

Physical Properties and Antifungal Activity of Red Galangal (*Alpinia purpurata* K. Schum) Rhizome Extract in Oil-in-Water-Based Cream Against *Trichophyton rubrum*

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Abstract

Trichophyton rubrum is a common fungus that causes a dermatophyte infection known as tinea cruris. More than a fifth of the population in Indonesia suffers from this infection. *Alpinia purpurata* K. Schum or well known as red galangal is said to have antifungal properties because of its composition, which includes compounds such as volatile oil, acetylchavicol, flavonoids, and phenols. This study was aimed to determine the antifungal activity of a oil-in-water-based cream containing red galangal rhizome extract (RGRE) against *T. rubrum*. RGRE was obtained by macerating the rhizomes in a solvent of 96% ethanol and evaporating until a thick extract was obtained. The extract was then formulated into an oil/water-based cream at concentrations of 10%, 15%, and 20%. The creams were evaluated for their physical properties, including organoleptic properties, homogeneity, pH, viscosity, adhesivity, and spreadability. The antifungal activity was tested using the agar diffusion method against *T. rubrum*. Data on the physical properties of the creams and their antifungal activity were analyzed using a one-way ANOVA test with a 95% confidence level. The results showed that creams with higher RGRE concentrations exhibited decreased spreadability and pH values but increased the viscosity and adhesivity test. The antifungal activity increased with higher RGE concentrations, starting at 10%, 15%, and 20%, with inhibition diameters of 12.33 mm, 18.83 mm, and 21.66 mm, respectively. Statistical analysis showed that RGE concentration affected the physical properties of the cream, including pH, spreadability, viscosity, adhesivity test as well as antifungal activity ($p < 0.05$). In conclusion, the water-in-oil-based cream containing 20% RGRE exhibits favorable physical properties and antifungal activity.

Keywords: cream, dermatophyte, diffusion method, viscosity, Zingiberaceae

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Introduction

Dermatophytosis or also known as ringworm, is a fungal infection which affects the stratum corneum of the skin, hair, and nails in humans [1]. It commonly results in a red, itchy, scaly, circular rash and hair loss may occur in the area affected. Dermatophytosis can be more invasive in immunocompromised patients, affecting the dermis and subcutaneous tissues [2]. Dermatophytosis is caused by dermatophyte. There are three types of dermatophytes: *Trichophyton*, *Microsporum*, and *Epidermophyton* [3]. The incidence of each type of dermatophyte depends on the type of fungus and geographical differences. The year-round humid air provides the ideal conditions for this mold to thrive.

Trichophyton rubrum is the most common fungus that causes fungal infections, particularly dermatophytosis. This infection is often referred to as tinea cruris and presents as an itchy, well-defined rash with scaly, red edges. *T. rubrum* attacks the stratum corneum, the outermost layer of the epidermis, and infects the skin [4]. The groin and genital regions are the most commonly affected areas, followed by the vulva. In Indonesia, the prevalence rate of skin diseases caused by *T. rubrum* is about 20-25% [5]

Several countries, including Indonesia, have long used plants for traditional medicinal purposes to treat various diseases, including fungal infections [6]. Currently, the use of herbs as alternative medicines is increasing due to their fewer side effects, ease of use, and affordability compared to chemical drugs [7]. One plant often used by communities to treat dermatophytosis is the red galangal rhizome by pressing and applying it to the affected area.

The red galangal rhizome comes from a plant with the scientific name *Alpinia purpurata* K. Schum., which belongs to Zingiberaceae family. The rhizome contains an essential oil composed of 48% methyl sinamate, 20%-30% sineol, and eugenol [8], [9]. The rhizome also contains flavonoids (galangin, kaempferide, and alpinin), galangol, terpenoids, saponins, tannins, and phenols [10], [11]. Some researchers reported that RGRE exhibited antifungal properties [12], [13]. The chloroform extract of red galangal affects the growth of the fungus *T. rubrum* with minimal inhibitory concentration of 20 mg/mL [14]. A concentration of 10% RGRE inhibited the growth of *Microsporum canis*, *Microsporum gypseum*, and *Trichophyton mentagrophytes* [15].

The use of extracts directly on the skin is not practical, it is necessary to develop dosage forms that are suitable for the skin. One suitable alternative topical dosage form is cream. Cream can provide a soft, shiny and moisturizing effect on the skin [16]. The oil/water-based cream was chosen because this type has the advantage of providing an optimum effect because it is able to increase the concentration gradient of the active substance that penetrates the skin that absorption increases [17]. Otherwise, this study was intended to determine the physical properties and the antifungal activity of RGRE in the oil/water-based cream, to find out the optimal RGRE oil/water-based cream formulation meeting the physical properties and good antifungal activity against *T. rubrum*.

