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OPTIMIZATION OF GREEN SYNTHESIS OF GOLD NANOPARTICLES USING CHILI FRUIT EXTRACTS (*Capsicum* Sp.)

Anggraeni In Oktavia[1,2], Retno Mastuti[1], A'liyatur Rosyidah[3], Estri Laras Arumingtyas[1]*

[1] 1Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, East Java, Indonesia, 65145
[2] Health Polytechnic of Putra Indonesia Malang, Malang, Indonesia
[3] Vaccine and Drug Research Center, National Research and Innovation Agency (BRIN), Bogor, Indonesia

e-mail:
[1] inoktaviaa@gmail.com
[2] mastuti7@ub.ac.id
[3] aliyatur.rosyidah@brin.go.id
[4] laras@ub.ac.id

ABSTRACT

The use of plant extracts as reductants in green synthesis of gold metal nanoparticles (AuNPs) requires optimal conditions of temperature, extract concentration and pH. The objective of this research is to identify the optimal synthesis conditions for producing gold nanoparticles (AuNPs). The reducing agents employed are extracts from three varieties of chili peppers: Katokkon, Cakra Hijau, and Paprika Merah. Variations of plant extract concentrations of 10%, 15%, and 20%, incubation temperatures of 25°C, 37.5°C, and 50°C, and pH levels of 5, 7, and 9. The optimization formula design was determined using Box-Behnken, Design Expert version 13.0. The formation of AuNPs was indicated by a change in the color of the solution from yellow to pink to purple. Surface plasmon resonance was observed in the range of 200-800 nm using a microplate reader. Chili, Katokkon, Cakra Hijau and Red Paprika extracts were able to synthesize Au into AuNPs. The optimal formula for synthesizing AuNPs using Katokkon and Red Paprika extracts at a concentration of 10% while Ckra Hijau at a concentration of 15%. The incubation temperature used was 37.5°C for Katokkon extract and 25°C for Cakra Hijau and Red Pepper extract. The pH level used for the synthesis of AuNPs was pH 5 for all chili pepper extracts.

Keywords: Chili, Green Synthesis, Gold Nanoparticle.

Introduction

Nanoparticles are defined as ultrafine particles with dimensions between 1 and 100 nanometers (Fadillah & Arumsari, 2022). The development of nanoparticle-based drug formulations has the opportunity to overcome and treat diseases because of the properties possessed by nanoparticles, which are able to improve the drug delivery system (Alaqad & Saleh, 2016). One of the most rapidly developed nanoparticles is gold nanoparticles (AuNPs). Gold is an inert and oxidation-resistant substance, and AuNPs can be applied to the health field including drug transportation, chemical and biological imaging, catalysis, therapy, and diagnostics (Mikhailova, 2021).

Nanoparticle synthesis is achieved through two main approaches: top-down and bottom-up methods. The top-down approach involves breaking down large materials into nanoscale particles, while the bottom-up method builds nanoparticles from molecular precursor (Ijaz et al., 2020). Various synthesis techniques, such as chemical reduction, laser ablation, and green synthesis using biological materials, have been developed to produce nanoparticles with controlled size and shape, which are of critical importance in various applications (Willian et al., 2021). Chemical synthesis of metal nanoparticles has several limitations, namely the use of hazardous inorganic reagents such as sodium borohydride or sodium hydroxide to reduce metal ions (Manikam et al., 2011).

Efforts to reduce the use of hazardous chemicals have been developed with a biologically-based metal nanoparticle synthesis method known as green synthesis. Through this approach, the biosynthesis of metal nanoparticles is carried out using plants, microorganisms and algae (Iravani, 2011; Kumar & Barathiraja, 2021). Some of the advantages of green synthesis of metal nanoparticles are that it costs less, reduces pollution and improves environmental safety and human health (Ying et al., 2022). Green synthesis of metal nanoparticles provides the advantages of a non-toxic, biocompatible and economical process (Çalışkan et al., 2020).

