Trop J Nat Prod Res, March 2024; 8(3):6525-6536 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Formulation and Antioxidant Activity Test of *Centella asiatica* Herba Extract and *Moringa oleifera* Leaves Extract as An Anti-Aging Emulgel

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ARTICLE INFO

Article history:
Received 13 November 2023
Revised 18 March 2024
Accepted 23 March 2024
Published online 01 April 2024

ABSTRACT

Asiaticoside in *Centella asiatica* L. and β-sitosterol in *Moringa oleifera* exhibit antioxidant properties, making them potential active ingredients in anti-aging formulations. Emulgel, a combination of emulsion and hydrogel systems, offers specialized structures that enhance the stability of delivery systems or bioactive ingredients. Proper excipients, including emulsifiers, bases, and gelling agents like Carbopol 940 and stearic acid, are crucial for maintaining emulgel stability. This study aimed to optimize Carbopol and stearic acid concentrations in an emulgel formula containing Gotu kola and Moringa leaf extracts for optimal physical quality and antioxidant activity. Three emulgel formulas (F1: 0.5% Carbopol; 4% stearic acid, F2: 1% Carbopol; 3.5% stearic acid, F3: 1.5% Carbopol; 3% stearic acid) were tested for organoleptic properties, homogeneity, pH, centrifugation, spreadability, adhesion, hedonic response, and antioxidant activity (measured using the DPPH technique). All formulas showed changes in shape and minor oil appearance during centrifugation, indicating texture instability due to low emulsion system stability. Despite this, all formulations were homogeneous with stable pH, spreadability, and adhesion. Panelists favored F1 followed by F3, with none preferring F2 in hedonic tests. Emulgel F1 demonstrated optimal physical quality and panelist preference, while emulgel F3, with the lowest stearic acid concentration (3%), exhibited the highest antioxidant activity. Emulgel F3 holds promise for further development as an anti-aging cosmetic product, combining superior physical quality with high antioxidant efficacy.

***Keywords*:** anti-aging, *Centella asiatica*, emulgel, *Moringa oleifera*, stearic acid

Introduction

Free radicals have been known to cause skin cancer, photodamage, and accelerated aging resulting from excessive exposure to sunlight and oxidative stress. The human body produces antioxidants in limited quantities, which may not suffice to counteract the daily production of free radicals. Hence, external antioxidant intake is required to combat free radicals.[1] Antioxidants neutralize free radicals and stop the oxidation of biological molecules such as lipids, proteins, and nucleic acids, protecting the skin from further damage and slowing aging. Furthermore, dietary adjustments and topical preparations with various active components, such as antioxidants, counteract these harmful effects.[2,3]

Topical antioxidants improve the skin's biophysical parameters, making the skin healthy and free from infections and conditions associated with oxidative stress.[4,5] Developing effective and stable topical formulations faces several challenges, including efficient skin and epidermal penetration and maintaining antioxidant potency by minimizing oxidative damage due to ionization, pH extremes, high storage temperatures, and metal ions.[6,7]

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Citation: Suena NMDS, Rahayu LRP, Wijaya SC, Antari NP, Adrianta KA. Formulation and Antioxidant Activity Test of *Centella asiatica* Herba Extract and *Moringa oleifera* Leaves Extract as An Anti-Aging Emulgel. Trop J Nat Prod Res. 2024; 8(3):6525-6536. https://doi.org/10.26538/tjnpr/v8i3.9

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Typically, topical formulations like creams and gels are chosen to address skin issues such as premature aging. These formulations, including creams, gels, and face masks, are commonly used by the public. Generally, these dosage forms' formulation and physical quality should conform to standards and exhibit good physical quality. However, these topical dosage forms still have their limitations. Gels struggle with delivering hydrophobic drugs, which are easily lost when exposed to sweat or water.[8] Creams require a heating process during manufacturing and can break down if the formula is incorrect,[9] while ointment (hydrocarbon-based) shows greasiness as well as phase inversion.[10]

An innovative approach is needed to address the limitations of the previously mentioned topical dosage forms, such as utilizing an emulgel—a gel-based emulsion.[8] Emulgel formation utilizes a combination of hydrogel and emulsion systems to create concrete structures that enhance the stability of the bioactive ingredient or delivery method. Other advantages of emulgel are incorporating hydrophobic drugs, better loading capacity, better stability, production feasibility, low dosage form cost, and improved patient compliance.[11–14] Common excipients used in emulgel include Carbopol 940 and stearic acid. Frequently utilized as a gelling agent, Carbopol quickly disperses in water at room temperature, exhibits a wide viscosity range, and achieves the desired gel base viscosity with only a small concentration.[15,16] On the other hand, stearic acid is a versatile excipient commonly used in topical formulations and widely used in cosmetics. Stearic acid is frequently used in cream formulations when partially neutralized with alkalis or triethanolamine. This results in the generation of a creamy base when combined with 5-15 times its weight in aqueous liquid. The appearance and plasticity of the cream depend on the proportion of alkali utilized.[16] To maintain the stability of emulgel preparations, it is crucial to use the appropriate excipients at

the correct concentration. It includes emulsifiers, bases, and gelling agents.[17] Carbopol and stearic acid function as gelling agents and bases, which determine the stability of the emulgel.

As technology progresses, various plants have demonstrated anti-inflammatory, antioxidant, anticancer, and other beneficial effects in numerous studies. Asiaticoside in *Centella asiatica* L. (also known as Gotu kola) and *β*-sitosterol in *Moringa oleifera* have antioxidant activity, a potential active ingredient in anti-aging dosage forms.[18–22] Gotu kola contains vitamins C and B, with its main active ingredient being triterpenoid glycosides like asiatic acid, asiaticoside, madecassic acid, madecassoside, sitosterol, kaempferol, and polyacetylene compounds.[19] This plant is recognized for its antioxidant and ethanol extract antioxidant activity against the DPPH free radical of 79.49 (%), corresponding to 6.059±0.022 mg ascorbic acid equivalent/g DW has been reported.[23] Moreover, Moringa plants, particularly their leaves, contain high antioxidants, including crucial phenolic bioactive compounds like flavonoids (quercetin, kaempferol, isorhamnetin, and apigenin).[24] Research has indicated that Moringa leaves contain *β*-sitosterol at 90 mg/g, total phenolics at 8 µg/mL, flavonoids at 27 µg/mL, and high antioxidant activity (69.72±1.15%) of the ethanol extract.[25,26] The Gotu kola herb extract and Moringa leaf extract have antioxidant activity. Combining the two extracts has the potential to increase their antioxidant activity. Studies have shown that combining extracts increases antioxidant activity compared to each extract alone. 30 A study by Reubun *et al*. showed that combining *Moringa oleifera* and *Centella asiatica* extracts can help cure Alzheimer's disease more effectively.[31] This study will combine Gotu kola and Moringa leaf extracts to formulate an emulgel with improved antioxidant activity. Previous studies have shown promising results for this combination of active ingredients.

