



Research Article

Utilization of Emulgel Watermelon (*Citrullus lanatus*) Flesh Extract as a Topical Antioxidant

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Abstract

The flesh of watermelon (*Citrullus lanatus*) contains carotenoid compounds that act as antioxidants. The purpose of this study was to determine the variation in the concentration of carbopol 940 on physical properties, irritation tests, and the stability of emulgel antioxidants against temperature and storage time. Evaluation of the physical properties of *C. lanatus* pulp extract emulgel includes organoleptic, homogeneity, dosage pH, dispersion, adhesion, emulsion type, viscosity as well as hedonic test and irritation test. Antioxidant stability testing of emulgel was carried out for 28 days at three temperature conditions: 4, 25, and 40°C and tested on days 0, 7, 14, 21, and 28 with the DPPH method. The results of the physical properties evaluation meet the requirements with pH values of 5.50–5.57, dispersion 5–6 cm, adhesion <4 seconds, viscosity 5624–15443 cPs, F2 and F3 hedonic tests are preferred by researchers, and irritation tests of all formulas show no irritation symptoms to all refiners. The results of antioxidant stability of emulgel after storage on the 28th day showed an average result of IC₅₀ temperature of 4°C (112.4547 ± 0.1432 mg/L), 25°C (119.3170 ± 0.1966 mg/L), and 40°C (124.1554 ± 0.1317 mg/L). The results of stability analysis show that temperature and storage duration affect antioxidant stability. The higher the temperature and duration of storage, the antioxidant stability of emulgel decreases. Storage of *C. lanatus* flesh extract emulgel at 4°C was able to maintain antioxidant activity for 28 days of storage.

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INTRODUCTION

Watermelon (*Citrullus lanatus*) is a popular fruit rich in various health-promoting nutrients¹. Beyond its well-known water content (approximately 91.45%), *C. lanatus* flesh also boasts a valuable nutritional profile, including vitamin C, thiamine, riboflavin, niacin, carbohydrates, fiber, and sugars (0.15% fat, 7.55% carbohydrates, 0.4% fiber, and 6.2% sugar)². Importantly, *C. lanatus* possesses potent antioxidant properties due to the presence of carotenoid compounds like lycopene, phytoene, phytofluene, beta-carotene, and lutein³. These bioactive components contribute to its ability to combat free radicals and potentially mitigate cellular damage. Studies have demonstrated the significant antioxidant activity of *C. lanatus* flesh extract, with Mariani *et al.*² reporting a value of 16.619 mg/L. Given its established antioxidant properties, *C. lanatus* flesh presents a promising material for the development of skincare cosmetic products, particularly in the form of emulgels. *Citrullus lanatus* flesh extract has gained interest for its potential use in dermatological applications due to its unique properties. These include thixotropy, emolliency, a non-greasy feel, ease of spreading, lack of staining, long shelf life, water solubility, environmental friendliness, transparency, and a pleasant appearance⁴. Emulgels, a type of emulsion formulation (oil-in-water [O/W] or water-in-oil [W/O]), are increasingly utilized for drug delivery. When a gelling agent is incorporated

into the emulsion, it transforms into a gel-like structure (emulgel). Notably, the concentration of the gelling agent significantly impacts the resulting viscosity of the emulgel⁵. This study investigates the effects of varying carbopol 940 concentrations on the physical properties, irritation potential, and stability of emulgel formulations containing *C. lanatus* flesh extract.

Carbopol 940, a commonly used gelling agent, significantly influences the consistency of emulgel formulations. Studies by Habiba *et al.*⁶ demonstrated that varying carbopol 940 concentrations (0.5%, 0.75%, 1%, and 1.25%) in olive oil emulgel with moringa leaf extract resulted in distinct consistency variations, ranging from less viscous to quite dense and viscous. Notably, 1% carbopol 940 concentration has been shown to yield formulations with desirable physical properties and stability⁷. Beyond physical characteristics, ensuring the safety and user acceptance of topical formulations is crucial⁸. Irritation tests are essential to evaluate the potential for skin irritation caused by the emulgel extract⁹. Additionally, hedonic testing allows for the assessment of user preference regarding the formulation's sensory attributes¹⁰.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay was selected for its simplicity, speed, sensitivity, and minimal sample requirement¹¹. This method measures the ability of the emulgel to scavenge free radicals, indirectly reflecting its antioxidant activity. DPPH is a stable free radical at room temperature that accepts an electron or hydrogen atom from an antioxidant compound, resulting in the formation of a stable molecule and a reduction in absorbance at 517 nm¹². Similar to findings by Wulansari *et al.*¹³, who reported a decrease in tamarind leaf extract's antioxidant capacity during storage at varying temperatures, we hypothesized that storage temperature and duration might influence the stability of the *C. lanatus* extract's antioxidant activity within the emulgel. Therefore, we evaluated the antioxidant stability of the emulgel formulations under different storage conditions. The emulgels were stored at 4°C, 25°C, and 40°C for 28 days. Antioxidant activity was assessed using the DPPH method at predetermined time points (days 0, 7, 14, 21, and 28) to investigate the combined effects of temperature and storage time. Based on the established knowledge that antioxidants are susceptible to oxidation¹⁴, and the potential impact of temperature and storage on the activity of natural antioxidants like those found in *C. lanatus* extract¹⁵, this study investigated the influence of carbopol 940 concentration on the physical properties, irritation potential, and, most importantly, the stability of the emulgel's antioxidant activity during storage at varying temperatures and durations.

