JURNAL ILMU KEFARMASIAN INDONESIA
Indonesian Journal of Pharmaceutical Sciences
ISSN p-ISSN 1693-1831 (Print) e-ISSN 2614-6495 (Online) PUBLISHER : Faculty of Pharmacy Universitas Pancasila

The antiglycation activity test of peel off mask containing gold nanoparticles synthesized from kacip fatima extract (*Labisia pumilla*)

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Received: 19 September 2024 / Accepted: 10 October 2025

ABSTRACT: Skin aging, which is influenced by intrinsic and extrinsic factors, can accelerate skin damage, with one of the main mechanisms being the glycation reaction that results in Advanced Glycation End Products (AGEs). AGEs are the result of indirect reaction between reducing sugars and amino acids, lipids, or proteins. This reaction is also known as the glycation reaction or the Maillard reaction. This research aims to develop a peel-off mask based on gold nanoparticles synthesized with Kacip Fatima extract as an anti-aging agent. Gold nanoparticles have the potential to inhibit the formation of AGEs and improve skin quality. In this study, gold nanoparticles were synthesized by the green synthesis method using Kacip Fatima extract as a reducing agent. The method used for gold characterization is by measuring the wavelength and absorbance, polydispersity index, and zeta potential value of gold nanoparticles. The method used for antiglycation is by using microplate reader, which is stored for 3 days and tested with excitation wavelength of 365 nm and emission wavelength of 415-445 nm. The characterization results showed that the gold nanoparticles had an average size of about 79.61 nm at week 0 and increased to 101.1 nm at week 9. Glycation testing revealed that masks with 20% gold nanoparticles inhibited glycation by up to 95%, higher than the concentration of 10%. This study successfully synthesized gold nanoparticles using Kacip Fatimah extract as a reducing agent, with particles ranging from 79.61 nm to 101.1 nm over 9 weeks. The nanoparticles showed antiglycation effects, with 10% nanoparticles inhibiting glycation by 78%, suggesting potential for environmentally friendly cosmetic applications like peel-off masks.

KEYWORDS: Glycation; gold nanoparticles; peel mask.

INTRODUCTION

Skin aging process is a complex biological process influenced by two main factors: intrinsic and extrinsic. Intrinsic factors occur naturally with age, while extrinsic factors occur faster due to exposure from the environment one key biochemical mechanism contributing to visible aging sign being the glycation of dermal protein [1]. This extrinsic factor is a major concern in the cosmetics industry, especially in the development of anti-aging products.

One of the processes that accelerate skin aging is the glycation reaction. Glycation reaction is the reaction between glucose and amino acids that resulting Schiff bases and Amadori products. This reaction continues in the formation of *Advanced Glycation End Products (AGEs)*. AGEs can be accumulated in the skin and lead to collagen crosslink, reducing it's flexibility and turnover, thereby contributing to wringkle formation and skin stiffness [2],[4].

The Glycation process is closely related to oxidative stress, as the formation of AGEs generetes to Reactive Oxygen Species (ROS), which are oxygen free radicals that are highly reactive and can cause oxidative stress, which further damage skin cells and acelerate aging.

Efforts to overcome premature aging have been carried out with various methods, one of which is the use of cosmetic products, including face masks. Among various topical anti-aging formulations, peel-off mask offer controlled film formation and efficient delivery of active ingredients to skin that forms a film layer on

How to cite this article: Ong PS, Abdulah S, Vardhani AK. "The Antiglycation activity test of peel off mask containing gold Nanoparticles synthesized from Kacip fatima extract (*Labisia pumilla*)". JIFI. 2025. 23(2): 382-389.

https://doi.org/10.35814/jifi.v23i2.1594
ISSN: 2614-6495

the skin of the face and can be peeled off without water when dry. This type of mask has the advantage of exfoliating and also maintaining skin moisture [3].

The use of gold nanoparticles in cosmetic products is increasingly in demand due to their superior effect as an anti-aging, mainly due to their ability as an antiglycation. Gold nanoparticles inhibit the formation of AGEs by reducing the reaction between glucose and amino acids, thereby reducing the formation of Schiff bases [4]. Free radicals are aggressive, reactive and unstable, triggering the activation of inducible nitric oxide synthase (iNOS) which will form peroxynitrite. This compound can damage lipids and DNA in the skin.

Gold nanoparticles can be synthesized through various methods, one of which is by the green synthesis method using plant extracts. This method is safer and more environmentally friendly than physical and chemical synthesis methods. The Kacip Fatima plant, for example, has a high content of flavonoids that act as antioxidants, making it a potential reducer for the synthesis of gold nanoparticles [5].

