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# Production and Organoleptic Test of Hand Sanitizer *Nanogold, Nanosilver,* and Eucalyptus Oil in the Pandemic Covid-19

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**ABSTRACT:** Hand sanitizer is an antiseptic that contains alcohol and other active ingredients that can prevent and inhibit microbial infections. The use of hand sanitizer has increased during the COVID-19 pandemic. Hand sanitizer used continuously can cause damage to the skin because it contains harsh chemicals, such as alcohol and peroxide. Therefore, there needs to be innovation in hand sanitizer during the Covid-19 pandemic, ranging from antioxidants, antimicrobials to antivirals. The purpose of this study was to produce and conduct organoleptic tests on hand sanitizer with the addition of *nanogold*, *nanosilver*, and eucalyptus oil. The formulations used in the manufacture of this hand sanitizer have been in accordance with those set by WHO with the addition of the active ingredients *nanogold*, *nanosilver*, and eucalyptus oil. The addition of *nanogold* functions as an antioxidant to keep the skin healthy. *Nanosilver* and eucalyptus oil function as an antimicrobial to antiviral, which is suitable in the formulation of hand sanitizer during the COVID-19 pandemic. The results of the research at the organoleptic test stage showed that hand sanitizer with the addition of *nanogold, nanosilver*, and eucalyptus oil were preferred by the panelists with an average value of 3.45 in the color category, 3.95 in the fragrance category, and 3.50 in the absorption level category.

**KEYWORDS:** Covid-19; Eucalyptus oil; Hand sanitizer; *Nanogold*; *Nanosilver*.

## INTRODUCTION

At the end of 2019, the world was shocked by a severe outbreak of unknown causes. This started from a report from the city of Wuhan in China to the World Health Organization (WHO) that many of its citizens were infected with this disease. This disease is growing rapidly and has spread to almost all parts of the world. On February 11, 2020, the World Health Organization (WHO) announced the name of this disease as *Corona Disease Virus* (Covid-19) caused by the SARS-CoV-2 virus, previously referred to as 2019-n CoV. It was declared a pandemic on March 12, 2020 [1]. This virus can be transmitted to other people through respiratory secretions and contact with surface objects or with other people [2].

In early 2020, this virus began to enter Indonesia. This made so many people sick that hospitals were overcrowded, and health workers were starting to get overwhelmed. In the end, the government appealed to every citizen to start being careful and always maintain cleanliness and health. With the current pandemic, everyone is starting to be careful in maintaining personal and environmental hygiene.

Hand sanitizer is one of the antiseptics that is often used by the public as a more practical handwash medium [3]. The use of hand sanitizer is increasing during the *new normal*. Many people carry hand sanitizer every day, especially when traveling. This is solely done by the community to maintain their own health, as well as the health of others. Hand sanitizer needs innovation with antimicrobials to antivirals. Thus, the hand sanitizer product that will be produced will have different characteristics from the hand sanitizer in general. The hand sanitizer formula that has been set by WHO is 70% alcohol and 3% peroxide. It is not friendly to our skin, considering that we often use it. Skin that is continuously exposed to harsh chemicals, such as alcohol and peroxide, will be very dangerous for skin health.

*Nanogold* is an inorganic metal nanoparticle with a size of 5-400 nm and has unique physicochemical properties, and is superior to the bulk [4]. *Nanogold* is known to have a high antioxidant ability which is used to ward off free radicals. Other benefits *Nanogold* are in cosmetic formulations can be used to restore skin damaged by harmful chemicals to normal conditions [5]. *Nanosilver* is known to have an excellent antibacterial ability and can be applied in everyday life. Silver ions can inhibit bacterial replication by denaturing bacterial DNA, which causes damage and death of bacteria [6]. *Nanosilver* is antibacterial, antifungal, and antiviral because silver ions are highly toxic to bacterial cells, viruses, and eukaryotic microorganisms [7]. *Nanosilver* is also

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resistant to changes in temperature and humidity compared to conventional antimicrobials and does not induce bacterial resistance [8].

*Eucalyptus* sp is an essential plant used for various health products or pharmaceuticals because it contains the compound 1-8-cineol [9]. Eucalyptus oil has an active ingredient called *eucalyptol* or 1-8-cineol. This active ingredient is known to have antiviral properties. In an *in silico*, *eucalyptol* or 1,8-cineol has the potential to inhibit Covid-19 infection. This inhibition is carried out by binding to the Covid-19 proteinase. The Mpro-*eucalyptol* will form hydrophobic interactions, hydrogen bond interactions, and strong ionic interactions. 1,8-Sineol *in silico* can inhibit virus replication by binding to spike proteins or proteins from the coronavirus [10].

