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Application of Gold Nanoparticles Synthesized with Malva Nut Gum as Reducing Agent for scFv Conjugates in a Colorimetric Chikungunya Virus Detection

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ABSTRACT

Gold nanoparticles have good surface plasmon properties in the presence of free electrons on their surface. The presence of free electrons on the surface of the plasmon causes gold nanoparticles to be able to bind to various kinds of biomolecules and proteins, including single-chain variable fragments (scFv). In this study, gold nanoparticle conjugates with Chikungunya antiviral scFv have been successfully prepared with the aim of developing a colorimetric method for rapid detection of envelope protein Chikungunya virus (E2 CHIKV). The manufacture of gold nanoparticles was carried out using malva nut gum and trisodium citrate as reducing agents. The results showed the formation of the AuNP-scFv conjugate which was characterized by a shift in the maximum wavelength for gold nanoparticles synthesized using malva nut gum as a reducing agent from 532.89 nm to 536.62 nm. Similarly, for gold nanoparticles synthesized using trisodium citrate as a reducing agent, the maximum wavelength shifted from 528.99 nm to 531.95 nm. The detection result of E2 CHIKV protein using the AuNP-scFv conjugate has not shown a positive result.

INTRODUCTION

Chikungunya is an infectious disease caused by the Chikungunya virus (CHIKV), which is transmitted through the bite of the Aedes mosquito. Complications of Chikungunya disease that result in death are rare, but it is still possible to cause bleeding [1]. Chikungunya virus can be recognized from its antibody. Usually, antibodies against CHIKV begin to develop at the end of the first week after infection and peak levels are reached after 3-5 weeks after the onset of the disease [2]. This causes chikungunya disease to take a long time to detect.

Rapid detection of the chikungunya virus can be done using a diagnostic kit. There are already commercially available diagnostics, namely the OnSite® Chikungunya IgM Rapid Test and the SD Bioline CHIK IgM rapid test. The diagnostic kit was tested for sensitivity to CHIKV in Indonesia which belongs to the Asian phylogroup. The results of his research showed that the OnSite® Chikungunya IgM Rapid Test had a sensitivity of 20.5%, while the SD Bioline CHIK IgM rapid test had a sensitivity of 50.8% [14]. From these results, it can be concluded that these imported rapid tests that have been sold commercially do not have a high sensitivity to Indonesian Chikungunya originating from the Asian CHIKV phylogroup. Therefore, it is necessary to develop a diagnostic kit that can detect the Indonesian Chikungunya virus which belongs to the Asian CHIKV phylogroup.

Rapid detection systems that are

sensitive to Chikungunya antigen and can easily be performed using antibody fragments, such as the single-chain variable fragment (scFv). Gold nanoparticles conjugated to scFv as a detector for anti-rabbit IgG have high sensitivity so it can be used as a promising detection tool for protein analysis and clinical diagnostics [8]. For example, the AuNP conjugated with PVY antibody has high effectiveness and sensitivity in detecting Potato Virus Y [15].

Gold nanoparticles are suitable for biological applications because of their unique physical and chemical properties. Currently, the application of gold nanoparticles has developed on a more varied biomedical scale such as use as a biosensor, immunoassay, genomics, photo-thermolysis of cancer cells, detection of microorganisms, drug delivery for chemotherapy, optical imaging, monitoring of biological tissues and cells by utilizing resonance scattering. Gold nanoparticles are very significant for use in the biomedical field because they are easy to manufacture, have the ability to interact with living cells or tissues and are not disturbed by other materials, and have low toxicity. Gold nanoparticles have surface convenience for bioconjugation with various bioreceptors due to their extraordinary optical properties related to localization of plasmon resonance. The use of gold nanoparticles as an immunosensor can increase sensitivity and accuracy, and is easily made into a mini-sized detection device [3].

