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Synthesis of Gold Nanoparticles Using Pegagan Leaf Extract as Reductor and Tyrosinase Enzyme Inhibitor Activity Test

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ABSTRACT

Introduction: *Nanoparticles ranging from 1-100 nm in diameter can be synthesized using green synthesis methods, which utilize environmentally friendly, non-toxic, economical, and easily manageable resources to promote sustainable nanotechnology development. Pegagan contains saponins, triterpenoids, flavonoids, and tannins that serve as antioxidants for the skin, offering anti-inflammatory and anti-aging benefits. This study aims to synthesize and characterize gold nanoparticles using pegagan leaf extract, and evaluate its potential as an inhibitor of the tyrosinase enzyme involved in melanin formation in human skin.*
Methods: *Synthesis was conducted by mixing $HAuCl_4$ at a concentration of 2 mM with liquid extract of pegagan leaves. The synthesized products were characterized using UV-Vis spectrophotometer, spectrofluorometer, and Particle Size Analyzer to observe the characteristics of the formed nanoparticles. Additionally, the inhibition activity of the tyrosinase enzyme was tested using a microplate reader at a maximum wavelength of 490 nm.*
Results: *During the synthesis of gold nanoparticles, there was a color change in the solution from yellow to purple. Measurements using UV-Vis spectrophotometer showed a spectrum with a peak wavelength at 533 nm, but no fluorescence was observed. Gold nanoparticles measured using PSA ranged in size from 24.36 to 91.28 nm. The inhibition activity test of the CA-AuNPs solution yielded an IC_{50} of -2,286.202 µg/ml, while the positive control solution using kojic acid showed an IC_{50} of 31.615 µg/ml.*
Conclusion: *Gold nanoparticles can be synthesized using pegagan extract as a reducer, but they do not exhibit potential as inhibitors of the tyrosinase enzyme.*

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1. INTRODUCTION (Font 10, Times New Roman, Spacing 1.15)

Background

Recent advancements in nanotechnology have drawn significant attention from scientists due to their vast potential applications. Nanotechnology offers many advantages, including smaller particle sizes, unique properties, and the ability to integrate with other technologies. Nanoparticles, with diameters ranging from 1 to 100 nm, exhibit properties that differ from bulk materials. These include higher surface area-to-volume ratios, which make nanoparticles valuable for a variety of uses such as diagnostic probes, optoelectronics, catalysis, biological sensing, and therapeutic applications (Menon et al., 2017).

A large number of researchers have focused on synthesizing metal nanoparticles, including gold (Au), silver (Ag), platinum (Pt), and selenium (Se). **Gold nanoparticles (AuNPs)** are of particular interest due to their unique physical and chemical properties, which are not observed in bulk gold. These nanoparticles

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have found applications in biomedicine, bioimaging, cancer therapy, and drug delivery, among others (Srivastava & Mukhopadhyay, 2015).

However, traditional chemical and physical synthesis methods for nanoparticles are costly, hazardous, and lead to environmental pollution. In response, biological methods, also known as **green synthesis**, have emerged as safer, more eco-friendly alternatives. This approach utilizes plants, which contain organic compounds such as terpenoids, flavonoids, and tannins, to reduce metal ions to nanoparticles. Green synthesis of gold nanoparticles is not only environmentally friendly but also minimizes the need for toxic chemicals (Yenni Octaviana & Muhammad Zakir, 2020).

This study focuses on **Centella asiatica** (Pegagan), a plant known for its rich content of bioactive compounds, including saponins and triterpenoids, which have shown significant antioxidant and anti-inflammatory effects. Pegagan is traditionally used for wound healing, anti-aging, and skin care, making it an ideal candidate for the green synthesis of gold nanoparticles (Fernenda et al., 2023).

Problem Formulation

This research aims to synthesize gold nanoparticles using **Pegagan leaf extract** as a reducing agent and to evaluate its potential as an inhibitor of **tyrosinase**, an enzyme involved in melanin formation. The research questions are as follows:

1. Can **Pegagan leaf extract** function as a reducing agent in the synthesis of gold nanoparticles?
2. What are the characteristics of the gold nanoparticles synthesized using Pegagan leaf extract?
3. How effective are the flavonoids absorbed by gold nanoparticles in inhibiting tyrosinase activity?

Objectives

The objectives of this study are:

1. To determine whether **Pegagan leaf extract** can act as a reducing agent in the synthesis of gold nanoparticles.
2. To characterize the gold nanoparticles synthesized using Pegagan leaf extract.
3. To evaluate the inhibitory activity of flavonoids absorbed by the gold nanoparticles on tyrosinase.