In this study, gold nanoparticles will be synthesized using the green synthesis method by utilizing quercetin in Kacip Fatima extract as a reducing agent and characterized of nanoparticles. These nanoparticles will be formulated into peel-off mask preparations that function as anti-aging. The antiglycation activity of peel-off mask gel will be tested *in vitro* [6].

▪ MATERIALS AND METHODS

Material

The materials used in this study include Kacip Fatimah Extract (Labisia pumila) (Javaplant, Indonesia), HAuCl4 30% solution (Sigma Aldrich, United Kingdom), Sodium Citrate (Merck, Germany), Sodium Azide (NaN3) (Merck, Germany), Aminoguanidine hydrochloride (Merck, Germany), Polyvinyl Alcohol (PVA) (Merck, Germany), Bovine Serum Albumin (BSA) (Merck, Germany), Fructose (Merck, Germany), Deionized Alcohol, aquadest, aquabidest, Sodium Nitrite (Merck, Germany), Sodium Carbonate (BASF, Germany), Sodium Hydroxide (BASF, Germany), Aluminum Chloride (BASF, Germany), and Methanol (BASF, Germany).

Equipment

The equipment used in this study includes analytical scales (Accu-Lab), Cole-Parmer Viscometer (Ametek, USA), analytical scales (Mettler Toledo, USA), microplate reader (Promega, USA), UV-VIS Spectrophotometer (Shimadzu, Japan), Particle Size Analyzer (PSA) Malvern Zeta Sizer Nano ZS (Malvern Instruments, UK), hot plate (IKA C-MAG, Germany), homogenizer, magnetic stirrer (IKA C-MAG, Germany), micropipette (Eppendorf, Germany), microplate (Corning, USA), Jenway 550 pH Meter, Texture Analyzer, 2-8°C temperature refrigerator (LG, South Korea), oven (Memmert, Germany), and glassware (Pyrex).

Methods

Synthesis of gold nanoparticles with kacip fatimah extract (*Labisia pumila*)

Firstly, HAuCl4 1 mM was prepared by diluting 113.6 µL of 30% HAuCl4 solution with double distilled water until 100 mL. Then, 5% of Kacip Fatimah extract was prepared by dissolving 1.48 grams dried simplicia (equivalent to a concentration of 5% extract) and dissolved in 10 mL of double distilled water. Gold nanoparticle was synthesized with a total of 5 mL of 1 mM HAuCl4 solution was added to erlenmeyer, then 200 µL of 5% Kacip Fatimah extract solution was added. The mixture is stirred using a magnetic stirrer at 300 rpm for 30 minutes. When the color changes from pale yellow to dark purple, 200 µL of sodium citrate is added and stirred again at 300 rpm for 1 minute [7].

Characterization of gold nanoparticles

Gold nanoparticles was characterized using UV-Vis spectrophotometer, Particle Size Analyzer, and TEM. The absorbance and wavelength of gold nanoparticles were measured using a UV-Vis spectrophotometer. Sample preparation was carried out by adding 500 µL of gold nanoparticles to a 5 mL measuring flask, then adding double distilled water to the limit. The sample was inserted into a ±3 mL cuvette for analysis at a wavelength of 300-800 nm. Particle size, particle size distribution, polydispersity index value, and zeta potential of gold nanoparticles were measured using the Malvern Zeta Sizer Nano ZS Particle Size Analyzer

(PSA) using the Dynamic Light Scattering (DLS) method. A total of 10 µL of gold nanoparticles were dissolved in a 10 mL measuring flask, double distilled water was added to the limit, and then 1 mL of the sample was inserted into the cuvette for testing. Transmission Electron Microscopy (TEM) is used to observe the structure of gold nanoparticle [8].

Formulation of peel-off mask gel

Peel-off masks are formulated in three variations of preparations with different gold nanoparticle content. The first preparation contains 10% gold nanoparticles, the second preparation contains 20%, and the third preparation is made without gold nanoparticles as negative control for glycation testing. These three formulas are prepared by a similar method, with differences in the content of active substances of gold nanoparticles.

Table 2.1 Formulation of active ingredients for mask preparations.