The addition of *nanosilver* and eucalyptus oil will strengthen the effectiveness of the antivirus against the *coronavirus*. Likewise, the addition of *nanogold* in the hand sanitizer will make the skin healthier. With these facts, it has supported research on "Production and Organoleptic Test of Hand sanitizer *Nanogold, Nanosilver* and Eucalyptus Oil in the Pandemic Covid-19".

## MATERIALS AND METHODS

### Tools

1000 ml beaker glass, hot plate, analytical balance Ohaus, test tube, 50 measuring cup ml, 100 ml beaker glass, stirrer, watch glass, dropper, *hand sanitizer*, micropipette, funnel, 50 ml volumetric flask, spatula, blue tip, Shimadzu 1800 UV-Vis Spectrophotometer, and Transmission Electron Microscope (TEM).

### Materials

$AgNO_3$, $HAuCl_4$, sodium citrate, *aquadest*, aquaregia, ethanol pa, DPPH powder, hand sanitizer basic, and eucalyptus oil solution.

### Method

#### *Synthesis and Characterization of Nanogold*

Synthesis of *nanogold* was made from a yellow solution of $HAuCl_4$ 1000 ppm. $HAuCl_4$ will be diluted six times as much as 100 ml with concentrations of 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm, and 30 ppm. 100 mL of distilled water is heated in a beaker until it boils. Next, 0.5 mL, 1 mL, 1.5 mL, 2 mL, 2.5 mL, and 3 mL of $HAuCl_4$ were added to each beaker. While heated, the reducing agent sodium citrate was added at each concentration of 2 grams. Then the solution is stirred while observing the color change that occurs. Heating is stopped when a burgundy color has formed. This indicates that nanogold was formed in this synthesis. The six *nanogold* with different concentrations were then characterized using a Transmission Electron Microscope (TEM) [11].

#### *Synthesis of Nanosilver Concentration of 20 ppm*

Synthesis of *nanosilver* was made from a colorless 1000 ppm $AgNO_3$ solution.. 100 mL of distilled water is heated in a beaker until it boils. Then $AgNO_3$ 2 ml while heated, added 2 grams of sodium citrate as a reducing agent. The solution is stirred while observing the changes that occur. Heating is stopped when the solution color changes to yellow [12].

#### *Antioxidant Activity Test*

0.002 gram of DPPH powder was put into a 50 ml volumetric flask, and ethanol was added to the mark, then shaken until homogeneous to obtain a DPPH solution with a concentration of 0.04%. Next, the DPPH solution was incubated for 30 minutes in a dark place at room temperature. After that, the DPPH solution was measured using a UV-Vis spectrophotometer at a wavelength of 400-800 nm. So that the data obtained for the maximum wavelength (λ) of DPPH which will be used to measure the absorbance of *nanogold*.

Next, the *nanogold* samples at each concentration were put into six dark bottles, and 0.04% DPPH solution was added in a 2:1 ratio. After that, the solution was shaken and incubated for 30 minutes at room temperature. Then, the solution was measured with a UV-vis spectrophotometer at a maximum wavelength of DPPH. The absorbance value of the solution was recorded, and the percent reduction (%) of free radicals was calculated [13].

#### *Making Hand Sanitizer*

Hand sanitizer basic, as much as 540 ml is poured into a 1000 ml beaker. Then, 30 ml of *nanogold*, 30 ml of *nanosilver*, and 5 drops of eucalyptus oil were added. All the ingredients were then stirred until homogeneous. After homogenization, hand sanitizer is immediately packaged.

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***Organoleptic Test***

This test method uses the human senses as the main tool for measuring product acceptance [14]. The senses used in assessing this product are the senses of sight, touch, and smell. In this test, consumers will be given a questionnaire which is used as a tool to obtain data. This questionnaire contains a scoring table and a list of questions to be filled out by the panelists. The organoleptic test on hand sanitizer was carried out by 20 panelists who had used hand sanitizer in Surabaya. The types of hand sanitizers compared include basic hand sanitizer (A) and hand sanitizer after adding *nanogold*, *nanosilver*, and eucalyptus oil (B). This test was carried out for three days and is used routinely on each product. Panelists' assessments are written in the form of a hedonic scale of 1-4, with the level of preference increasing as the number increases (1 = dislike, 2 = dislike, 3 = like, 4 = like very much). Organoleptic assessment parameters include color, fragrance, and absorption rate [15].