Significance of the Study

The significance of this research lies in its potential contributions to the field of nanotechnology and pharmacology. By utilizing an environmentally friendly approach (green synthesis) to produce gold nanoparticles using Pegagan leaf extract, this study aims to provide insights into sustainable nanoparticle production methods. Additionally, the evaluation of gold nanoparticles as tyrosinase inhibitors could contribute to the development of new natural compounds for skin care and treatment.

Gap Analysis and Hypothesis

While green synthesis methods have been explored for the production of gold nanoparticles, few studies have focused on **Pegagan leaf extract** as a reducing agent. Previous research has shown that gold and silver nanoparticles synthesized from plant extracts, including **Panax ginseng** (Jiménez Pérez et al., 2017) and **Terminalia arjuna** (Rakhi & Gopal, 2012), exhibit significant biological activities, such as antioxidant, antimicrobial, and tyrosinase inhibition properties. However, the potential of **Centella asiatica** in this area remains underexplored.

The hypothesis of this study is that **Pegagan leaf extract** can effectively reduce **$HAuCl_4$** to form gold nanoparticles, and the **flavonoids** absorbed onto the surface of these nanoparticles will exhibit inhibitory activity against **tyrosinase**. These nanoparticles could thus have applications in skin whitening, offering an alternative to synthetic chemicals currently used in cosmetic products.

2. RESEARCH METHOD (Font 10, Times New Roman, Spacing 1.15)

Describe This study utilized an experimental research design aimed at synthesizing gold nanoparticles (AuNPs) using **Pegagan leaf extract** (Centella asiatica) as a reducing agent. The primary objective was to investigate the reduction of gold ions ($HAuCl_4$) into gold nanoparticles and evaluate their potential as

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tyrosinase inhibitors. The experimental procedure involved synthesizing the gold nanoparticles, characterizing their properties, and testing their tyrosinase inhibitory activity.

The first step in the process was the preparation of **Pegagan leaf extract**. Fresh Pegagan leaves were thoroughly washed, air-dried, and ground into a fine powder. A **10% (w/v)** extract was prepared by adding the powdered leaves to distilled water, followed by boiling for 30 minutes and filtering to obtain the extract. The next step was the synthesis of gold nanoparticles by adding the **Pegagan leaf extract** to an aqueous solution of **$HAuCl_4$**. The reduction of gold ions occurred at room temperature under constant stirring. The synthesis was monitored using a **UV-Vis spectrophotometer**, which confirmed the formation of gold nanoparticles by detecting a characteristic **surface plasmon resonance (SPR)** peak. The reduction reaction can be represented by the equation:

$$HAuCl_4\,(aq) + \text{Reducing agent (Pegagan extract)} \rightarrow AuNPs\,(solid) + \text{By-products}$$

The next phase involved characterizing the synthesized gold nanoparticles to assess their size, morphology, and stability. **UV-Vis Spectroscopy** was used to record absorption spectra, with a peak around **530 nm** indicating successful nanoparticle formation. The **Transmission Electron Microscope (TEM)** provided images of the nanoparticles, revealing their shape and size distribution, while **Dynamic Light Scattering (DLS)** was used to measure the particle size and determine the **zeta potential**, providing information about the stability of the nanoparticles.

The final phase of the research involved testing the **tyrosinase inhibitory activity** of the gold nanoparticles. The activity was measured using a tyrosinase enzyme assay, with **L-DOPA** as the substrate. The gold nanoparticles were incubated with the enzyme, and the change in absorbance at 490 nm was measured to determine the degree of inhibition. The inhibition percentage was calculated using the following formula:

$$\frac{[(A-B)-(C-D)]}{A-B} \text{ X } 100\%$$

Description:
A = Absorption of enzyme + solution without inhibitor (buffer + enzyme + substrate)
B = Absorption of solution without enzyme, without inhibitor (buffer + substrate)
C = Absorption of enzyme + inhibitor solution (buffer + enzyme + substrate + inhibitor)
D = Absorption of inhibitor solution without enzyme (buffer + substrate + inhibitor)

A **positive control** (kojic acid), known for its tyrosinase inhibitory activity, was used for comparison. The data obtained from the inhibition assay were analyzed statistically using **SPSS software**, with a **One-way ANOVA** applied to determine if there were significant differences in the tyrosinase inhibition at varying nanoparticle concentrations.

Throughout the study, all experimental protocols were performed in accordance with standard laboratory practices, using high-quality reagents from reputable suppliers. No human or animal subjects were involved, ensuring full compliance with ethical standards for scientific research.

3. RESULT AND DISCUSSIONS (Font 10, Times New Roman, Spacing 1.15)

When presenting results in a table or figure, do not repeat all those contents in the text. Present only the summary of the text. Describe only new and important aspects of the study. Do not repeat all information from results section or any section above. Present limitations of the study. Write the issues that are new or unsolved, for future research. This section consists of the information on What/How the presented data were produced, no raw data should be present in the article. The produced data are presented in tables, or figures with an explanation of what is the result/findings from the work.