Based on this, in this study the detection of Chikungunya virus epitope envelope 2 protein was carried out using scFv-anti CHIKV. The use of scFv in this study is expected to be a detection system that has high effectiveness and sensitivity. Chikungunya virus will bind specifically to scFv-anti CHIKV through antigen-antibody recognition. ScFv conjugated with gold nanoparticles (AuNP). There are 2 synthesized gold nanoparticles, namely using the general procedure with trisodium citrate as a reducing agent and with another alternative using a slot gel reducing agent. The use of clay gel in the synthesis of gold nanoparticles can produce AuNP which is more stable for a long time, namely 23 days [4]. The use of the placer is expected to produce a more stable conjugate. The conjugation of scFv with gold nanoparticles can be characterized using a UV-Visible spectrophotometer. The AuNP-scFv conjugate will bind to the CHIKV envelope protein 2 (E2 CHIKV) to detect the Chikungunya virus. These antigen-antibody interactions can be identified by carrying out colorimetric tests. The positive results of this test are indicated by a change in the color of the test sample and a shift in the maximum wavelength.

MATERIALS AND METHOD

Materials and Reagents

The materials used in this study were gold powder, aqua regia with a ratio of HCl and HNO_3 (3:1), malva nut, trisodium citrate 5%, distilled water, DDH_2O, L-Rhamnosa, Luria Bertani medium (LB), Terrific Broth medium (TB), *E. coli* BL21 (DE3) bacteria containing recombinant plasmid, kanamycin, TSE buffer, scFv anti-CHIKV (0.07 mg/mL), 5mM PBS solution pH 7 .2, 0.1 mg/mL BSA solution, and 0.1 mg/mL CHIKV E2 protein.

Preparation of Malva Nut Gum Extraction

The malva nut gum was made through an extraction process by soaking 95 g of dried fruit in 800 mL of water and heating it for one hour until the fruit cracked and released the gel. The gel is separated from the skin and seeds, then drained until no more water drips. After that, the gel was blended until smooth and used as a bioreductor as well as a bio stabilizer in the synthesis of gold nanoparticles [4].

Preparation of 0.5 Mm Tetrachloroauric Acid ($HAuCl_4$) Solution

A 0.5 mM $HAuCl_4$ solution was prepared by dissolving 0.0493 g of gold powder in 4 mL aqua regia with the assistance of stirring and heating until bubbles appeared, then added DDH_2O to a volume of 500 mL and homogenized. Next, 0.5 mM $HAuCl_4$ solution was shaken and can be used immediately. Aqua regia is a solution made from a mixture of concentrated hydrochloric acid (HCl) and concentrated nitric acid (HNO_3) in a ratio of 3:1 [5].

Synthesis of Gold Nanoparticles Using Malva Nut Gum Reductor

20 mL of 0.5 mM $HAuCl_4$ solution was added to 3 mL of the tempayang gel. The synthesis of gold nanoparticles was carried out by heating using a microwave for 60 seconds. The color of the solution will change to cherry red [6].

Synthesis of Gold Nanoparticles Using Trisodium Citrate Reducing Agent

10 mL of 0.15 mM $HAuCl_4$ solution was heated at 100°C. After the solution boils, quickly add 1 mL of 5% trisodium citrate while stirring constantly using a magnetic stirrer. The solution was heated again for ±5 minutes until the color of the solution changed to reddish-purple [7].

Characterization of Gold Nanoparticles

To confirm the results of the gold nanoparticles that have been made, characterization was carried out using UV-Visible spectrophotometry to determine the maximum wavelength of the nanoparticles [5].

Production of scFv Anti-Chikungunya Recombinant Protein Using Terrific Broth (Tb) Medium

