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The In-vivo Safety and Efficacy Test of Antiaging Serum Containing Gold Nanoparticle Synthesized Using *Sida rhombifolia* Extract

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Abstract

Advance glycation end products (AGEs) are the basic root cause of endogenous aging. AGEs causing fragmentations and crosslinked collagen which damaging the skin integrity and mechanical properties resulting in reduced skin elasticity. Gold nanoparticles (AuNP) has been synthetized using Sida rhombifolia (Sidaguri) extract. This study aimed to determine the efficacy of antiaging serum containing AuNP synthesized using Sidaguri extract. AuNP was produced by reducing $HAuCl_4$ solution using Sidaguri extract. 10% of AuNP colloid was formulated into the serum. 19 woman applied patch containing serum and base placebo to perform irritation test followed by provocative test if there is any reddish reaction found. 16 women showed no signs of irritation and 3 women showed a reddish reaction, the provocative test showed no signs of irritation. Then efficacy test performed to 18 women by applying 1 drop of the serum at one side of the forearm and base placebo serum at the other side, after passing patch test. The antiaging serum had the ability to increase skin collagen (64.72 ± 27.11%) and skin elasticity (64.11 ± 11.67%) after 8 weeks of use, twice a day. There was a significant increase in skin collagen and elasticity index (P-value < 0.0001).

I. Introduction

Aging could be defined as a progressive accumulation of damage that leads to impaired function of the cells, tissues, and organs. Skin aging is a process influenced by genetics and environmental factors, and is largely affected by the accumulation of damage caused by UV radiation (Kim *et al.*, 2012). Skin aging is usually associated with increased wrinkles, saggy, and sluggish skin (Jenkins, 2002). Reduced skin firmness and elasticity are the results of damage to collagen or elastin in the dermis due to the formation of glycation proteins that have abnormalities in the cross-linking process (Yagi and Yonei, 2018). The presence of glycation proteins or AGEs could be inhibited through several mechanisms, one of which is the inhibition of the initial glycation reaction. Gold nanoparticles are one of the synthetic AGEs inhibitors that can interrupt the glycation reaction at an early stage. They are one of the synthetic AGEs inhibitors that can inhibit the glycation reaction at an early stage. They can inhibit the formation of AGEs by competing competitively with reducing sugars in the binding of free amino acid groups at the initial stage (Kim *et al.*, 2012; Liu *et al.*, 2014). In this research, gold nanoparticles were synthesized with Sidaguri or *Sida rhombifolia* (*S. rhombifolia*) extracts as bio-reductor. *S. rhombifolia* is a wild plant found in tropical climates country, Its abundant presence is often used in Indonesian traditional medicine (Silalahi, 2020) as an antioxidant, antiinflammation, and analgesic (Dhalwal, Deshpande and Purohit, 2007). Sidaguri has a total phenol content of 1672.54 mg GAE/100 g and 227.84 mg of flavonoids (Waluyo and Sutriyo, 2022). The content of flavonoids also plays an important role in reducing gold ions into gold metal and these properties play an important role in synthesizing the gold nanoparticles process (Makarov *et al.*, 2014; Teimuri-mofrad *et al.*, 2017). In a previous study, gold nanoparticles synthesized using the green synthesis method utilizing *S. rhombifolia* plant extract had a better anti-glycation effect (83.872%) compared to aminoguanidine (79.793%). In a further study serum containing 10% gold nanoparticles synthesized with Sidaguri extract was reported to be non-irritating and possessing anti-aging activity in vitro (Idris, 2019; Waluyo and Sutriyo, 2022). Based on that, researchers continue to study regarding the safety and anti-aging activity

on human for further insight of AuNP, synthesized using *S. rhombifolia*, potential as an anti-aging cosmetic.

II. Research Method

II.1 Materials

In this study, serum containing 10% gold nanoparticles synthesized with *S. rhombifolia* was used (Waluyo and Sutriyo, 2022), with the equipment of an 8 mm patch disc (Finn chambers AQUA, USA), skin analyzer (EH 900U Qianhe, China), and Oneswab Alcohol Swab (Onemed, Indonesia).

II.2 Methods

II.2.1 Product Evaluation on Skin

This clinical trial was approved by the Ethics Committee of University of Indonesia (protocol number 20 - 01 - 0068, version: 01, February 2020). The samples in this study included 18 healthy women aged 24 - 35 years.

