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Synthesis, Characterization, and Antifungal Activity Test of Nanosilver in Body Lotion Preparation

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ABSTRACT: Nanosilver is a nanoparticle known for its good antifungal ability. This study aims to determine the synthesis process and characterization of nanosilver produced and the antifungal activity of nanosilver in body lotion preparations. Body lotion in this study was made using a body lotion base which includes stearic acid, cetyl alcohol, TEA, glycerin, olive oil and distilled water. F1, F2, and F3 have nanosilver content of 10%, 15% and 20%, respectively. F4, as the positive control, contained methylparaben and F5 as a negative control. The body lotion produced in this study has a colour, aroma and texture that panellists prefer. The body lotion produced does not irritate the skin, is homogeneous, has good spreadability and has a pH value of 7. Nanosilver in this study was synthesized using a chemical reduction method with sodium citrate as a reducer and stabilizer. The synthesized nanosilver was characterized using a Transmission electron Microscope (TEM) and UV-Vis spectrophotometer to determine the nanosilver's shape, size and stability. The nanosilver produced has a round shape with an average particle diameter of 34.793 nm and has good stability because it has a wavelength absorption peak that is not large. Namely, at week-0, nanosilver has an absorption peak at a wavelength of 415.20 nm, and week-8 has an absorption peak at a wavelength of 423 nm. The antifungal activity test using the direct microscope count (DMC) method was conducted for eight weeks. Until week 8, it showed that each formulation experienced an increase in fungal hyphae, reaching 34.4% for F1, 21.2% for F2, 19.8% for F3, 16.8% for F4 and 46% for F5. From the results of this study, it can be seen that nanosilver can suppress fungal activity in body lotion preparations.

KEYWORDS: Antifungal, Body lotion, DMC, nanosilver, TEM, UV-vis spectrophotometer.

INTRODUCTION

The skin is the body part that has direct contact with the environment. Poor environmental conditions such as pollution and ultraviolet radiation cause the skin balance to be disturbed, causing the skin to become scaly, dry and rough [1]. Pollution causes an increase in Transepidermal Water Loss (TEWL) due to damage to the skin barrier by pollution. Increased TEWL results in skin hydration ability [2]. Meanwhile, exposure to ultraviolet light causes skin cells to absorb radiation, induce oxidative stress and produce reactive oxygen species (ROS) that cause DNA and cell wall damage. As a result, collagen is damaged by matrix metalloproteinase (MMP) enzymes, and the elastin structure becomes irregular, causing the skin to become rough and dry [3]. To overcome these problems, it is necessary to have cosmetics that can hydrate the skin, such as body lotion [4].

Body lotion reduces the evaporation of excess water on the skin and attracts water in the air to enter the stratum corneum [4]. The constituent components of body lotion include moisturizers, emulsifiers, solvents, fragrances, preservatives and active ingredients [5]. One of the constituent components of body lotion is a preservative that extends the product's shelf life by slowing microbial growth [6]. The most common microbial contamination is fungal contamination [7]. Fungi cause the ability of cosmetic products to maintain their quality to be disrupted [8]. One of the preservatives often used in cosmetic products is parabens [6]. The use of parabens is often controversial. Parabens in cosmetics can cause metabolic changes, and in pregnant women, parabens will impact the conceived fetus or babies who are still breastfeeding [9]. Using parabens with the wrong concentration can cause skin allergies and other adverse effects [10]. The controversial use of parabens on human health has caused cosmetics manufacturers and scientists to innovate in finding paraben substitutes [11]. An example of a material that can be used as a candidate for paraben replacement is nanosilver [12].

Nanosilver is a nano-sized material known for its antimicrobial ability [12]. Nanosilver has a large surface area to volume ratio, ease of conjugation with various ligands to obtain desired properties, toxicity to pathogens, catalytic applications and cytotoxicity against cancer cells [13]. The antimicrobial properties of nanosilver result from its surface area and volume ratio and the interaction of nanosilver with sulfur and phosphorus in cells [14]. Nanosilver acts as an antimicrobial by attaching to the microbial cell wall and interacting with membrane proteins. Nanosilver causes damage to the cell membrane, leading to leakage of cell contents.