Production of anti-chikungunya scFv recombinant protein was carried out using Terrific Broth (TB) medium added with kanamycin (an antibiotic) to inhibit the growth of bacteria other than E. coli BL21 (DE3) which contains recombinant plasmid. Production was carried out by growing 200 µL of E. coli BL21 (DE3) colonies containing recombinant plasmid in 5 mL of liquid LB medium containing kanamycin. Then incubated at 37°C at 150 rpm for 18 hours. A total of 5 mL of culture media that had been incubated was put into TB medium with a volume of 250 mL. Then added kanamycin with a concentration of 100 mg/mL as much as 250 µL. The media was incubated at 37°C with a speed of 150 rpm. The absorbance was checked with a UV-Visible spectrophotometer at a wavelength of 600 nm until an absorbance of 1 was obtained. When the absorbance was 1, 615 µL L-Rhamnosa 1 M was added to the medium. The medium was incubated again for 48 hours at 37°C at 150 rpm. The fermented medium was centrifuged at 3000 g for 20 minutes. The pellet and supernatant are separated. The pellet was resuspended in TSE buffer and vortexed until the pellet was homogeneously suspended to determine protein expression in the periplasm. Next, the suspension was centrifuged at 20,000 g for 30 minutes. The supernatant resulting from centrifugation was separated. The supernatant and pellet were then characterized using SDS-PAGE to determine the presence of expressed scFv protein. Next, the concentration of scFv was determined using the ImageJ application with the BSA standard.

Gold Nanoparticle Conjugation With scFv

Gold nanoparticles with a volume of 1 mL were centrifuged at 13000 rpm for 40 minutes. The pellets formed were separated from the supernatant. The pellet was resuspended with an equal volume of PBS solution (5 mM pH 7.2). A total of 72 µL scFv (0.07 mg/mL) was added to the solution. Then the solution was incubated for 12 hours. The solution was centrifuged again at 4500 rpm at 4°C for 30 minutes. The pellets formed were resuspended using PBS solution (5 mM pH 7.2). This conjugation is carried out using a procedure that has been carried out based on research [8]. This procedure is carried out for each gold nanoparticle that has been made in the previous stage. To confirm AuNP-scFv conjugation, the solution was characterized using a visible spectrophotometer.

CHIKV E2 Protein Detection

CHIKV E2 protein with a concentration of 0.1 mg/mL was reacted with AuNP-scFv conjugate while stirring. The volume ratio of E2 CHIKV protein and AuNP-scFv conjugate reacted was 1:1. After 30 minutes, a color change will occur which can be measured using a visible spectrophotometer [8].

Bovine Serum Albumin Test

Bovine Serum Albumin (BSA) solution with a concentration of 0.1 mg/mL reacted with AuNP-scFv conjugate while

stirring. The volume ratio of the BSA solution and the AuNP-scFv conjugate that was reacted was 1:1. After 30 minutes, the maximum wavelength of the solution was measured using a visible spectrophotometer.

RESULTS AND DISCUSSION

Malva Nut Gum Extraction

The malva nut gum was extracted by soaking the nuts in water and heating it for 1 hour. During this process, the nuts crackled, and the gel expands. The gel is then separated from the skin, then drained until there is no more water left. Then the gel was mashed using a blender. The refined gel was then used as a reducing agent as well as a biostabilizer in the synthesis of gold nanoparticles.

Preparation of 0.5 mM Tetrachloroauric Acid ($HAuCl_4$) Solution

Gold is a metal that is difficult to dissolve, therefore the dissolution process is carried out using aqua regia which is a suitable solvent to dissolve gold metal. In the preparation of $HAuCl_4$ solution, a redox reaction occurs, namely AuO ions are oxidized to Au^{3+} ions and tetrachloroaurate (III) anions are formed [6]. The following is the reaction that occurs during the process of making $HAuCl_4$ solution:

$Au_{(s)} + HNO_{3(aq)} + 4HCl_{(aq)} \rightarrow HAuCl_{4(aq)} + NO_{2(g)} + 3H_2O_{(l)} + H_{2(g)}$

In the preparation of the $HAuCl_4$ solution, H_2 gas is produced which is characterized by the appearance of bubbles when the reaction takes place and NO_2 gas which is brown in color. The process of making $HAuCl_4$ solution is carried out with the help of heating and stirring. The heating process is needed because the reaction cannot take place spontaneously and to accelerate the release of H_2 and NO_2 gases, so that the $HAuCl_4$ solution does not contain nitrogen oxides. Heating was carried out until all the gold powder had completely dissolved into a clear yellow solution and the remaining acid in the aqua regia had evaporated which was indicated by the absence of NO_2 gas being formed and white vapor with a pungent odor which was a sign of chlorine gas being formed so that inside the solution is left only $HAuCl_4$ and water. The solution is diluted using DDH2O to reduce the content of other metals that may be dissolved in the $HAuCl_4$ solution.