Green synthesis of metal nanoparticles can involve plant extracts, one of which is chili fruit extract. Compounds in chili fruit have bioactivity and function as AuNPs reducers. Chili Fruit Plants are plants whose fruits contain capsaicinoid compounds, phenolic compounds, ascorbic acid (vitamin C), flavonoids, carotenoids and alkaloids (Ertani et al., 2014; Lu et al., 2017; Pola et al., 2020; Yap et al., 2022). Capsaicinoids consist of dihydrocapsaicin, nordihydrocapsaicin, homodi-hydrocapsaicin, homocapsaicin (Chamikara et al., 2016; Tewksbury et al., 2006). Capsaicinoids, especially capsaicin, have potential in the health sector, including antioxidant and anti-cancer bioactivity (Basith et al., 2016; Tejeda et al., 2022). In addition, capsaicin can also be utilized as a reducing agent in the synthesis of plant extract-based metal nanoparticles. Metal nanoparticles reduced using capsaicin will

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increase the therapeutic potential. Silver nanoparticles reduced using chili fruit extract produced particle sizes of 20-30 nm (Amruthraj et al., 2015). Capsaicin-coated silver nanoparticles have the ability to inhibit the formation of serum albumin amyloid fibrils associated with various diseases (Anand et al., 2016).

Synthesizing AuNPs using plant extracts requires optimum conditions. Temperature, extract concentration and pH affect the green synthesis process of AuNPs (Abu-Elghait et al., 2023). Differences in reducers affect these optimum conditions (Sarsfield et al., 2021). This study aims to determine the optimum temperature, extract concentration and pH conditions in the synthesis of AuNPs using reducing chili fruit extract from three varieties that have different capsaicin content, namely Katokkon, Cakra Hijau and Paprika Merah.

Methodology

This research was conducted in July 2024 at the Laboratory of Physiology, Plant Tissue Culture, Brawijaya University Malang. The chili fruit used in this study is Katokkon (*Capsicum chinense* Jacq), Cakra Hijau (*Capsicum frutescens* Linn), dan Paprika Merah (*Capsicum annuum* L., Var. Special). obtained from traditional markets in Indonesia. Extraction of chili fruits was carried out using maceration method and followed by sonication based on Kusnadi et al., 2019. Each chili fruit was washed to remove dust and dirt, then dried using an oven (Memmert Oven UN55) at 60°C. After the fruit was dried, the chili fruit was pulverized into powder using a grinder (Ossel). Then, 3 grams of chili powder was mixed in 300 mL of 70% ethanol in a 500 mL Erlenmeyer flask for 24 hours. The mixture was then extracted using an ultrasonic sonicator (PS-30A) with a frequency of 20 kHz for 15 minutes. The solution was then filtered with Whatman grade No. 1 filter paper to obtain the appropriate extract (Kusnadi et al., 2019) with modifications. Furthermore, each chili extract from different species was made into 10%, 15%, and 20% concentrations by diluting it using aquadestilata.

Optimization of AuNPs using different concentrations of chili fruit extract, incubation temperature and pH. The optimization formula design was determined using Box-Behnken, Design Expert version 13.0 (Table 1). Gold solution was prepared with a concentration of 1 mM. Gold nanoparticles were synthesized by mixing 1 mL of $HAuCl_4$ solution with 1mL of chili fruit extract solution of each concentration. The mixture was incubated using a shaker incubator at 25°C, 37.5°C and 50°C and pH 5, 7, 9 for 3 hours to observe the color change from pale yellow to pink or dark purple. The AuNPs solution was observed using a microplate reader at a wavelength of 200-800 nm. The resulting AuNPs solution was stored at 4°C for use in the next assay (Khanzada et al., 2021; Patil et al., 2023) with modifications.

Result and Discussion

The chili fruit extract from each type of chili is brownish yellow in color (Figure 1). The formation of gold nanoparticles reduced using chili extracts is indicated by a change in the color of the mixture of $HAuCl_4$ solution and extracts from yellow to pink to purple (Figure 2).

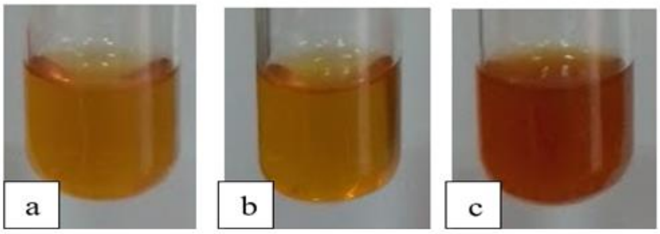

Figure 1. Extract Color a. Katokkon b. Cakra Hijau c. Red Pepper

Table 1. AuNPs optimization formula and position on microplate

tranNo.	pH	Temperature (°C)	Concentration %	Position on Microplate		
				Katokkon	Cakra Hijau	Paprika Merah
1	7	25	10%	A1	C1	E1
2	5	25	15%	**A2**	**C2**	**E2**
3	9	25	15%	A3	C3	E3
4	7	25	20%	A4	C4	E4
5	5	37,5	10%	**A5**	**C5**	E5
6	9	37,5	10%	A6	C6	E6
7	7	37,5	15%	A7	C7	E7
8	5	37,5	20%	A8	**C8**	E8
9	9	37,5	20%	A9	C9	E9
10	5	50	15%	**A10**	**C10**	E10
11	9	50	15%	A11	C11	E11