## RESULT AND DISCUSSION

***Synthesis and Characterization of Nanogold***

*Nanogold* was synthesized using a chemical method (*bottom-up*). The three basic ingredients used in the synthesis of *nanogold* were distilled water, sodium citrate, and$_4$ 1000 ppm sodium citrate functions as a reducing agent that will reduce gold metal ions ($Au^{3+}$) to gold atoms that are not charged ($Au^0$). Sodium citrate, when in water, will undergo hydrolysis, which will produce citric acid. This citric acid will then reduce gold metal ions ($Au^{3+}$) from the $HAuCl_4$ and produce gold atoms that have no charge ($Au^0$). Another function of adding sodium citrate is as a stabilizing agent (*capping agent*). The negative charge of the citrate ion will be absorbed by the surface of the *nanogold* so that the *nanogold* will repel each other because of the negative charge around the surface. This can prevent the aggregation of *nanogold*. The reactions that occur in the *nanogold* synthesis process are as follows:

$2HAuCl_4$ (l) + $3C_6H_8O_7$ (l) → 2Au (s) + $3C_5H_6O_5$ (l) + 8HCl (aq) + $3CO_2$ (g) [16].

The color change that occurs in the synthesis of nanogold starts from a colorless solution, then becomes dark blue, and the last is burgundy. At the beginning of the synthesis of *nanogold*, the solution was still a colorless solution because the gold atoms had not interacted with each other. Then during the synthesis process, the gold atoms with each other will start to interact slowly, which produces a dark blue color. The color change continued until the solution turned burgundy. This indicates that *the cluster* has reached nano size [17]. The color change in the solution during the *nanogold* synthesis process indicates the growth *cluster* is getting bigger.

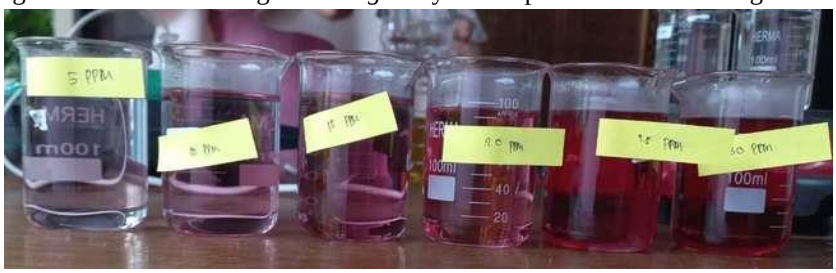

**Figure 1.** Nanogold synthesis results with concentrations of 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm, and 30 ppm

***Morphological Characterization Nanogold Using (Transmission Electron Microscope) TEM***

*Nanogold* with a concentration of 20 ppm that has been synthesized and then characterized by using the Transmission Electron Microscope (TEM). The purpose of characterization using TEM is to determine the shape and size of *nanogold*. The results of the characterization using TEM, which shows the shape of *nanogold*, can be seen in Figure 2.

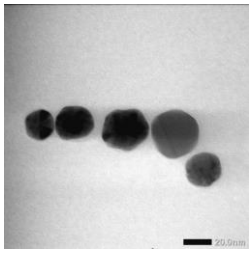

**Figure 2.** The results of TEM photos of nanogold at a concentration of 20 ppm

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**Figure 2** shows that *nanogold* with a concentration of 20 ppm has a *spherical* (round). Then the diameter of the *nanogold* in the image was measured using the ImageJ application, which produces the data in table 1 below:

**Table 1.** Size of Nanogold that has been measured using the imageJ application.

| Label | Size (nm) | |
|---|---|---|
| | 1 | 2 |
| 1 | 24,012 | 22,950 |
| 2 | 31,487 | 33,847 |
| 3 | 31,555 | 29,193 |
| 4 | 26,798 | 22,906 |
| 5 | 20,983 | 20,349 |

The measurement of the diameter of the *nanogold* was carried out twice vertically and horizontally. Each measurement result states the maximum and minimum measurement assumptions. Based on table 1, it can be seen that the diameter of *nanogold* is in the range of 20-33 nm. The diameter *nanogold* is 20,349 nm, and the *nanogold* is 33,847 nm. From these data, the average diameter *nanogold* is 26,408 nm. This shows that the gold particles have a size in the nanoscale because they are at a value of <100 nm.