Synthesis of Gold Nanoparticles using Malva Nut Gum Reductor

Malva nut gum can be used as a reducing agent in the synthesis of gold nanoparticles because it has characteristics similar to gum arabic. Gum arabic is known as a harmless natural polysaccharide, obtained from the bark and stems of the acacia tree. Gum arabic consists of 3 parts, namely arabinogalactan (AG), arabinogalactan protein (AGP), and glycoprotein (GP). Because the amino acids that are mostly considered as reducing agents are contained in gum arabic, especially in the AGP and AG moieties, gum arabic has been proven to be used as a bioreductor in the synthesis of gold nanoparticles [9].

Gum arabic has a hydrophilic polysaccharide framework and a network of hydrophobic glycoproteins which will form an effective layer around the gold nanoparticles. Binding of the complex structure of polysaccharides and proteins to gum arabic can be effective and irreversibly bound to gold nanoparticles in the protein matrix to produce non-toxic and stable nanoparticle construction [10].

One of the components in malva nut gum that can act as a reducing agent is basorin. Au^{3+} ions in $HAuCl_4$ solution will be reduced by reducing compounds contained in the gel where it forms Au0 ions. Synthesis of gold nanoparticles using biological materials tends to take a long time. The synthesis of nanoparticles using a malva nut gum takes 4 hours [4]. Therefore, in this study the heating was carried out using a microwave because it only required a much shorter time.

Microwave synthesis of nanoparticles with bioreductors is a very promising method. Microwave heating requires shorter reaction times, reduces energy consumption and better results are obtained by preventing agglomeration in the formation of particles [11]. The $HAuCl_4$ solution that had been added to the gel was heated in a microwave. In this study, the Samsung ME731K microwave was used. Heating the $HAuCl_4$ solution that had been

added to the gel was carried out for 60 seconds.

In the synthesis of gold nanoparticles using a malva nut gum reductor with a concentration of 3% w/v, gold nanoparticles with a size of 74-106 nm are produced and can be stable for 23 days [4]. From the results of the synthesis in this study, red gold nanoparticles were obtained as shown in **Figure 1**. The maximum wavelength of gold nanoparticles synthesized using a malva nut gum reductor was 532.89 nm with an absorbance of 1.360. The resulting gold nanoparticles have a longer stability of 53 days which is indicated by the color of the solution which does not change and there is no precipitate.

Figure 1. Result of Synthesis of Gold Nanoparticles with Malva Nut Gum Reducing Agent

Synthesis of Gold Nanoparticles Using Trisodium Citrate Reducing Agent

In this study, the synthesis of gold nanoparticles was also carried out with the reducing agent triodium citrate as a comparison with AuNP which had been synthesized with the reducing agent in the jar gel. Gold nanoparticles are generally unstable, so a stabilizer is needed to prevent aggregation in the formation of gold nanoparticles. The reducing agent trisodium citrate used in this study acts as a stabilizer because it has the properties of a buffer solution. The negative charge of the citrate ion will be adsorbed on the surface of the gold nanoparticles resulting in a repulsive force between the gold nanoparticles due to the negative charge around the surface. This can prevent the aggregation of gold nanoparticles. Trisodium citrate is a salt derived from a weak acid (citric acid) and a strong base (NaOH) as shown in the reaction equation below:

$Na_3C_6H_5O_{7(s)} + 3H_2O_{(aq)} \rightarrow C_6H_8O_{7(aq)} + 3NaOH_{(aq)}$

Citric acid obtained from the decomposition of trisodium citrate acts as a weak reducing agent, so it can reduce Au3+ ions to Au0. Au^{3+} ions will repel each other due to the influence of similar charges. After the Au^{3+} ions are reduced to Au^0, the Au charges combine and interact with each other through metal-to-metal bonds to form nanometer-sized particles. The equation for the reaction that occurs can be seen as follows:

$HAuCl_{4(aq)} + 3C_6H_8O_{7(aq)} \rightarrow 2Au_{(aq)} + 3C_5H_6O_{5(aq)} + 8HCl_{(aq)} + 3CO_{2(g)}$

The results of gold nanoparticles synthesized using trisodium citrate produced a reddish-purple solution as shown in Figure 2. The maximum wavelength of gold nanoparticles synthesized using trisodium citrate as a reducing agent was 528.99 nm with an absorbance of 0.462.