12	7	50	20%	A12	C12	E12
13	7	50	10%	B1	D1	F1
14	5	25	10%	B2	**D2**	**F2**

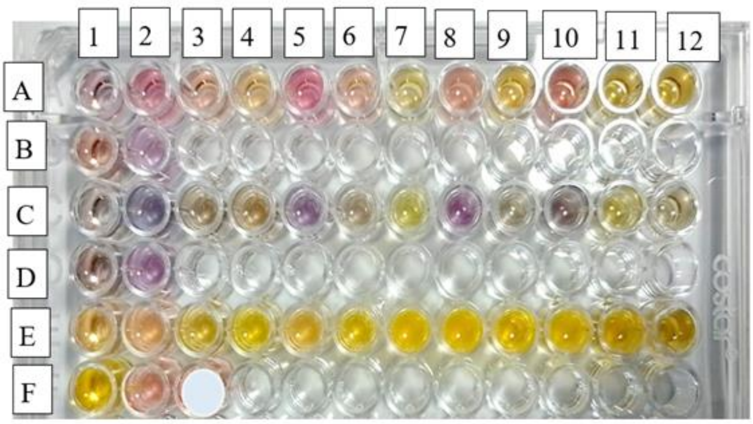

Figure 2. Color change of colloidal AuNPs synthesized using chili extracts of three varieties with different extract concentrations, pH and temperature. The sequence of images is described in table 1.

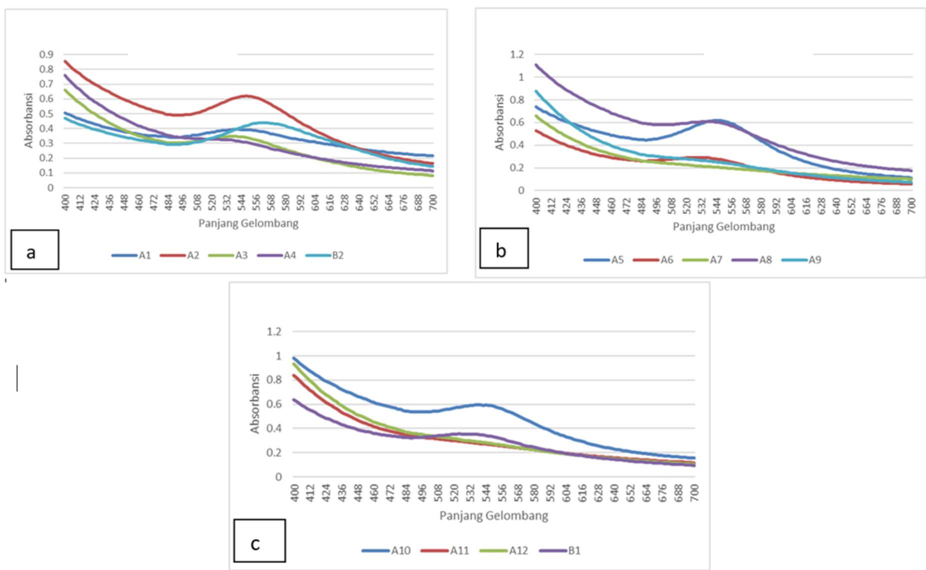

Figure 3. Wavelength and absorbance of AuNPs reduced using Katokkon chili extract at temperature a. 25°C; b. 37.5°C; c. 50°C

Kotokkon extract was able to reduce $HAuCl_4$ to AuNPs at 25°C, pH 5, 15% extract concentration produced a wavelength of 548 nm and an absorbance of 0.6195 (A2) ((Figure 3a). At 37.5°C, pH 5, 10% extract concentration produced a wavelength of 544 nm and absorbance of 0.6181 (A5) while at pH 5, 20% extract concentration produced a wavelength of 536 nm and absorbance of 0.609 (8)

(Figure 3b). At 50°C, pH 5, 15% extract concentration produced a wavelength of 538 nm and an absorbance of 0.5942 (A10) (Figure 3c).