MATERIALS AND METHODS

Materials

The following instruments were used in this study: analytical balance (Ohaus), pH meter (Hanna Instruments), Anton Paar viscometer (ViscoQC 300), oven (Mettler), UV-Vis spectrophotometer (Shimadzu), climatic chamber (LNEYA), optical microscope (Novel), hotplate (Corning), micropipettes (Across Pro), Karl Fischer Titrator (Aquacounter AQV-300), UV Box Thin-layer chromatography (TLC) spotting display (Camag), homogenizer (Heidolph), adhesion tester, dispersion tester, TLC chamber, refrigerator (AQUA), and blender (Advan). Standard laboratory glassware was also used. *Citrullus lanatus* was obtained from the Kramat Jati main market, East Jakarta, Indonesia, and identified as *Citrullus lanatus* (Thunb.) Matsum. & Nakai by the National Research and Innovation Agency of the Republic of Indonesia, Cibinong (accession number B-579/II.6.2/IR.01.02/4/2023). The extract was prepared at the Indonesian Medicinal and Aromatic Crops Research Institute, Bogor. Other materials used include carbopol 940 (Dwilab Mandiri), triethanolamine (Dwilab Mandiri), paraffin liquid (Dwilab Mandiri), tween 80 (Dwilab Mandiri), propylene glycol (Dwilab Mandiri), methylparaben (Kimia Jaya Laboran), propylparaben (Kimia Jaya Laboran), span 80 (Labsains Chemical Center), distilled water (Fragrant Chemical), beta-carotene (Sigma Aldrich), DPPH (Sigma Aldrich), TLC plate GF₂₅₄ (Merck), absolute methanol (Merck), vitamin C (Merck), methylene blue (Kimia Jaya Laboran), magnesium powder, and concentrated HCl (Universitas Muhammadiyah Prof. DR. HAMKA).

Methods

Preparation of *C. lanatus* flesh extract

Ripe *C. lanatus* fruits were obtained and thoroughly washed. The fruits were dissected to separate the red flesh, white flesh, and rind. The red flesh was cut into small pieces and manually pressed to remove excess moisture. Subsequently, the red

flesh was oven-dried at 50°C for 48 hours (2 × 24 hours). The dried material was then pulverized using a blender to obtain a fine powder for extraction¹. A mass of 150.432 g of the red flesh powder was subjected to maceration extraction using 96% ethanol as the solvent. The mixture was macerated for 48 hours (2 × 24 hours) with constant agitation. The extract was then separated from the residue by filtration using filter paper. The residue was re-extracted with fresh 96% ethanol using the same maceration conditions. The combined filtrates from both maceration steps were concentrated using a rotary evaporator to remove the solvent and obtain a concentrated *C. lanatus* flesh extract.

Extract evaluation

The organoleptic properties (appearance, color, and odor) of the *C. lanatus* flesh extract were evaluated visually¹⁶. A qualitative screening for flavonoids was performed using a standard method¹⁷. Briefly, 0.5 g of extract was dissolved in 10 mL of hot methanol. Subsequently, 0.1 g of Mg powder and five drops of concentrated HCl were added. The formation of an orange, pink, or dark red color that persisted for at least three minutes was considered indicative of the presence of flavonoids. Thin-layer chromatography was employed to identify beta-carotene compounds. A mobile phase of chloroform : ethanol (1 : 1) and a stationary phase of silica gel 60 F₂₅₄ were used⁵. Moisture content of the extract was determined using an automated Karl Fischer titrator. Approximately 0.05 g of the sample was weighed and introduced into the instrument for analysis¹⁶.