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In addition, Ag^+ ions released in the cell attack the respiratory chain and cause oxidative stress. As a result, there is an increase in reactive oxygen species (ROS), which results in protein damage and inhibition of cell growth [15].

Nanosilver has a solid antifungal effect on fungal strains [16]. Nanosilver has a robust inhibitory ability against Candida albicans fungi [8]. Nanosilver causes changes on the surface of Candida albicans, namely by forming holes in the cell wall and cell membrane [17]. Nanosilver can be used in cosmetic formulations because nanosilver has low skin irritation potential [18]. Absorption of nanosilver in the body can be through 3 routes, namely through the skin, through the respiratory system and orally (through the digestive system) [19]. Compared to other routes, nanosilver absorption through the skin is low because the skin has a protective layer that makes nanosilver unable to penetrate the deepest layers of the skin. It has been reported that nanosilver penetrating the skin barrier is only found in the stratum corneum, epidermis and dermis (with the least amount - none) [20].

MATERIAL

A. Tools

1L volumetric flask, beaker, hot plate, stirrer, magnetic stirrer, analytical balance, body lotion container, porcelain cup, stirrer, Olympus CX23 microscope, Shimadzu 1800 UV-Vis spectrophotometer, and Transmission electron Microscope (TEM) tool set.

B. Materials

$AgNO_3$, sodium citrate, stearic acid, cetyl alcohol, glycerin, TEA, methylparaben, olive oil and distilled water.

METHODS

A. Synthesis and characterization of nanosilver

The synthesis process begins with the preparation of 1000 ppm $AgNO_3$ solution. The solution dissolved 1.573 grams of $AgNO_3$ into 1000 mL of distilled water until homogeneous. In the next step, 1000 mL of distilled water is heated until the temperature reaches ± 100 ℃. Add 2 grams of sodium citrate and 20 mL of 1000 ppm $AgNO_3$ solution. Homogenize the mixture. Wait until there is a color change to a stable yellow color. The solution is a 20 ppm nanosilver solution. The nanosilver that has been formed is then characterized using TEM to determine the nanosilver's size and morphology and UV-Vis spectrophotometry to determine the stability of the nanosilver.

B. Making body lotion

Body lotions are made using the following formulations:

Table 1. Body lotion formulation

Material	Function	Formulation (%) (b/b)				
		F1	F2	F3	F4	F5
Nanosilver	Antifungal	10	15	20	-	-
Stearic acid	Emulgator	2,5	2,5	2,5	2,5	2,5
Cetyl alcohol	Emollients	5	5	5	5	5
Gliserin	Humectants	5	5	5	5	5
TEA	Emulgator	2	2	2	2	2
Nipagin	Preservatives	-	-	-	0,1	-
Olive oil	Active ingredients	5	5	5	5	5
aquades	Solvent	62,5	57,5	52,5	72,4	72.5

The oil phase (stearic acid and cetyl alcohol) and water phase (distilled water, glycerin, TEA and nipagin) were heated separately at the same temperature of ±70℃. After heating, put the oil phase into a mortar and add the water phase with rapid stirring. If an emulsion has formed, continue stirring using a mixer machine. Add nanosilver and olive oil, then homogenize again.

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C. *Body lotion tests*

Body lotion that has been formed is carried out several tests, such as organoleptic, irritation, homogeneity, pH, and spreadability tests.

- ***Organoleptic test***

  The organoleptic test was conducted by 30 panelists who observed and assessed the body lotion's physical condition, including color, aroma, and texture.

- ***Irritation test***

  The irritation test was conducted by 30 panelists who observed and assessed the presence or absence of side effects from using body lotion on the skin. The side effects include skin redness, itching and roughness.

- ***Homogeneity test***

  Homogeneity test is done by taking body lotion samples at the top, middle and bottom. The sample is applied to the object glass and observed to determine whether there is clumping.