Materials and Methods

Materials

Red galangal rhizome obtained from Merapi Farma Herbal Yogyakarta, *T. rubrum* ATCC, Sabouraud Dextrose Agar (SDA) (Oxoid), NaCl (E. Merck), ethanol 96% (E. Merck), stearic acid (Bratachem), liquid paraffin (Bratachem), triethanolamine (TEA) (Bratachem), propyl paraben (Bratachem), and vaseline alba (Bratachem).

The tools used were digital balance (Ohaus, Pioneer®), oven (Binder), autoclave (Hirayama Hiclave HVE-50®), incubator (Binder), biosafety cabinet (Monmouth Guardian MSC TI 200®), micropipette (Soccorex), pH meter (Ohaus Starter 300®), adhesivity test kit, spreadability kit, viscosimeter (Rheosys Merlin VR II), waterbath (Memmert®).

Methods

Extraction of red galangal rhizome

The extraction of the red galangal rhizome was carried out using the previous method with slight modifications [18]. A total of 250 grams of dried galangal rhizome powder was extracted using the maceration method with 1,500 ml of 96% ethanol for five days. The macerate was filtered, and the residue was macerated again with 1,000 mL of 96% ethanol for two days. The first and second filtrations were then combined and evaporated with a rotary evaporator at 50 rpm and 40°C until a thick extract was obtained.

Characterization of RGRE

The obtained RGRE was examined organoleptically, including odor, taste, and color. The yield of the extract was calculated by taking the ratio of the thick extract obtained to the weight of the starting material, then multiplying by 100. The drying shrinkage was performed using the moisture balance analyzer. RGRE was dried at 105 °C for 30 minutes [19].

Table 1. Formula RGRE in oil/water-based cream

Ingredients	Cream base	F1 (10%)	F2 (15%)	F3 (20%)
RGRE (g)	0	2	3	4
Vaseline alba (g)	4	4	4	4
Liquid paraffin (g)	2	2	2	2
Stearic acid (g)	2	2	2	2
TEA (g)	0.4	0.4	0.4	0.4
Methyl paraben (g)	0.06	0.06	0.06	0.06
Propyl paraben (g)	0.006	0.006	0.006	0.006
Distilled water (g)	Ad 20	Ad 20	Ad 20	Ad 20

Formulation of RGRE on M/A type base cream

The composition of the oil-in-water-based cream was presented in Table 1. The ingredients in the aqueous phase, including glycerin, methyl paraben, TEA, and distilled water, were heated in a water bath at a controlled temperature of

70-75°C. The oil phase ingredients, including stearic acid, cetyl alcohol, and propyl paraben, were melted at the same temperature as the water phase. After melting the oil phase, it was slowly added to the water phase with constant stirring in a warm mortar. After forming the cream and cooling it, RGRE at concentrations of 10%, 15%, and 20% was added and stirred homogeneously to mix the cream base with the active substance.

Organoleptic test of RGRE cream

Organoleptic test of the cream was carried out by visually observing the cream preparation. The components observed include the color, odor, and shape of the cream [20].

Homogeneity test of RGRE cream

The homogeneity test was performed by applying a small amount of cream to a watch glass and spreading it thinly. The cream is considered homogeneous if there are no lumps in the smearing results and no coarse grains are present on the glass [21]. This test was replicated three times for each formula.

pH test of RGRE cream

The pH of the cream was determined using pHmeter. This test was replicated three times for each formula.

Adhesivity test of RGRE cream

A 0.5 g sample of cream was placed on the glass surface, and a 1 kg load was applied for five minutes. Then, the 1 kg load was lowered. The glazed object was then removed with an 80 g puller load. The time it took to remove the glazed object was recorded as the cream's adhesivity ability. The test was replicated three times.

Spreadability test of RGRE cream

Amount 0.5 g of cream was placed on the center of a glass and covered with glass that has been weighed for five minutes. Next, add a 50-gram weight and record the diameter of the spread. Then, the test was continued by adding another 100-gram load. The diameters of the spread were calculated by measuring the average diameter of several sides. The spreadability test was replicated three times [22].