Citrullus lanatus flesh extract antioxidant activity test

Citrullus lanatus flesh (10 mg) was weighed and dissolved in pro-analysis grade methanol in a 100 mL volumetric flask to prepare a 100 mg/L stock solution. The stock solution (600, 1000, 1400, 1800, or 2200 µL) was pipetted using a micropipette and diluted further with methanol to a final volume of 10 mL in separate volumetric flasks. This resulted in solutions with final concentrations of 6, 10, 14, 18, and 22 mg/L, respectively. Two milliliters of each extract concentration solution were pipetted into separate vials, followed by the addition of 2 mL of 0.1 mM DPPH solution. The vials were incubated in the dark for 30 minutes. The absorbance of each sample was then measured at 517 nm using a UV-Vis spectrophotometer. Three replicates were performed for each concentration. Vitamin C solutions (4, 5.5, 7, 8.5, and 10 mg/L) were prepared and used as a positive control following the same procedure¹⁸. The results of the extract solutions were compared to the vitamin C standard curve to determine the antioxidant activity.

Citrullus lanatus flesh extract emulgel formulation

The formulation for the *C. lanatus* flesh extract emulgel is detailed in **Table I**. Carbopol 940 was dispersed in twenty parts (w/w) purified water and allowed to hydrate for 24 hours. Triethanolamine was then gradually added under constant stirring until the gel base reached a pH of 6. Methylparaben and propylparaben were weighed, dissolved in propylene glycol, and incorporated into the gel base with continuous stirring until a homogenous mixture was obtained. An oil phase was prepared by combining liquid paraffin and span 80 in a separate container on a water bath set to 70°C. The water phase was prepared by mixing tween 80 with distilled water on a separate water bath set to 70°C. Both phases were stirred continuously throughout this process. The oil and water phases were then combined and homogenized using a suitable homogenizer. The homogenized emulsion was subsequently incorporated into the prepared gel base with continuous stirring until a uniform emulgel formed. Finally, the *C. lanatus* flesh extract was added to the emulgel and stirred until thoroughly homogeneous¹⁹.

Table I. *Citrullus lanatus* flesh extract emulgel formula.

Materials	Formula (%)				Function
	F1	F2	F3	F4	
<i>Citrullus lanatus</i> flesh extract	0.25	0.25	0.25	0.25	Active substances
Carbopol 940	0.5	0.75	1	1.25	Gelling agent
TEA	0.18	0.55	0.8	1.02	Extermination
Propylene glycol	10	10	10	10	Humectants
Methylparaben	0.18	0.18	0.18	0.18	Preservatives
Propylparaben	0.02	0.02	0.02	0.02	Preservatives
Liquid paraffin	5	5	5	5	Oil phase
Span 80	1.4	1.4	1.4	1.4	Emulsifiers
Tween 80	3.6	3.6	3.6	3.6	Emulsifiers
Distilled water	ad 100	ad 100	ad 100	ad 100	Solvent

Evaluation of *C. lanatus* flesh extract emulgel formulation

Organoleptic test: The emulgel formulations were subjected to a visual evaluation of color, odor, and texture using the naked eye²⁰.

Homogeneity test: Approximately 0.1 g of each emulgel formulation was weighed and spread evenly in a thin layer onto a clean, transparent glass slide. The spread emulgel was visually inspected for homogeneity, ensuring the absence of coarse particles or uneven distribution²¹.

pH test: The pH of the emulgel formulations was measured using a calibrated pH meter. Prior to measurement, the pH meter was calibrated with standard solutions of pH 4 and 7. About 10 g of each emulgel sample were weighed and transferred to a beaker. The pH electrode was immersed into the sample until a stable reading was obtained on the meter. The displayed pH value was then recorded²².

Viscosity test: The viscosity of the emulgel formulations was determined using an Anton Paar viscometer. A 200 mL beaker was filled with a well-mixed sample of the emulgel. The appropriate spindle (specify size and model number) was carefully immersed into the sample, ensuring the fill line on the spindle was submerged. The viscometer was then turned on, and the spindle was rotated at a speed of 100 rpm. The viscosity readings were recorded in centipoise (cPs) after the readings stabilized.

Dispersion test: The spreadability of the emulgel formulations was evaluated using a simple method. A transparent glass plate was placed on a millimeter block paper base. About 0.5 g of the emulgel sample was spread uniformly onto the glass plate. A second transparent glass plate was carefully placed on top of the sample, and a weight of 50, 100, or 150 g was applied for one minute to ensure consistent contact. Following this, the upper glass plate was removed, and the diameter of the spread emulgel was measured using the underlying millimeter block paper. This procedure was repeated for each weight (50, 100, and 150 g) with fresh emulgel samples²³.

