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Effectiveness of Nanogold-Nanosilver Hand Soap on Hand Skin Using Statistical Test Method

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Abstract – The effectiveness of nanogold-nanosilver hand soap on hand skin using the statistical test method "paired sample T-test" by the application of SPSS gets a Sig. 2 - tailed value = 0. Based on theory, if the value is less than 0,05 it indicates that there is a significant difference in the effectiveness of using nanogold-nanosilver hand soap which is better than using hand soap in general. The organoleptic test of the color, scent, and texture indicators of the two soaps (between standard hand soap and nanogold-nanosilver hand soap) are on average liked by respondents, and also showed an increase of 24% the condition of the hand skin became more moist and by 19% the skin of the hands became smoother. The ratio of the mixture of nanogold-nanosilver hand soap is 7: 2: 1. Ratio 7 is hand soap in general, ratio 2 is nanosilver 20 ppm, and ratio 1 is nanogold 20 ppm, this is based on the results of the most stable concentration characteristics test at a concentration is 20 ppm with an average nanoparticle size is 21,205 nm and known to have 9 clusters. The results of the nanogold antioxidant activity test resulted in an IC50 value of 13,4691 ppm.

Keywords – Covid, organoleptic test, antioxidant, nanoparticle, cluster

I. Introduction

The use of handwashing soap has been intensified since the emergence of a new and dangerous virus, which threatens human lives around the world. The virus is called 2019-nCoV which originated in Wuhan City (China). According to WHO (*World Health Organization*), at least 45,171 cases have been reported in early 2020, with a total death toll of 1,115 people [1]. *Corona Virus Disease-19* (Covid-19) is a disease term caused by *Severe Acute Respiratory Syndrome Coronavirus* 2 (SARS-CoV-2). The development of Covid-19 is very fast and spread throughout the world, resulting in a global pandemic. With the potential for sufferers to experience life-threatening conditions around 20% [2].

With this potential, it resulted in the issuance of regulations in each country to take precautions. These actions are called health protocols, which function so as not to multiply or increase victims due to exposure to the Covid-19 virus [3]. Activities in the health protocol include wearing masks, avoiding leaving the house, avoiding crowds, maintaining distance, always maintaining cleanliness, and always washing hands with soap even though they are inside the house [3,4].

That way, using soap every day and every time is an obligation for everyone. Where soap serves to clean fat (dirt). Just like soap in general, hand soap is formulated more specifically and focuses on cleaning dirt, especially to prevent the spread of germs, viruses, or bacteria on the hands. Meanwhile, frequent hand washing with soap can cause damage and irritation to the skin, such as reddened, dry, and peeling skin [5].

The damage caused by too much handwashing occurs due to the absorption of excess oil on the skin, which in turn reduces the skin's moisture. If the skin moisture is reduced, the skin will be easily irritated. Unhealthy skin like this can interfere with performance in daily activities [6]. Thus, additional active ingredients are needed that can work optimally without damaging the skin surface and can regenerate damaged skin quickly. The composition can be found in nanoparticles such as gold (nanogold) and silver (nanosilver).

Nanogold in hand soap formulations functions as an agent that moisturizes and softens the skin because it is an antiaging material commonly used in cosmetic products [7]. In its nano size, it can facilitate the skin regeneration process faster because it can provide the highest catalytic effect as a collagen-forming catalyst [8]. Meanwhile, nanosilver in hand soap additives is useful for providing high antimicrobial effects because it has properties that tend to be stable. Therefore, nanosilver has been developed and used in modern cosmetic formulations [9].

The addition of gold and silver nanoparticles in hand soap formulations is considered safe for skin health because nanogold and nanosilver do not easily oxidize like other metal properties, and even tend to be more reduced so they are considered safe [9,10]. So, based on this description, this study refers to the comparison of the effectiveness of standard hand soap with nanogold-nanosilver hand soap on hand skin using statistical test methods.

II. Experimental Section

2.1 Materials

The materials used in this study are 1000 ppm $HAuCl_4$ solution, 1000 ppm $AgNO_3$ solution, Sodium Citrate, DPPH solids, distilled water (aquades), 96% ethanol, and ready-to-use hand washing soap (standard hand soap).

