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FORMULATION AND CHARACTERIZATION OF ANTIFUNGAL SHAMPOO WITH THE ADDITION OF RED GINGER EXTRACT

Abstract: Red ginger has been proven to be efficacious as an antifungal with active ingredients such as eugenol, kaempferol, galangin, and acetoxychavicol acetate. The use of the red ginger extract in shampoo products is expected to increase the added value of red ginger other than as an effort to find alternative sources of medicinal preparations that are safer for people with scalp infections. This study aims to (1) determine the effectiveness of shampoo with the addition of red ginger extract on fungi that cause scalp infections, (2) determine the effect of adding red ginger extract on the characteristics of shampoo products, and (3) determine consumer preferences for shampoo products with the addition of red ginger extract. In this study, a shampoo formulation was carried out with the addition of several concentrations of red ginger extract. The design used was a single factor Completely Randomized Design with 4 variations in the concentration of red ginger extract of 0.5%, 1%, 2%, and 3%. A formula was made without the addition of ginger extract as a control. Parameters observed included pH, water content, free alkali content, emulsion stability, viscosity, inhibition, and organoleptic tests. The results showed that the shampoo with the addition of red ginger extract was quite effective in inhibiting the fungi *Trichophyton mentagrophytes* and *Microsporum canis* with inhibition area diameters of 29-34 mm and 32.3-36 mm, respectively. The addition of red ginger extract turned out to affect the characteristics of the shampoo, pH value, water content, free alkali content, and red ginger emulsion stability according to SNI standards (06-2692-1992). Based on the characteristics of the shampoo, product stability, and preference test, the recommended red ginger extract shampoo formula is added with a red ginger extract concentration of 0.5%.

Key words: Red ginger, shampoo, antifungal, *T. mentagrophytes*, *M. canis*.

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Introduction

Red ginger is one type of spice that has been widely used as a phytopharmacology product. Empirically, red ginger can treat stomach disorders, fever, swollen spleen, ear inflammation, bronchitis, rheumatism, and strong drugs (aphrodisiacs).

Furthermore, red ginger is also an antifungal and antibacterial, commonly used to treat eczema, tinea versicolor, ulcers, and sores.

The antifungal properties of the red ginger extract have been scientifically proven. The results of a study conducted by Hernani et al (2017) explain that

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red ginger extract can inhibit the growth of fungi that cause skin diseases, namely *Trichophyton mentagrophytes* and *Microsporum canis*. Red ginger extract applied in the ointment can inhibit *Trichophyton mentagrophytes* by 34.67mm and *Microsporum canis* by 39.33 mm. Moreover, Sundari and Winarno (2017) suggest that several dosage forms of the red ginger extract can inhibit the growth of 5 (five) types of fungi, namely: *Trichophyton rubrum*, *Trichophyton ajelloi*, *Trichophyton mentagrophytes*, *Microsporum gypseum*, and *Epidermophyton floccosum*.

Scalp infections caused by fungi are common in the community. Fungal infections that attack the scalp area (*Tinea capitis*) can cause scalp ringworm or better known as ringworm infection, as well as the appearance of dandruff or dandruff symptoms. Fungal skin infections not only attack the people of Indonesia but also people around the world. Data from health institutions in the United States records that more than 12 million people annually contract skin diseases caused by fungi (Windono and Sutaryadi, 2017). According to Elewiski (2017), children who live in big cities are very susceptible to suffering from scalp infections. Even in developed countries such as America and Canada, it is estimated that 15-25% of children aged 5 to 10 years experience it. The mode of transmission is very easy, causing the number of people with this infection to continue to grow. Antifungal compounds for fungi that cause scalp infections that are widely used nowadays are synthetic antifungal compounds such as zinc-pyrithione (ZPT), sulfur, selenium, ketoconazole, and clotrimazole. The use of synthetic antifungal ingredients turns out to have side effects, making hair dry and cracked (Marzuki, 2017). Synthetic antifungal agents also cause resistance to certain fungi. Several ethanolic extracts from the findings, especially red ginger, galangal, bitter ginger, and white turmeric from Kalimantan, have been shown to inhibit the activity of pathogenic fungi, including strains that are resistant to synthetic antifungals such as amphotericin and ketoconazole (Fickter et al., 2017).