Ingredients	Formula I (%)	Formula II (%)
Gold nanoparticles	10	20
Polyvinyl Alcohol	8	8
Polyvinyl Pyrrolidone	2	2
Propylene Glycol	5	5
Xanthan Gum	0.5	0.5
Euxyl PE	0.5	0.5
Denatured Alcohol	20	20
Deionized Water	Up to 100	Up to 100

The preparation of the mask base was started by mixing Xanthan Gum with Propyleneglycol to form masses I. Masses II was prepared by dissolving Polyvinyl Alcohol (PVA) in hot water, then stirring using ultraturax at a speed of 1000 rpm until dissolved. After cooling, denaturation alcohol is added little by little while stirring, then Polyvinyl Pyroli-done (PVP) is added and stirred until homogeneous. After that, Euxyl PE is inserted and stirred again until homogeneous. Masses I is then added to masses II while stirring until well mixed. Finally, Essential Oils are added and stirred until homogeneous. Gold nanoparticles that have been synthesized using Kacip Fatima extract are added to the base of the mask according to the predetermined formulation. The mixture is stirred using ultraturax for 5 minutes until homogeneous mixing.

Antiglycation activity test of peel-off mask gel

BSA solution was prepared by dissolving 0.1 g of BSA in aquabidest up to a volume of 10 mL. A 0.5 M fructose solution was made by dissolving 0.9 g of fructose in aquabidest up to a volume of 10 mL. Sodium azide solution (0.1 M) was prepared by dissolving 0.065 g of sodium azide in aquabidest up to a volume of 10 mL. PBS solution with pH 7.4 was made by mixing 50 mL of PBS with 100 µL of 0.1 M sodium azide solution and stirring until homogeneous. A 5% (0.45 M) aminoguanidine hydrochloride solution was prepared by dissolving 0.5 g of aminoguanidine in 10 mL of aquabidest. Test solutions were prepared in 8 microplate wells, including gold nanoparticle test solution, Kacip Fatimah extract solution, positive control with aminoguanidine, and negative control without aminoguanidine because aminoguanidine works by interacting and acting as a trap for carbonyl group thereby can be anti-glycation agent. Blank solutions excluded fructose. After incubation, fluorescence intensity was analyzed using a microplate reader with an excitation wavelength of 365 nm and emission of 415-445 nm [9].

Table 2.2 Components of Antiglycation test materials.

Substance	20 µL Solution	BSA Solution	PBS Solution	Fructose Solution
Nanoparticles Test Solution	Nanoparticles	50 µL	80 µL	50 µL
Preparation (10 or 20%)	Preparation	50 µL	80 µL	50 µL
Positive Control Solution	Aminoguanidine	50 µL	80 µL	50 µL
Negative Control Solution	-	50 µL	80 µL	50 µL
Blank for Nanoparticles Test	Nanoparticles	50 µL	80 µL	-
Blank for Preparation	Preparation Base	50 µL	80 µL	-
Blank for Positive Control	Aminoguanidine	50 µL	80 µL	-
Blank for Negative Control	-	50 µL	80 µL	-

- **RESULTS**

Synthesis of gold nanoparticles

The results of the synthesis showed that gold nanoparticles were successfully formed, characterized by a dark purple color, indicating the presence of gold nanoparticles as the active substance. The preparation had a distinctive lavender scent derived from the essential oil used during the formulation process. Additionally, the texture was relatively thick due to the addition of xanthan gum as a thickening agent in the formulation. The purple color produced served as an indicator of the successful synthesis of gold nanoparticles, aligning with the expected optical characteristics of this process.

Characterization of gold nanoparticles

Spectrophotometer UV-Vis

The absorbance and wavelength of the synthesized gold nanoparticles were measured using a UV-Vis spectrophotometer. Sample preparation involved transferring 500 µL of gold nanoparticles into a 5 mL volumetric flask, followed by the addition of aquabidest to the calibration mark. Subsequently, the prepared sample was placed into a cuvette with a volume of approximately 3 mL for analysis. Measurements were conducted within a wavelength range of 300–800 nm, ensuring accurate characterization of the optical properties of the nanoparticles

Particle Size Analyzer

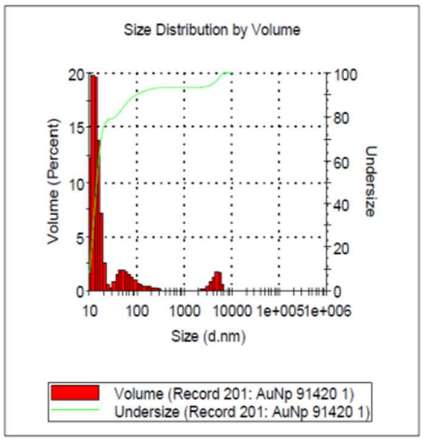

Figure 3.1 Particle Size Distribution.