II.2.2 Participant Criteria

The participants of this study included 18 healthy women aged 24 - 35 years. Age range was created wide enough to give insight regarding the benefit of the product however this is also a potential bias. Therefore, researchers were baselining the all participant first then each participant become a control for their own to reduce bias. The participants were requested to discontinue applying other cosmetic products to the forearm's skin within a week before and during the study. An informed consent was obtained from all participants. The exclusion criteria were women with skin disorders such as psoriasis, acne, allergies, wounds, scars, and other skin diseases; pregnant, menopausal, lactating, and smoker women; women suffering from illness, taking oral or topical medications that affect skin conditions; and unwillingness to follow the research.

II.2.3 Irritation Test

Irritation test must be conducted 1-week before the efficacy test, volunteers were not allowed to apply cosmetics and drugs that affect the skin condition on the back and inner upper arm, the location of the irritation and efficacy test will be carried out. In addition, the volunteers were given a similar kind of soap to be used on the back (irritation test) and upper arms (efficacy test) (Wunderlich, 2011; Lazzarini, Duarte and Ferreira, 2013). The irritation test was carried out by the clinical researcher before and after patch application. Volunteers will be tested using both anti-aging serum containing gold nanoparticles and serum base. The test sample was dripped onto the disc patch, the patch was then affixed to the upper back area which has been cleaned with an alcohol swab before. After 48 hours, the patch was removed and observed at 15 minutes, 24 hours and 48 hours after removal (Lazzarini, Duarte and Ferreira, 2013). Evaluation of skin reaction was calculated using Equation (1). The assessment was established according to Table 1.

$$\text{Response} = \frac{\sum (\text{score X respondents number})}{4 \text{ X subject number}} \text{ X } 100 \text{ X } \frac{1}{2} \quad (1)$$

If any reaction was found during irritation test, provocative test should be performed to ensure that the reaction was caused by the test product. Test carried out for 1 week after volunteer applying the test product in an area 3 cm above the antecubital fossae and monitored daily (Draelos, 2011).

Table 1. Scoring system of irritation reaction

Symbol	Level	Clinical responses [a]
-	0	Negative reaction
+	1	There is slight erythema, either spotty or diffuse
++	2	Moderate uniform erythema
+++	3	Intense erythema with edema
++++	4	Intense erythema with edema and vesicles

[a]clinical responses based on literature (An *et al.*, 2014)

II.2.4 Collagen Content and Skin Elasticity Test

The efficacy test was conducted after the volunteers reported to passing the irritation test. Volunteers were assessed for their collagen and elasticity levels as a baseline before applying the serum to the area of the test. The volunteer will receive 2 test samples, namely serum containing gold nanoparticles and serum base. Both products were applied as much as 1 drop (± 300 mg) on the right arm and left arm. The products applied to the right and left arms were randomized and the volunteers will not be informed the content of each product (blinded). Both products were applied every day, 2 times a day in the morning and evening. The trial was tested for 8 weeks with observation points before use and the 2nd, 4th, 6th, and 8th weeks after use. Observation of the benefits of the preparation was carried out by observing changes in collagen content and skin elasticity using the EH-900U skin analyzer.

II.2.5 Statistical Analysis

Data analysis performed using GraphPad Prism version 8.0.2.263. The data analyzed in this study is the result of EH-900U skin analyzer reading for each parameter. Prior to carrying out the comparative analysis, the normality of the data obtained was assessed first using Shapiro Wilk method. The analysis was comparing the mean value of each results bar from the control and placebo groups at each measurement time using two-way ANOVA method to observe the probability of significant changes occurring in the control and placebo groups.

III. Results and Discussion

Irritation skin test observed after 24 hours, 1 volunteer showed 2 papules and 4 other volunteers showed redness around the test area. These five

volunteers were asked to postpone the efficacy test and were observed for the next 24 hours. After the next 24 hours, 3 volunteers were still found to have a reddish reaction (without papules). To ascertain whether the volunteers were sensitive to the test product, a provocative test was carried out. The test was applied the product in an area 3 cm above the antecubital fossae for 1 week and the volunteers were monitored daily. During 1 week of testing, no irritated volunteers were found. Based on calculations with Equation (1), the initial response value for the skin irritation test was 1.97 (Table 2), indicating that serum is a moderate irritant. After the provocative test, a response value of 0.00 was obtained and this indicated that the serum was included in a substance that was not or slightly irritating (Draelos, 2011; An *et al.*, 2014). Therefore, all volunteers were passed the skin irritation test and qualified to performed efficacy test. The volunteers that showed reddish reaction was suspected sensitive to the patch even though we already used hypoallergenic patch.