3.1. Univariat

Table1. Characterization results using PSA

Peak	particle size (nm)
1	24,36 – 91,28
2	105,7 – 615,1

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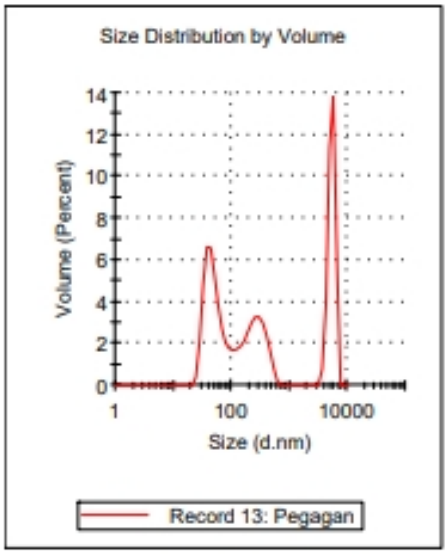
3	3580 – 6439

Based on the measurement results table using the Particle Size Analyzer (PSA), there are three identified peaks, namely the first peak with a size range of 24.36–91.28 nm, the second peak with a size range of 105.7–615.1 nm, and the third peak with a size range of 3580–6439 nm. In the first peak, the particle size is already in the nanoparticle scale, ranging from 1-100 nm. However, to achieve optimal gold nanoparticles, the desired size is usually between 20-30 nm. The particle aggregation process can cause a significant increase in nanoparticle size.

Several factors can cause particle aggregation, including color changes during the synthesis process, where heating is not immediately stopped, resulting in an increase in particle size. Conversely, if the heating and stirring conditions of gold nanoparticles do not reach optimal stability, it can inhibit the formation of perfect nanoparticles. These conditions can be influenced by storing samples at unstable temperatures, which can potentially cause precipitation and also affect the results of nanoparticle synthesis.



Gambar 1. Characterization results using PSA

Table 2. Results of kojic acid inhibition test

Sample	concentrationi (µg/ml)	% Inhibition	linear regression
Asam Kojat	80	96,944	y= 1,2466x + 10,588 R^2 = 0,9207
	60	93,653	
	40	73,437	
	20	38,223	
	0	0	

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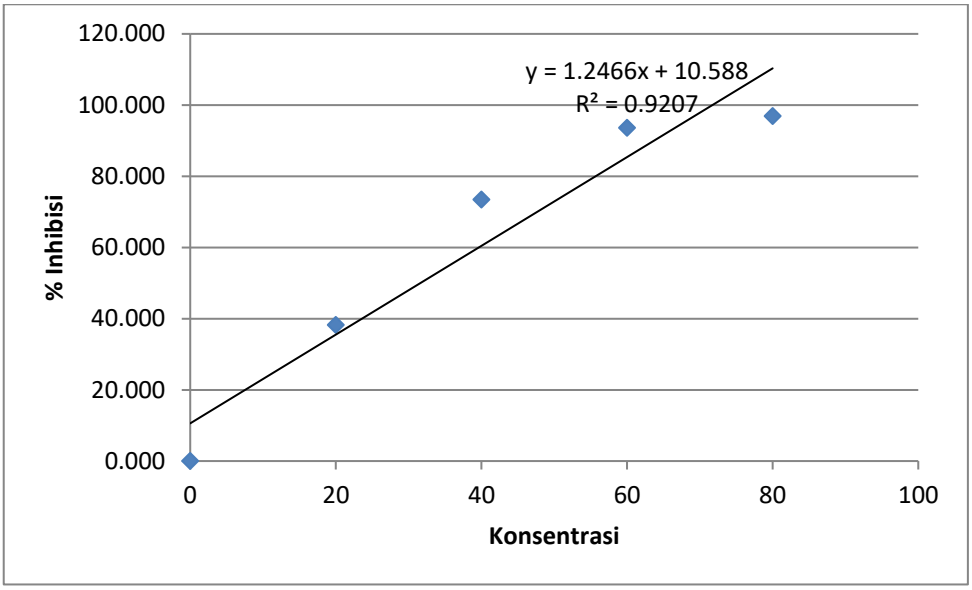

Figure 2. Relationship between the concentration of positive control kojic acid and % inhibition of tyrosinase enzyme

The inhibition percentage results obtained were then used to calculate the IC50 value. The IC50 value of the kojic acid sample by the L-tyrosinase substrate was 31.615 µg/ml with a linear regression equation of y = 1.2466x + 10.588 and an R2 value of 0.9207. The R2 value can be considered poor, possibly due to inaccurate pipetting (Table 2).