The side effects caused by synthetic antifungal ingredients have prompted the emergence of various studies to obtain safer antifungal ingredients. Ilyas et al (2017) have conducted a study on the use of ethyl p-methoxycinnamate *Kaempferia galanga* rhizome as an anti-dandruff in shampoo using test bacteria of *Candida albicans* and *Mentagrophytes*. The results

showed that shampoo containing 2% ethyl p-methoxycinnamate was the best anti-dandruff shampoo. One source of vegetable ingredients that have anti-fungal properties is red ginger. The efficacy of ginger as an antifungal agent is due to its chemical content such as basonin, eugenol, galangal, galangol, and acetoxychavicol acetate (ACA). According to De Pooter et al, (2017) ACA compounds are antifungal and anticarcinogenic. ACA levels in fresh red ginger essential oil are between 0.5-1%.

Given its potential as an effective antifungal, it is supported by its high productivity. Red ginger allows it to be developed as a commercial antifungal product. The use of the red ginger extract in shampoo products is expected to increase the added value of red ginger other than as an effort to find alternative sources of medicinal preparations that are safer for people with scalp infections. Thus, the objectives of the study were (1) to determine the effectiveness of shampoo with the addition of red ginger extract on fungi that cause scalp infections, (2) to determine the effect of adding the red ginger extract to the characteristics of shampoo products, and (3) to determine consumer acceptance of shampoo products with red ginger extract.

Materials and Method

Materials and equipment

The raw materials used were 10 months red ginger rhizome which was obtained from the Bogor Spice and Medicinal Plant Research Center. The study was conducted at the Laboratory of the Indonesian Agricultural Research and Development Center in Bogor. Chemicals used include 60% ethyl acetate, 12% maltodextrin, sodium lauryl ether sulfate, Cocamidopropyl betaine, NaCl, citric acid Diethanolamine (DEA), bromidox, deionized water, alcohol, and perfume.

Method

Preparation of red ginger simplicial

Red ginger rhizome was washed, chopped with a thickness of about 5-7 mm to obtain the ideal thickness of dried simplicial, about 3-5 mm. The red ginger rhizome was dried at a temperature of 50 - 60°C for approximately 12 hours, then crushed with a 0.25 mm grinder (50 mesh). The red ginger powder produced was analyzed for water content, ash content, acid insoluble ash content, ethanol-soluble extract content, and water-soluble extract content.

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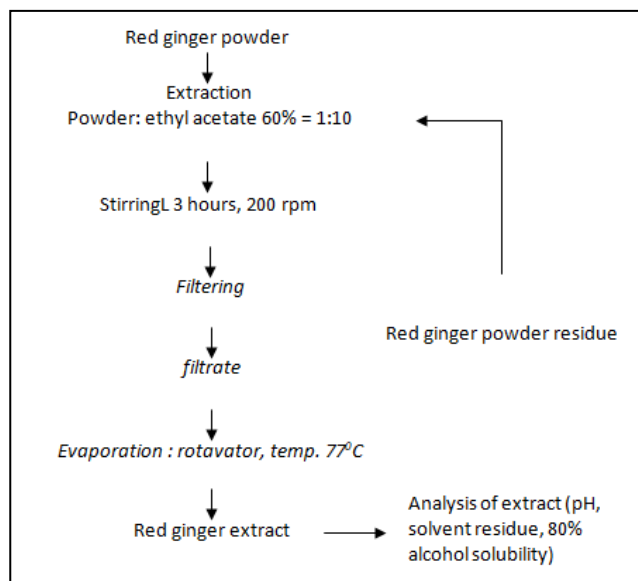