- ***pH test***

  pH test is carried out by dipping a universal indicator into the sample.

- ***Spreadability test***

  The spreadability test is done by weighing 0.5 grams of body lotion. Place it on a glass that has been weighed and cover it with glass that has been weighed as well. Wait up to 1 minute and measure the resulting diameter. Add 50 grams of weight, wait 1 minute and measure the diameter. Change the weight to 100 grams, wait 1 minute and measure the diameter.

D. *Antifungal activity test*

Antifungal activity test using Direct Microscope Count (DMC) method. 0.5 grams of sample was placed on an object glass marked to separate each spot. Cover with cover glass and observe the fungal hyphae using a 40x and 100x magnification microscope. Observations were made from week 0 to week 8. Capture the image produced by the microscope using Optilab software and count the number of hyphae produced.

RESULT AND DISCUSSION

A. *Synthesis and characterization of nanosilver*

The synthesis of nanosilver in this study used a chemical reduction method. In general, chemical reduction methods require three components, namely metal precursors, reducing agents and stabilizers [21]. The metal precursor used in this study is $AgNO_3$. Reducing agents and stabilizers can use the same compounds, such as sodium citrate [22]. The reducing agent lowers the metal ion's oxidation state to zero [23]. In this case, sodium citrate as a reducing agent will reduce silver ions (Ag^+) to Ag^0 [22]. As a stabilizer, sodium citrate controls the growth and agglomeration of nanoparticles [24]. The reaction formed in this nanosilver synthesis process is:

$2Ag^+ + C_6H_5O_7^{3-} \rightarrow 2Ag^0 + C_5H_4O_5^{2-} + H^+ + CO_2$ [25].

Figure 1. Synthesized nanosilver

The nanosilver that has been formed is then characterized using a TEM instrument and UV-Vis Spectrophotometer. Characterization using TEM aims to determine the shape and particle size of the resulting nanosilver.

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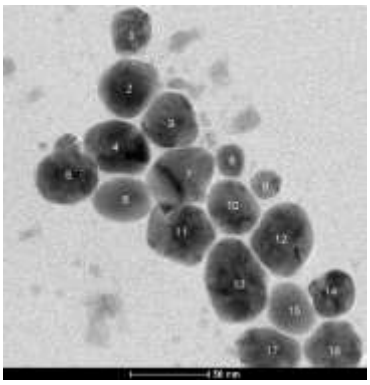

Figure 2. TEM results from the image of 20 ppm nanosilver

From the characterization results using TEM, it can be concluded that the nanosilver produced is spherical. The results obtained are by previous studies, which state that if the use of sodium citrate reducers, then the nanosilver obtained tends to be round [26] [21] [27]. The following is the diameter size of the nanosilver particles produced:

Table 2. Diameter size of nanosilver particles

Label	Diameter (nm)
1	25.732
2	36.394
3	34.264
4	38.967
5	39.746
6	21.648
7	44.466
8	35.779
9	19.181
10	30.430
11	39.571
12	41.580
13	53.930
14	27.378
15	27.261
16	37.977
17	37.182
average	**34,793**

From the table, it can be seen that the average size of the nanosilver particles is 34.793 nm. These results are to the theory that the size of nanoparticles ranges from 1-100 nm [28].

Characterization using a UV-vis spectrophotometer aims to determine the stability of the synthesized nanosilver. Nanosilver was characterized at a wavelength of 350-550 nm [29]. Using a UV-vis spectrophotometer, characterization was done twice, namely at week 0 and week 8. Checking for two times aims to determine whether there is a shift to a larger wavelength because if there is a significant shift in the absorption peak, it shows that the stability of the nanosilver is low [8]. At week 0, the absorption peak was produced at a wavelength of 415.20 nm; at week 8, the absorption peak was produced at 423.30 nm.

