2018 Nanogolds Influence on Antioxidant Activity

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Nanogold's Influence on Antioxidant Activity of Green Tea Extracts in the Framework of New Essential Ingredients Discovery in Cosmetic Formulation

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Abstract. The circulation of cosmetic containing hazardous materials especially mercury has occurred in Indonesia and other countries. The impact has also been felt by the wider community. So far efforts have been made, among others, by searching for and finding new essential ingredients in cosmetic formulas. Mercury in cosmetics leads to the formation of free radicals in the body that must be resisted with antioxidants. Nanogold has been shown to have antioxidant activity. Similarly, green tea extracts are also reported to possess antioxidant activity. Their combined form in cosmetic formulas are expected to increase antioxidant activity. Thus, this study will examine antioxidant activity of nanogold combined with green tea extract. This is particularly important with regard to the application of both in cosmetic formulas. The results obtained show that antioxidant activity of green tea extract of 20 ppm are 91,16%. Antioxidant activity of green tea extract with nanogold in 5, 10, 15, 20, 25, and 35 ppm are 90,63; 90,77; 90,90; 91,42; 91,56; 91,42, and 91,42 respectively. To sum up, the combination of nanogold and green tea extract gives no significant impact towards antioxidant activity.

1. Introduction

Cosmetics containing hazardous materials, especially mercury, have been found in the market and are widely distributed in both urban and rural communities. This can be known from the research related mercury contents in facial cream in 2012 where 5 of 6 facial creams were positively analyzed containing mercury [1]. The increase occurs in 2017 where 39 samples out of 45 positively contain mercury [2]. The case of mercury containing cosmetics is not only in Indonesia but covers almost all countries in Southeast Asia, India, and other countries [3].

The impact has also been felt by the public at large, especially when the termination of cosmetics [4]. Mercury is a very powerful antimicrobial material, so that at the beginning of use all acne is healed and the skin becomes smooth [5]. At the time of the discontinuation of cosmetics, acne will appear even much more. Mercury also suppresses melanocyte cells so these cells not to excrete melanin that its skin pigment [6]. In the early of use the skin show very white and satisfying users. At the time of discontinuation of cosmetics, hyperpigmentation that occurs because melanocyte cells become uncontrolled produce melanin. It does not stop here, the damage of cells and tissues continues directly due to the use of mercury [7]. Mercury is the source of free radicals in the human body. Free radicals attack all important organs and cause damage organ function [8].

So far, efforts have been made, among others, by searching for and finding new essential ingredients in cosmetic formulas. Mercury in cosmetics leads to the formation of free radicals in the body that must be resisted with antioxidants [4]. New materials as antioxidants are tested for their activity using

artificial free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) [9]. DPPH includes stable artificial free radicals that are stable so that when new materials of antioxidants can reduce this free radicals was certainly also able to reduce the actual free radical. The quantitative price of the antioxidant power is indicated by the magnitude the free radical damping expressed in percent of damping [10].

Nanogold has been shown have antioxidant activity. Percent damping of nanogold to DPPH at 20 ppm was 56.85% and at 25 ppm 66.27% [11]. Using Nanogold as cosmetics is selected concentration of 20 ppm because it has a higher colloidal stability. Drops of free radicals by nanogold are still ongoing because nanogold was not damaged after give an activity. This was evidenced by the increase in the damping percentage that reaches 76.01% in measurements after one hour and becomes 94.01 after two hours. The mechanism of action of nanogold as antioxidants is different from organic antioxidants. Nanogold works as a catalyst that conducting catalytical process where its returns to original state after the catalytical process ends. Than the catalyst or nanogold in order prepare for subsequent catalytic processing without any damage [12].

The green tea is a plant containing catechins that are the dominant antioxidant compounds found in this plant [13]. Green tea extract provides a protective effect of skin damage due to oxidation, photoaging and preventing premature aging. The use of green tea extract topically can provide a smoothing or moisturizing effect on facial skin, and can reduce black stains [14]. Green tea extracts were also reported to have antioxidant activity through activity testing using DPPH [15]. Green tea extract has an antioxidant activity of 91.39% in concentrations of 193 mg / L or 193 ppm [13]. When compared to nanogold that have 56.85% damping activity in concentrations of only 20 ppm nanogold looks superior to antioxidant material [10].