Figure 1 - Flowchart of the red ginger simplicial extraction process

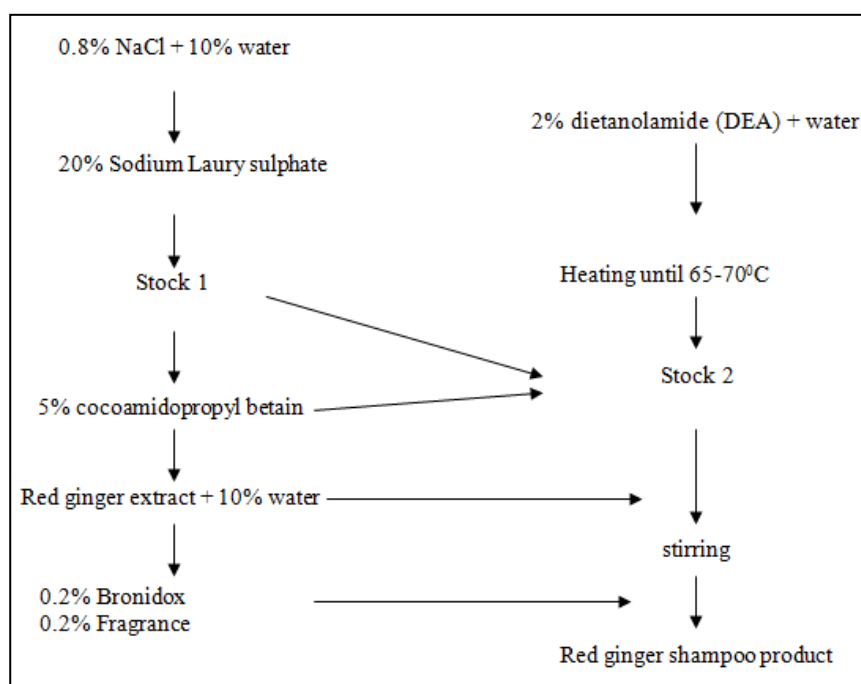


Figure 2 - Flowchart of making red ginger shampoo

Extraction

Extraction of red ginger powder was carried out by the repeated maceration method. The red ginger powder which had been mixed with 60% ethyl acetate solvent with a ratio of material:solvent = 1:10 was placed in a container and closed then stirred at 200 rpm for 3 hours. After being that, filtering was carried out until the filtrate was obtained. The remaining filtered dregs were extracted again with the same process, then the filtrate obtained was combined. The filtrate was evaporated using a rotary evaporator at a

temperature of 77 °C until a concentrated extract was obtained. The overall steps of the red ginger powder extraction process are presented in Figure 1.

The obtained extraction was analyzed for pH, residual solvent, and solubility in 80% alcohol. The extract was dried using a spray dryer with the addition of 12% maltodextrin which had been dissolved in water and 96% ethyl acetate.

Shampoo Formula

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The shampoo was formulated based on the formula of Ismayanti (2017). The shampoo made was given the addition of red ginger extract with various concentration levels of 0.5%, 1%, 2%, and 3%. The stages of making shampoo are presented in Figure 2. The experimental design used was a single factor completely randomized design, with 4 variations of red ginger extract concentration and 3 replications.

Shampoo Analysis

Analysis of the shampoo made includes the following:

- Shampoo characteristics are pH, viscosity, water content, free alkali, and emulsion stability. Product stability was tested using the acceleration method of Connors et al (2017). In this test, shampoo was placed in an incubator at 50°C for a month. Storage under these conditions is equivalent to storage at room temperature for 9 months. Observations were made for 5 days which included observing the pH value and viscosity of the shampoo.
- Antifungal activity was tested using the good diffusion method (Brock and Madigan, 2017).
- The preference test included an assessment of the appearance, aroma, viscosity, amount of foam, and impression after use by 30 panelists (Laksono, 2017). Panelists were asked to rate their level of preference for the above parameters by giving a score from dislike to like (1 to 5).

Results and Discussion

Quality Characteristics of Simplicia and Extract of Red Ginger

The quality characteristics of the red ginger Simplicia used in this study are presented in Table 2. Extraction of the simplicial with 60% ethyl acetate solvent resulted in 10.65% red ginger extract. The solubility test of the extract in 80% alcohol gave a score of 1:30, indicating that the extract can dissolve well in alcohol (Anon, 2017).

Shampoo Formula

The results of the analysis of the characteristics of the shampoo formula with the addition of red ginger extract are presented in Table 3. The shampoo characteristics of these formulas meet the quality standards, except for the viscosity of the shampoo produced with the addition of 3% red ginger extract.

pH Value

The higher the concentration of addition of red ginger extract, the pH value of the shampoo produced tends to decrease. The pH value is still under the range of quality requirements set by SNI, between 5.0 to 9.0. The results of the analysis of variance showed that the concentration of red ginger extract had a significant effect on the pH value of the shampoo produced. The addition of acidic red ginger extract, with a pH of 4.31, resulted in a decrease in the pH of the shampoo.