During the skin irritation test 3 volunteers showed a reddish reaction (without papules), therefore a provocative test was carried out. A provocative test on volunteers suspected of being sensitive to cosmetics. This test was conducted to confirm a positive result from a patch test of a cosmetic product containing ingredients suspected of causing contact dermatitis. The provocative test was chosen over the open patch test because the open patch test was carried out if the test substance was suspected to be irritating, whereas the HET-CAM test reported that the serum containing gold nanoparticle was non-irritant (Draelos, 2011; Waluyo and Sutriyo, 2022). Based on the final results of the provocative test, volunteers were not sensitive to the cosmetics but sensitive to the patch even though researchers already use hypoallergic patch.

Efficacy test of collagen content and skin elasticity observed after 8 weeks of serum containing gold nanoparticles application, the average collagen level was 64.72 ± 27.11%, while the average collagen level when using a placebo was 31.11 ± 16.68%. The normality test was performed using the Shapiro-Wilk test and it was found that the data for both groups had an abnormal or non-parametric distribution. Data obtained during the study has wide range of collagen level per respondent, therefore standard deviation is quite big. The Wilcoxon test was carried out to compare the value of collagen levels between sample and placebo group every two week. At week 8, p value of 0.0005 *** ($p < 0.05$) was obtained. This shows that there was a significant difference at week 8 where the test group gave a significantly higher results of collagen level value than the control group. Analysis using 2-way ANOVA with post-hoc Sidak was also performed, it was confirmed that the significance different between sample group and placebo group occurred after 8 weeks of using the serum as shown in Figure 1.

Table 2. Irritation skin result

Product	N	Irritated Volunteer			Response Score	Primary Irritation Index
		15 min	24 h	48 h		
Gold nanoparticle containing serum sample (serum test)	19	5	3	3	1.97	Mild irritation
Serum base placebo (control)	19	5	3	3	1.97	Mild irritation

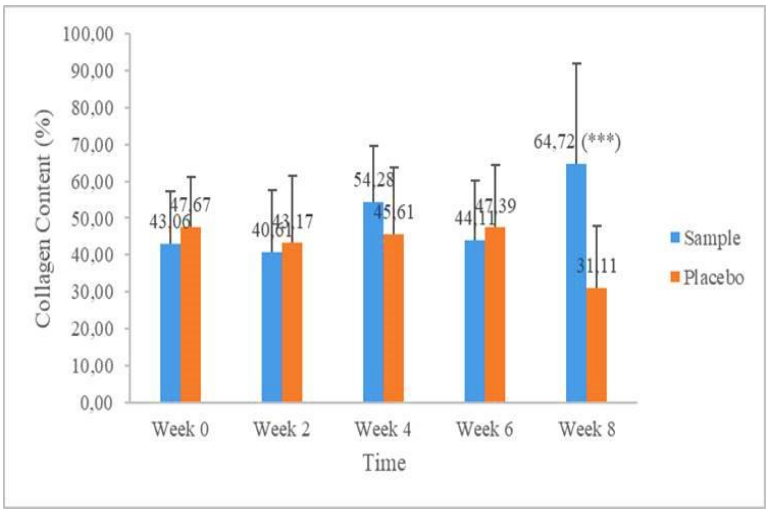

Figure 1. Skin collagen levels in the sample and placebo groups

Furthermore, the average skin elasticity was 64.11 ± 11.67%, while the average skin elasticity when using placebo was 38.67 ± 21.85%. The normality test was tested using the Shapiro Wilk test and it was found that the data for placebo groups were an abnormal or non-parametric distribution meanwhile the sample groups showed a normal or parametric distribution. A comparison of changes every two weeks between the two test groups was carried out using 2-way ANOVA with post-hoc Sidak and it was found that a significant difference between the test group and the control group only occurred after 8 weeks of serum use, this can be seen in Figure 2. This shows that a new significant difference was seen when the serum was used for 8 weeks with a higher increase in the sample group compared to the placebo group.