Sohail *et al*.[3] reported that the emulgel formulation based on lycopene significantly improved skin hydration and elasticity over 12 weeks. Additionally, compared to the placebo formulation, there were significant reductions in erythema, melanin, and sebum content. So far, researchers have not found any reports regarding the topical effects of lycopene-based emulgel on human volunteers. This supports the importance of formulating emulgel dosage forms with anti-aging potential. Previous studies on emulgel formulations as antioxidants have demonstrated their potential to promote topical anti-aging, limit oxidative stress-related skin disorders, and prevent premature aging process.[32–34]

In this study, an emulgel dosage form was formulated by combining extracts from Gotu kola and Moringa leaves, intended for anti-aging purposes, and subjected to physical quality testing. Numerous studies have been conducted on the antioxidant activity of Gotu kola extract and Moringa leaf extract, as evidenced by published scientific articles. One study has even formulated Moringa leaf extract into an emulgel.[34] No research has published results on emulgel formulations using active ingredients from a combination of Gotu Kola and Moringa leaf extracts, specifically for anti-aging purposes. This research aimed to determine the appropriate concentration of Carbopol and stearic acid in the emulgel formula to produce a dosage form with the best physical quality and antioxidant activity. The objective was to assess the emulgel's physical quality in line with established standards, thereby addressing the limitations of other topical dosage forms. It represents a novel advancement in pharmaceutical dosage forms aimed at combating aging. In addition to using a combination of Gotu kola herb extract and Moringa leaf extract as the active ingredients, the novelty of this study lies in the process of making this emulgel, which differs from that of previous researchers, specifically in the development and mixing of the gelling agent with the emulsion base.

Materials and Methods

Materials include, Carbopol 940 (PT. Brataco, Indonesia); propylene glycol (PT. Karunia Sejahtera Abadi SABA KIMIA, Indonesia); stearic acid (PT. Brataco, Indonesia); coconut oil (PT. Barco, Indonesia); triethanolamine (TEA) (PT. Brataco, Indonesia); methylparaben (PT. Karunia Sejahtera Abadi SABA KIMIA, Indonesia); propylparaben (PT. Karunia Sejahtera Abadi SABA KIMIA, Indonesia); dimethicone (PT. Brataco, Indonesia); distilled water (UD. Sekawan Bali Sejahtera, Indonesia); green tea fragrance (Fadjar Kimia, Indonesia); and 70% ethanol (PT. Brataco, Indonesia). Others are DPPH powder (PT. Smart Lab, Indonesia), standard vitamin C (Merck KGaA, Germany), and methanol (Merck KGaA, Germany). The tools include a Universal pH indicator strip (Macherey-Nagel, Germany), analytical balance (Ohaus pioneer, PA 224C), Digital weighing Balance (ACIS BC-500), rotary evaporator (BUCHI R-300), water bath (MEMMERT GmbH+Co., KG, Germany), various laboratory glassware (Pyrex), and Elmasonic S 40 H Ultrasonicator (Hans Schmidbauer GmbH & Co.KG, Germany).

Plant material and preparation

The plant sample *Centella asiatica* L.(Gotu kola) was collected in January 2023 from Karangasem district, Bali province, Indonesia, while *Moringa oleifera* leaves were collected from Denpasar and Tabanan district, Bali province, Indonesia. The plants were identified by "Eka Karya" Botanical Garden Characterization Laboratories, National Research and Innovation Agency through e-Layanan Sains, Badan Riset dan Inovasi Nasional at Indonesia, and voucher specimen no. 77400 for *Moringa oleifera* Lam. and 77401 for *Centella asiatica* (L.) Urb. were assigned. The collected Gotu kola and Moringa leaves were washed and sorted wet. They were air-dried under shade and ground into powder using a blender.[34]

Extract Preparation

The powdered samples were macerated with 70% ethanol solvent in a ratio of 1:6 by ultrasonication (Elma Sonic®) for 30 minutes at 50°C at a frequency of 50 kHz. The extract was left in a macerator for 24 hours at room temperature and then filtered with a Buncher funnel. The filtrate was evaporated in a rotary evaporator at 50°C and then in a water bath to obtain a thick extract.[35] The percentage yield of each extract was calculated from formula 1:

$$\%\text{yield } \frac{\text{weight of extract (final)}}{\text{weight of Simplicial powder (initial)}} x100\% \quad \text{.....(1)}$$

Preparation of the emulgel

Three emulgel formulations were developed with varying concentrations of Carbopol and stearic acid, as outlined in Table 1. These formulations include F1 (0.5% Carbopol and 4% stearic acid), F2 (1% Carbopol and 3.5% stearic acid), and F3 (1.5% Carbopol and 3% stearic acid). The gel base was prepared by heating distilled water in a Beaker in a water bath. Crushed Carbopol was gradually added to the water while stirring until fully dispersed, resulting in a gel base that was allowed to stand for 24 hours. Subsequently, the gel base was reheated in a water bath while simultaneously preparing the emulsion mass.

The emulsion mass was created by preparing the oil phase, which included stearic acid and dimethicone, and adding coconut oil in a glass Beaker. The aqueous phase comprised triethanolamine dissolved in distilled water in another glass Beaker. Both phases were melted and heated separately at 60°C-70°C. Upon reaching the designated temperature, the oil phase was slowly incorporated into the water phase, with constant stirring until homogenous, yielding an emulsion mass.

Preservatives, methylparaben and propylparaben, were pulverized in a separate mortar and dissolved in propylene glycol. The heated gel base was transferred to the mortar and ground for homogeneity. The preservative mixture was introduced to the gel base and ground until homogeneous. Subsequently, the emulsion mass was gradually added to the gel base while grinding constantly to form an emulgel base. Extracts of Gotu kola herb and Moringa leaf were crushed in a separate mortar to a smooth consistency. The extract mixture was then slowly added to the emulgel base while grinding until homogeneous. The fragrance was crushed until homogeneous and evenly distributed and was added to emulgel. The product was packed into containers and labeled.

