

Dyah Ayuwati Waluyo-  
EVALUATION OF ANTI-  
GLYCATION EFFECT AND  
SAFETY OF SERUM ANTI-AGING  
FORMULATION CONTAINING  
GOLD NANOPARTICLES (AuNP)  
USING SIDAGURI EXTRACT  
(SIDA RHOMBIFOLIA)

---

**Submission date:** 24 Jun 2021 01:53PM (UTC+0700)

**Submission ID:** 1611460175

**File name:** Artikel\_untuk\_uji\_kemiripan\_-\_Dyah\_Ayuwati\_Waluyo.docx (1.48M)

**Word count:** 2653

**Character count:** 14661

## EVALUATION OF ANTI-GLYCATION EFFECT AND SAFETY OF SERUM ANTI-AGING FORMULATION CONTAINING GOLD NANOPARTICLES (AuNP) USING SIDAGURI EXTRACT (*SIDA RHOMBIFOLIA*)

### ABSTRACT

**Objective:** The purpose of this study is to determine the anti-aging properties and safety of serum-containing gold nanoparticles (AuNP) using Sidaguri extract (*Sida rhombifolia*) through their anti-glycation effect.

**Methods:** The anti-aging effect of serum was performed *in-vitro* by measuring advance glycation end products (AGEs) formed during incubation using a Microplate reader, and safety of serum was performed using hen's egg chorioallantoic membrane (HET-CAM) method using White Leghorn egg.

**Results:** The study showed that serum formulation had an anti-glycation effect with inhibition percentages are  $68.20 \pm 6.86\%$  and  $74.83 \pm 19.91\%$  for a serum containing 10% and 20% gold nanoparticles and little to no irritation potency for both serum formulations with RI value 0.0 and 0.0, respectively.

**Conclusion:** Due to both their anti-glycation effect and irritation behavior, serum formulation containing gold nanoparticle synthesized using Sidaguri extracts could be utilized as anti-aging cosmetics in the future.

**Keywords:** gold nanoparticle, Sidaguri extract, glycation, HET-CAM, safety

## INTRODUCTION

*Aging* is defined as accumulative damage that could lead to cell, tissue, and organ disruption, which happened progressively. This aging occurs in all organs, including the skin. One of the causes of skin aging is the presence of advanced glycation end products (AGEs). Glycation reaction is a non-enzymatic reaction between reducing sugar and free amino acid in the protein resulting in AGEs production. The reduced free radical production could inhibit AGEs in the initial phase by reducing free radical production to reduce the formation of carbonyl or dicarbonyl groups, blocking in the propagation phase by blocking the dicarbonyl or carbonyl groups of sugar and detoxification of reactive dicarbonyl metabolite [20].

AuNP is one of the anti-glycating agents that inhibit AGEs production by competitively binding reducing sugar in binding free amino groups of lysine and arginine that are important for glycation. By this description, AuNP could be categorized as an anti-glycating agent that inhibits in the initial phase [8]. The ability of AuNP had been proven scientifically. The gold nanoparticle has an anti-glycation inhibition activity on collagen as much as  $56.3 \pm 4.2\%$  compared to Aminoguanidin  $64.0 \pm 5.7\%$  [8]. AuNP synthesized using Sidaguri extract, resulting in  $83.372\%$  inhibition activity compared to Aminoguanidine with  $79.793\%$  inhibition activity [6]. AuNP is an anti-aging agent on cosmetics due to its anti-glycation activity.

AuNP could be synthesized using plant extract (green synthesis method). The advantages of green synthesis are relatively safe for the environment, reproducible, and cost-effective, and more stable product material. Green synthesis has been performed using plant extract, vitamins, biodegradable polymers, and microorganisms [18]. In extract plants, the ability to synthesize nanoparticles is due to their ability to reduce metal ions. Their reductive capacity relied on the hyper-accumulating and reductive capacity. Flavonoid could undergo tautomeric transformation to the keto-form; this leads to releasing the reactive hydrogen atom that is important to reduce metal ions in gold nanoparticle from  $Au^{3+}$  ion to Au [10]. Sidaguri is one of the wild plants found massively in a tropical environment, including Indonesia. Sidaguri has a reduction capability due to its antioxidant activity [2]. The availability and the content lead to the usage of Sidaguri extract to synthesize gold nanoparticles.