To further clarify the antioxidant activity of the material it is necessary to test the activity of green tea extract and nanogold at the same concentration. In this study both will be tested its antioxidant activity separately and simultaneously. In addition, also will be conducted related research to answer the problem of how the effect of adding nanogold to antioxidant activity of green tea extract. This is particularly important with regard to the application of both in cosmetic formulas.

2. Material and Method

Materials used in this study include: Nanogold 20 ppm, made from HauCl4 base material that is reduced with sodium citrate. To obtain nanogold 20 ppm HauCl4 1000 ppm base solution of 20 ml diluted to 1000 ml. This solution is heated to boiling and added 2 g of sodium citrate. The color change from yellow, to colorless, then turns red to explain the process of reduction from Au3 + to Au + and finally to Au in colloidal form. Nanogold 20 ppm then tested its antioxidant activity.

The green extract is made by heating 1000 ml of water and incorporating 20 mg of dried green tea leaf powder. At first green tea extract pale green, after allowed to cool the color increases thick. This shows the process of extraction more perfect. The active substances of dissolved green tea in water are increasing. This green tea extract is then tested for its antioxidant activity. In this activity test required free radical artificial 2,2-diphenyl-1-picrylhydrazyl (DPPH).

The instruments used in the antioxidant activity test of both nanogold, green tea extract and the combination of both are UV-visible Spectrophotometers. The decrease in UV absorption of DPPH compounds indicates the occurrence of the free radical damping process. The difference in absorption compared with the initial absorption multiplied by 100% is the percentage of damping which also shows the quantity of antioxidant activity quantitatively. The greater the percentage of the damping the higher the antioxidant activity of antioxidant material.

The methods used to answer the problem are:

Nanogold Synthesis

The first step in this research is by heating aquades 200 mL to boiling. Adding sodium citrate as much as 0.3 gram and 1000 ml HAuCl4 solution of 7 ppm, 6 mL, 5 mL, 4 mL, 3 mL, 2 mL, 1 mL for each concentration of 35 ppm, 30 ppm, 25 ppm, 20 ppm , 15 ppm, 10 ppm and 5 ppm. The mixture is stirred using a stirrer at 500 rpm. The heating is stopped when the solution turns into a burgundy color.

Green tea extract 20 ppm

100 grams of green tea is put into a glass of chemistry. Adding aquades at 100°C until the solution is ± 1cm above the sample. Stirring green tea extract and wait for up to 15 minutes. Filter the solution

using a vacuum pump. Insert the filtrate into the freeze dryer tool until the dry extract is obtained. A total of 2 mg of dried extract was diluted with 100 mL warm aquades using 100 mL measuring flask. The solution is shaken until homogeneous so that the solution concentration of green tea extract 20 ppm.

Testing the Effect of Nanogold Addition on Antioxidant Activity of Green Tea Extract

DPPH powder 4 mg was put into a 100 mL measuring flask, dissolved with ethanol on a 100 mL measuring flask. Shake until homogeneous and placed in dark space. Measured using a UV-Vis Spectrophotometer instrument at a wavelength of 400-600 nm to obtain the maximum wavelength of DPPH. 5 mL solution of green tea extract 20 ppm was inserted into 7 dark bottles, then 5 mL nanogold 5, 10, 15, 20, 25, 30, 35 ppm and 100 mP DPPH solution added 10 mL. Shuffled to homogeneous and measured absorbance using the UV-Vis Spectrophotometer instrument at maximum DPPH wavelength.