2.2 Instrumentation

The instrument used are UV-Vis Spectrophotometer (Shimadzu Pharmaspec UV- 1700) and TEM Jeol Type JEM 1400 (*Transmission Electron Microscopy*). UV-Vis Spectrophotometer was used to calculate the absorbance value of the antioxidant test and measure the wavelength for the stability characteristic test of the nanogold. TEM is used to observe and know the shape and size of the colloidal nanogold.

2.3 Procedure

Nanogold Synthesis

The synthesis of nanogold concentrations of 5, 10, 15, 20, 25, and 30 ppm was made by heating 100 mL of aquades in a glass beaker until boiling, then entering each $HAuCl_4$ solution 1000 ppm sequentially as much as 0.5 mL, 1 mL, 1.5 mL, 2 mL, 2.5 mL, and 3 mL. Then put as much as 2 grams of sodium citrate in each solution in a beaker glass. Stir and wait until the color of the solution changes, originally yellow, to a concentrated red color [10].

Nanosilver Synthesis

The synthesis of nanosilver with a concentration of 20 ppm was made by heating 100 mL of aquades in a glass beaker until boiling, then adding 2 mL of 1000 ppm $AgNO_3$ solution. Then put 2 grams of sodium citrate while stirring and wait until the solution changes color to yellow [10].

Antioxidant Activity Test of Nanogold

In this antioxidant activity test, a DPPH solution is needed as a control solution with a concentration of 0.004%, by dissolving 0.002 grams of DPPH solids into a 50 mL volumetric flask. Then the DPPH solution was incubated for 30 minutes at room temperature with the condition of the volumetric flask covered by aluminum foil. This testing process uses a UV Vis Spectrophotometer instrument and how works by reading the absorbance value of the sample solution and DPPH solution at the maximum wavelength of the DPPH solution. The sample solution is a mixture of 2 mL of nanogold solution and 1 mL of 0.004% DPPH solution [11].

Nanogold Characterization Test

There are two characterization tests on nanogold, called stability characterization and size characterization [12]. Where stability characterization requires UV-Vis Spectrophotometer to measure the stability of the nanogold based on changes in wavelength (λ) of the first nanogold synthesis results at each concentration until storage for 7 days [10]. Then size characterization is done to observe and know the shape and size of the nanogold with TEM up to 50 nm image scale [12].

Organoleptic Test of Hand Soap

This organoleptic test was conducted in January 2022 and had 20 panelists of various ages from Watulimo District, Trenggalek Regency, East Java, Indonesia. With indicators to observe the color, smell, and texture, then see changes in results after use such as moisture, and smoothness on the hands [13], and in this test, standard hand washing soap is needed as a comparison of 20 mL. Then nanogold and nanosilver-based hand soap are put into a bottle of 20 mL with a consecutive ratio of basic liquid hand soap: nanogold: nanosilver is 7: 1: 2 [10,12]. Finally, the results of the data from this organoleptic test will be processed using basic statistical test methods and statistics test with the SPSS (*Statistical Program for Social Science*) application.

III. Results And Discussion

3.1 Nanogold Synthesis

The process of making nanogold synthesis uses a commonly used method, namely the Turkevich method, with concentrations of 5, 10, 15, 20, 25, and 30 ppm. The basic material for making it uses 1000 ppm $HAuCl_4$ solution (tetra chloroauric acid) with the reducing agent sodium citrate. This is because sodium citrate is a reducing agent that acts as a stabilizer so that gold aggregates are not formed in the resulting nanogold solution. Compared to other synthesis methods, such as electrochemical methods, this synthesis method is considered faster, simpler, and easier to apply [14].

$HAuCl_4$ solution is a yellow solution, when it is boiled together with aquades, then added with sodium citrate, the solution will change color to intense red, this indicates that a reaction is taking place where gold nanoparticles are formed [10].

Fig. 1 Nanogold synthesis results

Based on Fig. 1, the synthesis results are solid red, this is because the synthesis results are good or not aggregated if they have a final color of red or solid red. If the synthesis results are blue, then the solution is experiencing aggregation caused by changes in the temperature of the material in it [10]. The reactions that occur in this nanogold synthesis are:

$$HAuCl_4 .3H_2O \leftrightarrow AuCl_4^- + H_3O^+$$

$$AuCl_4^- + \text{Sodium Citrate} \leftrightarrow \text{Nanogold} \qquad [14]$$

3.2 Nanosilver Synthesis

The process of synthesizing nanosilver with a concentration of 20 ppm uses a chemical reduction method. The basic material used 1000 ppm $AgNO_3$ solution with sodium citrate as the reducing agent. Sodium citrate has a negative charge that can be adsorbed by nanosilver, resulting in a repulsive force between silver particles, which can then prevent agglomeration. Therefore, the use of reducing agents such as sodium citrate has resulted in a colloidal synthesis of silver nanoparticles that tend to be more stable than using other reducing agents [12].