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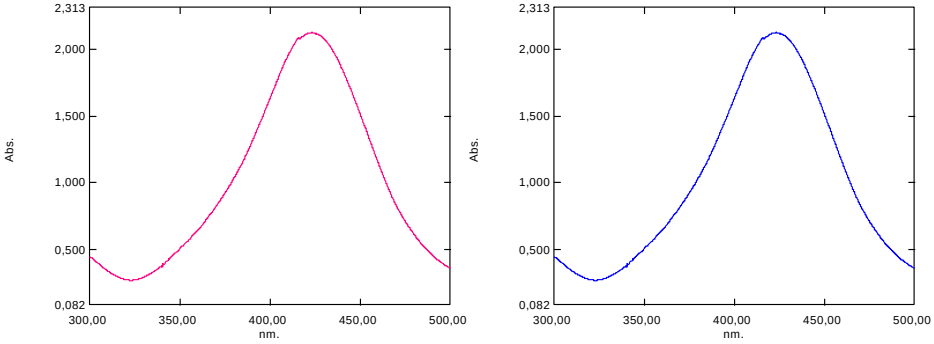

Figure 3. Characterization results with uv-vis spectrophotometer at week 0 and week 8

The uv-vis spectrophotometer characterization showed no major shift in absorption peak from week 0 to week 8, indicating that the nanosilver produced showed good stability.

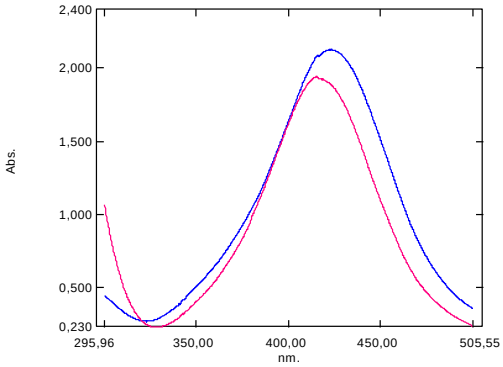

Figure 4. The shift of nanosilver absorption peak at week 0 and week 8

B. Body lotion testing

The resulting body lotion will be subjected to several tests, including organoleptic, irritation, homogeneity, spreadability, and pH.

Organoleptic test: Organoleptic test was conducted by 30 panelists on five body lotion formulations. Panelists test based on their preference for the body lotion's physical appearance, including color, aroma and texture. Each panelist gave an assessment with the value criteria (1) stated immensely dislike, (2) stated dislike, (3) stated quite like, (4) stated like and (5) stated very like. Based on the results of the organoleptic test that has been carried out, the following results are obtained:

- **Color**

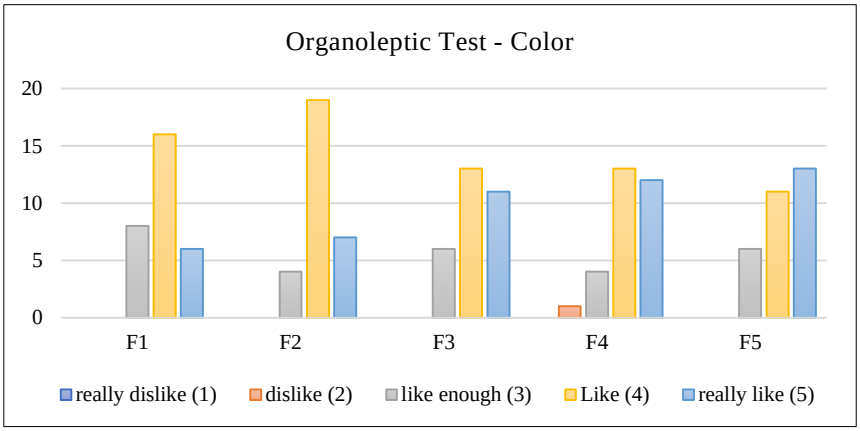

Figure 5. Organoleptic test result diagram - color

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In F1, 20% of the 30 panelists stated that they liked it, 27% stated that they quite liked it, and 53% stated that they liked it. The resulting average value is 3.93, included in the moderately preferred category. In F2, 13% of the panelists stated that they liked it, 23% stated that they liked it, and 64% stated that they liked it. The resulting average value is 4.1, included in the preferred category. In F3, 20% of the panelists stated that they liked it, 37% stated that they liked it, and 20% stated that they quite liked it. The average value obtained was 4.17, included in the preferred category. In F4, 3% of the panelists stated that they did not like it, 13% stated that they quite liked it, 40% stated that they liked it, and 44% stated that they liked it. The average value obtained is 4.2, included in the preferred category. In F5, 20% of panelists stated that they moderately liked it, 37% stated that they liked it, and 43% stated that they liked it. The average value obtained for F5 is 4.23, classified as favorable.

- **Scent**

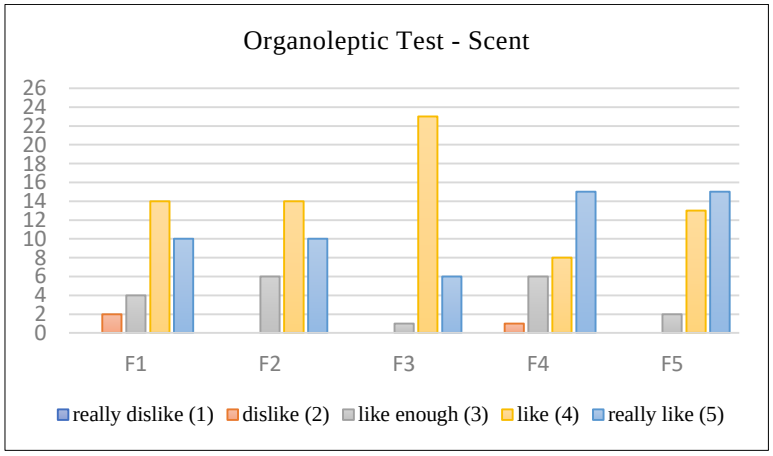

Figure 6. Organoleptic test result diagram - scent

In F1, 7% of panelists said that they did not like it, 13% said that they liked it, 33% said that panelists liked it, and 47% said that they liked it. The average value obtained for F1 is 4.067, so it is included in the preferred category. For F2, 20% of panelists stated that they quite liked it, 33% stated that they liked it, and 47% stated that they liked it. The average value obtained for F2 is 4.133, included in the preferred category. For F3, 3% of panelists said they liked it, 20% said they liked it, and 77% said it. The average value for F3 is 4.17, so it is classified as a preferred category. In F4, the results showed that 3% of panelists stated that they did not like it, 20% stated that they quite liked it, 27% stated that they liked it, and 50% stated that they liked it. The average value for F4 is 4.23, so it is included in the preferred category. Furthermore, for F5, the results showed that as many as 7% stated that they quite liked it, 43% stated that they liked it, and 50% stated that they liked it. The average value obtained for F5 is 4.43, classified as favorable.

- **Texture**

In organoleptic testing of the texture part of the body lotion, the results showed that in F1, 7% of panelists stated that they did not like it, 13% stated that they quite liked it, 23% stated that panelists liked it and 57% stated that they liked it. So an average value of 3.967 was obtained. This value is included in the quite liked category. In F2, 3% of panelists stated that they did not like it, 13% stated that they quite liked it, 37% stated that panelists liked it, and 47% stated that they liked it. The average value obtained for F2 is 4.17, included in the preferred category. For F3, 7% of panelists stated that they did not like it, 13% stated that they quite liked it, 20% stated that panelists liked it, and 60% stated that they liked it. The average value falls into the moderately favorable category. In F4, the results showed that 7% of panelists stated that they did not like it, 13% stated that they quite liked it, 23% stated that panelists liked it, and 57% stated that they liked it. The average value obtained is 3.967, included in the moderately preferred category. Then for F5, the results showed that 3% of panelists stated that they did not like it, 13% stated that they quite liked it, 40% stated that panelists liked it, and 44% stated that they liked it. So an average value of 4.233 was obtained. This value is classified in the preferred category.