In this study, a gold nanoparticle was synthesized using Sidaguri extracts and formulated into the serum, subjected to an in-vitro anti-glycation test and irritancy test. An anti-glycation inhibition test was performed to optimize the formulation dose by observing the ability of serum formulation to inhibit AGEs formed during the test. Meanwhile, the irritancy test was performed using the HET-CAM test to classify further the material based on its irritancy characteristics.

## MATERIAL AND METHODS

### Material and Chemicals

Material used during the study are Gold nanoparticle synthesized using Sidaguri extract, Xanthan Gum (Deosen Biochemical (Ordos) Ltd., China), Phenoxethanol, Glycerin (Palm-Oleo Sdn, Malaysia), Bovine Serum Albumin (BSA) (Sigma Aldrich, England), Fructose (Merck,

Germany), Phosphate Buffer Saline (Merck, Germany), Sodium Azide (Merck, Germany), Aminoguanidine HCl (Shandong Zhi Shang Chemical, China), White Leghorn Egg (Balitnak, Indonesia), Sodium Chloride (NaCl) 0.9% (B. Braun, Malaysia), Sodium hydroxide, distilled water and double distilled water.

The tools used are analytical weigh, Ika Eurostar High Speed Mixer (IMMlab, France), pHmeter (Eutetech Instrument, Singapore), Microplate reader GloMax (Promega Corporation, Madison, WI, USA), Micropipette (Eppendorf), 96 well plate (Costar), Viscometer Cole-Parmer with spindle L3 and L4 (Cole Parmer, USA), hot plate and glassware.

### Serum Anti-Aging Formulation

AuNP was synthesized using Sidaguri extract; AuNP was prepared by reacting HAuCl<sub>4</sub> and Sidaguri extract for 150 minutes, then Sodium citrate for another minute. Then, the final product of AuNP was formulated into serum-containing xanthan gum as a thickening agent, phenoxyethanol as a preservative, and glycerine as a humectant.

Firstly, the serum basis was optimized by varying the concentration of xanthan gum, as listed in table 1. The serum was prepared by dispersing xanthan gum to the third-quarter volume of distilled water user and let the xanthan gum out until a transparent film was formed. hen, glycerine and phenoxyethanol were added into the gel, and the mixture was stirred with a mixer at 1000 rpm until a homogeneous mixture obtained while adding the remaining distilled water. The serum basis was evaluated its organoleptic, pH, viscosity, and homogeneity.

**Table 1.** Formula of serum anti-aging basis

Material	Formula (%w/w)		
	F1	F2	F3
Xanthan Gum	0.50	0.75	1.00
Glycerin	3.00	3.00	3.00
Phenoxyethanol	0.80	0.80	0.80
Distilled water	Ad 100	Ad 100	Ad 100

After optimizing the serum basis, two concentrations of AuNP were added to the optimized serum basis as listed in table 2. The two formulas have tested the activity against AGEs formation using anti-glycation test.

**Table 2.** Formula of serum anti-aging containing AuNP

Material	Formula (%w/w)	
	F2.1	F2.2
AuNP	10	20
Serum basis	Ad 100	Ad 100

### Anti-glycation Inhibition Test

The dosage of AuNP used in serum was optimized using an anti-glycation test. This study used modification methods by Spinola and Sutriyo [6, 16]. The sample used in this test is aminoguanidine HCl, serum formulation containing 10% AuNP (F2.1), and serum containing 20% AuNP (F2.2).