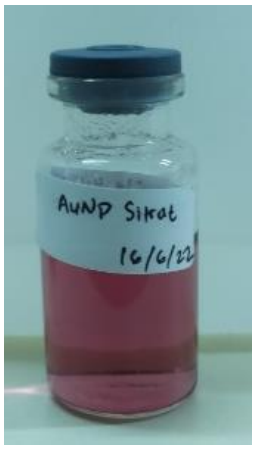

Figure 2. Result of Synthesis of Gold Nanoparticles with Trisodium Citrate Reducing Agent

Production of Anti-Chikungunya scFv Recombinant Protein Using Terrific Broth (TB) Media

The scFv used in this study was produced using E. coli BL21 (DE3) bacteria containing the plasmid pd861-pelb-scfv-chikv-linkc. The plasmid used was composed of kanamycin resistance gene,

ori, strong ribosome binding site (RBS), rhaBAD (rhamnose-inducible) promoter, signal peptide, and scFv anti-Chikungunya virus coding gene (scFv-CHIKV). The production of scFv protein in this study was carried out using Terrific Broth (TB) media. The addition of kanamycin in the media and production process functions as an antibiotic so that the growth of other bacteria does not occur. The choice of kanamycin as an antibiotic because the plasmid contains kanamycin antibiotic selection markers. TB media that had been added to the transformant E. coli BL21 (DE3) culture and kanamycin were incubated and checked for absorbance at a maximum wavelength of 600 nm until OD = 1 was obtained. The achievement of absorbance 1 indicated that bacterial growth had entered the exponential phase and the optimum time for inducer was added. The added L-Rhamnosa functions as an inducer to help protein production. The scFv plasmid contains a signal peptide to secrete proteins into the periplasmic membrane. Peptide signals will help deliver proteins from the cytoplasm to the periplasm and then outside the cell (extracellular). The process of taking scFv is also carried out from pellets produced using TB media. The pellet was resuspended in TSE buffer to increase the extracellular osmotic pressure so that the cells could shrink and secrete proteins in the bacterial periplasm. Characterization of the formed scFv protein was carried out using SDS-PAGE.

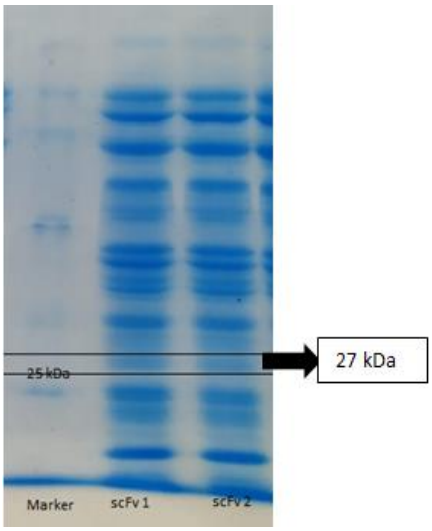

Figure 3. SDS-PAGE result of *scFv* Pellet by TB Medium (periplasma fraction)

Based on **Figure 3**, the SDS-PAGE results show a band around 27 kDa. This indicates that scFv has been expressed in the periplasm of E. coli grown in TB media. Protein concentration was determined using ImageJ with the BSA standard curve shown in **Figure 4**.