Physical quality testing

The emulgel's physical quality was evaluated, including organoleptic properties, homogeneity, mechanical stability (centrifugation), pH tests, adhesion, and spreadability. The physical quality tests were performed on each formulation on days 1, 7, and 28, and each test was replicated three times.

Organoleptic properties were observed directly in the shape/consistency, color, and aroma of the emulgel dosage form produced.[36] Homogeneity was observed by applying 1 g of emulgel to a glass object, covering it with another, and then observing whether the dosage form was homogeneous and the surface was uniformly smooth. The dosage form is said to be homogeneous when there are no lumps and coarse particles.[37] Mechanic stability was observed by weighing 5 grams of emulgel, centrifuging at 5000 rpm for 30 minutes, and then observing whether separation existed. Emulgel is considered stable if no separation occurs.[38,39] The pH test was performed by diluting 0.5 g of emulgel with 5 mL of distilled water (1:10) and then immersing the Universal Indicator pH strips. The pH strips were used because they give a more straightforward, cheaper, and quicker way to obtain pH readings than a pH meter, which does not require pre-calibration.[40] After dipping well, the color change on the strips was matched to the universal indicator to determine the pH of the emulgel.[41]

A spreadability test was performed by weighing 0.5 g of emulgel and placing it on a square glass measuring 10x10 cm^2. It was then covered with a glass of the same size and known weight, left for 1 minute, and the diameter of the emulgel spread was measured. The procedure was repeated for each additional weight of 50 g, 100 g, and 150 g.[37] The adhesion test was performed by weighing 0.5 g of emulgel, placing it on a square glass measuring 10 x 10 cm^2, and then covering it with the same glass. The glass was then mounted on a test fixture, subjected to a 1 kg load for 5 minutes, and removed with an 80 g load. The time taken for the two pieces of glass to be released was recorded.[42]

Hedonic Test

The ethical approval for the hedonic test on humans was obtained from the Health Research Ethics Committee of Denpasar Health Polytechnic, with the ethical approval number LB.02.03/EA/KEPK/0137/2023. The hedonic evaluation was performed using a questionnaire on 30 participants aged 15-30 in good health. The participants in the sample were selected through convenience sampling. Participants who withdrew from the study were excluded. Inclusion criteria required participants to be willing to participate after receiving information about the research, to have good use of their five senses, to not be experts in hedonic testing (i.e. common people), and to have used similar products before.

Thirty participants were recruited for this hedonic test that aimed at gathering respondents' opinions via social research methodologies.[43] Participants were instructed not to apply the dosage form to their skin but to palpate it using fingers only during evaluation. Participants evaluated the dosage forms based on their level of preference using a hedonic rating scale ranging from 1 to 5, with corresponding criteria as outlined as follows.[44] A rating of 'Strong Dislike' signified a negative experience, leading the participant to express no desire to retry the dosage form. 'Dislike' indicated a negative experience in trying the dosage form. 'Somewhat Dislike' suggested that the participant found the dosage form acceptable but not as satisfactory as a commonly used similar product. 'Like' implied that the dosage form was comparable to the commonly used similar product. 'Very Like' indicated that the dosage form surpassed the commonly used similar product in terms of preference.

Antioxidant Activity Test

The DPPH standard solution was prepared by accurately weighing 4 mg of DPPH powder into a 100 mL volumetric flask. Methanol p.a. was added to the volumetric flask and vortexed. It was then made up to volume by adding more to make a final concentration of 40 ppm DPPH standard solution.

The emulgel test solution was prepared by weighing 50 mg of each emulgel formula. This was dissolved in ethanol and adequately mixed. The volume was then adjusted to 50 mL (1000 ppm). Concentration variations of 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm were prepared. For the ascorbic acid comparison solution, 5 mg of ascorbic acid was dissolved in ethanol p.a. while stirring, homogenized, and the volume adjusted to 50 mL (100 ppm). Concentration variations of 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm were prepared.

The antioxidant activity of the emulgel was assessed by pipetting 2 mL of the sample solution from various concentrations (20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm) into a test tube containing 2 mL of 40 ppm DPPH solution, respectively. The mixture was incubated at room temperature in the dark for 30 minutes, and the absorbance was measured at the maximum wavelength of 517 nm using a UV-Vis spectrophotometer.[45]

Similarly, the antioxidant activity of the ascorbic acid was determined by pipetting 2 mL of ascorbic acid solution from various concentrations (1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm) into a test tube containing 2 mL of 40 ppm DPPH solution, respectively, followed by incubation at room temperature for 30 minutes in the dark place. The absorbance was subsequently measured at the maximum wavelength using a UV-Vis spectrophotometer at 517 nm. The IC_{50} value was obtained from the linear regression equation between %inhibition and concentration against DPPH. The percentage of inhibition was calculated using the following equation.

$$\%\text{inhibition} = \frac{\text{DPPH absorbance} - \text{sample test (emulgel) absorbance}}{\text{DPPH absorbance}} \text{x} 100\%. \quad ...(2)^{45}$$

Table 1: The formula for preparing Gotu kola extract and Moringa leaf extract Emulgel.

Ingredients	Concentration (%)			Function
	F1	F2	F3	
Gotu kola extract	5	5	5	Active ingredient
Moringa leaf extract	5	5	5	Active ingredient
Carbopol 940	0.5	1	1.5	Gelling agent
Propylene glycol	10	10	10	Humectant
Stearic acid	4	3.5	3	Oily base
Coconut oil	10	10	10	Penetration enhancers
Triethanolamine	2	2	2	Alkalizing agent and emulsifier
Dimethicone	2	2	2	Antifoaming agent and water repellent agent
Methylparaben	0.1	0.1	0.1	Preservative
Propylparaben	0.3	0.3	0.3	Preservative
Green tea fragrance	0.6	0.6	0.6	Fragrance
Distilled water	60.5	60.5	60.5	Vehicle

The IC_{50} value was obtained by calculating linear regression using Microsoft Excel, with the sample concentration (ppm) as the abscissa (x-axis) and the % inhibition value as the ordinate (y-axis). The AAI (Antioxidant Activity Index) value was calculated using the following formula.

$$\text{AAI value} = \frac{\text{DPPH concentration (ppm)}}{\text{sample } IC_{50} \text{ value}} \quad \ldots\ldots(3)$$

Data Analysis

Statistical data analysis was performed on the spreadability, adhesion, and hedonic tests. SPSS software was used for all statistical analyses, with a 95% confidence level. First, a normality test was performed to determine the data distribution from the spreadability, adhesion, and hedonic tests, followed by a one-way ANOVA post hoc LSD test. Where necessary, a non-parametric test, the Kruskal-Wallis post hoc Mann-Whitney test, was performed on the spreadability data, and the hedonic test results were analyzed descriptively by considering the median, minimum, and maximum values.