There are two solutions, a test and a blank solution. The differences between these two solutions are the blank solution did not contain fructose 0.5 M. The test solution was prepared by mixing 50  $\mu$ L Bovine Serum Albumin 10 mg/mL (BSA), 50  $\mu$ L fructose 0.5 M, 80  $\mu$ L phosphate buffer saline (PBS) pH 7.4 containing 0.2% sodium azide ( $\text{NaN}_3$ ) and 20  $\mu$ L of a sample in the microplate. A blank solution was prepared by mixing 50  $\mu$ L Bovine Serum Albumin 10 mg/L (BSA), 80  $\mu$ L phosphate buffer saline (PBS) pH 7.4 containing 0.2% Sodium Azide ( $\text{NaN}_3$ ), and 20  $\mu$ L of a sample in the microplate.

Then, the microplate was incubated at 37°C for seven days. The intensity of each mixture was analyzed using a microplate reader by measuring the intensity of the fluorescent at 415-445 nm emission and 365 nm excitation. The percentage of inhibition was calculated using equation 1 [16]. All test and the blank solution was prepared duplo or triple.

$$\%inhibisi = \frac{(F_c - F_{cb}) - (F_s - F_{sb})}{(F_c - F_{cb})} \times 100\%$$

equation 1

- F<sub>c</sub> : The fluorescence intensity of the negative control solution
- F<sub>cb</sub> : The fluorescence intensity of the negative control's blank solution
- F<sub>s</sub> : The fluorescence intensity of the test solution
- F<sub>sb</sub> : The fluorescence intensity of the sample test's blank solution

### Irritancy Test Using HET-CAM method

HET-CAM method performed using a fertile chicken egg from White Leghorn species. Eggs used must be fresh (stored no longer than seven days) with a weight range of 45 – 70 grams. The egg should be washed thoroughly, then incubated until day eight after laid at temperature 37.8±0.3°C. The eggs were placed with CAM membrane in the top position, and the eggs must be rotated 180° every day. On day 8, the eggs were checked using candling light to separate the fertile and sterile eggs. On day 9, the fertile eggs were tested with the sample test.

The eggs were divided into five groups of treatment. Group 1 was given NaCl 0.9% (negative control), group 2 was given with NaOH 0.1 N (positive control), group 3 was given serum basis, group 4 was given serum containing 10% AuNP, and group 5 was given serum containing 20% AuNP.

As much as 0.3 mL sample test was applied into CAM membrane. After sample test application, the time of hemorrhage, lysis, and coagulation occurred will be noted [15, 17]. Irritation index (RI) will be calculated using equation 2, the mean of RI will be used to determine the irritancy level of testing material. Irritation classification could be seen in the table 2.

$$RI = \frac{301 - \text{secH}}{300} x5 + \frac{301 - \text{secL}}{300} x7 + \frac{301 - \text{secC}}{300} x9$$

equation 2

secH : time when hemorrhage happened (in seconds)

secL : time when lysis happened (in seconds)

secC : time when coagulation happened (in seconds)

**Table 3.** Material irritation classification

Irritation Index (RI value)	Material Classification
0-0.9	Non-irritant
1-4.9	Weak to little irritant
5-8.9	Medium irritant
9-21	Strong to severe irritant

[source : (Sahu et al. 2014)]

## RESULTS

### Serum Formulation

The obtained basis serum had a clear gel-like cosmetic form and was found to be homogenous on all formulas. The differences in xanthan gum influence pH and obtained viscosity. Viscosity obtained for formula F3 was measured with different spindle due to the stark differences in viscosity with F1 and F2 that has less viscous. F3 was measured with spindle L3 meanwhile, F1 and F2 were measured with spindle L4. All serum formulation evaluation results could be seen in table 3. Based on the appearances and consistency, the considered formula was formula F1 and F3.