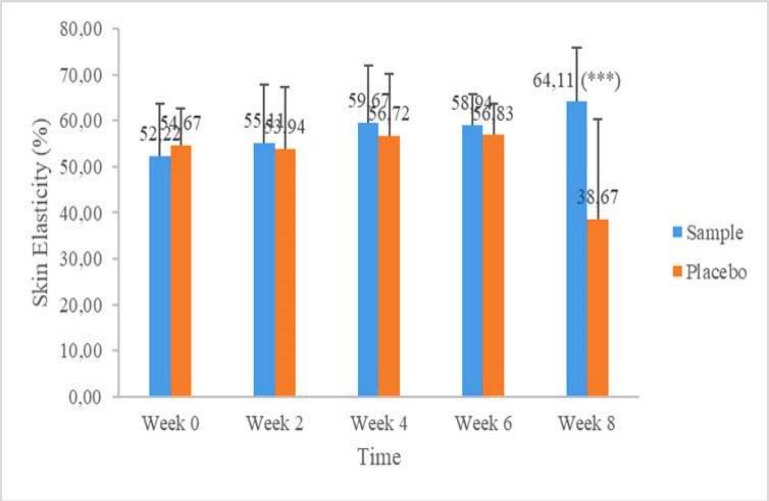

Figure 2. Skin elasticity in the sample and placebo groups

Data obtained during study showed a great variation, it suspected due to respondent wide range of ages and the variety of works each respondent has. Respondent aged around 34 years old has low collagen level and skin elasticity level. The efficacy test reported that the serum containing gold nanoparticles has the ability to increase collagen levels and skin elasticity within 8 weeks. Increased levels of collagen can occur because gold nanoparticles are able to prevent the formation of AGEs which can degrade collagen and cause protein aggregation. Collagen, one of the important components in the extracellular matrix, is prone to enzymatic chemical modification. Cross-linking mediated by AGEs can occur between the collagen helices between lysine and histidine or arginine, and the effect of this cross-linking is the formation of new proteins that are resistant to enzymatic degradation (McKay, Priyadarsini and Karamichos, 2019). Glycated collagen fibrils also affect the process of removing old collagen fibrils results in the reduction of new collagen fibrils formation (Putte, Schrijver and Moortgat, 2016). Gold nanoparticles are able to inhibit the occurrence of glycation reactions. Therefore, they are able to inhibit the decrease in collagen levels that occur due to glycation reactions.

The content of elastin in the skin is only in small amounts, around 2%, but the damage to elastin causes significant changes to the skin, such as the formation of wrinkles and sagging of the skin. Decreased skin elasticity with increasing age can be due to various factors, such as the decreased function of fibroblasts which causes reduced production of extracellular matrixes, such as fibronectin, collagen, and elastin. In addition, collagen and elastin also decrease due to the effects of oxidation and glycation. Glycation reactions can occur at any time and in any layer of the skin, but if it is controlled there will be better results. AGE accumulation, the result of the glycation reaction, in the dermis layer can cause the skin to turn yellow and Nε-carboxymethyl lysine (CML) can target collagen fibers, whereas collagen together with elastin fibers are responsible for maintaining skin elasticity. So, glycation causes a decrease in the movement of skin tissue because it causes the formation of crosslinks in type 1 collagen and elastin (Yonei, Takabe and Yagi, 2015). Incubation of collagen fibrils with methylglyoxal, a reducing sugar, showed no effect on fibril stiffness but resulted in higher fibril tension. In addition, another study found that glycated collagen fibrils make the skin stiffer and reduce its ability to withstand mechanical stress (Van Putte, De Schrijver and Moortgat, 2016). Gold nanoparticles have been shown to inhibit the glycation reaction. Therefore, the increase in skin elasticity after applying serum containing gold nanoparticles potentially caused by the activity of gold nanoparticles which are able to inhibit glycation so that the possibility of crosslinking in collagen and elastin can be reduced.

In addition, this is also supported by an increase in collagen levels in the administration of

serum containing gold nanoparticles as shown in Figure 2. Furthermore, researchers decided not to purify the AuNP colloidal to gain benefit from the flavonoid content in *S. rhombifolia* extract. Phenolic and flavonoid content of *S. rhombifolia* was mainly used as bio reductor to reduce Au^{3+} to Au metal in AuNP synthesis process (Makarov *et al.*, 2014). However, flavonoid content in *S. rhombifolia* also has the potential to be an anti-aging agent through senolytic, senomorphic and antisenescence activity. Senescent cells secreted the senescence-related secretion phenotypes (SASPs) are aging factors, some flavonoids can kill senescent kills. Senomorphic activity was referring to flavonoid has the ability to suppressing SASP or proinflammatory secrete. Other antisenescence activities are flavonoid has antioxidant effect that able to reduce radical oxygen species in senescent cells (Fan *et al.*, 2022).

IV. Conclusions

AuNP serum was concluded safe to use as there was no irritative reaction from volunteer and the serum has an anti-aging activity proven by the increase of collagen content level and skin elasticity level during 8 weeks of serum usage.

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