Adhesion test: The adhesive properties of the emulgel formulations were evaluated using a modified version of a previously described method²⁴. Briefly, 0.25 g of each emulgel sample was applied to two pre-designated glass slides. A 1 kg weight was placed on top of the slides for 5 minutes to ensure uniform contact. The slides were then secured onto the testing apparatus, and an additional 80 g load was applied. The time taken for the emulgel to detach from the glass slides was recorded for each sample.

Phase separation (cycling test): The stability of the emulgel formulations against phase separation was evaluated using a thermal cycling test. Each formulation underwent six cycles over a 24-day period. Each cycle involved storing the emulgel for 48 hours at 4°C, followed by 48 hours at 45°C. After each cycle (every 96 hours), the emulgels were visually inspected for any signs of phase separation (creaming, sedimentation, or cracking)²⁵.

Determination of emulsion type: The type of the emulgel was determined using the methylene blue dye dilution method²⁰. Briefly, 0.5 g of emulgel was spread onto a microscope slide. A drop of 1% methylene blue solution was then added to the emulgel sample on the slide. The slide was covered with a coverslip, and the distribution of the dye was observed under a microscope at a suitable magnification. If the dye diffused evenly throughout the emulgel, the emulsion was classified as O/W. Conversely, if the dye remained localized as discrete blue specks within the emulgel, the emulsion was classified as W/O.

Hedonic test: A hedonic test was conducted to evaluate the color, aroma, and texture of the prepared *C. lanatus* flesh extract emulgel²⁶. Twenty student volunteers from the Faculty of Pharmacy and Science, Universitas Muhammadiyah Prof. DR. HAMKA, participated in the study.

Irritation test: Following approval from the Ethics Committee of Universitas Muhammadiyah Prof. DR. HAMKA number 03/23.10/02922 dated 2023, a closed patch test was conducted to assess the irritation potential of the emulgel formulations. Twenty healthy volunteers participated in this study. The emulgel was applied to a designated area (approximately 2 cm diameter) on the upper arm of each volunteer. The application site was then occluded with a waterproof plaster for 24 hours. During this period, the application sites were visually observed for signs of irritation at 4-hour intervals²⁷.

Antioxidant stability of *C. lanatus* flesh extract emulgel formulation

Emulgel formulations were stored at controlled temperatures of 4°C, 25°C, and 40°C for a period of 28 days. To assess the impact of storage on antioxidant stability, the DPPH method was employed on the emulgel samples at predetermined time points: days 0, 7, 14, 21, and 28²⁸.

Preparation of 0.1 mM DPPH solution: A 0.1 mM DPPH solution was prepared by dissolving 3.9432 mg of DPPH powder in analytical grade methanol. The solution was then brought to a final volume of 100 mL with additional methanol in a volumetric flask¹⁸.

Determination of the maximum wavelength of DPPH: The maximum absorption wavelength (λ_{\max}) of the DPPH solution was determined. Briefly, 2 mL of 0.1 mM DPPH solution in methanol was added to a cuvette. The absorbance was scanned across a wavelength range of 400-800 nm using a UV-Vis spectrophotometer to identify the λ_{\max} of DPPH¹⁸.

*Measurement of antioxidant activity of *C. lanatus* flesh extract emulgel formulation:* As much as 10 mg of emulgel were dissolved in methanol in a 10 mL volumetric flask to obtain a stock solution of 1000 mg/L. A series of five dilutions were prepared from the stock solution to achieve final concentrations of 45, 65, 85, 105, and 125 mg/L. Briefly, aliquots of 450, 650, 850, 1050, and 1250 μ L of the stock solution were transferred to separate volumetric flasks and diluted to 10 mL with methanol. About 2 mL of each diluted solution were then combined with 2 mL of 0.1 mM DPPH solution and incubated in the dark for 30 minutes. The absorbance of each sample was measured at the λ_{\max} of DPPH using a UV-Vis spectrophotometer. All measurements were performed in triplicate¹⁸.