This method is made by heating 100 mL of distilled water in a *glass beaker* until boiling, the boiling process is needed to help accelerate the reduction process. Then added 2 mL of 1000 ppm $AgNO_3$ solution and 2 grams of sodium citrate while stirring and wait until the solution changes color to yellow [10].

Fig. 2 Results of nanosilver synthesis

The characteristic of the formation of colloidal nanosilver is the final result of the synthesis which changes color to yellow [15]. The reactions that occur in this synthesis process are:

$$4Ag^{+} + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^{0} + C_6H_5O_7H_3 + 3Na^{+} + H^{+} + O_2$$ [15].

3.3 Antioxidant Activity Test of Nanogold

An antioxidant activity test is used to determine the IC50 value or *inhibition concentration.* Where the value can determine how much reduction or inhibitory activity of nanogold against free radical compounds [16]. This process uses the DPPH method because this method is easier to apply and DPPH solids themselves are easy to obtain [17]. The required DPPH solution has a concentration of 0.004% and is obtained from dissolving 0.002 grams of DPPH solids into a 50 mL volumetric flask. Then the DPPH solution was incubated for 30 minutes at room temperature with the condition of the volumetric flask covered by aluminum foil [11].

This DPPH compound is sensitive to light, so a cover such as aluminum foil is needed to prevent damage [17]. Then a sample solution or mixed solution was made from 2 mL of nanogold solution and 1 mL of 0.004% DPPH solution. Then tested using a UV Vis Spectrophotometer instrument and how it works is by reading the absorbance value of the sample solution and DPPH solution as a control solution at the maximum wavelength of DPPH solution [11]. The maximum wavelength is 517 nm [18].

Table 1**.** DPPH Absorbance and Sample Absorbance in each Concentration

Concentration (ppm)	Absorbance	
	DPPH	Sample
5		0,4831
10		0,4481
15	0,8695	0,4204
20		0,4114
25		0,3794
30		0,3319

Through the absorbance value, the percentage of silencing can be determined (Table 2) and a graph will be obtained where the IC50 value can be calculated (Fig. 3).

$$\% \text{ inhibition } = \frac{Absorbance_{\ DPPH} - Absorban \quad _{Sample}}{Absorbance_{\ DPPH}} \ x\ 100\%$$ [11]

Table 2**.** Inhibition results at each concentration (ppm)

Concentration (ppm)	Inhibition (%)
5	0,4831
10	0,4481
15	0,4204
20	0,4114
25	0,3794
30	0,3319

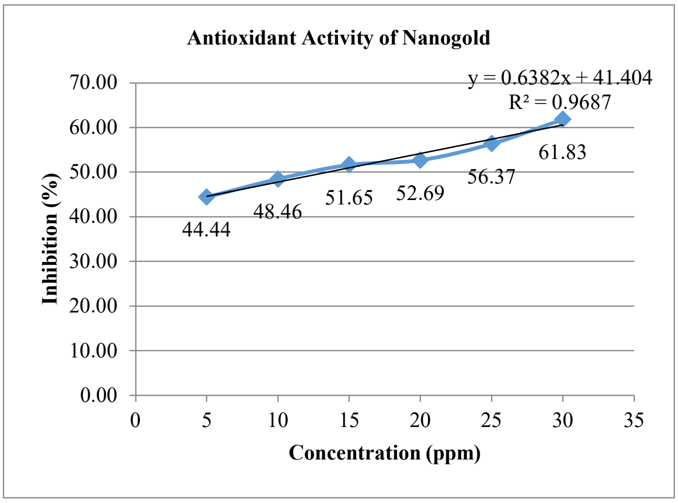

Fig 1. Antioxidant activity curve of nanogold based on percentage inhibition with concentration (ppm)

The calculation of the IC50 value is known in the linear regression equation (Y = bx + a) of the curve Fig. 3, by replacing Y with the number 50. Then the X value or the value of the IC50 will be obtained [16,18]. Thus, the IC50 value obtained in the nanogold antioxidant activity test is 13.4691 ppm. This category of antioxidant activity is considered very strong, this is based on the indicators of IC50, there are 5; 1) very strong group, worth < 50 ppm, 2) strong group, worth 50-100 ppm, 3) medium group, worth 100-150 ppm, 4) weak group, worth 150-200 ppm, 5) and very weak group, worth > 200 ppm [11]. Thus, the smaller the IC50 value produced, the stronger the antioxidant activity of a product in counteracting free radicals, and vice versa [17].