The measurements were performed using a microplate reader at a wavelength of 490 nm. In this study, the concentrations of kojic acid used were 80, 60, 40, 20, and 0 µg/ml, respectively. From these concentrations of kojic acid, the inhibition percentages obtained were 96.944, 93.653, 73.437, 38.223, and 0%, respectively. Based on Table 2, it can be seen that the higher the concentration of kojic acid used, the greater the percentage of inhibition obtained. This is also reflected in the absorbance value of kojic acid, where the higher the concentration of kojic acid, the lower the absorbance value and the lower the intensity of the brown color.

3.2. Bivariat

Table 3. Relationship Between Self-Efficacy, Motivation and Level of Independence

Sample	concentrationi (µg/ml)	% Inhibition	linear regression
	413,33	10,202	
Solution	165,33	3,620	y= -0,0158x + 13,878
CA-AuNPs	66,13	11,801	R^2 = 0,2114
	26,45	19,370	
	10,58	13,634	

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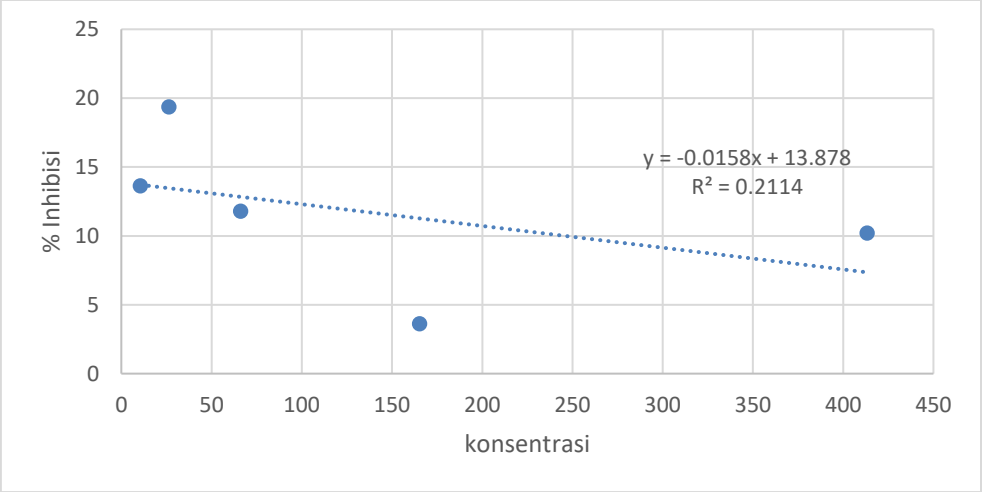

Figure 3. Relationship between sample concentration and % inhibition of tyrosinase enzyme

The inhibition percentage results obtained were then used to calculate the IC50 value. The IC50 value of the CA-AuNPs solution sample by the L-Tyrosinase substrate was 2.286.202 µg/ml with a linear regression equation of y = -0.0158x + 13.878 (Table 3).

Meanwhile, the sample concentrations of the CA-AuNPs solution with a series of concentrations were 413.33, 165.33, 66.13, 26.45, and 10.58 µg/ml, respectively. From this series of sample concentrations, the inhibition percentages obtained were 10.202, 15.184, 22.383, 29.044, and 23.996%, respectively. Based on the test results, the IC50 value was found to be -2,286.202 µg/ml. The inhibition mechanism that occurs is competitive inhibition of L-tyrosine oxidation by the tyrosinase enzyme, where the 3-hydroxy-4-keto part of the flavonoid structure acts as a copper (Cu) metal chelator from the tyrosinase enzyme structure.

4. CONCLUSION AND RECOMMENDATION (Font 10, Times New Roman)

Conclusion

Based on this study, it can be concluded that:

1. The synthesis of gold nanoparticles using gotu kola leaf extract as a reducing agent successfully formed gold nanoparticles.
2. Characterization results showed the presence of gold nanoparticles with an absorbance at a wavelength of 533 nm of 0.772 based on UV-Vis spectrophotometry. The particle size produced ranged from 24.36 to 91.28 nm based on characterization with PSA. However, gold nanoparticles did not show fluorescence based on fluorescence spectrophotometry.
3. In the tyrosinase enzyme inhibitor activity test, the results showed that the CA-AuNPs solution sample that absorbed flavonoid compounds did not have the potential as a tyrosinase enzyme inhibitor, with an IC50 value of -2,286.202 µg/ml. In contrast, kojic acid showed potential as a tyrosinase enzyme inhibitor with an IC50 value of 31.615 µg/ml.

Recommendations

This research can be continued to apply gold nanoparticles in various fields. Plants with significant tyrosinase enzyme inhibitor activity are needed, and tests of tyrosinase enzyme inhibitor activity from plant extracts that have not been made into gold nanoparticles should be conducted for use as a comparison. Attention should be paid to several important factors that can affect the results, such as pH, temperature, and storage conditions. It is also necessary to test the tyrosinase enzyme inhibitor activity using gotu kola leaf extracts.

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