Data analysis

The antioxidant activity of the emulgel formulations was determined by calculating their % inhibition of DPPH radical oxidation according to **Equation 1**. This equation utilizes the absorbance values of the control DPPH solution ($A_{\text{abs control}}$) and the DPPH solution containing the sample ($A_{\text{abs sample}}$). The half-maximal inhibitory concentration (IC_{50}) value, representing the sample concentration required to inhibit 50% of DPPH activity, was determined using a linear regression equation derived from the formula ($y = a \pm bx$)²⁹. The categorization of antioxidant activity followed the scale established by Blois³⁰: very strong (<50 mg/L), strong (50-100 mg/L), medium (100-150 mg/L), low (150-200 mg/L), and very low (>200 mg/L).

$$\%inhibition = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100\% \quad [1]$$

RESULTS AND DISCUSSION

The extraction process yielded 24.28% (w/w) of *C. lanatus* flesh extract, as detailed in **Table II**. The extract exhibited a characteristic watermelon odor, a thick consistency, and a brownish-red color, as shown in **Figure 1**. The moisture content of the extract, determined using a moisture test, was 18.1816%, which falls within the established range (5-30%) for viscous extracts³¹. Phytochemical screening revealed the presence of flavonoids *C. lanatus* flesh extract. The formation of a red-orange color upon addition of HCl and Mg powder serves as a characteristic test for flavonoid compounds (**Figure 2**). This color change indicates a reduction of the flavonoid components, further supporting their presence in the extract³².

Table II. *Citrullus lanatus* flesh extract evaluation results.

Extract evaluation	Result
Yield (%)	24.28
Organoleptic	Form: viscous Color: brownish red Smell: typical of watermelon
Phytochemical screening (flavonoid)	Red-orange (positive flavonoids)
Water content (%)	18.18
TLC	Rf extract: 0.71 Rf beta-carotene: 0.76

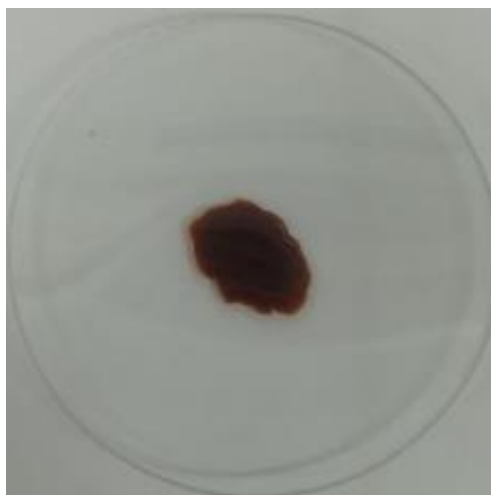


Figure 1. *Citrullus lanatus* flesh extract.



Figure 2. Flavonoid test results.

Thin-layer chromatography analysis was performed to identify the presence of beta-carotene in the *C. lanatus* flesh extract. Under visible and UV light at 254 and 366 nm, the extract exhibited an Rf value of 0.71, while the reference standard beta-carotene displayed an Rf value of 0.76 (Figure 3). Since the Rf values of the extract and standard fell within a close range (0 to 1)³³, the presence of beta-carotene in the *C. lanatus* flesh extract is confirmed.

Prior to emulgel preparation, the antioxidant activity of *C. lanatus* flesh extract was evaluated using the DPPH free radical scavenging assay and compared to vitamin C, a readily available and cost-effective standard with established strong antioxidant properties³⁴. The carotenoid content of the extract was also determined using a UV-Vis spectrophotometer to identify the presence of these antioxidant pigments. Carotenoids typically exhibit absorption peaks within the 400-550 nm range³⁵. Analysis of the *C. lanatus* flesh extract revealed three peaks at 502, 470, and 444 nm, suggesting the presence of carotenoid compounds.

The DPPH assay relies on the ability of antioxidants to scavenge the stable free radical DPPH, resulting in a color change from purple to yellow and a decrease in absorbance at the λ_{\max} of DPPH (typically around 515-520 nm)^{36,37}. In this study, the λ_{\max} of the DPPH solution was determined to be 516 nm. To minimize light and oxygen exposure, known to degrade the DPPH solution³⁸, it was stored in the dark and protected with aluminum foil. Following the method described by Molyneux³⁷, samples containing the extract and DPPH solution were incubated for 30 minutes to allow for the characteristic slow-moving reaction between antioxidants and free radicals, evident by the color change.