Viscosity test of RGRE cream

A total of 0.5 grams of cream was weighed and placed on the Rheosys Merlin VR II viscometer plate at temperature of 25°C. It was placed in the center of the 30 mm parallel plate with a 1.00 mm gap. The tool setting was 10 points, and the rotating speed was between 1 and 35 rpm. The delay time was 10 seconds, and the integration time was 0.2 seconds [22].

Emulsion type test of RGRE cream

Testing was carried out using the dilution method. 0.5 grams of the cream preparation was weighed. Then, the cream was dissolved in distilled water. If the cream cannot be diluted with water, then it is an oil-in-water (O/W) emulsion. If the cream can be diluted with water, then it is a water-in-oil based emulsion [23].

Antifungal activity test of RGRE cream

One loop of *T. rubrum* fungal colony was added to 0.9% NaCl solution. Then, it was compared to the McFarland standard of 10^8 CFU/ml [24]. A sterile cotton swab was dipped into *T. rubrum* fungal suspension and spread it onto the SDA media. Then, 5 wells were created on the media surface. Each well was filled up with a base cream as a negative control and a treatment control (F1, F2, and F3 creams), as well as a positive control (2% ketoconazole cream), with each cream weighing 100 mg. Then, the inoculated plate was incubated at a controlled temperature of 32°C for 4 days. After that, the diameters of the inhibition zone around the wells were measured. The experiment was done in triplicate.

Data analysis

The data were analyzed using one-way ANOVA followed by Duncan's test with SPSS 17.0 software. If the data were not normally or homogeneously distributed, the Kruskal-Wallis test was performed, followed by the Mann-Whitney test, to determine the differences among the cream formulas.

Results and Discussion

Characteristic of RGRE

Table 2 shows that the odor of RGRE is typical of galangal, and its color is blackish brown. The taste of RGRE is slightly bitter. The yield of RGRE in this study was 4.96%. Both external and endogenous factors affect the yield that is obtained. Anatomical and physiological traits of a plant, such as differences in the chemical makeup of various plant sections and developmental phases, are referred to as endogenous factors. Conversely, exogenous factors—like light, rain fall, and soil—are associated with the environment [25], [26]. The extraction process and conditions, including temperature, stirring, time, and solvent, also have an impact on yield. The yield is also influenced by the sample size and solvent type [27]. Contact and interaction between the sample and the solvent are enhanced by a reduced sample surface area. The drying shrinkage results obtained for RGRE ($5.86 \pm 0.1054\%$) meet the requirement of less than 10% [19]. Low moisture content prevents the possibility of extracts being contaminated with fungi, molds and bacteria, otherwise if the moisture content is high, it can cause the extract to be easily overgrown with fungi, molds and bacteria so that it will reduce the biological activity of the extract during storage [28].

Table 2. Characteristics of RGRE

Observation	Result
Color, odor, taste	Brown, pungent, slightly bitter
Yield	4.96%
Water shrinkage	$5.86 \pm 0.105\%$

Physical properties of RGRE cream

The cream preparation evaluation test in this study was carried out to assess the physical quality of the cream as a topical treatment. The organoleptic test aims to evaluate the cream's appearance, smell, and color. The results of the organoleptic test can be seen in Table 3. The organoleptic of cream base looked different because it is odorless and semi solid white in color due to the absence of extracts. Our results showed that extracts concentration affected the consistency of the cream dosage form. Formulation of F3 had the thickest consistency due to the addition of the extract with the highest concentration of 20%. The higher the extract concentration, the thicker the cream and the darker its color. The cream's smell will also be more pungent or sharp.

Table 3. The organoleptic test and homogeneity of RGRE cream

Formulation	Odor	Color	Consistency	Homogeneity
Cream base	No smell	White	Soft	Homogenous
F1	Weak pungent	Yellow	Light thick	Homogenous
F2	Slightly pungent	Brownish Yellow	Thick	Homogenous
F3	Pungent	Brown	Thicker	Homogenous

The purpose of this homogeneity test is to determine if the active substances and ingredients were mixed well (homogeneous). A cream is considered homogeneous if there are no lumps in the smearing results and no coarse grains on the glass surface [21]. The result of homogeneity showed no lumps on the glass surface; it means the homogeneity test met the requirements for a good cream.

The purpose of this pH test evaluation was to determine the safety of applying topical cream preparations. Topical preparations should have a pH close to the normal pH of the skin (pH 4.5-6) [22]. According to some experts [29], the skin's true pH may be closer to 6. A pH that is too alkaline causes the skin to become scaly, and a pH that is too acidic triggers skin irritation [30]. The results of the pH test are shown in Table 3. Formulation of F1, F2, and F3, as well as the cream base, all meet the requirements because they fall within the pH parameter range of 4.5-6.5.