Results and Discussion

The yields of Gotu kola herb and Moringa leaf extracts were 14.27% and 17.5%, respectively. The yield value indicates the extraction method's efficiency in extracting secondary metabolites from plant materials.[46,47] The extraction of Gotu kola herb and Moringa leaves was performed using the ultrasound-assisted extraction (UAE) method due to its higher effectiveness and efficiency. The use of ultrasonic waves helps to increase the penetration of the solvent into the cell membrane wall, thereby producing more secondary metabolites and increasing the extract yield compared to conventional extraction methods such as maceration.[46,48] This was evidenced by the high yield of 14.27% for the Gotu kola herb extract and 17.5% for the Moringa leaf extract. Both extracts met the requirements outlined in the Indonesian Herbal Pharmacopoeia, which recommended extract yield of not less than 7.3% for Gotu kola herb and <9.2% for Moringa leaf extract.[49] Both extract yield values were higher than those obtained by other researchers using conventional maceration methods.[50,51] The solvent used in the extraction was 70% ethanol because of its good ability to penetrate both lipophilic and hydrophilic matrixes and cellular membranes to interact with plant metabolites.[52] The result of the emulgel formulation of Gotu kola herb and Moringa leaf extract with three formulations containing different concentrations of Carbopol and stearic acid, namely F1 (0.5%; 4%), F2 (1%; 3.5%) and F3 (1.5%; 3%) is shown in Figure 1. Physical observation of the formulated emulgel for 28 days showed no changes in color and aroma but did exhibit changes in its shape on days 7 and 28. A small amount of oil appeared on the surface of F2 and F3, but it did not affect the homogeneity of the emulgel. After 28 days of storage, the three emulgel formulations remained homogeneous (Figure 2). Also, the pH of the three formulations was constant after 28 days of storage. In the centrifugation test, the three formulas showed separation, indicating that they were less stable [53,54] (Figure 3). The physical quality observation results of the three Emulgel formulas are shown in Table 2. The three emulgel formulas remained homogeneous throughout the 28-day testing period, as indicated by the absence of any coarse particles or lumps in all three formulas.[14,53,54] The combination of extracts in each formula resulted in a light green to brownish-green color. The green tea aroma of each formula resulted from using fragrances in emulgels, which enhances the acceptance of panelists or consumers. The observed results indicate that on day 7, a small amount of oil was visible on the surface of emulgels F2 and F3, which increased on day 28, leading to the separation of all three formulas after centrifugation (Table 2). These changes in organoleptic and mechanical stability may be attributed to the inconsistent strength of excipients, primarily emulsifiers, affecting the stability of the emulsion system and resulting in alterations to the dosage form.[55,56] The oil that formed was likely due to suboptimal excipient concentration, excessive use of coconut oil as a penetration enhancer, and the use of crude extracts as the active ingredient so that each extract still contained different compounds of varying polarity that were extracted during the extraction process.[57,58] To enhance the stability and consistency of the preparation, additional emulsifiers can be combined with stearic acid and triethanolamine. For instance, cetyl alcohol can be added as a stiffening agent.[16] The use of a combination of emulsifiers can enhance the stability of the emulsion system and improve the consistency of the preparation, thereby preventing phase separation.[59]

Each excipient has a concentration range and unique interactions. When partially neutralized with an alkali such as triethanolamine (TEA), stearic acid can function as an emulsifier, forming a creamy base suitable for the formulation of topical dosage forms, such as emulgels with an emulsion system, as demonstrated in this study. The alkali ratio impacts topical dosage forms containing stearic acid's physical characteristics, appearance, and plasticity.[16] Therefore, it is necessary to utilize stearic acid and TEA in optimal concentrations to produce stable and effective emulgel dosage forms. Additionally, Carbopol also has a similar interaction with stearic acid and TEA.

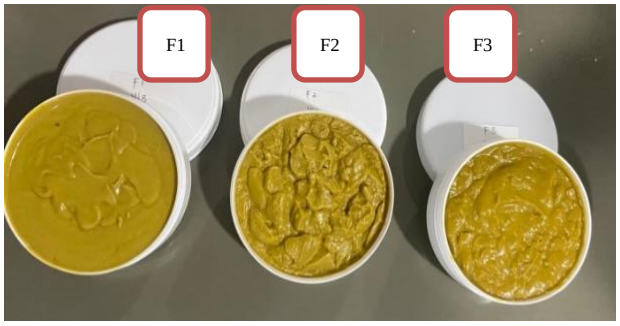

Figure 1: The appearance of the Gotu kola herb and Moringa leaf extracts emulgel dosage form.

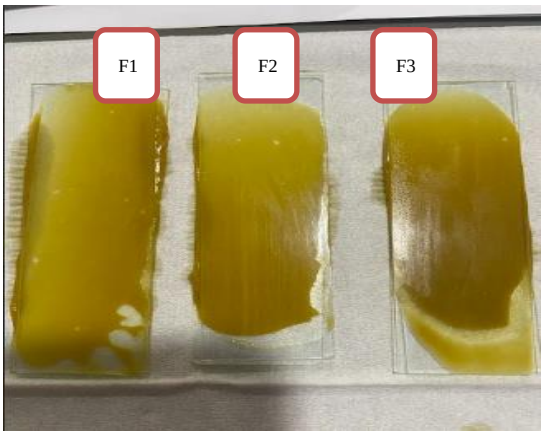

Figure 2: The homogeneity test results after 28 days.