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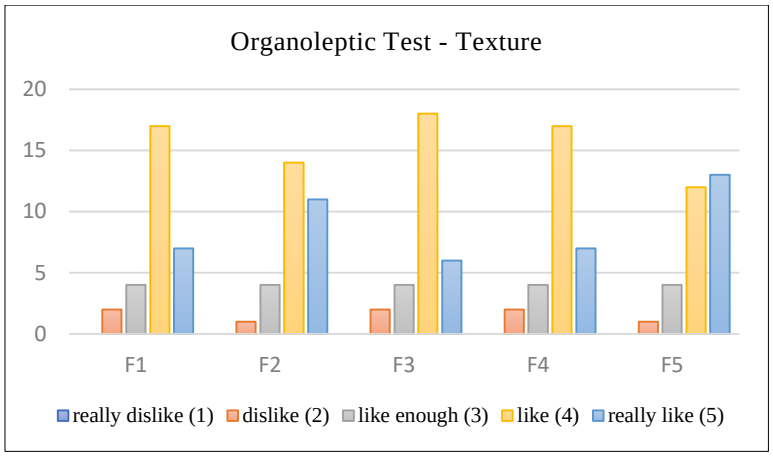

Figure 7. Organoleptic test result diagram - texture

Irritation test: The irritation test was conducted by 30 panelists. Each panelist applied each body lotion formulation to their skin. After the body lotion samples were applied to the skin, the panelists tested whether there were signs of irritation, such as skin redness, itching and roughness. Based on the irritation test conducted on 30 panelists, all panelists stated that no irritation occurred when the body lotion samples were applied to the skin.

Homogeneity test: A homogeneity test was conducted to determine how homogeneous or evenly mixed the ingredients in the body lotion [30]. A homogeneity test was conducted on the five body lotion formulations (F1, F2, F3, F4 and F5). A homogeneity test is done by observing whether there are coarse particles on the glass object [31]. The following are the results of the homogeneity test of body lotion preparations:

Table 3. Result of body lotion homogeneity test

Sample	Homogeneity
F1	Homogenous
F2	Homogenous
F3	Homogenous
F4	Homogenous
F5	Homogenous

Based on the homogeneity test that has been done, the five body lotion formulations do not have any coarse particles. So, it can be concluded that the five formulations of body lotion preparations are homogeneous.

Spreadability test: The spreadability test was conducted to determine the spreadability of the lotion on the skin surface [32]. The spread diameter of a good body lotion ranges from 5-7 cm [33]. The measurement results of the spreadability test of each lotion formulation can be seen in the following table.

Table 4. Results of the spreadability test

	Body lotion formulation					Average (cm)
	F1	F2	F3	F4	F5	
Glass	6,3	6,2	6,4	6,0	6,3	6,24
50 gram	7,0	7,2	7,3	6,9	7,2	7,12
100 gram	7,4	7,6	7,8	7,5	7,9	7,58

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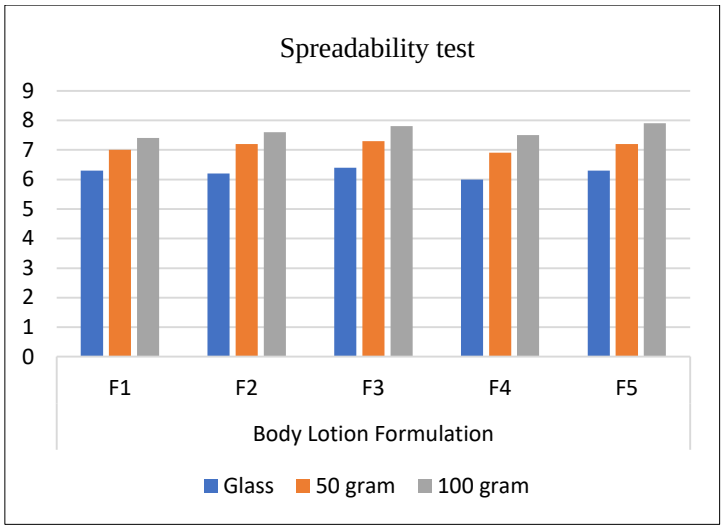