Cakra Hijau extract is able to reduce $HAuCl_4$ into AuNPs at 25°C, pH 5, 10% extract concentration produces a peak wavelength of 562 nm and absorbance of 0.6423 (D2) while at 15% extract concentration produces a peak wavelength of 582 nm, and absorbance of 0.6809 (C2)((Figure 4a). At 37.5°C, pH 5, 10% extract concentration produced a peak wavelength of 572 nm and absorbance of 0.5421 (C5) while at 20% extract concentration produced a peak wavelength of 576 nm and absorbance of 0.5834 (C8) (Figure 4b). At 50°C, pH 5, 15% extract concentration produced a peak wavelength of 576 nm and absorbance of 0.5654(C10) (Figure 4c).

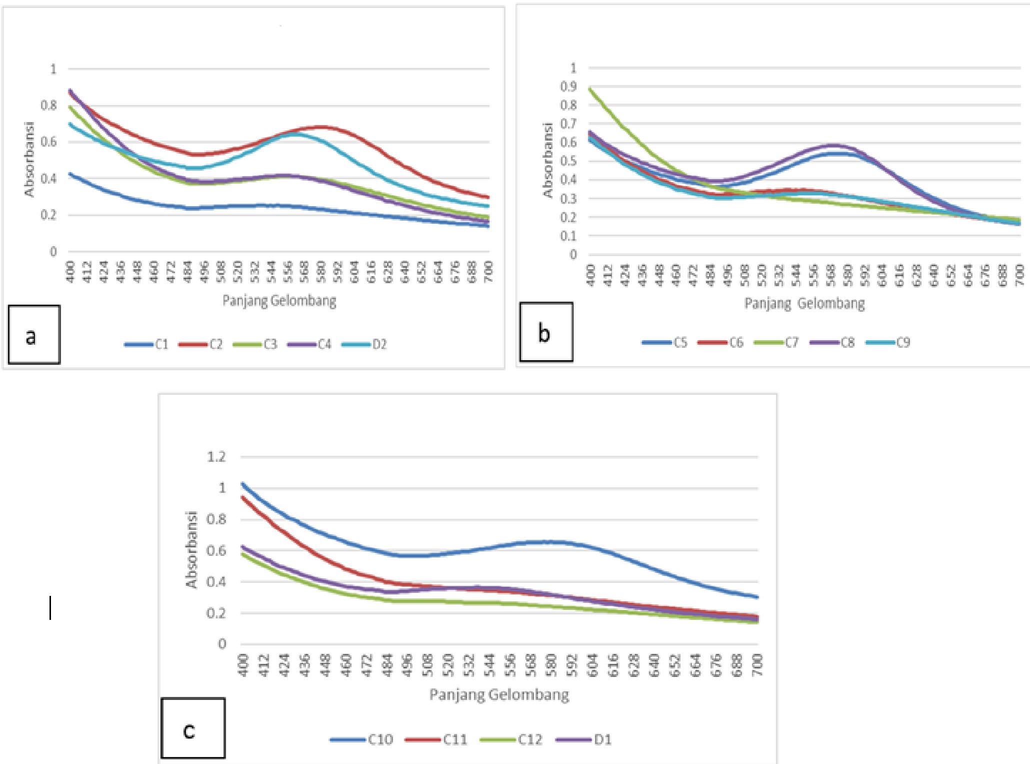

Figure 4. Wavelength and absorbance of AuNPs reduced using Cakra Hijau extract at temperatures a. 25°C; b. 37°C and 50°C.

Paprika Merah extract was able to reduce $HAuCl_4$ into AuNPs at 25°C, pH 5, 10% extract concentration produced a peak wavelength of 540 nm, and absorbance of 0.5751 (A2) while at 15% extract concentration produced a peak wavelength of 534 nm and absorbance of 0.595 (F2) (Figure 5a). Synthesized AuNPs with various concentrations of red pepper extract and pH at 37.5°C and 50ºC did not form a wavelength peak (Figures 5b and 5c). The absence of a wavelength peak indicates the absence of AuNPs.