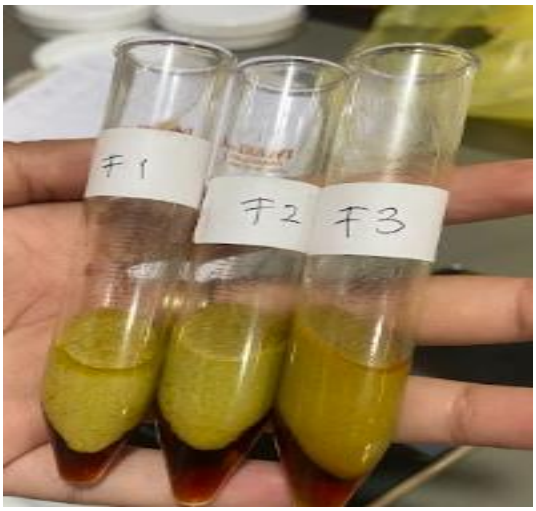

Figure 3: The centrifugation test results after 28 days.

Table 2: The physical quality observation results of Gotu kola herb and Moringa leaves extract emulgel.

Characteristics	Formula	Test Results, Day-		
		1	7	28
Shape	F1	T-	T-	T-
	F2	T	TO	TO
	F3	T+	TO+	TO+
Color	F1	LG	LG	LG
	F2	LG	LG	LG
	F3	BG	BG	BG
Aroma	F1	G	G	G
	F2	G	G	G
	F3	G	G	G
Homogeneity	F1	HM	HM	HM
	F2	HM	HM	HM
	F3	HM	HM	HM
pH	F1	6	6	6
	F2	6	6	6
	F3	6	6	6
Mechanic stability	F1	U	U	U
(centrifugation)	F2	U	U	U
	F3	U	U	U

Description:
F: Formula
LG: Light Green
BG: Brownish Green
T-: Slightly viscous and stiff.
T: Thick but a bit stiff.
T+: Thick and soft.
TO: Thick but a bit stiff and there is a little oil.
TO+: Thick and soft and there is oil.
G: Green tea aroma
HM: Homogeneous
U: unstable (separation occurred)

Carbopol dispersed in water creates an acidic colloidal dispersion, which, when neutralized, yields a highly viscous gel. Amino acids, potassium hydroxide, sodium bicarbonate, sodium hydroxide, and organic amines such as TEA are effective neutralizing agents for Carbopol.[16] TEA is particularly significant in emulgel formulation, as it influences stearic acids and Carbopol. Emulgel formulations vary only in the concentration of Carbopol and stearic acid, with no change in the concentration of TEA. Thus, as both stearic acid and Carbopol concentrations increased, the proportion of TEA became insufficient to adequately interact with these components,[15] ultimately causing instability in the Emulgel as observed through the separation of oils upon centrifugation.

The high concentration (10%) of coconut oil, which acts as a penetration enhancer, is also considered to cause separation in the emulgel. However, Čižinauskas *et al*. showed that using coconut oil as a penetration enhancer at a 10% (w/w) concentration did not significantly increase the flux of dihydroquercetin as a penetration enhancer.[60] However, another study suggests that less than 3% coconut oil can enhance penetration in emulgel foam dosage forms.[61] Therefore, the 10% concentration of coconut oil used in this gel formulation may have been too high and less effectively homogenized by existing emulsifiers and gelling agents. A combination of emulsifiers could be used to improve the stability of emulsion system formulations. This assumption is supported by further evidence indicating the presence of oil in the dosage form, including the 2.47% fat content found in Moringa leaves[62] and 0.05% essential oil in Gotu Kola herb extract.[63] Additionally, it should be noted that environmental factors such as temperature, light, and air may impact the stability of dosage forms.[64]

The pH test results indicate that the three formulas have a stable pH of 6 for 28 days of the study period (Table 2), which corresponds to the pH range in different areas of the skin, 4.1-7.4.[65] This pH value demonstrates that the emulgel produced is similar to the skin's physiological pH and is unlikely to have any adverse effects when used. If the pH of the emulgel dosage form is too acidic, it can cause skin irritation. Conversely, if the pH of the dosage form is too alkaline, it can lead to skin dryness.[66] It is important to note how pH values play a crucial role in the efficacy of the emulgel. The pH value influences the physical stability of the emulgel by affecting Carbopol and stearic acid, which are excipients sensitive to pH changes[16] Additionally, the pH value impacts the stability of the active ingredients present in the dosage form.[67]

Based on the observation of the spreadability test for 28 days, it was found that the three emulgel formulations had a spreadability that met the requirements for good spreadability, namely 5-7 cm[68] (Figure 4). Although the adhesion results varied for the three emulgel formulations, they could adhere for more than 4 seconds to meet what is considered good adhesion[69] (Figure 5) (Table 3). The topical spreadability of all three formulations falls within the range of 5-7 cm, indicating good spreadability[68,70] (Table 3). The spreadability of a formulation indicates how easily it will spread when applied to the skin, improving skin penetration.[71] A cream with better spreadability will have a larger surface area in touch with the skin, aiding in the even dispersion of the active ingredients.[70] Figure 4 presents a graph revealing fluctuations in spreadability over 28 days, with F3 exhibiting the highest spreadability, followed by F1 and F2. These fluctuations in spreadability may be due to the storage temperature of the formulation. This problem arises from a lack of complete temperature control during storage, thus negatively impacting the spreadability of the dosage form. As time goes on, the viscosity of the preparation becomes low, which makes the strength in binding water weak, as a result of which the preparation undergoes syneresis - a process of liquid discharge from the gel.[72] Syneresis can be reduced or avoided by taking steps like designing the packaging to hide potential syneresis areas, minimizing external pressure, and maintaining the product at a constant temperature.[73]

The physical quality test's descriptive data indicates that all preparations were similar. Researchers cannot use descriptive analysis

to determine the best formula because the test data varies slightly in several parameters. Therefore, to determine if there were differences in test results among the three formulas, researchers require the assistance of statistical tests. Differences in means among more than two test groups were observed using ANOVA and Kruskal-Wallis tests. ANOVA is typically used when the research data qualifies for parametric tests, while Kruskal-Wallis is used when the available data does not qualify for a parametric test. There was no significant difference in spreadability between the formulas on day 1, with a p-value of 0.267 according to Kruskal-Wallis's test. However, the One-Way ANOVA reveals a marked difference in spreadability among formulas on the 7th and 28th days, with p-values of <0.001 and 0.020, respectively. The post hoc LSD analysis revealed that on day 7, F3 exhibited significantly different spreadability among the three formulas with a p-value of <0.001. On day 28, F2 and F3 were found to be significantly different from F1, with a p-value of 0.007.