Table 1. Quality characteristics of red ginger simplicial (content, percentage, c/p)

Parameter	Percentage (%)
Water content (% w/w)	7.80
Ash content	6.12
Ash content that is not soluble in acid	2.93
Water-soluble essence	31.22
Ethanol soluble essence	21.6

Viscosity

The viscosity values of the shampoo formula with concentrations of red ginger extract of 0.5%, 1%, and 2% were respectively 1185.2546.5 and 3960. Those results meet the standards, while shampoos with a red ginger extract concentration of 3% had a viscosity value beyond the standard of shampoo viscosity according to Schmitt and William (2017); i.e., 400-400 cP. The results of the analysis of diversity showed that the concentration of red ginger extract had a very significant effect on the viscosity value of the shampoo produced. The addition of red ginger extract in the shampoo formulation increased the value of dissolved solids in the shampoo, thereby increasing the viscosity value.

Free Alkali

The results of the analysis of free alkali levels in shampoos containing red ginger extract showed that

all shampoo formulas did not contain free alkali compounds which were calculated as NaOH. The free alkali content greatly affects the quality of shampoo. Thus, the concentration must be considered. Shampoos with high free alkali content will cause the pH of the shampoo to become alkaline and will cause scalp irritation, dry hair, and breakage (Toaha, 2017).

Water Content

According to Indonesian National Standard or SNI (1992), the maximum shampoo water content is 95.5%. The results of the analysis of the water content of the shampoo with red ginger extract showed that the higher the red ginger extract concentration, the lower the water content. Table 3 presents that at the concentrations of 0.5%, 1%, 2%, and 3%, the water content was 80%, 79.5%, 79.0%, and 77.5% respectively. The results of the analysis of diversity showed that the concentration of red ginger extract

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had a significant effect on the value of water content. The addition of red ginger extract in the shampoo formula results in an increase in the value of dissolved

solids. The decrease in water content is also affected by the reduction in the concentration of deionized water as a solvent.

Table 2. Results of characteristic analysis of red ginger extract shampoo

Addition of red ginger extract (%)	pH	viscosity (cP)	water content (%)	free alkali (%)	emulsion stability (%)
0.5	7.58 c	1185 a	80.0 b	0	21.24 a
1.0	7.24bc	2546 b	79.5 b	0	22.48 b
2.0	6.67 b	3960 c	79.0 b	0	22.65 b
3.0	5.95 a	8486 d	77.5 a	0	23.29 c
0	7.66	793	80.5	0	20.58
<i>Merang Shampoo</i>	6.05	3960	83.0	0	18.88
<i>Natur Shampoo</i>	7.61	8680	83.0	0	19.35
Standard	5.0 9.0*	400 4000*	max,95.5**	0*	-

Description: *SNI 06-2692-1992

Source ** Schmitt and William

Emulsion Stability

The results of the analysis of diversity showed that the concentration of red ginger extract had a significant effect on the stability value of the shampoo emulsion. The lowest value of emulsion stability was shown by shampoo with 0.5% red ginger extract, 21.24%. the highest emulsion stability was obtained by shampoo with 3% red ginger extract, 23.29%. Compared with commercial shampoos namely Merang and Natur, the red ginger shampoo from the study had a higher stability value. Merang and Natur emulsion stability values were 19.35% and 18.8%, respectively. The high value of emulsion stability in the research shampoo can also be seen from the appearance of the shampoo which looks thicker. This is probably due to the high concentration of NaCl in the shampoo formula which functions as a thickening agent. Another factor is the occurrence of coagulation and flocculation of the shampoo emulsion from the research findings.

The increase in the value of emulsion stability due to the increase in the concentration of red ginger extract may be due to differences in water content in the shampoo due to differences in extract concentrations. The greater the concentration of the extract added, the smaller the water content and vice versa. The increasing water content with the lower concentration of red ginger extract causes faster separation of the dispersed phase and the dispersing phase. According to Suryani et al (2017), the faster the phase separation, the lower the level of emulsion stability.