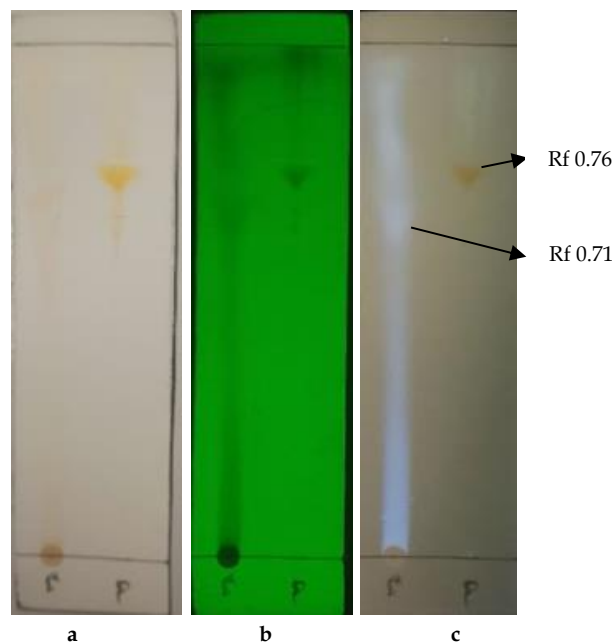


Figure 3. TLC results in visible light (a), UV 254 nm (b), and UV 366 nm (c). *Citrullus lanatus* flesh extract (S) and beta-carotene (P).

The DPPH assay results (Table III) revealed an IC_{50} value of 21.3876 mg/L for the *C. lanatus* flesh extract, compared to 8.6484 mg/L for vitamin C. Both the extract and vitamin C fall within the category of very strong antioxidants ($IC_{50} < 50$ mg/L) based on established classifications³⁰. However, the extract exhibited a higher IC_{50} value than vitamin C, which is likely due to the presence of various secondary metabolites with antioxidant properties within the extract, compared to the pure compound nature of vitamin C. Mariani *et al.*² reported a *C. lanatus* flesh extract IC_{50} value of 16.619 mg/L, highlighting potential variations in antioxidant activity arising from differences in plant growth location and sample treatment methods. The concentration of *C. lanatus* flesh extract incorporated into the emulgel formulations was 100-fold higher than the determined IC_{50} value of 2138.76 mg/L, translating to approximately 0.21% of the total emulgel weight³⁹. This concentration was subsequently increased to 0.25% to potentially mitigate potential reductions in antioxidant activity arising from interactions with the emulgel base components⁴⁰.

Table III. *Citrullus lanatus* flesh extract and vitamin C antioxidant activity.

Sample	IC_{50} (mg/L)
<i>Citrullus lanatus</i> flesh extract	21.3876
Vitamin C	8.6484

The physical properties of the *C. lanatus* flesh extract emulgel formulations (F1-F4; Figure 4) are summarized in Table IV. All formulations exhibited a yellowish-white color and lacked stickiness. However, consistency varied across formulations: F1 had a slightly watery consistency, F2 possessed an emulgel-like consistency, F3 displayed a slightly viscous consistency, and F4 exhibited the most viscous consistency. This demonstrates that increasing carbopol 940 concentration significantly impacted consistency ($p < 0.05$) but did not affect odor or color. Homogeneity testing revealed that all formulations met the desired specifications, with no uneven color distribution or coarse particles observed. This ensures uniform distribution of active ingredients across each application³.

pH testing is required to determine the degree of acidity of the preparation as it relates to consumer acceptance of a product⁴¹. The pH of the emulgels ranged from 5.50 to 5.57, exhibiting a slight decrease compared to the initial pH of 6 after the addition of the *C. lanatus* extract (weak acid, pH 5.31)³. However, these values remained within the normal human skin pH range (4.5 to 6.5)⁴². One-way ANOVA analysis showed no significant difference in pH between the formulations, indicating that carbopol 940 concentration did not influence pH.

Viscosity testing revealed a significant difference ($p < 0.05$) between the formulations. As expected, F4, containing the highest carbopol concentration (1.25%), displayed the highest viscosity, resulting in a thicker emulgel. All formulations met the standard viscosity range (6000-50000 cPs) for emulgel preparations according to SNI 16-4399-1996. The spreadability

(dispersion) of the emulgels decreased with increasing carbopol 940 concentration ($p < 0.05$). This aligns with the observed consistency variations, as higher carbopol concentrations lead to increased viscosity and reduced spreadability⁴³. Nonetheless, all formulations met the dispersion criteria of 5 to 7 cm⁴⁴.

Adhesion time also increased with increasing carbopol concentration ($p < 0.05$). F4, with the highest carbopol content, exhibited the longest adhesion time. This is attributed to the more viscous nature of F4 compared to the other formulations. All formulations met the minimum adhesion requirement of 4 seconds for topical preparations⁴⁵. The emulgels demonstrated good physical stability throughout six storage cycles at varying temperatures, with no observable phase separation between the oil and water phases. This suggests stability under both high and low temperatures⁴⁶. Microscopic examination (4x/0.1 magnification) confirmed that all formulations (F1-F4) were O/W emulsions. As shown in Figure 5, the even distribution of methylene blue within each preparation confirms this classification.