These results show that the spreadability of the emulgel dosage forms was influenced by the concentration of stearic acid and Carbopol. A reduction in the stearic acid concentration leads to a decrease in viscosity, ultimately causing an increase in spreadability. F3 possesses the lowest stearic acid concentration, resulting in higher spreadability. Despite F1 containing the highest amount of stearic acid, its spreadability is not the lowest. Rather, F2 displays the weakest spreadability. When combined with stearic acid, it is essential to consider the appropriate Carbopol concentration to obtain emulgel dosage forms with optimal spreadability. Additionally, higher Carbopol concentrations result in thicker dosage forms due to increased viscosity, leading to lower spreadability. Therefore, selecting the correct Carbopol concentration is crucial to ensure emulgel dosage forms have the desired spreadability.[74]

The adhesion of the three formulas meets the requirements for good adhesion, which is more than 4 seconds,[69,75] as shown in Table 3. Adhesion and spreadability are inversely related, which means that dosage forms with a lower spreadability will have a longer adhesion time and vice versa.[71] A dosage form with a longer adhesion time will be more effective in absorption of the active ingredient.[76] From Figure 5, the graph shows a fluctuation in adhesion as a function of storage temperature, which affects the spreadability since the spreadability results were related to the adhesion results in the physical quality test of the dosage form.

Table 3: The Spreadability and Adhesion of The Gotu kola herb and Moringa leaves Extracts Emulgel

Formula	Replications	Day-1	Day-7	Day-28
Spreadability (cm)				
F1	R1	7.28	5.67	6.96
	R2	5.80	5.75	7.29
	R3	7.84	6.50	6.25
		D: 7.28 (5.80-7.84)^{a}*	M: 5.97±0.458^{b}	M: 6.83±0.531^{d}
F2	R1	6.25	5.52	6.00
	R2	6.00	5.80	5.50
	R3	6.25	5.20	6.16
		D: 6.25 (6.00-6.25)^{a}*	M: 5.51±0.300^{b}	M: 5.89±0.344^{e}
F3	R1	7.56	8.12	7.02
	R2	7.02	8.41	7.56
	R3	7.28	7.56	8.41
		D: 7.29 (7.02-7.56)^{a}*	M: 8.03±0.432^{c}	M: 7.66±0.701^{d}
Adhesion (second)				
F1	R1	33	193	32
	R2	51	236	38
	R3	29	334	48
		M: 37.67±11.71^{f}	M: 254.33±72.27^{i}	D: 38 (32-48)^{k}*
F2	R1	232	201	26
	R2	192	227	105
	R3	203	204	113
		M: 209.00±20.67^{g}	M: 210.67±14.22^{i}	D:105 (26-113)^{k}*
F3	R1	6	58	13
	R2	9	46	10
	R3	8	90	31
		M: 7.67±1.52^{h}	M: 64.67±22.75^{j}	D: 13 (10-31)^{k}*

Description:
(*): data were not normally distributed nor homogenous.
D: the value of Median (Minimum-Maximum)
M: Mean plus minus Standard Deviation ($\bar{x} \pm SD$)
Different superscript letters in the same column in the spreadability and adhesion data represent significant differences at $p < 0.05$

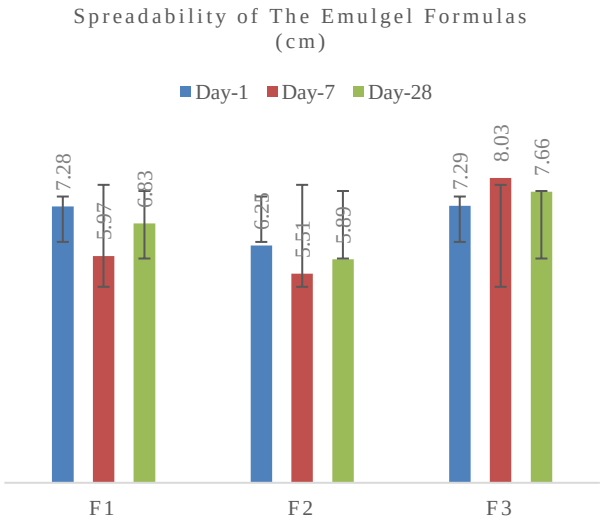

Figure 4: The Graph of Emulgels Spreadability from Day-1 to Day-28

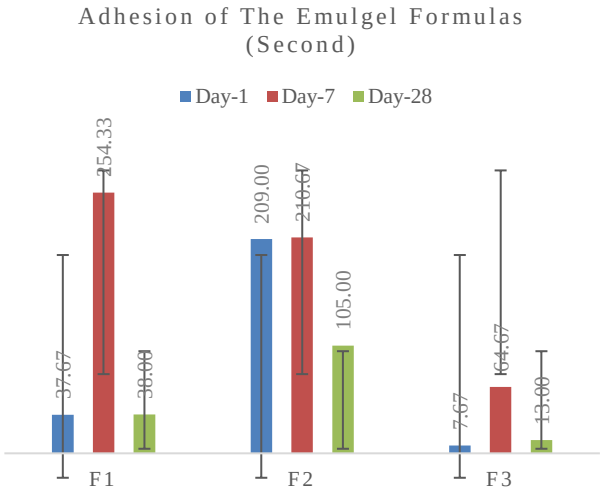

Figure 5: The Graph of Emulgels Adhesion form Day-1 to Day-28

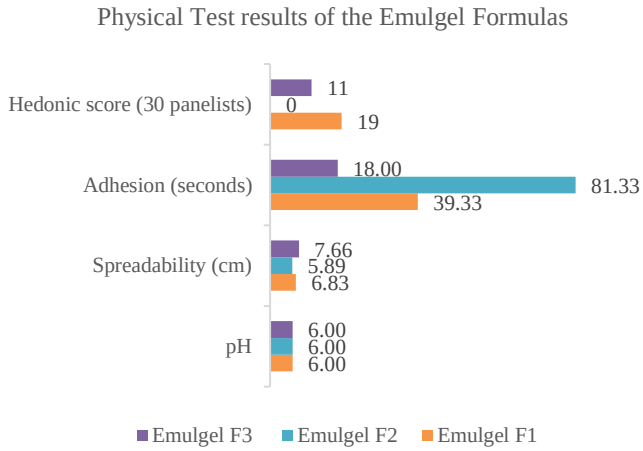

Figure 6: The Graph of Physical Test Results of The Emulgel Formulas at Day 28

The outcomes from conducting the One-Way ANOVA examination for adhesion on day 1 and day 7 demonstrated a significant difference in each formulation with a p-value <0.001 and 0.005, respectively. The Kruskal Wallis test on day 28 indicated no significant difference in adhesion between each formula with a p-value of 0.113. The post hoc LSD analysis of adhesion on day 1 revealed that the F2 formula exhibited significantly different adhesion than the other two, with a p-value <0.001. The post hoc LSD analysis results indicate that F3 has significantly different adhesion compared to the other two formulas on day 7, with p values of 0.002 and 0.007.