***Synthesis of Nanosilver concentration of 20 ppm***

*Nanosilver* with a concentration of 20 ppm was synthesized using *bottom-up*. The three basic ingredients for the synthesis of *nanosilver*, aquadest 1000 ppm $AgNO_3$ mother liquor, *and* sodium citrate. The function of the addition of sodium citrate is to reduce silver metal ions $Ag^+$ to silver atoms $Ag^0$. The main problem in the synthesis of *nanosilver* is agglomeration which can cause the *nanosilver* to become larger. Agglomeration can be prevented by synthesizing at a low concentration of 20 ppm. At low concentrations, agglomeration will be difficult to occur. The reactions that occur in the synthesis of *nanosilver* are as follows:

$4Ag^+ + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^0 + C_6H_5O_7H_3 + 3Na^+ + H^+ + O_2$ [18].

The color change that occurs in the synthesis of *nanosilver* starts from a colorless solution to a yellow solution. The yellow color in the solution indicates that *nanosilver* has been formed [19].

**Figure 3.** The results of the synthesis of nanosilver with a concentration of 20 ppm

***Antioxidant Activity Test***

*Nanogold* with various concentrations that have been synthesized and then tested for antioxidant activity. This test aims to determine the ability of *nanogold* to reduce free radicals. In testing this antioxidant activity using the DPPH (*1.1-diphenyl-2-picryhydrazyl*) method and analyzed using the Uv-Vis Spectrophotometer instrument. DPPH (*1.1-diphenyl-2-picryhydrazyl*) is a stable free radical compound [20]. This method was chosen because the process is easy, simple, fast, inexpensive, and requires only a small sample due to its high sensitivity [21]. This test consists of three stages, namely: measuring the maximum wavelength (λ) of DPPH, measuring the absorbance value of *nanogold*, and measuring the absorbance value of *nanogold* that has been added with DPPH. The purpose of storing DPPH in a dark place is to prevent the dissolution of the DPPH solution because it is easily oxidized [22]. Incubation is carried out for 30 minutes so that DPPH can react perfectly with the sample [23].The maximum wavelength (λ) of 0.04% DPPH solution was measured at a wavelength of 400-800 nm using a UV-Vis Spectrophotometer. The results of the analysis of the Uv-Vis Spectrophotometer showing the maximum wavelength (λ) of DPPH can be seen in Figure 4.

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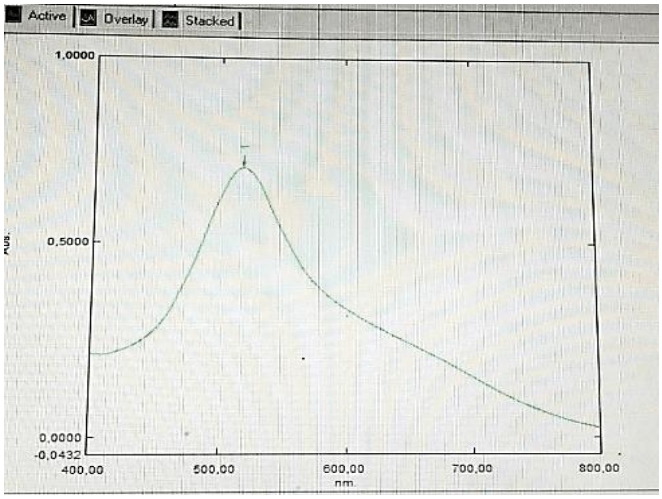


**Figure 4.** The maximum wavelength (λ) of DPPH is at 400-800 nm

From Figure 4, the maximum DPPH wavelength (λ) data is 517 nm. The maximum wavelength (λ) of DPPH data is used as a reference to measure the absorbance value of DPPH, the absorbance value of *nanogold* before adding DPPH, and the absorbance value of *nanogold* after adding DPPH. The result of measuring the absorbance value of DPPH is 0.8690. While the results of measuring the absorbance value of *nanogold* before and after adding DPPH can be seen in table 2-3.

