilizer in the form of PVP K90 was added. The concentration variations of the PVP K90 stabilizer used were 0.5%, 1%, 2% and 5%. After going through all the characterizations of nanoparticles in the form of visual color observations, maximum wavelength using UV-Vis spectrophotometry, measurements with a Particle Size Analyzer (PSA) and confirmation with a polarizing microscope, the best concentration that meets the standards for all characterizations is gold nanoparticles with the addition of 1% PVP K90 stabilizer. more stable than other concentrations.

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