Based on the test results over 28 days, F2 exhibits the highest adhesion despite having the lowest spreadability. Following it in the ranking were F1 and F3, with the latter having the highest spreadability and, thus, the lowest adhesion. However, on the seventh day, F1 exhibited the most excellent adhesion. On day 7, there was a change in the formulation, resulting in the release of oil in F2 and F3. Longer storage times can increase the potential for emulgel instability. One such instability is the occurrence of syneresis, where the liquid is released to the surface of the preparation, in addition to the release of oily components due to the emulsifier's failure to maintain the stability of the emulsion system. Syneresis during storage is caused by the aggregation between the polymer chains of the gelling agent, which continues to occur. This aggregation is induced by the movement of the polymer chains in the gel system.[77] These instabilities can impact the spreadability and adhesion of the formulas.

The hedonic test results showed that 30 panelists had their respective evaluations of the three emulgel formulas. The texture, non-sticky impression, color, and aroma of the emulgel formulas were the four characteristics that panelists rated in the hedonic test (Table 4). The formula ranking results showed that the most preferred formula was F1, with 19 panelists; the preferred formula was F3, with 11 panelists; and the least preferred formula was F2, with 0 panelists. F1 is the most preferred because the texture and color were lovely, and even the panelists liked the aroma because it was similar to the panelists' favorite fragrance. In addition, F1 was more appealing to the eye and easy to apply.

Meanwhile, F3 was chosen as the preferred formula because the panelists liked the texture of the dosage form. After all, it was thick and soft, light, easy to apply, and a reasonably attractive color and distinctive smell. Figure 6 summarizes the result of physical tests on the Gotu kola herb and Moringa leaf extract emulgel formulas on day 28.

The median hedonic results in Table 4 illustrate the varying median values of texture characteristics, with F1 and F3 having a median of 4 and F2 having a median of 3. These results were consistent with the Kruskal Wallis Hedonic test findings, demonstrating that each formula's texture exhibits a significant difference with a p-value <0.001. The texture with the highest Mean Rank value is owned by F3, which has a value of 61.13. Following that, F1 has a Mean Rank value of 40.65, and F2 has a Mean Rank value of 34.72. Therefore, panelists preferred the texture of F3 the most as determined by median (minimum-maximum), Mean Rank, and Kruskal-Wallis post hoc test. Panelists preferred the texture of formula F3 due to its superior ease of application compared to other formulas. This ease of application, in turn, was influenced by its spreadability. Good spreadability is essential for easy application of the emulgel dosage form without requiring excessive pressure that would cause the wider surface to come into contact with the skin. It ensures that the dosage form spreads and is distributed evenly.[78] This outcome aligns with the physical quality testing conducted, indicating that F3 has a high spreadability, consistent with the panelists' perception.

The color characteristics of each formula had distinct median values, with F1 having the highest median value, followed by F2 and F3. The color of each formula displays a significant difference, with a p-value <0.001, as per the findings of the Kruskal Wallis Hedonic test. The color with the highest Mean Rank value was F1, which scored 65.12. Following that was F2, with a Mean Rank value of 39.38, and finally, F3, with a Mean Rank value of 32.00. Additionally, based on the median value (minimum-maximum), Mean Rank, and Kruskal Wallis test with post hoc analysis, F1 was deemed the most preferred color formula by the panelists. Therefore, F1 yields the color most favored by

the panelists. The color appearance of the dosage form is affected by changes in the concentration of Carbopol and stearic acid, even though each formula has the same extract concentration. The non-sticky impression and aroma characteristics yielded similar median values across the three formulas. It complies with the Kruskal Wallis Hedonic test results, which showed no significant variations in non-sticky impression and aroma between each formula, with p-values of 0.321 and 0.412, respectively. It indicates variations in Carbopol and stearic acid concentrations do not affect the scent with equal concentration in each formula. The variation in viscosity levels noticed in the spreadability and stickiness of the three formulas had no impact on the non-sticky sensation perceived by the panelists.

The hedonic testing and ranking results indicated that F1 was preferred by 19 panelists, F3 by 11, and F2 by none. The panelists' preferences indicate that F1 is the top-rated formula, with F3 and F2 following closely behind in second and third place, respectively.[79] This study used a sample of homogeneous panelists with comparable age ranges to ensure consistent assessment results. It may be essential to perform hedonic tests again with a more diverse group of panelists, including those aged 17-45.

Based on the results of the physical quality tests, including organoleptic tests, mechanical stability (centrifugation), pH, spreadability, and adhesiveness, it can be concluded that F1 has the best physical quality when compared to F2 and F3. Similarly, the results of the hedonic test indicated that panelists favored F1 the most. When determining the optimal formula, priority was given to physical quality criteria over panelist preference in the hedonic test. This is because the physical quality test is evaluated based on various physical quality standards that ensure the preparation has good physical quality. Meanwhile, the hedonic test only assesses physical appearance using the five senses and the perception of panelists with varying tastes. Formulators need to design preparations that meet consumer interest while maintaining quality standards. The study found that the physical quality and hedonic test results were consistent, indicating that F1 was the optimal formula for both physical quality and panelist preference.