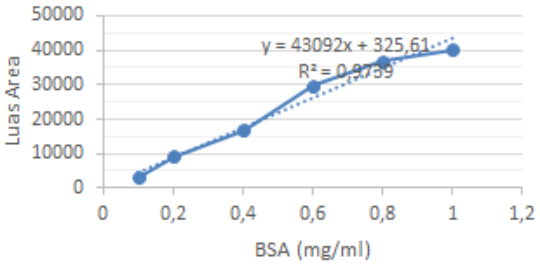

Figure 4. BSA Standard Curve

The obtained SDS-PAGE scFv band was determined for its area using the ImageJ application. Obtained area of 3,876. The area results were then entered into the BSA Standard curve in **Figure 4**, so that an anti-CHIKV scFv concentration of 0.07 mg/mL was obtained.

Gold Nanoparticle Conjugation with scFv

Gold nanoparticles have excellent surface plasmon properties. Surface plasmons are electron oscillations on metal surfaces that allow the binding of various bioreceptors such as proteins, DNA/RNA, enzymes, and antibodies [12]. Gold nanoparticles have free electrons on their surface so they can interact with various biomolecules such as scFv.

Conjugation was carried out between the gold nanoparticles that had been produced from synthesis using a reducing agent in a malva nut gum or trisodium citrate solution with scFv anti-CHIKV (0.07 mg/mL). The scFv used in this study was anti-CHIKV scFv which had been engineered to contain cysteine in its linker amino acid composition. This is to allow scFv to bind to the surface of the gold nanoparticles. Cysteine is a non-essential amino acid that contains a sulfhydryl (-SH) group.

The binding that occurs between gold nanoparticles and scFv is included in a chemical interaction, namely chemisorption through thiol derivatives as shown in **Figure 5** C). Free thiol or sulfhydryl groups on scFv will form covalent bonds with

electrons on the surface of gold nanoparticles [13].

Identification of the occurrence of conjugation between AuNP and scFv can be seen by the change in color of the solution and a shift in the maximum wavelength.

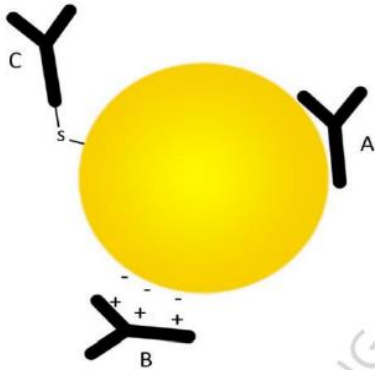

Figure 5. Interactions between Biomolecules and Gold Nanoparticle Surfaces. A) Hydrophobic Interactions B) Ionic Interactions C) Covalent Bonds [13].

Malva Nut Gum scFv-AuNP Conjugates

From the results of the AuNP conjugate analysis process with scFv anti-CHIKV 0.07 mg/mL, there was a shift in the maximum wavelength from 532.89 to 536.62 nm. In addition, there was a color change from red to purple. This shows a positive result that the conjugation process between the AuNP and scFv has been successful.

Trisodium Citrate scFv-AuNP Conjugates

From the results of the analysis process on the synthesized AuNP conjugate using trisodium citrate with scFv anti-CHIKV 0.07 mg/mL there was a shift in the maximum wavelength from 528.99 nm to 531.95 nm and the color changed to purple. This showed a positive result that there was a conjugation process between AuNP synthesized using trisodium citrate as a reducing agent and scFv.

Based on these results, it can be said that conjugation of gold nanoparticles synthesized using malva nut gum or trisodium citrate reducing agents can occur. However, when compared between the two, the use of gold nanoparticles synthesized from a malva nut gum reducing agent has a better stability. The gold nanoparticles synthesized using a malva nut gum reducing agent remained stable for more than 30 days, whereas using trisodium citrate produced AuNP which was only stable for a few days. This can be seen from the color of the conjugate produced by the AuNP trisodium citrate reductant which is slightly cloudy due to the aggregates formed. Therefore, the use of AuNPs synthesized using a malva nut gum reducing agent is more promising for use in the conjugation process. However, it is still necessary to optimize the conjugation conditions so that the resulting conjugate does not differ much from the initial (red) gold nanoparticles.