The particle size measurement in this study was conducted using a Particle Size Analyzer (PSA), where the size of gold nanoparticles is influenced by the type and concentration of plant metabolites during the synthesis process [12]. Plant Plant contents that can be used for phytomining include bioactive polyphenols, alkaloids, proteins, sugars, phenolic acid, terpenoids, etc. Differences in content and concentration are factor that affect the yield, shape and size of nanoparticles. Variation in plant extract concentration, metal salt

concentration, solvent, pH as well as time and temperature will affect quanity, dimension and morphology of nanoparticles [1].

At week 0, the average size of the nanoparticles was 79.61 nm, with size distributions based on volume of 10.2 nm (10%) and 14.2 nm (50%). This measurement reflects the initial characteristics of the nanoparticles without considering changes in subsequent weeks. The polydispersity index (PdI) indicates the degree of particle size distribution, which is related to the stability of the nanoparticles. The larger polydispersity index, the more unstable it will becomes due to the increasing aggregation of particles. Generally the PdI index range from 0 to 1. PdI values between 0.05-0.08 indicate monodispersion, while values between 0.08-0.5 indicate moderate polydispersion [10].

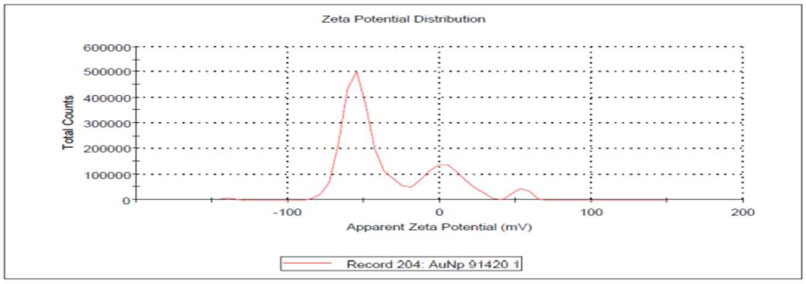

Figure 3.2 Potential zeta values.

The zeta potential value reflects the repulsive forces between particle charges and serves as an indicator of nanoparticle suspension stability. Nanoparticles with zeta potential values ranging from -30 mV to +30 mV are generally considered unstable and prone to flocculation. In week 0, the gold nanoparticles had a zeta potential of -34.8 mV, signifying strong stability. However, this value declined to -26.8 mV in week 4 and further to -21.4 mV in week 9, indicating a progressive loss of stability. This reduction may be influenced by various factors, including pH fluctuations, changes in ionic strength, and temperature variations [11].

Figure 4.4 Transmission Electron Microscopy (TEM)
Integrated Laboratory Testing Services of Diponegoro University.
Accessed May 2022, from labterpadu.undip.ac.id

Transmission Electron Microscopy (TEM) was employed to observe the morphology and structural details of the gold nanoparticles. This technique operates by transmitting electrons through the sample using an electron gun, which directs the beam through a condenser lens. Upon interacting with the sample, the electrons are directed to three additional lenses: the objective lens, which limits the deviation in resolution; the intermediate lens, which amplifies the objective lens; and the projector lens, which forms an image on the fluorescent screen displayed on a monitor. This high-resolution imaging provides precise visualization of the nanoparticles' shape and size.

Analysis glycation test

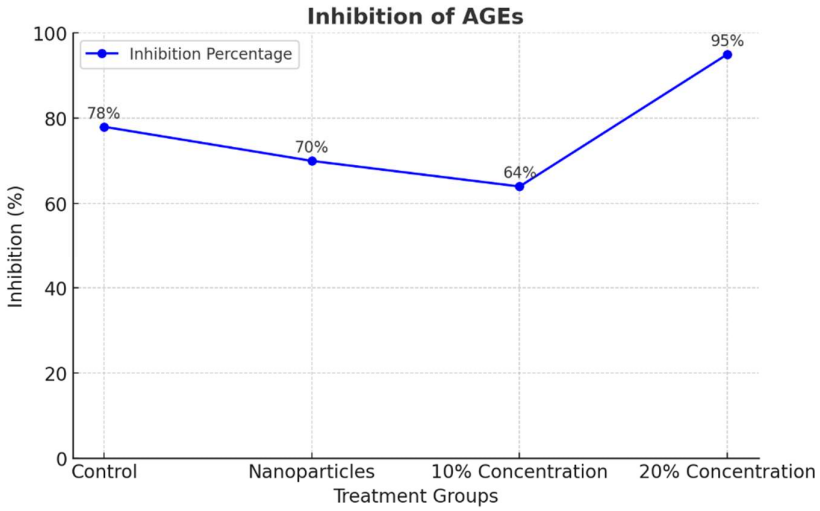

Figure 4.5 Percent glycation ınhibition.