The parameter used for analyzing antioxidant activity with the DPPH method is IC_{50}, which represents the concentration required to reduce 50% of DPPH.[80] The IC_{50} and AAI results are presented in Table 5. Specifically, F1 demonstrates a strong antioxidant activity with an IC_{50} value of 74.33 ppm, F2 also exhibits a strong activity with an IC_{50} of 50.59 ppm, and F3 displays a very strong activity with an IC_{50} of 39.65 ppm.[81] Furthermore, we obtained the IC_{50} value of ascorbic acid as a comparison standard, which falls under the very strong category because it is a standard and potent antioxidant. To standardize antioxidant testing results based on the DPPH method, we used the Antioxidant Activity Index (AAI).[82] The AAI was determined by comparing the concentration of the DPPH solution with the IC_{50} concentration of the sample. The results for the test were as follows: F1 at 0.54 medium category, F2 at 0.79 medium category, and F3 at 1.01 strong category. In contrast, ascorbic acid demonstrated a very strong category result of 8.25.[82,83] Thus, according to the results of the calculations performed in the IC_{50} and AAI tests, the best antioxidant activity among the three emulgel formulas was F3, with IC_{50} of 39.65 ppm, which means that at this concentration, the dosage form can reduce the concentration of free radicals (DPPH) by 50%. Data for measuring IC_{50} and AAI values of emulgels of Gotu kola and Moringa leaf extracts and standard ascorbic acid are presented in Table 5 and Figure 6. In each formulation tested for antioxidant activity, the concentration of Carbopol varies from the lowest to the highest level, and the concentration of stearic acid varies from the highest to the lowest level. The recommended concentration range of Carbopol as a gelling agent is 0.5% - 2%.[16] This study found that the high antioxidant activity was F3 with Carbopol content of 1.5% and stearic acid of 3%. According to research conducted by Destrina,[84] Carbopol concentration variation did not affect the antioxidant activity of jackfruit leaf extract in a gel dosage form. In this study, Carbopol affected the viscosity of emulgel of Gotu kola herb and Moringa leaf,[85] but did not affect the antioxidant activity of the emulgel, which is in line with the research conducted by Destrina,[84] as previously mentioned.

Table 4: The Hedonic Test Result of The Gotu kola herb and Moringa leaves Extracts Emulgel

Hedonic characteristics	Formula	N	Median (Minimum-Maximum)
Texture	F1	30	4.00 (1-5)^{a*}
	F2	30	3.00 (2-5)^{a*}
	F3	30	4.00 (3-5)^{b*}
Non-Sticky Impression	F1	30	4.00 (1-5)^{c*}
	F2	30	4.00 (2-5)^{c*}
	F3	30	4.00 (2-5)^{c*}
Color	F1	30	5.00 (2-5)^{d*}
	F2	30	3.50 (1-5)^{e*}
	F3	30	3.00 (1-5)^{e*}
Aroma	F1	30	4.00 (2-5)^{f*}
	F2	30	4.00 (2-5)^{f*}
	F3	30	4.00 (2-5)^{f*}

Description:
(*): data were not normally distributed.
Different superscript letters in the same column in each hedonic characteristic data represent significant differences at $p < 0.05$.

Table 5: The result of IC_{50} and AAI value of The Gotu kola herb and Moringa leaves Extracts Emulgel and Ascorbic Acid

Sample	IC_{50} (ppm)	Category	AAI	Category
Emulgel F1	74.33	Strong	0.54	Moderate
Emulgel F2	50.59	Strong	0.79	Moderate
Emulgel F3	39.65	Very Strong	1.01	Strong
Ascorbic Acid	4.85	Very Strong	8.25	Very Strong

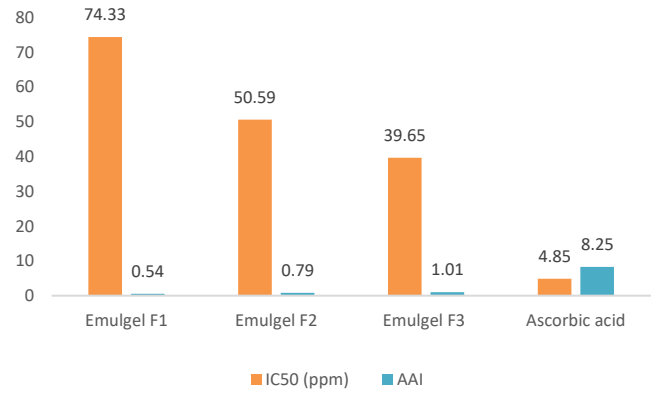

Figure 7: The IC_{50} and Antioxidant Activity Index (AAI) of the Emulgel Formulas Compared to Ascorbic acid

A practical range for stearic acid concentration in topical formulation is 1% to 20%.[16] Emulgel F3, with the lowest level of stearic acid (3%) among the three formulations, showed the highest level of antioxidant activity compared to the others. A reduction in the concentration of stearic acid results in a decrease in viscosity, leading to a more easily spreadable preparation. It was demonstrated in F3, which exhibited the best spreadability due to its softer consistency. The preparation's softer consistency is anticipated to improve the active ingredient's release.[71,86–88] Binder *et al*. showed that drug penetration decreases slightly as the viscosity of hydrogels increases.[89] F3 has the lowest stearic acid content, resulting in lower viscosity and the highest antioxidant activity, as indicated by its lowest IC_{50}. This is because F3 releases active substances more efficiently, inhibiting the DPPH. In contrast, F1, with the highest stearic acid concentration, exhibited the highest IC_{50}, indicating weaker antioxidant activity.

Based on study findings, the emulgel formulation of extracts from Gotu kola herb and Moringa leaves has demonstrated potent antioxidant activity. Premature or natural aging involves the skin's reduced tissue function caused by oxidative stress when the number of free radicals in the body outnumbers available antioxidants.[1] Oxidative stress may arise from various factors, including sun exposure. Sunlight containing UV A and B rays may lead to oxidative stress. Hence, external antioxidants are necessary to reinforce combat against free radicals alongside the body's natural supply.[25] The emulgel formulated in this study shows the potential to be an external source of antioxidants that can help reduce the number of free radicals in the body, thereby helping to prevent premature aging. Formula F3, which has the highest antioxidant activity, is the emulgel formula that needs further improvement and adjustment for better physical stability and quality while maintaining its antioxidant activity.

Conclusion

The study's shortcomings include observing emulgel physical stability briefly and the small number of formula modifications. More extended stability observations, formula modifications, and the employment of approved equipment are essential components of future formula development. Emulgel F1, with a content of 0.5% Carbopol and 4% stearic acid, was the optimal formula for both physical quality and panelist preference. Meanwhile, emulgel F3, with a content of 1.5% Carbopol and 3% stearic acid, showed the highest level of antioxidant activity compared to the others. In order to maintain its antioxidant activity while having better physical stability and quality and high acceptability for consumers, the emulgel formula with the highest antioxidant activity, formula F3, needs to be further optimized and developed as an anti-aging cosmetic product.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

We express our gratitude to the Faculty of Pharmacy's laboratory at Universitas Mahasaraswati Denpasar for granting us access to their research facilities and resources.

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