CHIKV E2 Protein Detection

The E2 protein is the main antigenic site for antibody binding. The E2 protein used in this study has a concentration of 0.1 mg/mL. In this study, the maximum wavelength shift was obtained for the gold nanoparticle conjugates synthesized using a malva gum reductor with scFv, which was 529.60 nm and the color changed to faded gray. The gold nanoparticle conjugate synthesized using citrate reductor, the maximum wavelength shifted to 529.11 nm and the color changed to faded gray.

These results indicate that the shift in the maximum wavelength that occurs does not indicate a positive result between the reaction of the AuNP-scFv conjugate with E2 protein. This is because the added E2 does not interact with scFv but instead interacts with gold nanoparticles because they have a much stronger binding capacity with proteins. The surface of the gold nanoparticles should be blocked first so that the parts that are not bound to scFv cannot bind to other compounds.

Bovine Serum Albumin Test

The results of measurements using a visible spectrophotometer in this test showed that there was a shift in the maximum wavelength that was the same as in the detection of E2 protein. The BSA test using gold nanoparticle conjugates synthesized using a malva nut gum reducing agent with scFv showed a maximum wavelength at 528.86 nm. The color change of the solution is also the same as the detection of E2 protein, which is faded gray.

Tests with conjugates of AuNP synthesized using trisodium citrate as a reducing agent also showed the same thing. The BSA test shows a shift in the maximum

wavelength to 529.23 nm. The color change of the solution is also the same as the E2 protein detection.

Based on these results it can be concluded that neither E2 nor BSA could yet be distinguished for its interaction with the anti-CHIKV AuNP-scFv conjugate. This can happen because on the AuNP surface there are still free electrons which can also bind with other compounds so that both E2 and BSA added with the conjugate actually interact with the gold nanoparticles and form other aggregates. BSA can bond covalently to the gold nanoparticle surface because it also contains cysteine. Therefore, the positive result of the maximum wavelength shift is due to the interaction between BSA and gold nanoparticles. Based on these results, it is necessary to add a blocking agent when AuNP has been conjugated with scFv, so that AuNP will not bind to other molecules or proteins.

The change in maximum wavelength and overall color in this study is shown in **Table 1** and **Table 2**.

Table 1. Changes in Maximum Wavelength of Gold Nanoparticles From Synthesis to Final Testing

Type of AuNP	Max. Wavelenght (nm)		
	After Synthesized	Preparation + *scFv*	
		+E2	+BSA
AuNP - Malva nut gum	532,891,360 Abs	536,62 0,212 Abs	529,60 0,279 Abs	528,86 0,188 Abs
AuNP - Citrate	528,990,462 Abs	531,950,245 Abs	529,11 0,225 Abs	529,230,233 Abs

Table 2. Color Changes of Gold Nanoparticles From Synthesis to Final Testing

Type of AuNP	Color Indicator		
	After Synthesized	Preparation + *scFv*	
		+E2	+BSA
AuNP-Malva nut gum	 Red Cherry	 Faded Purple	 Faded Gray	 Faded Gray
AuNP-Citrate	 Reddish Purple	 Faded Purple	 Faded Gray	 Faded Gray

CONCLUSION

The interaction of gold nanoparticles synthesized using the malva nut gum as reducing agent and conjugated with scFv anti-CHIKV showed positive results marked by a shift in the maximum wavelength from 532.89 nm to 536.62 nm and a change in the color of the solution from red to purple. Likewise, the interaction of gold nanoparticles synthesized using trisodium citrate as a reducing agent conjugated with scFv anti CHIKV showed positive results marked by a shift in the maximum wavelength from 528.99 nm to 531.95 nm and a change in color of the solution from reddish purple to purple. Detection of E2 protein using conjugates of AuNP synthesized with trisodium citrate as a reducing agent and a placer gel has not shown positive results. The use of gold nanoparticles synthesized with malva nut gum has better stability than trisodium citrate. This can be seen from the conjugate solution and after

adding E2 and BSA the color remains clear, whereas using AuNP synthesized with trisodium citrate produces a slightly cloudy solution because aggregates are formed.