▪ DISCUSSION

The results of particle size analysis showed that the synthesized gold nanoparticles changed in size over time. In the first week (week 0), the average size of the nanoparticles was 79.61 nm, which slightly increased to 95.08 nm in week 4 and 101.1 nm in week 9. This increase in size can indicate the occurrence of particle aggregation that can be affected by environmental factors such as pH and temperature present during the synthesis process [12]. This aggregation process can affect the performance of nanoparticles in certain applications, because the larger the particle size, the lower its diffusion and penetration ability in biological systems.

The Polydispersity Index (PdI) values exhibit variations that correspond to changes in nanoparticle size. At the initial measurement in week 0, the PdI was recorded at 0.287, classifying it as moderately polydisperse. However, the PdI increased to 0.370 by week 4 and further to 0.395 by week 9, suggesting a gradual decline in the stability of gold nanoparticles over time. This rise in PdI may result from fluctuations in particle size due to aggregation, which signifies a progressive reduction in nanoparticle stability [13]. Nonetheless, the PdI value remains within the moderate polydispersive range, which suggests that nanoparticles can still function in a wide range of applications despite the decrease in stability for examples for cosmetics product especially anti-aging product.

Measuring zeta potential also provides important information regarding the stability of nanoparticles. The potential zeta value of gold nanoparticles at week 0 is -34.8 mV, which indicates good stability because the nanoparticles have a fairly strong repulsive force. However, the potential zeta value decreased to -26.8 mV in week 4 and -21.4 mV in week 9, signaling a decrease in stability over time. This decrease can be caused by factors such as pH, ionic strength, and temperature influencing the interaction between the nanoparticles and the surrounding media, which in turn leads to the possibility of flocculation of the nanoparticles [11]). Nonetheless, the still fairly low potential zeta value indicates that the nanoparticles remained stable over the observed time span.

Morphological and size analysis of gold nanoparticles through Transmission Electron Microscopy (TEM) provides a clearer picture of the shape and size distribution of the particles. The TEM results showed that most gold nanoparticles had a spherical or almost spherical shape, which was consistent with previous findings using the Particle Size Analyzer method. Despite the increase in particle size, gold nanoparticles retain the desired morphology for applications in medicine and cosmetics [14].

Glycation testing showed significant results in terms of the ability of gold nanoparticles to inhibit the formation of AGEs (Advanced Glycation End Products). Gold nanoparticles at a concentration of 20% showed

glycation inhibition of 95%, which is higher than the concentration of 10% which only reaches 70%. Gold nanoparticles can prevent premature aging by breaking the Schiff base reaction that forms ketoamine, witch ketoamine will become the main precursor of AGEs. The higher concentration of gold nanoparticles, the greater prevention of Schiff base formation.

These results show that gold nanoparticles have the potential as effective antiglycation agents, especially in cosmetic and therapeutic applications to prevent or reduce the formation of harmful AGEs [15]. This potential provides the basis for further research regarding its application in skin care products or the therapy of glycation-related diseases.

CONCLUSION

This study succeeded in synthesizing gold nanoparticles using Kacip Fatimah extract (Labisia pumila) as a reducing agent, which produced nanoparticles with an average size of about 79.61 nm in the first week, increasing to 101.1 nm in the ninth week. An increase in particle size indicates the presence of aggregations that are likely to be caused by interactions between particles or environmental influences. The polydispersity index (PdI) of nanoparticles shows a polydispersive size distribution, although it is still in the fairly stable category. Testing of the antiglycation effect showed that 10% gold nanoparticles provided the highest glycation inhibition (78%), followed by 20% gold nanoparticles (70%) and control (64%). These results show the potential of gold nanoparticles in inhibiting the glycation process, which can be useful for the development of cosmetic products, especially peel-off masks that can reduce the negative impact of glycation on the skin. Overall, this study shows that the synthesis of gold nanoparticles using Kacip Fatimah extract has the potential to be an environmentally friendly alternative in the cosmetics industry.

Acknowledgements: The authors would like to thank the Faculty of Pharmacy, Univercity of Indonesia, Jakarta for allowing this study to be conducted in their laboratory

Funding: This research was funded through personal funds.

Conflict of interest statement: The authors declared no conflict of interest

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