**Table 4.** Physical evaluation of serum basis

Formula	Homogeneity	pH	Viscosity (at 30 rpm)
F1	Homogenous	6.94±0.03	2019.53±32.07 cps (L3)
F2	Homogenous	6.71±0.04	3014.33±50.40 cps (L3)
F3	Homogenous	6.50±0.05	5508.77±91.08 cps (L4)

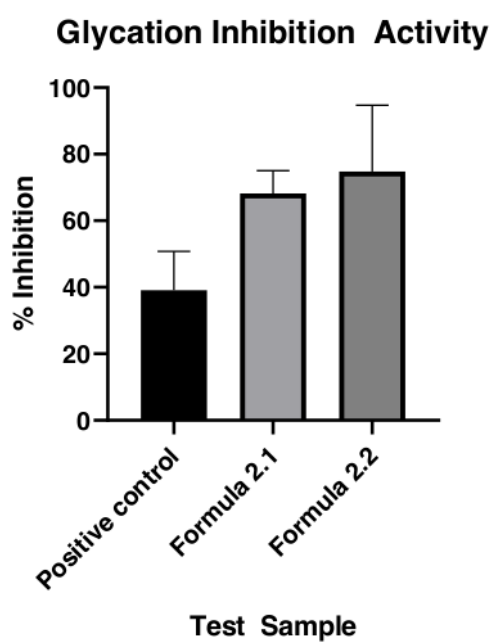
Note: Data given in mean±SD, n = 3

### Anti-glycation test

Anti-glycation inhibition for formula 10% are 68.20±6.86% meanwhile formula 20% resulting 74.83±19.91% inhibition with the positive control are 39.09±11.67%, raw data could be seen at table 5. Based on the statistical calculation, both formulas resulted in a significantly better inhibition activity (p<0.05) than the positive control. However, the comparison between formula 10% and formula 20% was not significantly different. Based on the comparison, the optimized dosage used for serum formulation was 10%.

**Table 5.** Fluorescence intensity of anti-glycation activity test

Sample	Fluorescence intensity		
	I	II	III
Negative control (test)	662	662,3	540,7
Negative control (blank)	200,4	249	230,8
Positive control (test)	324,9	349,6	363,8
Positive control (blank)	99,2	116,3	120,1
Formula 2.1 (test)	244,9	241,8	236,1
Formula 2.1 (blank)	134,6	103,8	NA
Formula 2.2 (test)	693,4	842,9	762,8
Formula 2.2 (blank)	696,5	612,5	699,8



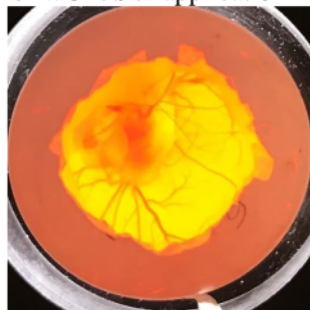
Note: Data shown as mean $\pm$ SD, n= 81(positive control, formula 2.2); n= 54 (formula 2.1)