The evaluation of adhesivity test aims to determine the cream preparation's ability to adhere to the skin. The longer the preparation remains attached, the more the drug will penetrate the skin. A good topical preparation adhesivity test lasts more than four seconds [31]. The ability to adhere for a long time shows that the cream's active ingredients allow for greater absorption into the skin and provide a better effect. As shown in Table 3, F3 cream has the longest adhesivity time, followed by galangal cream, F2 cream, and F1 cream. Formulation F1, F2, and F3 had adhesivity time of more than four seconds, while the base has an adhesivity time of three seconds and does not meet the adhesivity parameters. Factors that can affect adhesivity include the concentration of added active substances, temperature, stirring method, pH, particle size, and viscosity. Cream F3 contains 20% RGRE, which makes the cream more adhesive and thicker. The adhesivity of the cream is influenced by its viscosity; the thinner the consistency, the shorter the adhesivity. The statistical analysis of the adhesivity test results showed significant differences among the concentrations of RGRE cream.

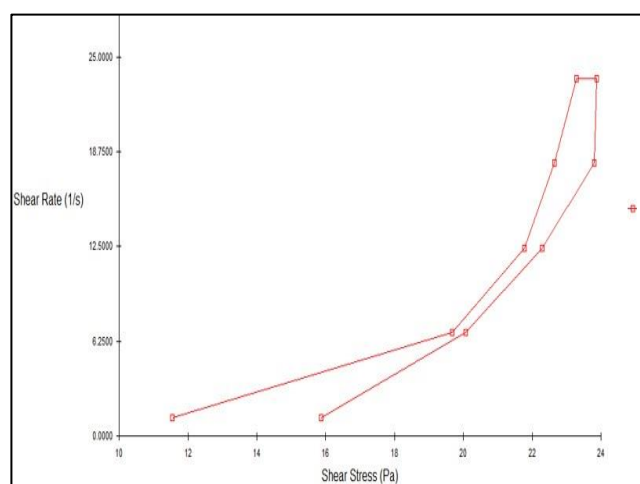
A spreadability test was performed on cream preparations to evaluate their ability to spread on the skin's surface. Creams require good spreadability to ensure better drug delivery. Spreadability should show a diameter between 5 and 7 cm [32]. Table 3 shows that creams F2 and F3 have the smallest diameter and do not meet the requirements because

their extract concentration is highest, making them thicker. The thicker the cream, the longer it takes to spread. Meanwhile, the basis cream and F1 cream formulations meet the spreadability testing requirements. Additionally, the viscosity of the cream increased, the spreadability decreased [33]. The adhesivity test revealed significant differences in adhesivity among the various RGRE cream concentrations.

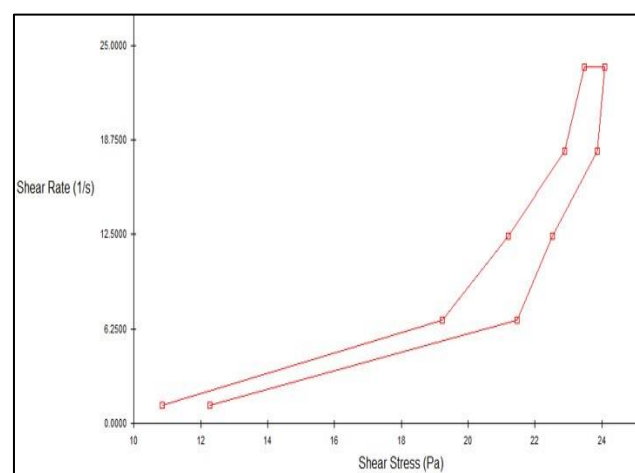
Table 4. Adhesivity test, spreadability and pH value of RGRE cream

Cream Formula	Adhesivity test (s)	Spreadability (cm)	pH
Creambase	30.00 ± 0.076	5.25 ± 0.053	6.45 ± 0.076
F1	45.90 ± 0.265	5.09 ± 0.036	6.41 ± 0.050
F2	50.06 ± 0.076	4.62 ± 0.058	5.51 ± 0.104
F3	60.03 ± 0.076	4.41 ± 0.058	5.11 ± 0.104