The antioxidant activity of nanogold is considered stronger than the antioxidant activity of aloe vera extract, which is 250.42 ppm, with a very weak category. Although aloe vera extract itself qualitatively has antioxidant activity because it contains vitamins A, C, and E [19].

3.4 Nanogold Characterization Test

In the characterization test, there are 2 processes, namely stability characterization and size characterization [12]. Where stability characterization requires UV-Vis Spectrophotometers to measure the stability of the nanogold based on changes in wavelength (λ) of the first nanogold synthesis results at each concentration in the storage for 7 days [10].

Table 3. Wavelength difference of nanogold at first synthesis and after storage for 7 days

Concentration (ppm)	**Wavelength**	
	Day One	**Seventh Day**
5	525,00	525,00
10	528,00	528,00
15	529,50	529,50
20	531,00	531,00
25	527,00	526,00
30	526,50	528,00

Based on Table 3. it can be seen that at concentrations of 5 ppm to 20 ppm, the resulting λ is stable, while at concentrations of 25 and 30 ppm, it experiences a decrease-increase and is considered less stable. The factor of instability is the synthesis process that is less than optimal in providing reducing agents, resulting in instability in the synthesis results [12], then another factor is the uneven storage temperature, resulting in signs of damage to the synthesis results [14].

The largest concentration of nanogold synthesis which is declared stable is 20 ppm, from this concentration that the second characterization is tested, namely size characterization. Where this serves to determine the shape and size of the nanogold colloid following the theory. This process requires a TEM with a 50 nm image scale. The way this tool works is the same as an electron microscope but on a microscopic, nanoscopic, or atomic scale [12].

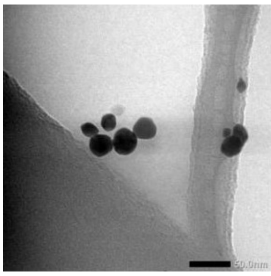

Fig 2. TEM test results of 20 ppm nanogold

In Fig. 4, it can be seen that the cluster shape of the gold nanoparticles is circular, with the number of clusters being 9.

Table 4. Size of each 20 ppm nanogold cluster

Cluster	Size (nm)	
	1	2
1	22.160	17.382
2	17.911	21.612
3	27.562	26.419
4	32.719	29.427
5	25.924	27.102
6	10.511	17.382
7	11.481	7.904
8	23.235	23.585
9	19.761	19.606

The average size of the nanogold cluster is 21,205 nm, the smallest size is 7,904 nm, then the largest size is 32,719 nm. Based on the average results, the size of the nanogold is stated by the theory, because the size of the nanoparticles themselves is in the range of 1-100 nm [12]. If the synthesis of nanogold using the turkevich method, then the results of nanoparticle size are obtained between 10-25 nm in diameter [14].

3.5 Organoleptic Test of Hand Soap

The organoleptic test was conducted in January 2022 and had 20 panelists of various ages from Watulimo District, Trenggalek Regency. Panelists have given each sample as much as 20 mL, called standard hand soap and nanogold-nanosilver hand soap with a consecutive ratio of soap: nanogold 20 ppm: nanosilver 20 ppm is 7: 1: 2 [10,12]. The test indicators are to observe color, scent, and texture, then see changes in results after use such as moisture, and smoothness on the hands [13].

Fig 3. Standard hand soap (yellow), nanogold-nanosilver hand soap (orange)

The color indicator in this test is a visual indicator, where the main attraction of a product comes from visible visuals. Where an attractive color will give a good first impression. Then the scent indicator will indicate whether or not a product is accepted, because scent or aroma is alluring, so it can increase or decrease the intensity of interest in a product. The onset of this scent is because it is volatile and slightly soluble in water or fat. Then the assessment of the texture indicator shows that if the texture is highly preferred, then the product is easy to apply. Meanwhile, indicators to see the effectiveness of the product can be seen in the percentage difference in hand skin condition if it changes. If the percentage states an increase, it is proven that the product is more effective than hand washing soap in general, and vice versa [13]. Each indicator in this test has an assessment category as shown in the table below [20]:

Table 5. Organoleptic test indicator assessment categories

Category	**Value**
Strongly Dislike	1
Dislikes	2
Enough Likes	3
Like	4
Very Favorable	5

From the organoleptic test of color, scent, and texture indicators, the data results are:

Table 6. Recapitulation of organoleptic test results for color, scent, and texture.