**Figure 1.** Result of glycation inhibition activity test

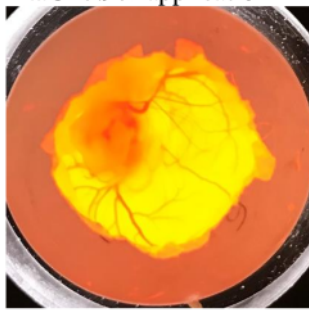


### Irritation test using HET-CAM test

A1. Before NaCl 0.9% application



A2. After NaCl 0.9% application



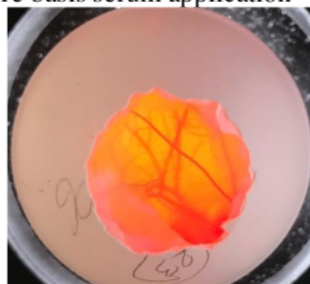
B1. Before NaOH application



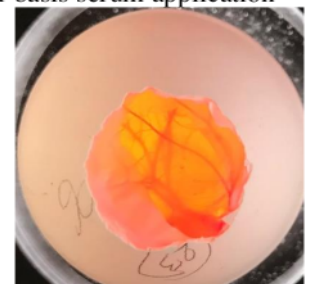
B2. After NaOH application



C1. Before basis serum application



C2. After basis serum application



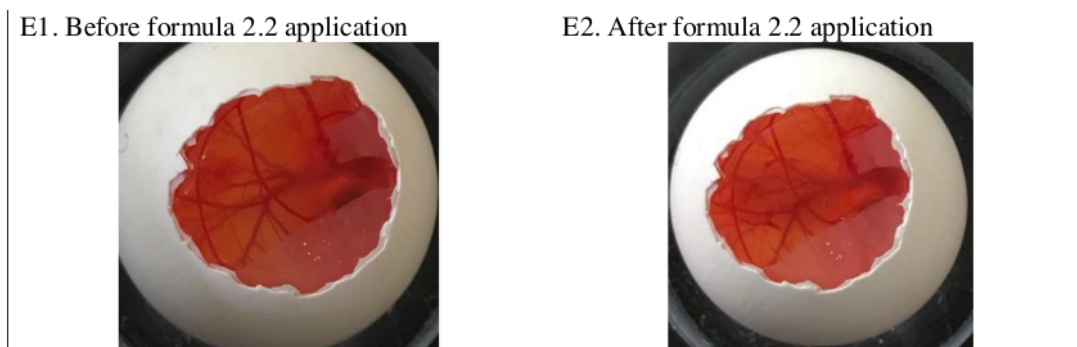
D1. Before formula 2.1 application



D2. After formula 2.1 application







**Figure 2.** Images of the egg during the HET-CAM test

There was no hemorrhage, lysis, and coagulation formed on the egg's CAM in groups 1, 3, 4, and 5. Hemorrhage, lysis, and coagulation were found in group 2, positive control. Hemorrhage formed on averagely the first 10s, followed by blood vessel lysis around 80 – 120s, and coagulation formed was varied on 170 – 300s (see fig 2. B2). All results of eye irritant potency could be seen in table 6 and figure 2.

**Table 6.** Result of irritant test

Test Solution	Irritation Score (RI) (Mean)	Irritant Classification
NaOH 0.9% (negative control)	0.0±0.00	Non-irritant
NaOH 0.1 N (positive control)	12.408±1.28	Strong to severe irritant
Serum basis	0.0±0.00	Non-irritant
Serum containing 10% AuNP	0.0±0.00	Non-irritant
Serum containing 20% AuNP	0.0±0.00	Non-irritant

Note: data given in mean±SD, n=4

## DISCUSSIONS

### Formulation and Evaluation of Anti-Aging Serum

AuNP was formulated into serum due to its high concentration than other cosmetics forms [11, 14]. The advantages of serum are easy and comfortable to use because of the minimum oil phase [3]. In this study, xanthan gum was used as a thickening agent due to its pseudo-plasticity characteristics so that the cosmetics would become smooth and soft [12]. Xanthan gum is non-toxic, not irritating when applied to the skin, and stable over an extensive range of pH values [9]. A low concentration of xanthan gum in an aqueous solution is highly viscous and stable in changes of heat, pH, and resistance to enzymatic degradation due to its structural rigidity [12].

F1 and F2 have almost the same appearances; however, F1 was less viscous. Therefore, we considered using the F2 due to the API (AuNP) liquid form and anticipating the final product not too watery, and the F2 was selected.

### Anti-glycation test

AGEs are glycation reaction products derived from a non-enzymatic reaction between sugar and free amino acid in the protein. Based on AGEs' chemical characteristics, AGEs could be classified into non-cross-linking and cross-linking. The cross-linking AGEs could be classified into fluorescent and non-fluorescent ones [19]. The inhibition mechanism of AGE synthesis could only delay or reduce AGE formation [20]. Aminoguanidine is one of the antiglycation agents usually used as a positive control during the antiglycation inhibition activity test. Aminoguanidine limited AGEs' formation by trapping products from early glycation, such as intermediate carbonyl compound [4].