**Table 2.** The absorbance value of nanogold before adding DPPH

| ***Nanogold* Concentration (ppm)** | **Absorbance** |
|---|---|
| 5 | 0.1201 |
| 10 | 0.1609 |
| 15 | 0.2314 |
| 20 | 0.2965 |
| 25 | 0.3771 |
| 30 | 0.4184 |

**Table 3.** The absorbance value of nanogold after adding DPPH

| ***Nanogold* Concentration + DPPH (ppm)** | **Absorbance** |
|---|---|
| 5 | 0.6033 |
| 10 | 0.6088 |
| 15 | 0.6516 |
| 20 | 0.7062 |
| 25 | 0.7561 |
| 30 | 0.7510 |

From this data, it will be used to calculate the percentage of free radical scavenging by nanogold using the following calculations:

$$\%\text{Inhibition} = \frac{DPPH\ Absorbance - Absorbance\ Sample}{Absorbance\ DPPH\ Absorbance} \text{ x } 100\%$$

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In the above calculation, the absorbance of the sample is the reduction of the absorbance value of *nanogold* after adding DPPH to the absorbance value of *nanogold* before adding DPPH. The results of calculations and graphs of free radical reduction from various concentrations of *nanogold* can be seen in table 4 and figure 5.

**Table 4.** Results of the calculation of %free radical inhibition by nanogold at each concentration

| Concentration (ppm) | Abs DPPH | Abs NG + DPPH | Abs NG | Abs sample | % inhibition |
|---|---|---|---|---|---|
| **5** | 0.869 | 0.6033 | 0.1201 | 0.4832 | 44.40 |
| **10** | 0.869 | 0.6088 | 0.1609 | 0.4479 | 48.46 |
| **15** | 0.869 | 0.6516 | 0.2314 | 0.4202 | 51.65 |
| **20** | 0.869 | 0.7062 | 0.2965 | 0.4097 | 52.85 |
| **25** | 0.869 | 0.7561 | 0.3771 | 0.379 | 56.39 |
| **30** | 0.869 | 0.751 | 0.4184 | 0.3326 | 61.73 |

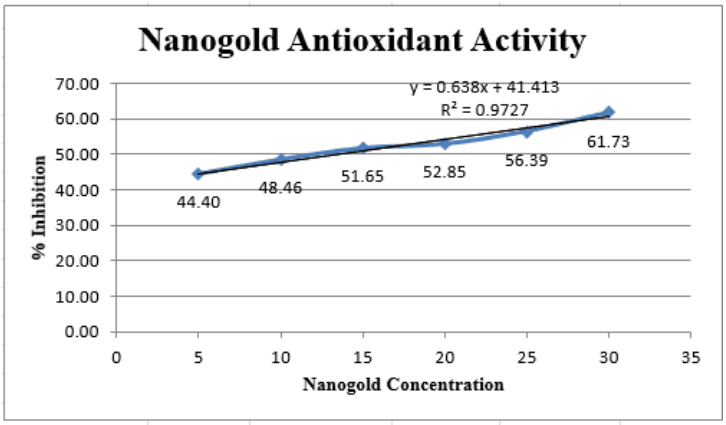


**Figure 5.** Graph of the relationship between nanogold at each concentration and the ability to reduce free radicals

Based on the graph above, it can be concluded that the higher the concentration of *nanogold*, the higher the ability of *nanogold* to reduce free radicals. This is because the higher the *nanogold*, the more *nanogold clusters* are formed. Thus, more and more *nanogold clusters* are able to reduce free radicals.

To determine the antioxidant activity of the DPPH method, the $IC_{50}$ (50% inhibition concentration) was calculated. $IC_{50}$ is the concentration of the sample solution that can inhibit free radical activity by 50% [24]. The $IC_{50}$ is calculated by using a linear regression equation that has been obtained from the graph of the relationship between *nanogold* and % attenuation. From the graph above, the linear regression equation is obtained, namely y = 0.638x + 41,413 with $R^2 = 0.9727$ . From the linear regression equation, the $IC_{50}$ by replacing the y variable with the number 50 so that the value of the x variable can be known. Variable y is the percentage of inhibition, and variable x is the sample concentration required to reduce 50% of DPPH free radical activity [25].

The $IC_{50}$ from the linear regression equation is 13.4531 ppm. A compound is a very strong antioxidant if it has an $IC_{50}$ value of <50 ppm. If a compound has an $IC_{50}$ between 50-100 ppm, then the compound is included in the category of strong antioxidants. For compounds that have $IC_{50}$ between 100-150 ppm, they are included in the category of moderate antioxidants. Compounds that have an $IC_{50}$ between 150-200 ppm are included in the category of weak antioxidants and compounds that have an $IC_{50}$ value of >200 ppm are included in the category of very weak antioxidants [26]. Based on the data that has been obtained, it can be seen that the antioxidant activity of *nanogold* is in the very strong category. This is because *nanogold* has an $IC_{50}$ of less than 50 ppm, which is 13.4531 ppm.