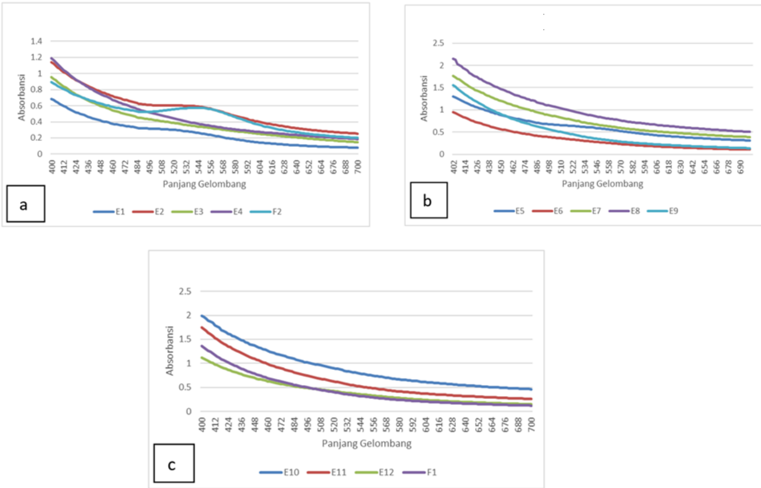

Figure 5. Wavelength and absorbance of gold nanoparticles reduced using Red Paprika chili extract at temperatures a. 25°C b. 37.5°C and 50°C.

Visually visible color changes are used as an initial identification of the formation of nanoparticles, as reported by researchers Rajeshkumar, 2016. The color change of the solution from yellow to pink to purple indicates the formation of AuNPs synthesized with chili fruit extract. In this study, the initial color of the $HAuCl_4$ solution is pale yellow, the color of the extract is dark yellow when the two solutions are reacted under optimal conditions, the color of the solution changes to pink to purple which shows the reduction ability of chili fruit extract which causes the reduction of $HAuCl_4$ to AuNPs (Botteon et al., 2021; Liu et al., 2019). The different colors of AuNPs, from light pink to dark red, depend on the size, shape and structural characteristics of the nanoparticles (Majumdar et al., 2016). In previous studies, the successful formation of AuNPs synthesized using chili extract and AuNPs synthesized using curcumin was at a wavelength of 500-600 nm (Dharman et al., 2023; Patil et al., 2023). In this study, AuNPs reduced with Katokkon, Cakra Hijau and Paprika Merah extracts showed wavelengths of 534-592 nm.

The successful synthesis of AuNPs using chili fruit extract is due to the metabolites in chili fruit extract, namely sugars, terpenoids, poyphenols, alkaloids, phenolic acids, and proteins. In these metabolites there are -OH groups involved in the reduction and stabilization of AuNPS. The presence of this -OH group causes Au^{3+} to be reduced to Au^0 (Patil et al., 2023; Patra & Moussawi, 2015). The concentration and composition of secondary metabolites in plant extracts affect the formation of AuNPs, which in turn affects the color and maximum wavelength of AuNPs. (Bharadwaj et al., 2021; Khan et al., 2022; Ying et al., 2022). The higher the concentration of the extract, the higher the gold reduction, resulting in more AuNPs and thus a higher wavelength peak (Sarsfield et al., 2021).

The synthesis of AuNPs using Katokkon, Cakra Hijau and Red Paprika fruit extracts with pH 5, 7 and 9 produced different wavelength peaks. The maximum wavelength (λ_max) of AuNPs can change with pH variation. AuNPs show a sharp absorption peak in the range of 500-600 nm, which is related to surface plasmon resonance (SPR). pH affects the stability and particle size of AuNPs (Biswas, 2021). At low pH, nanoparticles tend to be larger in size due to aggregation, which can shift the wavelength to higher values. Conversely, at higher pH, the nanoparticles tend to be smaller and well-dispersed, which can result in lower wavelength peaks (Sarsfield et al., 2021). Temperature can affect the variation of nanoparticle size thus affecting the wavelength peak. Based on reaction kinetics at high temperatures causes the reaction rate to increase due to higher energy collisions, so that the reduction of $HAuCl_4$ is greater and produces more AuNPs and is indicated by a higher wavelength peak (Sarsfield et al., 2021).

Conclusion

Chili Katokkon, Cakra Hijau and Paprika Merah extracts are able to synthesize Au into AuNPs. The optimum condition for the synthesis of AuNPs using Katokkon chili extract was at 10% extract concentration at 37°C incubation temperature. Optimum conditions for the synthesis of AuNPs using Cakra Hijau extract occurred at a concentration of 15% at an incubation temperature of 25°C. The optimum condition for the synthesis of AuNPs using Paprika Merah extract was at 10% extract concentration at 25°C incubation temperature. The pH level used to synthesize AuNPs using chili extract in each variety is 5. The wavelengths of Katokkon, Cakra Hijau and Paprika Merah AuNPs under optimal conditions are 544, 582 and 540 nm.

Declaration of Competing Interest

The authors declare that they have no competing interests

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