3. Result and Discussion

3.1 Nanogold Synthesis and Characterization

In this study, the synthesis of gold nanoparticles was made by heating aquades to boiling, then added 0of sodium citrat. The color of the solution is changed begins with a yellow colored solution becomes colorless and then turns into dark blue and red. Addition of sodium citrate serves as a reducing agent also as a stabilizer agent so that the nanogold solution is not aggregated. The negative charge of the citrate ion will be absorbed by the nanogold surface so that the gold particles will repel each other. The reaction is as follows:

$$2Au^{3+} + 4C_6H_5O_7^{3-} + 6H_2O \rightarrow 2Au + 4C_6H_8O_7 + 3O_2$$

When gold is in ionic form, Au3 + will repel each other because of the influence of similar charge. However, after being reduced by sodium citrate to Au0, the charge of Au atoms becomes neutral so that the atoms will get closer to each other through the bonds between the metals and the longer the cluster will grow to the nanometer size. The explanation can be illustrated as follows:

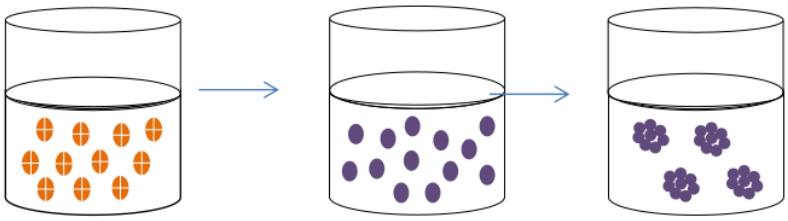

Fig 1. The Illustration of The Nanogold Cluster Growing

The color intensity resulting from nanogold synthesis of 5 ppm - 35 ppm is concentrated. This relates to the cluster diameter and the cluster distance density. The higher the concentration the narrower the distance between the clusters and the color will be stronger and the higher the nanogold concentration, the more clusters are formed so that the resulting color is stronger. The synthesis of gold nanoparticles was then characterized by UV-Vis spectrophotometer and obtained the maximum wavelength to be used to calculate the cluster diameter using the Brus equation as follows:

$$\frac{1240,6}{524} = 1,3 + \frac{14,84}{R^2}\left(\frac{1}{(0,25)^2} + \frac{1}{(0,25)^2}\right) - \frac{2,6}{6,5R}$$

Table 1. The Diameter of Nanogold cluster used Bruss Equation

No	Konsentrasi Nanogold (ppm)	λ maks (nm)	Diameter Cluster (nm)
1	5 ppm	524	20,90
2	10 ppm	521	20,95
3	15 ppm	526	20,99

4	20 ppm	524	20,90
5	25 ppm	522	20,82
6	30 ppm	527	22,05
7	35 ppm	525	20,95

3.2 Preparation and Characterization of Green Tea Extracts

The green tea extract solution begins by weighing the green tea leaf powder that has been dried and in the blender.Selection of solvent using aquades to extract green tea caused catechin compounds contained in green tea is polar and soluble in water. Catechins have more than one hydroxyl group so that the solubility in the water is greater. In addition, the aquades solvent is a safe solvent and is not toxic when applied to human skin. Analysis of green tea extract solution using UV-Vis Spectrophotometer showed that 20 ppm green tea extract solution had maximum wavelength absorption at 291 nm and absorbed value was 0.162.

3.3 Testing the Effect of Nanogold Addition on Antioxidant Activity of Green Tea Extract

The preparation of DPPH solution is by weighing a 4 mg DPPH black powder inserted into 100 mL measuring flask, dissolved with ethanol up to miniskus boundary mark. Shake until homogeneous and placed in dark space. Furthermore, it was measured using a UV-Vis Spectrophotometer instrument at a wavelength of 400-600 nm to obtain the maximum wavelength of DPPH. DPPH solution has a maximum absorption wavelength of 518 nm and absorbance of 0.758. This absorbance value will be used as an initial DPPH absorbance control.

Analysis of antioxidant activity was done by measuring the absorbance of green tea extract and nanogold tested with DPPH radicals. The absorption of DPPH will decrease and the intensity of purple color from DPPH will fade over time when reacted with antioxidants. This is because the antioxidant will contribute an atom H or Au to the DPPH radical so that the DPPH radical will become more stable. Calculation of free radical damping activity by nanogold and green tea extract at each concentration was done by entering absorbance data which have been analyzed using UV-Vis Spectrophotometer instrument into the following% damping formula:

$$\%\ \text{of dumping} = \frac{\text{Abs}_{\text{DPPH}} - \text{Abs}_{\text{sampel}}}{\text{Abs}_{\text{DPPH}}} \times 100\%$$

Based on the measurements of the UV-Vis Spectrophotometer at the maximum DPPH wavelength of 518 nm, there is a% damping as shown in the following table.