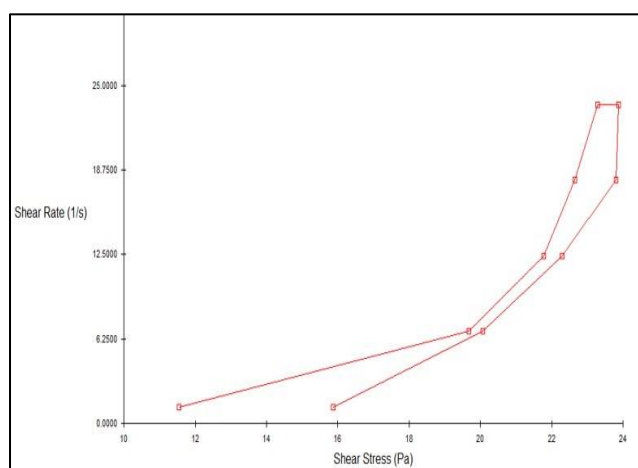
A viscosity test is performed to determine the viscosity and flow rate of a particle in a preparation. The greater the viscosity, the greater the force required for the preparation to flow. The purpose of the test is to determine the flow rate of the preparation produced by the cream. The smaller the viscosity value, the thinner and softer the cream's consistency. Based on the calculation of the relationship between shear stress (SS) and shear rate (SR), the four formulations demonstrated pseudoplastic flow properties (Figure 1), as evidenced by an upward linear trend in the graph, because the correlation coefficient (r) value of the relationship between $\log$ SS vs. SR is greater than that of SS vs. SR, and the slope value (B) exceeds 1 [34]. Pseudoplastic flow, also called "shear thinning," occurs when a substance begins to flow as soon as force is applied. However, when a greater force is applied, the substance's viscosity actually decreases. This behavior is exhibited by most topically applied emulsions, allowing them to spread smoothly on the skin [35].



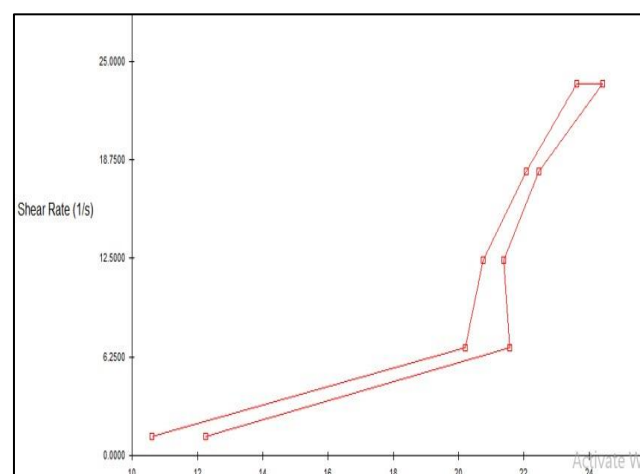
a. Creambase



b. F1



c. F2



d. F3

Figure 1. Rheogram of RGRE cream

The difference between the base cream and F1, F2, and F3 was the level of the active ingredient RGRE in each. The base cream contains no RGRE; F1 contains 2%; F2 contains 3%; and F3 contains 4%. As the concentration of RGRE increased, the cream's viscosity increased (Figure 2). Increasing the amount of extract decreased the water content and increased the cream's viscosity. Viscosity results showed that F3 cream has the highest viscosity because it was solid and thick due to the addition of 20% RGRE. The ideal viscosity range for semi-solid preparations is 2,000–50,000 cPs [36]. F1 and F2 did not meet the requirements for good viscosity within this range. However, some studies on cream preparations reported that the ideal viscosity is close to 10,000 cPs [37], [38]. The analysis of the viscosity test data showed that increasing the RGRE concentration in the cream increased its viscosity and affected its physical properties. F1 and F2 did not meet the good viscosity requirements refer to this range. From the analysis of the viscosity test data, it can be concluded that increasing the concentration of RGE in the cream can increase the cream's viscosity, thus affecting the cream's physical properties.