Stability Test during Storage

Stability of pH Value

The pH value of the shampoo during storage for 30 days (the equivalent of 9 months of storage at room temperature) slightly increased in the shampoo

without the addition of red ginger extract. On the other hand, in the shampoo added with red ginger extract, the pH value tends to decrease. The greater the addition of the extract, the lower the pH value. The red ginger extract which is acidic has the effect of decreasing the pH values of the shampoo during storage. The range of pH values in shampoo with the addition of red ginger extract was 7.25 to 5.65. This value is still following the quality requirements of the pH value set by the Indonesian National Standard, SNI (1992), 5.0 – 9.0.

Viscosity Stability

The value of the viscosity of the shampoo during storage with a measurement interval of five days showed a continuous increase until the 30th day. According to Connors et al (2017), storage for 30 days at 500C is equivalent to storage for 9 months at room temperature. The increase in viscosity on the first day and the 30th day respectively from 793.35 cP to 6213 cP on the 0% extract concentration; from 1185 cP to 8853.5 cP on the 0.5% extract concentration; from 2546.5 cP to 12780 cP on 1% extract concentration; from 3960 cP to 15100 cP on 2% extract concentration; and from 8486.5 cP to 16950 cP on 3% extract concentration. The range of shampoo viscosity values after storage of the five shampoo formulas was not following the quality requirements of the shampoo viscosity value. According to Schmitt and William (2017), the viscosity of a good shampoo has a value in the range of 400-4000 cP. The increase in the viscosity of red ginger shampoo during storage is probably due to the reduction in water content of the shampoo during storage and the reaction between a mixture of surfactants and electrolytes (NaCl) and essential oil from red ginger extract (Anon, 2017). There are two types of surfactants used in the red ginger shampoo formula, namely Sodium Lauryl Ether Sulfate (SLES)

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andCocoamidopropyl Betainewhich also function as foam stabilizers and thickeners (www.nzic.org.nz/chemprocessed/detergent). The percentage of SLES and Cocoamidopropyl Beaten in the shampoo formula was 12 and 4%, respectively, while in the ginger galangal shampoo it was 20 and 5% (http://www.chemistrystore.com/shampoo_formulas.htm).

To overcome the increase in the viscosity of the shampoo which is too high during storage, it is necessary to add a viscosity modifier of about 0.5% - 2% of the weight of the shampoo formula. Preferred viscosity regulators include propylene glycol, or alkali polyglycolide (United States Patent No.4,668,422).

Antifungal Power

The results of the antifungal shampoo test showed that there was an inhibition of the test fungi, *T.mentagropytes*, and *M.canis*, with the diameter of the inhibition area being 29-34 mm and 32.2-36 mm, respectively (Table 4). The difference was due to the character of the fungal spore wall and the speed of spore germination. According to Soltys (2017), *T.mentagropytes* has a thin spore wall and a very fast growth phase, while *M. canis* has a very thick spore wall and a slow growth phase. The level of viscosity of the shampoo affects the process of diffusion of antifungal substances into fungal cells. The higher the

level of viscosity of the shampoo, the process to achieve a balanced concentration between the solution outside the cell and the solution inside the cell will be longer. For comparison, the diameter of the inhibition area of anti-dandruff shampoo with the addition of ethyl p-methoxycinnamate compound from *Kaempferia galanga* rhizome of 2% gave an inhibitory area of 26.20 mm on *T.mentagropytes* (Ilyas et al.2017).

The antifungal power of red ginger shampoo is due to the red ginger extract containing various antifungal components such as phenolic components, including l-acetoxy chavicol acetate. According to Janssen and Scheffeer (2017), the active ingredient in the red ginger extract that acts as an antifungal is l-acetoxy chavicol acetate. These components have antifungal activity on *T.mentagropytes*, *T.rubrum*, *T.concentrium*, *Rhizopus stolonifera*, and *Aspergillus niger* with a concentration of 14 mg/ml. It was also reported that the active compound that acts as an antifungal and can inhibit *T.mentagropytes* in red ginger is a phenylpropanoid group compound, namely l'-acetoxichavicol acetate, l'-acetoxyeugenol acetate, and l'-hydroxychavicol acetate. A study conducted by Hernani et al (2017) reported that the three compounds were also identified in the purified red ginger extract, with high levels of the compound l'-acetoxichavicol acetate.