Hedonic testing indicated that respondents preferred formulations F2 and F3 based on color, aroma, texture, and ease of application²⁶. These findings suggest good user acceptance of the *C. lanatus* extract emulgel. Closed patch tests on volunteers' forearms revealed no irritation after 24-hour application of the emulgel preparations. This suggests good skin compatibility, likely due to the safe ingredients used and the pH remaining within the safe range for skin⁴⁷.

The results of this study demonstrate the successful development of stable *C. lanatus* flesh extract emulgels with varying carbopol 940 concentrations. While increasing carbopol concentration significantly impacted consistency and adhesion time, all formulations met the established criteria for physical properties. Notably, hedonic testing revealed user preference for formulations F2 and F3, suggesting good user acceptance. Importantly, the emulgels exhibited no irritation potential, indicating safety for topical application.



Figure 4. *Citrullus lanatus* flesh extract emulgel.

Table IV. *Citrullus lanatus* flesh extract emulgel evaluation results.

Physical evaluation	F1	F2	F3	F4
Organoleptic	Smell: distinctive Shape: rather watery Color: yellowish white	Smell: distinctive Shape: emulgel mass Color: yellowish white	Smell: distinctive Shape: slightly viscous Color: yellowish white	Smell: distinctive Shape: thick Color: yellowish white
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Ph	5.57 ± 0.0205	5.55 ± 0.0654	5.53 ± 0.0618	5.50 ± 0.0169
Viscosity (cPs)	5624.67 ± 83.2666	8423.67 ± 23.0940	15443.33 ± 582.8665	19070 ± 301.9934
Dispersion (cm)	6.18 ± 0.0750	5.81 ± 0.2346	5.55 ± 0.0704	5.25 ± 0.0625
Adhesion (seconds)	1.04 ± 0.0251	1.12 ± 0.0251	1.23 ± 0.0208	1.34 ± 0.0351
Cycling test	(-) No physical changes	(-) No physical changes	(-) No physical changes	(-) No physical changes
Emulsion type	O/W	O/W	O/W	O/W
Irritation test	No symptoms of irritation	No symptoms of irritation	No symptoms of irritation	No symptoms of irritation
Hedonic test (%)	93.75	100	100	80

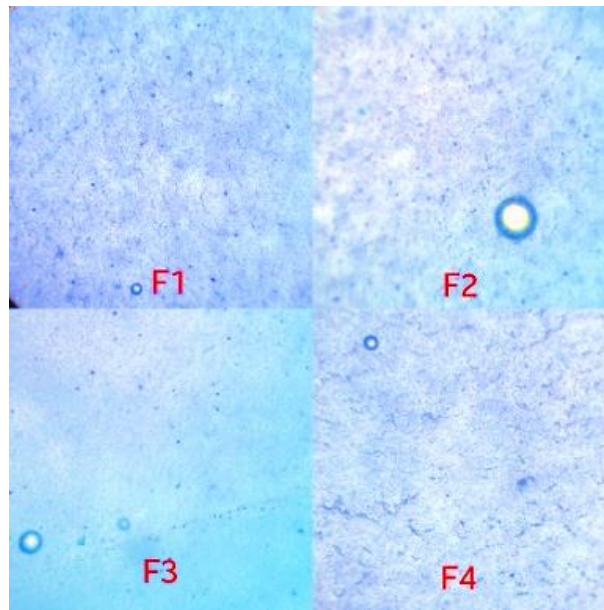


Figure 5. *Citrullus lanatus* flesh extract emulgel type results.

The F3 emulgel formulation with 1% carbopol 940 that exhibits appropriate physical properties was selected as the representative sample for antioxidant stability testing. As depicted in Figure 6, the IC_{50} value, a measure of antioxidant activity (lower IC_{50} indicates higher activity)³⁷, increased for emulgels stored at all three temperatures (4°C, 25°C, and 40°C) with increasing storage duration. This trend suggests a decrease in overall antioxidant activity during storage. The observed increase in IC_{50} values signifies a diminished capacity of the *C. lanatus* flesh extract within the emulgel to scavenge free radicals.

Two-way ANOVA analysis revealed a statistically significant interaction between storage temperature and storage time ($p < 0.05$). Specifically, the IC_{50} value increased with both longer storage duration and higher storage temperature. After 28 days, emulgels stored at 40°C exhibited the highest average IC_{50} values, while those stored at 4°C displayed the least pronounced decrease in activity. This phenomenon is likely attributed to the susceptibility of carotenoids, the primary antioxidant compounds in *C. lanatus* flesh, to degradation by heat, light, and oxygen. Carotenoid instability can lead to structural changes, such as the conversion from trans to cis isomers, rendering them more susceptible to oxidation⁴⁸.