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REFERENCES

[1] Tusila, E. D, "GambaranPengetahuan Masyarakat Dewasa Tentang Penyakit Chikungunya," 2016.

[2] Organization, W. H. (2017) Chikungunya. [Online]. https://www.who.int/news-room/factsheets/detail/chikungnya

[3] Raghavendra, R., Kanthadeivi, A., Satesh, K., & Aarrthy, M, "Diagnostic and Therapeutic Application of Gold Related papers," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 6, no. (0975–1491), p. 74–87, 2014.

[4] Lidya, B., Santoso, B., Yusuf, M., Alisyahbana, B., & Subroto, T, "Application of malva nut gum as bioreductor and stabilizer on gold nanoparticle synthesis : Preparation of Chikungunya immunoassay biosensor," p. 15, 2018.

[5] Wahyuni, M., & Sudrajat, M. S, "Skrining Aktivitas Bioreduktor beberapa Ekstrak Tanaman Asia untuk Sintesis Nanopartikel Emas," 2017.

[6] Rahma, D. E, "Sintesis Nanopartikel Emas Menggunakan Bioreduktor Ekstrak Daun Ketapang (Terminalia catappa) dengan Irradiasi Microwave," *UIN Maulana Malik Ibrahim*, 2019.

[7] Herlan, S., Anung, P., Hotman, L., Rien, R., Mujinah, Dede, K., Witarti, Hambali, & Abdul, M., "Sintesis Nanopartikel Emas Menggunakan Reduktor Trisodium SItrat," p. 021, 2014.

[8] Liu, Y., Liu, Y., Mernaugh, R. L., & Zeng, X, "Biosensors and Bioelectronics Single chain fragment variable recombinant antibody functionalized gold nanoparticles for a highly sensitive colorimetric immunoassay," vol. 24, p. 2853–2857, 2019.

[9] Wu, C. C., & Chen, D. H, "Facile green synthesis of gold nanoparticles with gum arabic as a stabilizing agent and reducing agent," *Gold Bulletin*, vol. 43, no. 4, pp. 234-240, 2010.

[10] Pujiyanto, A., Mujinah, M., Lubis, H., Witarti, W., Setiawan, H., Kurniasih, D., Purnamasari H, P., Sutriyo, S., & Mutalib, A, "Penggunaan Gum Arab Sebagai Stabilisator Nanopartikel Emas (AuNP) Untuk Diagnosis dan Terapi Kanker," 2014.

[11] Iravani, S., H. Korbekandi, S. V. Mirmohammadi & B. Zolfaghari, "Synthesis of Silver Nanoparticles: Chemical, Physical and Biological Method," *Research in Pharmaceutical Sciences*, vol. 9, no. 6, pp. 385-406, 2013.

[12] Wang, S., Li, W., Chang, K., Liu, J., Guo, Q., Sun, H., Jiang, M., Zhang, H., Chen, J., & Hu, J, "Localized surface plasmon resonance-based abscisic acid biosensor using aptamer functionalized gold nanoparticles," pp. 1-13, 2017.

[13] Jazayeri, M. H., Amani, H., Pourfatollah, A. A., Pazoki-toroudi, H., & Moghadam, B. S, "Various methods of gold nanoparticles (GNPs) conjugation to antibodies," *SBR*, 2016.

[14] Kosasih, H., Widjaja, S., Surya, E., Hadiwijaya, S. H., Butarbutar, D. P., Jaya, U. A., Nurhayati, Alisjahbana, B., & Williams, M, "Evaluation of two IgM rapid immunochromatographic tests during circulation of Asian lineagechikungunya virus," *Southeast Asian Journal of Tropical Medicine and Public Health*, vol. 43, no. 1, p. 55–61, 2012.

[15] Manzila Ifa, Tri Puji Priyanto & Fikri Hidayatulloh, "Konjugat Poliklonal

Antibodi Nanopartikel Emas untuk Deteksi Potato Virus Y," *Jurnal Fitopatologi Indonesia*, vol. 16, no. 2, pp. 87-93, Maret 2020.