Table 3. The antifungal test results of red ginger extract shampoo

Red ginger extract concentration (%)	Inhibitory zone diameter	
	<i>Trichophyton mentagrophytes</i>	<i>Microsporum canis</i>
0.5	33.5	33
1	34	32.2
2	32.7	34.7
3	29.4	36

Consumer Preference Test

Preference on the color/appearance of the shampoo

The average value of panelists' preference for the appearance of shampoos with various levels of red ginger extract concentration was 3 (ordinary), with the highest value being shampoo with 0.5% extract added. Friedman's nonparametric test results showed that the concentration of red ginger extract affected the panelists' preference for the appearance of the shampoo. The response of the panelists at the ordinary

level of preference (score 3) reached 50% and the level of liking (score 4) was 36.7%. This is due to the shampoo with 0.5% red ginger extract had lighter color than other shampoos which were more concentrated along with the addition of the red ginger extract concentration level.To overcome this, it is necessary to bleach the red ginger extract to neutralize the brown color without affecting the levels of active ingredients and the activity of red ginger extract as an antifungal.

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Table 4. The average score of the panelists' preference for the color of the red ginger shampoo

Parameters	Red ginger extract concentration			
	0.5 %	1.0 %	2.0 %	3.0 %
Color	3.33	3.10	3.12	2.7
Scent	2.97	3.27	3.20	2.63
Amount of foam	3.10	3.20	3.00	3.50
Thickness	3.37	3.20	3.03	2.80
Impression after use	3.12	3.20	3.12	3.03

Preference on the Scent of Shampoo

The panelists' preference scores for shampoo scents with various levels of red ginger extract concentration gave the results that shampoos with 1% and 2% red ginger extract concentrations were the shampoos that the panelists responded best to, with average scores of 3.27 and 3.2. Judging from the responses of the panelists on shampoos with 1% extract, 43.3% of panelists responded with a score of 4 (like) and only 6.7% responded to a score of 5 (love). To increase the level of preference for the scent of red ginger extract shampoo, it is necessary to increase the concentration of synthetic fragrances (perfume) added. The right synthetic fragrance will produce a good combination of the scent of red ginger spices.

Preference on the Thickness of Shampoo

Organoleptic research on the viscosity of shampoo was carried out visually and felt by the sense of touch (shampoo was poured into the hand). Friedman's nonparametric test results showed that the panelists' preference for shampoo viscosity was influenced by the concentration of red ginger extract. Based on the response by the panelists, shampoo with 0.5% red ginger extract obtained a score of 3 from 51% panelists and a score of 4 from 39% panelists.

Preference on the Shampoo Foam

The assessment of preference for shampoo foam was carried out by assessing the amount of foam produced after rinsing shampoo with water. The preference value for the amount of foam by the panelists ranged from 3.0 to 3.5. Based on the response by the panelists, the shampoo with 0.5% red ginger extract obtained a score of 3 from 63.3% panelists and a score of 4 from 23.3% of panelists. Friedman's nonparametric test results showed that the addition of red ginger extract in the shampoo did not affect the preference test results for the amount of shampoo foam. This is because the type and

concentration of surfactants that function as foam generators in all shampoo formulas are the same.

Preference on the Effect After Using Shampoo

The organoleptic assessment of the preference for the after-use effect was carried out by assessing the panelists' general preference for the shampoo used after rinsing with water. The results of Friedman's nonparametric analysis showed the addition of red ginger extract in the shampoo affected the results of the preference test on the impression after using the shampoo. Based on the response by the panelists, shampoo with 0.5% red ginger extract obtained a score of 3 from 63.3% of the panelists and a score of 4 from 26.7% of the panelists. In general, the panelists liked the shampoo with a light appearance or not too thick, a gentle scent, not too low nor too high viscosity, and not too slippery after rinsing with water.

1. The antifungal test results showed that the red ginger extract shampoo was effective enough to inhibit *T. mentagrophytes* and *M. canis* fungi with inhibition area diameters of 29-34 mm and 32.3-36mm, respectively.
2. The characteristics of the shampoo with the addition of antifungal ingredients red ginger extract meet the standards in terms of pH, viscosity, water content, and emulsion stability, except for the extract concentration of 3%.
3. The results of the stability test showed that at the end of 9 months of storage, the pH still met the standard but the viscosity did not meet the standard.
4. Based on the characteristics of the shampoo, product stability, and preference test, the recommended shampoo formula is a shampoo with the addition of 0.5% red ginger extract.

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