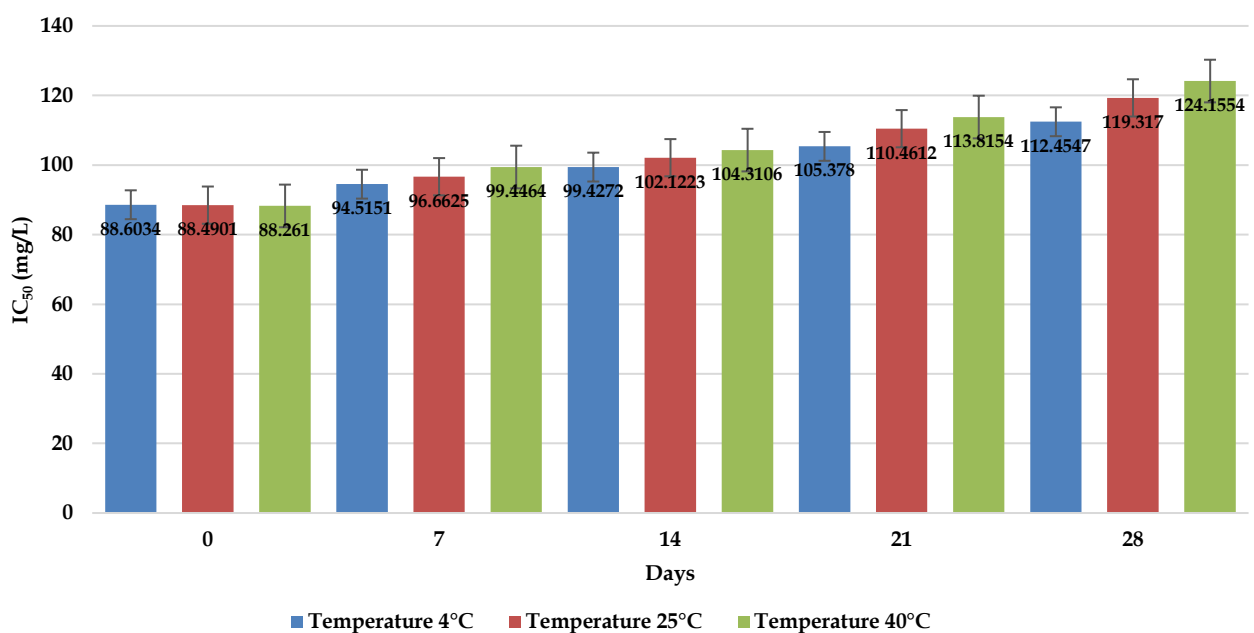


Figure 6. *Citrullus lanatus* flesh extract emulgel type results.

Carotenoids are susceptible to degradation through heat and oxidation, leading to the breakage of conjugated double bonds within their molecules⁴⁹. This process reduces their biological activity and is often observed as a decrease in carotenoid content. Our findings align with this established knowledge, as evidenced by the observed stability of the emulgel's antioxidant activity when stored at 4°C compared to higher temperatures (25°C and 40°C). Similar results were reported by Aryayustama *et al.*⁵⁰, who demonstrated that pandan fruit carotenoid extract maintained its content during storage at 4°C for four weeks, while degradation occurred at higher temperatures (28°C and 45°C).

The observed temperature dependence of carotenoid stability can be attributed to their inherent chemical properties. Lower temperatures minimize the rate of thermal decomposition and oxidative reactions, thereby preserving the integrity and bioactivity of these antioxidant compounds⁴⁹. Additionally, oxidation can lead to the formation of various degradation products with potentially altered or diminished biological activity compared to the parent carotenoid molecules⁵¹.

CONCLUSION

This study investigated the influence of carbopol 940 concentration on the physical properties, irritation potential, and stability of *C. lanatus* flesh extract emulgel formulations. While variations in carbopol 940 concentration impacted physical properties like dispersion, adhesion, and viscosity, no significant differences were observed in hedonic preference or irritation. Notably, storage temperature and duration significantly affected the antioxidant stability of the emulgel formulations. Higher temperatures and longer storage periods led to decreased antioxidant activity. Importantly, emulgels stored at 4°C maintained significant antioxidant activity throughout the 28-day study period.

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DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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