Table 2. Dumping percentage of Nanogold and green tea extract.

The concentration (ppm)	**% of dumping (hours)**				
	1 mnt	**1**	**2**	**3**	**4**
Green tea extract 20 ppm	91,16	91,95	91,69	92,61	93,14
Green tea extract 20 ppm + NG 5 ppm	90,63	91,95	92,08	92,35	93,67
Green tea extract20 ppm + NG 10 ppm	90,77	91,03	91,03	91,69	93,01
Green tea extract20 ppm + NG 15 ppm	90,90	91,82	92,22	92,22	93,67
Green tea extract 20 ppm + NG 20 ppm	91,42	92,08	92,35	92,74	93,40
Green tea extract 20 ppm + NG 25 ppm	91,56	92,08	93,01	93,54	82,85

Green tea extract 20 ppm + NG 30 ppm	91,42	91,95	93,01	92,88	77,04
Green tea extract 20 ppm + NG 35 ppm	91,42	92,22	92,74	93,40	77,04

Calculation percent of DPPH free radical damping by green tea extract samples added with nanogold at concentrations of 5-35 ppm and reacted with 0.005% DPPH solution at 518 nm wavelength for 1 minute, 1 hour, 2 hours, 3 hours, 4 hours. The timing of the interaction of the sample with DPPH is very influential on the decrease of DPPH absorbance. This suggests that the longer the interaction time, the more radical muted by nanogold and green tea extract. DPPH radical damping by the addition of nanogold increased during minute 1 to 3 hours. However, it dropped dramatically when the 4th hour added nanogold concentration of 25 ppm, 30 ppm and 35 ppm. This is because nanogold particles have aggregated to inhibit antioxidant activity at high concentrations of 25 ppm to 35 ppm. The increase was only demonstrated by the addition of nanogold at a concentration of 5 ppm to 20 ppm.

Based on the graph it can be seen that the concentration of 5 ppm to 20 ppm the percent of free radicals damping or scaffolding continues to be increase. The results of optimum and best concentrations were shown at concentrations of 20 ppm having an average percentage of damping 92.4%. Decrease in antioxidant activity was shown at concentrations of 25 ppm to 35 ppm. It is caused that in the higher concentration nanogold cluster will grow larger and aggregation. This condition caused nanogold not be compete to bind the DPPH (free radicals). One way anova analysis was used in this study to test the hypothesis that the addition of nanogold had an effect on the antioxidant activity of green tea extract. The result of anova test gives significant value p <0,05 which shows that there is a real influence between nanogold variable on green tea extract.

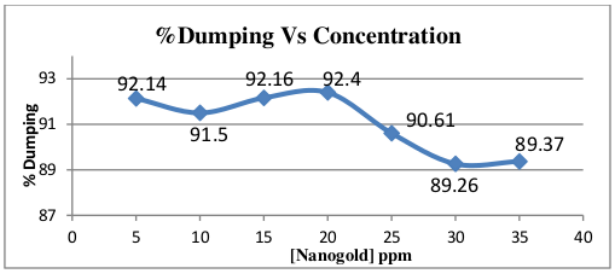

Fig 2. The percentage dumping green tea extract with nanogold

4. Conclusion

The results of antioxidant activity green tea extract 20 ppm was 91,16% that increased after 1 hour, 2 hours, 3 hours and 4 hours were 91,95%; 91.69%; 92.61% and 93.74%. The results obtained that antioxidant activity of green tea extract of 20 ppm were 91,16%. Than antioxidant activity of green tea extract with nanogold 5, 10, 15, 20, 25, 30 and 35 ppm are 90,63; 90,77; 90,90; 91,42; 91,56; 91,42, and 91,42. Nanogold various concentrations give increased the antioxidant activity of green tea extract was not significant. Nanogold various concentrations give unequal effects at the antioxidant activity of green tea extract. The effect at concentrations of 5, 10, 15 and 20 ppm nanogold decreased the antioxidant activity of green tea extract, while the concentration of 20 and 25 ppm of nanogold increased the antioxidant activity of green tea extract. In conclusion to increase the antioxidant activity in cosmetic formulas, the nanogold and green tea extract are included gradually or not simultaneously.

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