Bovine serum albumin (BSA)-fructose test was one method used for detecting AGEs formed by measuring fluorescence intensity at emission and excitation wavelengths of 415-445 and 365 nm [7]. Then as mentioned before, bovine serum albumin will be acting as a model protein and fructose as the glycating agent. The condition chosen for the incubation process was similar to the average human body (37°C, pH 7.4). To create pH 7.4 condition, PBS was added into the mixture. NaN<sub>3</sub> was added to the PBS solution to prevent yeast from growing during the incubation process. During incubation, the plate shall be covered with aluminium foil to prevent the mixture from dried. Incubation for seven days of the mixture was expected to produce fluorescent AGEs detected using a microplate reader [5]. Theoretically, the sample solution will result in higher intensity than the blank sample because there is little to no AGEs formation reaction in the blank. In pre-studies, gold nanoparticle synthesized with Sidaguri extract has been proven AuNP has higher inhibition activity, 83.87±4.39%, than Aminoguanidine, 79.79±3.20%, and there is a significant difference in their inhibition activity [6].

### HET-CAM test

HET-CAM test is used to classify material that is expected to be non-irritant or mildly irritant. HET-CAM evaluates the conjunctiva part of the eye [1, 15]. In this research, serum AuNP was predicted to be non-irritant material; therefore, usage of HET-CAM for eye irritation tests was performed. HET-CAM test principle observes and measures hemorrhage, lysis, and coagulation effects on the CAM when material test applied, the test used was using RTM (Reaction Time Method) [15]. Tests were performed on day-9 when nerve tissue and pain perception have not yet been formed [1].

Based on ICCVAM guidelines, the HET-CAM test is acceptable if the RI value of 0.9% NaCl as a negative control was 0.0 and the RI value of 0.1 N NaOH as a positive control was ranged between 10 and 19. In this experiment, all these requirements were met. RI value of 0.9% NaCl is 0.0 and RI value of 0.1N NaOH is 12.408. Thus, both basis serum and serum-containing AuUNP could be presumed non-irritant to the eye with an RI value 0.0.

## **CONCLUSION**

Serum anti-aging containing AuNP synthesized using Sidaguri extract has high anti-glycation inhibition activity compared to the positive control with dose optimized at 10%. Then, serum-containing AuNP synthesized with Sidaguri extract is a little to no irritant material. Considering the *in-vitro* activity and safety, AuNP synthesized from Sidaguri extract could be considered to be used as anti-aging cosmetics. However, the anti-aging activity and eye irritation characteristic of AuNP needs further study.

## **ACKNOWLEDGMENT**

Universitas Indonesia supported this work via PUTI SAINTEKES Q4 Grant program.

## **REFERENCES**

# Dyah Ayuwati Waluyo-EVALUATION OF ANTI-GLYCATION EFFECT AND SAFETY OF SERUM ANTI-AGING FORMULATION CONTAINING GOLD NANOPARTICLES (AuNP) USING SIDAGURI EXTRACT (SIDA RHOMBIFOLIA)

## ORIGINALITY REPORT

4%

SIMILARITY INDEX

3%

INTERNET SOURCES

1%

PUBLICATIONS

1%

STUDENT PAPERS

## PRIMARY SOURCES

1	Submitted to University of Glasgow Student Paper	1%
2	archive.org Internet Source	1%
3	www.freepatentsonline.com Internet Source	1%
4	www.jbc.org Internet Source	1%
5	www.njppp.com Internet Source	1%
6	Péter Budai, Éva Kormos, István Buda, Gergő Somody, József Lehel. "Comparative evaluation of HET-CAM and ICE methods for objective assessment of ocular irritation caused by selected pesticide products", Toxicology in Vitro, 2021 Publication	1%

---

Exclude quotes      On

Exclude matches      < 17 words

Exclude bibliography      On