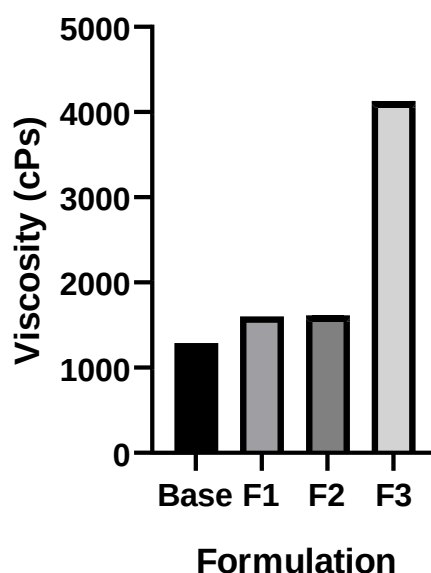


Figure 2. The viscosity of RGRE cream

Emulsion-type testing determines the type of emulsion in a cream preparation. To confirm the type of emulsion formed, distilled water was added to the cream and stirred. After stirring, it was found that the oil-in-water emulsion dispersed in water, confirming that the cream preparation was indeed an oil-in-water emulsion. The presence of a more water-soluble emulsifier, triethanolamine, caused this type of emulsion.

Antifungal activity of RGE cream

The antifungal activity test results show that RGE cream inhibits the fungus *T. rubrum* as shown in Table 5. The inhibition zone diameters, in order from largest to smallest, are F3, F2, F1, and F0. Higher concentrations of RGE cream resulted in greater inhibitory activity. However, the antifungal activity of cream F3 was weaker than that of the positive control (ketoconazole 2%). This finding supports the previous research, which reported that the RGRE was able to inhibit *M. canis*, *M. gypseum*, and *T. mentagrophytes*, though its effectiveness was lower than that of 2% ketoconazole [2]. The cream base without RGRE, which was used as a negative control, exhibited the least inhibitory effect on *T. rubrum*. The cream base exhibited antifungal activity because it contained parabens, which function as preservatives and inhibit the growth of *T. rubrum*. Statistical analyses revealed significant differences in the antifungal activities of F1, F2, and F3 compared to the negative control ($p < 0.05$). Significant differences were also observed among the base cream, F1, F2, F3, and ketoconazole cream. Administering RGRE in cream formulation at different concentrations affects its antifungal activity.

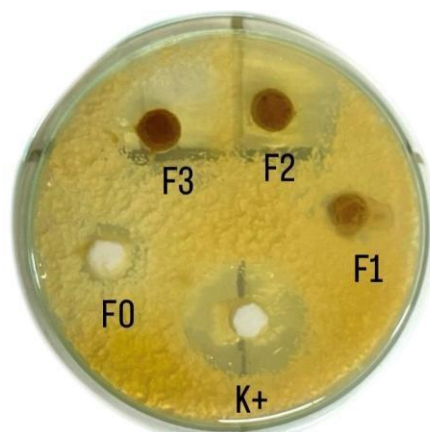


Figure 3. The antifungal activity of RGRE cream against *T. rubrum*

Table 5. Antifungal activity of RGRE cream against *T. rubrum*

Cream Formula	Diameter of inhibition zone (mm)
Base cream	$7.50 \pm 0.173^{\$}$
F1	$12.33 \pm 0.289^{*\$}$
F2	$18.83 \pm 0.289^{*\$}$
F3	$20.83 \pm 0.289^{*\$}$
Positive Control	$21.66 \pm 0.289^{*}$

*denotes significant different to negative control; \$denotes significant different to positive control

Several references mentioned that the essential oil of RGE contains 1,8-cineole and acetyl chavicol, which have antifungal and antibacterial properties [39]. Gas chromatography-mass spectrometry (GC-MS) analysis showed that the RGRE contains several fatty acids, including palmitic acid and stearic acid which contributes to its activity [18]. Furthermore, studies have demonstrated that palmitic acid also known as hexadecanoic acid, and other fatty acids exhibit antifungal activity against the yeast *C. albicans* [40], [41]. Additionally, palmitic acid inhibits several types of phytopathogenic fungi and other fungi, particularly in the process of inhibiting spore germination of some fungi [14]. A study reported that RGRE contained of flavonoids, tannins, quinones, and steroids/triterpenoids. Flavonoids and saponins can cause the outer cell membrane to leak and aggregate, which can hinder the growth of dermatophytes. They can also interfere with the fungal cytoplasmic membrane's ability to function [14].

Conclusion

RGRE concentration in the cream formulation affected the physical properties and antifungal activity of the cream. The higher concentration of RGRE in the cream was followed by an increase in its physical properties, including the viscosity, adhesivity, and antifungal activity. F3 was the optimal formula with a concentration of 20% RGRE in oil-in-water-based cream.

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Declarations

Author contribution : SM: proposed the concept, design, and methodology, drafting and revising article. SR: performing the research and analyzing data. SFF: reviewing the methodology and data.

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Ethics Declaration : We declare that the research work has fulfilled all relevant ethical guidelines including statements of approval/consent.

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