Panelists	**Soap Color**		**Soap Scent**		**Soap Texture**	
	Standard	**Nanogold Nanosilver**	**Standard**	**Nanogold Nanosilver**	**Standard**	**Nanogold Nanosilver**
1	5	4	4	5	2	4
2	5	3	4	4	3	3
3	5	4	5	3	3	5
4	5	3	5	5	4	2
5	4	4	2	5	5	3
6	5	5	5	4	4	4
7	4	4	4	4	5	5
8	5	4	5	4	4	2
9	3	4	3	4	3	3

Panelists	Soap Color		Soap Scent		Soap Texture	
	Standard	Nanogold Nanosilver	Standard	Nanogold Nanosilver	Standard	Nanogold Nanosilver
10	3	3	5	3	4	4
11	4	4	4	5	5	5
12	2	5	5	5	3	5
13	3	5	5	5	4	5
14	3	5	4	5	5	4
15	4	4	2	5	3	5
16	5	5	5	3	4	4
17	4	4	2	3	5	5
18	3	3	3	4	4	4
19	4	4	5	4	3	3
20	4	3	5	4	4	4
Average	4	4	4,1	4,2	3,85	3,95

It can be concluded that the color indicator has the same average of 4 in the like category, which indicates that both soaps have attractive colors. Then in the scent and texture indicators, only 0.1 difference is superior to nanogold-nanosilver soap and is in the enough like until like category, which indicates that nanogold-nanosilver soap is preferred in aroma and texture that is easy to apply.

Furthermore, the indicator of the moisture and smoothness of hands can be seen in different conditions in hand skin before using soap, after using standard hand soap for 3 days, and after using nanogold-nanosilver hand soap for 3 days [10], the results are obtained as in the following table:

Table 7. Recapitulation results in changes in moisture and smoothness of hands, before and after using nanogold-nanosilver hand soap

Panelists	Moisture			Smoothness		
	Before using soap	After using regular hand soap	After using nanogold-nanosilver hand soap	Before using soap	After using regular hand soap	After using nanogold-nanosilver hand soap
1	2	3	4	1	3	5
2	2	3	4	2	4	5
3	2	2	4	2	3	5
4	2	3	4	1	4	5
5	3	4	4	3	5	5
6	3	4	5	2	4	5

Panelists	Moisture			Smoothness		
	Before using soap	After using regular hand soap	After using nanogold-nanosilver hand soap	Before using soap	After using regular hand soap	After using nanogold-nanosilver hand soap
7	3	4	5	3	3	4
8	3	3	5	1	4	5
9	2	3	4	2	3	5
10	2	3	5	3	4	5
11	3	4	5	1	4	5
12	1	5	5	3	4	5
13	2	3	5	2	4	5
14	1	4	5	3	3	3
15	1	3	5	1	4	5
16	1	4	5	2	4	4
17	2	4	5	3	3	4
18	1	3	4	2	4	5
19	1	4	5	1	5	5
20	2	3	5	2	4	5
Average	1,95	3,45	4,65	2	3,8	4,75

Based on the results of Table 7. there are average results from 20 panelists for each category of hand condition after use, then the data can be presented in percentage form using the calculation:

$$\text{Data percentage} = \frac{\text{average value}}{\text{maximum value}} \text{ x } 100\% \qquad [21]$$

Thus, the average value for the hand moisture category before using soap is 1,95. Then divided by its maximum value of 5, and multiplied by 100%, resulting in a data percentage of 39%, for further percentage data, presented in Table 8.

Table 8**.** Percentage data of organoleptic test indicators on moisture and smoothness of hand skin.

Hand Skin Condition	Before using Soap (%)	After using Standard Handwashing Soap (%)	After using Nanogold-Nanosilver based Hand Soap (%)
Humidity	39	69	93
Subtlety	40	76	95

So, it can be seen that before using soap, the skin condition of the hands is not good, and with the use of hand washing soap there is an increase of about 30% in moisture and 36% in hand smoothness, then further increases after using nanogold-nanosilver-based hand washing soap about 24% in moisture and 19% in smoothness.