Figure 8. Diagram of body lotion spreadability test results

From the above data, it can be concluded that all lotion formulations have good spreadability. For each formulation does not have a significant difference in spreadability. Each body lotion formulation has the same content or lotion base size. Giving nanosilver does not significantly influence the spreadability of body lotion.

pH test: Based on the requirements of lotion preparations, SNI 16-4399-1996 states that the pH should range from 4.5 - 8.0. If a lotion has a pH outside the PH range, it can cause irritation, dry skin, scaly skin, skin feeling too slippery and impaired skin elasticity [34]. The following are the results of the PH test on body lotion preparations.

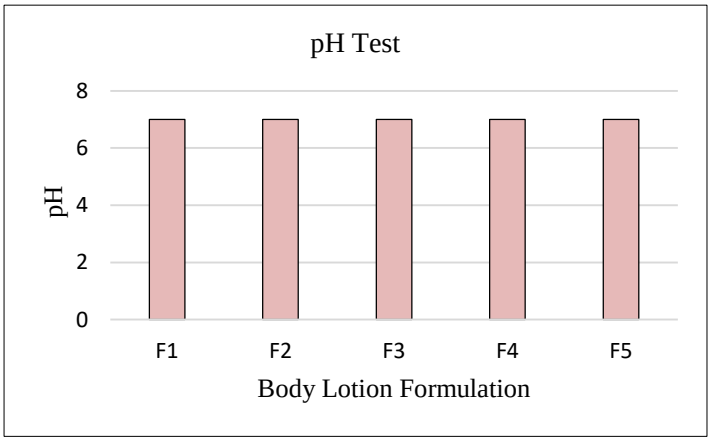

Figure 9. Diagram of the result of body lotion pH test

In this study, all body lotion formulations produced had a pH of 7.0. These pH results are by the recommended pH range for body lotion preparations. The pH value is influenced by the ingredients added to the body lotion preparation, such as TEA. TEA is alkaline, and the addition of TEA causes the pH of the resulting body lotion to approach an alkaline pH [35]. The addition of nanosilver does not affect the pH of the body lotion.

C. Antifungal Activity Test

The Nanosilver antifungal activity test was conducted on 5 body lotion formulations with different nanosilver content. F1 is a body lotion that has 10% nanosilver content, F2 is a body lotion that has 15% nanosilver content, F3 is a body lotion that has 20% nanosilver content, F4 is a body lotion that serves as a positive control where the body lotion in this formulation has methylparaben content, and F5 is a body lotion that has a function as a negative control where this body lotion has no preservative content.

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Antifungal activity testing in this study uses a direct microscopic calculation method. The test sample was taken about 0.5 grams, placed on a glass object, and then covered with a glass object cover. The sample was observed using a microscope at 10x and 40x magnification because the fungal hyphae can already be seen [36]. Optilab software was used in this test to assist in the image capture. The number of hyphae in each test sample was then calculated from the image. This test was carried out every week for eight weeks to see the addition of the number of hyphae that grew on the test sample.

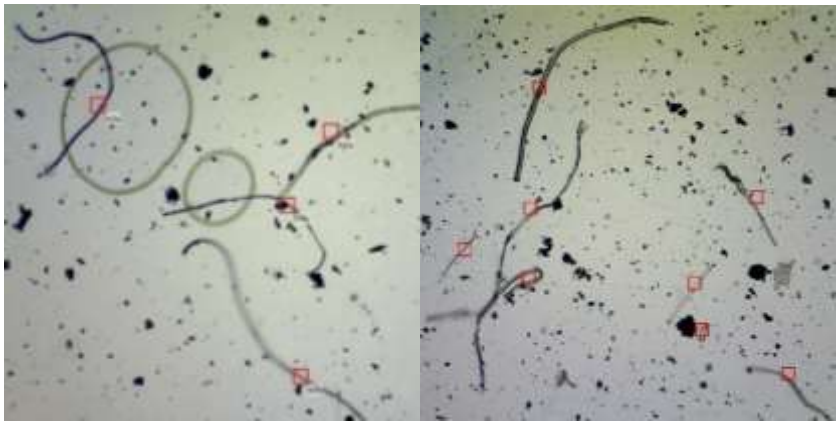

Figure 10. Observation results using a microscope

hyphae caught on the microscope were then counted using the following formula:

$$\% \text{ hyphae count} = \left(\frac{\text{hyphae count}}{\text{counting area}}\right) \text{ x } \left(\frac{\text{counting area}}{\text{total area}}\right) \text{ x } 100\%$$

From the tests that have been carried out, the following results are obtained:

Table 5. Antifungal activity test results

Sample	Percentage of Hyphae Count (%)								
	W-0	W-1	W-2	W-3	W-4	W-5	W-6	W-7	-8
10%	0	3,6	17,6	9,6	16,6	20,4	23,2	31,4	34,4
15%	0	2,8	13,4	7,6	12,8	14,4	16	18	21,2
20%	0	2,6	13,6	8	11,4	13,2	15	16,4	19,8
paraben	0	2,4	14,6	6,4	7,8	13	14,2	15,8	16,8
non-preservative	0	3,2	14,2	24	32,6	37,8	40,4	41,6	46

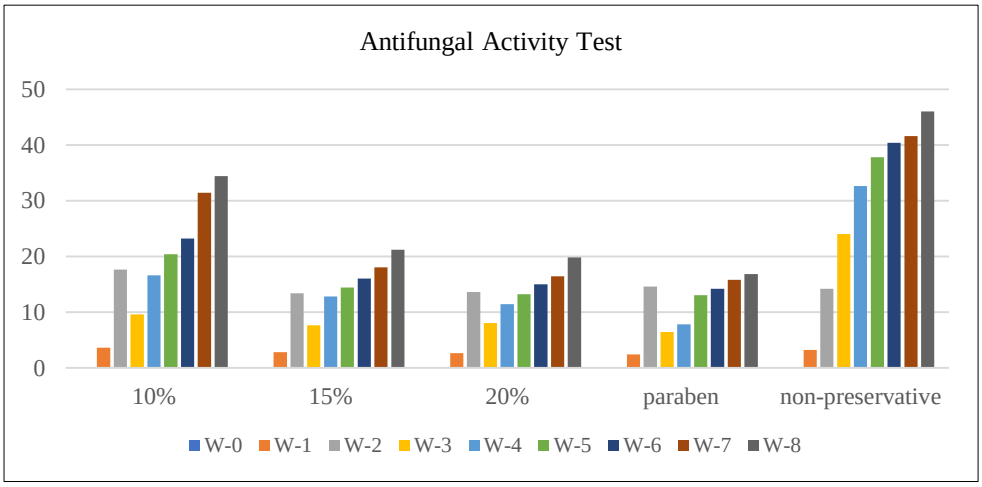

Figure 11. Diagram of antifungal activity test results

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The table and diagram show that F1 increased the number of hyphae by 34.4%, F2 by 21.2%, F3 by 19.8%, F4 by 16.8%, and F5 by 46%. Nanosilver is a nanomaterial with antifungal solid properties compared to other nanomaterials such as copper, zinc, magnesium, silicon, etc [37]. From the results of this test, it can be seen that F2 and F3, which have 15% and 20% nanosilver content, respectively, are good enough to suppress fungal activity in body lotion preparations.

CONCLUSION

Synthesis of nanosilver using a chemical reduction method with sodium citrate as a reducer and stabilizer produced spherical nanosilver with an average particle diameter of 34.793 nm. The characterization results using a spectrophotometer showed that the nanosilver produced had good stability due to the shift of absorption peaks that were not large at week 0 and week 8. The results of antifungal testing showed that F1 had an increase in the number of hyphae, reaching 34.4%, F2 by 21.2%, F3 by 19.8%, F4 by 16.8% and F5 by 46%.

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