***Organoleptic Test***

The organoleptic test stage for *hand sanitizers* was carried out on two products, namely *hand sanitizer* basic (**A**) and *hand sanitizer* with the addition of *nanogold*, *nanosilver* and eucalyptus oil (**B**).

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**Figure 6.** Hand sanitizer (A) and Nanogold Nanosilver and Eucalyptus Oil Hand sanitizer (B)

The organoleptic test assessment includes three categories, namely: color, fragrance, and absorption rate, which are assessed by 20 panelists. The average results of the assessments of the 20 panelists can be seen in the following table and bar chart:

**Table 5.** Average Value of Organoleptic Test (Color, Fragrance, Absorption Rate) on the quality of both hand sanitizer

| Parameter | Average Hedonic Test Value Sample | |
|---|---|---|
| | A | B |
| Color | 3.30 ± 0.571[a] | 3.45 ± 0.605[a] |
| Fragrance | 3.50 ± 0.827[a] | 3.95 ± 0.826[a] |
| Absorption rate | 2.95 ± 0.686[a] | 3.50 ± 0.607[b] |

Description: 1 = dislike; 2 = dislike; 3 = likes; 4 = very much like
ab = different letter notations on the same line indicating a significant difference (P < 0.05) at the Kruskal Wallis test level

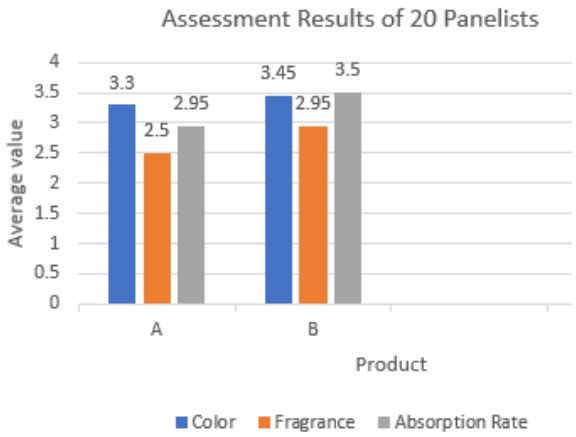


**Figure 7.** The results of the assessment of 20 panelists on the color, fragrance, and absorption rate of the two hand sanitizer

Based on **Table 5**, products hand sanitizer A and B in the color test showed that there was no significant difference (P > 0.05) at the Kruskal Wallis test level. In the fragrance test, the results also showed that there was no significant difference (P > 0.05) between products A and B at the Kruskal Wallis test level. The absorption rate test gave results indicating that there was a significant difference (P < 0.05) between product A and product B at the Kruskal Wallis test level.

The color assessment of product A produces an average value of 3.30 and product B of 3.45. The average results of products A and B fall into the like category. The **Figure 7** color assessment of product B has a higher average value than product A. Thus, it can be said that the color of product B is preferred by the panelists. The assessment of the fragrance category in product A resulted in an average value of 3.50 and product B of 3.75. The results of the average value of products A and B fall into the like category. Based on **Figure 7**, it can be seen that in the fragrance category, the average value of product B is higher than product A. Thus, it can be concluded that the fragrance of product B is preferred by the panelists.

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In the absorption level category assessment, product A produced an average value of 2.95 and product B of 3.50. The results of the average value of product A fall into the less favorable category. While the results of the average value of product B fall into the preferred category. **Figure 7** shows that the absorption rate assessment of product B has a higher average value than product A. Thus, it can be concluded that the level of absorption of product B is preferred by the panelists.

Organoleptic tests conducted by 20 panelists showed that product A 45% could cause the skin to feel smoother, 85% do not cause skin itching, 70% do not feel hot, and 85% do not cause redness and irritation of the skin.

Meanwhile, in the organoleptic test of product B, data showed that product B 90% could cause the skin to feel smoother, 95% do not cause skin itching, 95% do not feel hot, and 95% do not cause redness and irritation of the skin.

## CONCLUSIONS

Hand sanitizer with the best formulation is a hand sanitizer with the addition of *nanogold*, *nanosilver*, and eucalyptus oil. This is because hand sanitizer with the addition of *nanogold*, *nanosilver*, and eucalyptus oil have antioxidant activity that can reduce free radicals. And based on organoleptic tests, hand sanitizer containing *nanogold*, *nanosilver*, and eucalyptus oil were preferred by the panelists.

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