To know whether or not there is a significant difference between the use of nanogold-nanosilver hand soap and ordinary hand soap, then a statistical test was carried out with the paired sample T-Test method on SPSS for each hand skin condition data. This statistical test method serves to determine whether there is a significant change or shows a significant difference between the two variables (when using ordinary hand soap with nanogold-nanosilver hand soap) [21].

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Results using standard soap	3.45	20	0.686	0.153
	Results using nanogold nanosilver soap	4.65	20	0.489	0.109

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Results using standard soap & Results using nanogold nanosilver soap	20	0.494	0.027

Paired Samples Test

		Paired Differences							
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	95% Confidence Interval of the Difference Upper	t	df	Sig. (2-tailed)
Pair 1	Results using standard soap - Results using nanogold nanosilver soap	-0.950	0.605	0.135	-1.233	-0.667	-7.025	19	0.000

Fig 4. Statistical data of hand moisture indicators when using standard hand soap with nanogold-nanosilver-based hand soap

Based on statistical data, the average skin moisture condition using ordinary hand soap is 3.45 out of 20 data. While the average skin moisture condition using nanogold-nanosilver hand soap is 4.65 out of 20 data. Then the significance value (*2-tailed*) is 0, where $0 < 0.05$. It can be concluded that there is a significant difference between the initial variable (use of standard hand soap) and the final variable (use of nanogold-nanosilver hand soap).

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Results using standard soap	3.80	20	0.616	0.138
	Results using nanogold nanosilver soap	4.75	20	0.550	0.123

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Results using standard soap & Results using nanogold nanosilver soap	20	0.466	0.038

Paired Samples Test

		Paired Differences							
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
Pair 1	Results using standard soap - Results using nanogold nanosilver soap	-0.950	0.605	0.135	-1.233	-0.667	-7.025	19	0.000

Fig 5. Statistical data of hand smoothness indicators when using standard hand soap with nanogold-nanosilver-based hand soap

Based on statistical data, the average skin smoothness condition using ordinary hand soap is 3.80 out of 20 data. While the average skin moisture condition using nanogold-nanosilver hand soap is 4.75 out of 20 data. Then the significance value (*2-tailed*) is 0, where $0 < 0.05$.

In theory, if the Sig. 2-tailed is $p < 0.05$, then there is a significant difference. If the Sig. 2-tailed value is $p > 0.05$, then there is no significant difference [21]. So, it can be concluded that there is a significant difference (meaningful) between the initial variable (use of ordinary hand washing soap) and the final variable (use of nanogold-nanosilver hand washing soap).

IV. Conclusions

The nanogold synthesis has a solid red finish, while the nanosilver synthesis has a yellow finish. In the antioxidant activity test of nanogold, the IC50 value of nanogold is 13.4691 ppm and is classified as a very strong category. The concentration of nanogold that has stability for 7 days seen from the stable wavelength on the UV Vis Spectrophotometer instrument is 20 ppm. TEM test results from 20 ppm nanogold are known to have 9 clusters, with an average size of 21.205 nm, this is stated according to theory because the average nanogold size has a diameter of 10-25 nm. In the organoleptic test, the color, scent, and texture indicators of the two soaps (between standard hand soap and nanogold-nanosilver hand soap) are on average liked by respondents. While the indicators of moisture and smoothness of hand skin from when using standard hand soap to nanogold-nanosilver hand soap increased by 24% and 19%. Through statistical tests, it can be seen that the significance value (*2-tailed*) is 0, where $0 < 0.05$, which means that there is a significant difference between the initial variable (use of ordinary hand washing soap) and the final variable (use of nanogold-nanosilver hand washing soap). So, nanogold-nanosilver hand soap is considered more effective for cleaning hands and making skin more moist and smooth, without giving side effects of damage to the skin such as irritation or dry skin.

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VI. AUTHOR CONTRIBUTIONS

The authors, Putri Azizah K. F., conducted this experiment from the beginning until the finish, analyzed the results of each test data, and wrote the manuscript, while Titik Taufikurohmah made experimental procedures, provide direction towards appropriate data results, and revised this manuscript. This outcome of the manuscript has been approved by the authors.

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