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ANALYSIS OF TOTAL FLAVONOID CONTENT IN ETHANOL 96% EXTRACT OF PEGAGAN HERB (*Centella asiatica* L.) LIP HYDRATING FORMULATION USING UV-VIS SPECTROPHOTOMETRY

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ABSTRACT

The lips are prone to dryness due to the absence of oil glands and their vulnerability to moisture loss, which necessitates the use of a lip hydrating preparation to maintain hydration. Pegagan (*Centella asiatica* L.) contains bioactive compounds such as flavonoids, triterpenoids and vitamin C, which act as antioxidants and support skin repair. Previous research focused on the determination of flavonoid content in pure extracts. Therefore, the flavonoid content after formulation into cosmetic preparations has not been evaluated. This study aimed to determine the significant differences of varying concentrations of Pegagan (*Centella asiatica* L.) herb extract, specifically F1 (3%), F2 (5%) and F3 (7%) on the total flavonoid content in lip hydrating preparations using the UV-Vis spectrophotometer. Qualitative testing for flavonoids in the samples using 10% NaOH reagent produced a yellow color and H_2SO_4 produced a brown color, indicating that the samples were positive for flavonoids. Quantitative analysis was performed using UV-Vis spectrophotometry through $AlCl_3$ complexation with quercetin as a standard. The results of the quantitative analysis showed that the total flavonoid content of the samples was 6.24 mg QE/g for F1, 9.4237 mg QE/g for F2 and 10.6561 mg QE/g for F3. The results of the statistical analysis using SPSS indicated a significant differences of varying concentrations of Pegagan (*Centella asiatica* L.) herb extract on the total flavonoid content in the lip hydrating preparation, with the highest content obtained in F3 (7%).

Keywords : *Centella asiatica* L., flavonoid, lip hydrating, UV-Vis spectrophotometry

INTRODUCTION

Lips are susceptible to dryness and chapping due to environmental factors such as UV exposure, vitamin deficiency, dehydration and the habit of licking the lips. Cases of dry lips as a symptom of dehydration are reported to reach 51.90% daily (Abdulsalam et al., 2022). This condition triggers irritation and discomfort because the thin structure of the lips lacks oil glands, facilitating transepidermal water loss. The use of lip hydrating products serves as a solution to prevent lip damage and maintain health.

Lip hydrating products are effective in maintaining stratum corneum hydration and suppressing transepidermal water loss (Dahmer et al., 2023). These cosmetic products act as humectants and are often formulated with herbal extracts to maintain the integrity of the lip barrier through intensive hydration mechanisms and antioxidant activity. A highly potential natural ingredient is *Centella asiatica* (L.) herb extract.

The active components of pegagan extract consist of 57 secondary metabolites dominated by triterpenoids (asiaticoside, madecassoside, asiatic acid), flavonoids, volatile mono-sesquiterpenes and vitamin C (Kandasamy et al., 2023). These constituents support the efficacy of lip hydrating products through antioxidant, brightening, collagen-stimulating, skin barrier-strengthening and moisturizing activities.

The antioxidant activity of pegagan is largely influenced by flavonoid compounds. Extraction using 96% ethanol has been proven to yield the highest total flavonoid content (11.92 mg QE/g) compared to 50% (5.58 mg QE/g), 70% (5.24 mg QE/g) and 80% (6.67 mg QE/g) (Solikah et al., 2023). Furthermore, pegagan formulations exhibit SPF values of 4.7 (moderate) at 3% concentration, 7.6 (extra) at 5% and 11.7 (maximum) at 7% (Ramlah et al., 2025). An increase in flavonoid levels is proven to increase SPF values linearly due to the strong positive correlation between the two (Lahmadi et al., 2022)**.**

Total flavonoid content measurement is most effectively performed using UV-Vis spectrophotometry, preceded by qualitative phytochemical screening using 10% NaOH and H_2SO_4 (Inayah et al., 2024). This method is chosen for its simplicity, cost-effectiveness and validity, utilizing the principle of flavonoid complexation with $AlCl_3$, which induces a bathochromic effect (Widiyana, 2021). The total content is calculated using a standard curve equation, expressed in milligrams of quercetin equivalents per gram of sample (mg QE/g).

Previous studies have largely focused on the analysis of pure pegagan extract and have not evaluated its content in complex cosmetic preparations such as lip hydrating. This study aimed to determine the significant differences of varying concentrations of Pegagan herb extract at 3%, 5% and 7% on the total flavonoid content of lip hydrating preparations using UV-Vis spectrophotometry to ensure quality and provide novel data for the development of herbal cosmetics.

METHOD

Location and Study Period

The study was conducted at the Technology and Formulation Laboratory, Department of Pharmacy, Campus III, Poltekkes Kemenkes Surakarta. The research took place from September 2025 to June 2026.

Instruments and Materials

The instruments used in this study included a grinder, 80-mesh sieve, maceration vessel, aluminum foil, spatula, flannel cloth, porcelain dish (Herma®), funnel (Pyrex®), measuring cylinder (Pyrex®), water bath, analytical balance, mortar and pestle (Onemed®), watch glass, test tube (Iwaki®), micropipette, volumetric flask (Pyrex®), UV-Vis spectrophotometer, cuvette, vortex, beaker glass (Iwaki®) and lip hydrating packaging.

The materials prepared for this study included dried *Centella asiatica* (L.) herb, aquadest, cetostearyl alcohol, xanthan gum, glycerin, phenoxyethanol, iron oxides red, fragrance, quercetin standard, pro-analysis (p.a.) ethanol, 96% ethanol, 10% NaOH, 1 M CH_3COONa, 10% $AlCl_3$ and H_2SO_4.

Sample Preparation

This study used 500 grams of dried *Centella asiatica* (L.) herb purchased from Bina Agro Mandiri, located at Jalan Patangpuluhan No. 46, Ketangunggan, Wirobrajan, Yogyakarta, which is supported by a Certificate of Analysis (CoA). Plant identification was conducted at the Biology Laboratory, Faculty of Applied Science and Technology, Universitas Ahmad Dahlan, located at Jalan Ahmad Yani, Tamanan, Banguntapan, Bantul, Special Region of Yogyakarta, 55191.

Preparation of 96% Ethanol Extract of *Centella asiatica* (L.) Herb

Dried *Centella asiatica* (L.) herb of 500 grams was pulverized using a grinder and sieved through an 80-mesh screen (Putri et al., 2024). The resulting powder was stored in a

tightly sealed container to prevent contamination. The powder of 250 grams was then extracted by maceration using 1250 mL of 96% ethanol (1:5 ratio) in a maceration vessel. The mixture was stirred until homogeneous and macerated for 72 hours, with the solvent replaced every 24 hours (Solikah et al., 2023). The extract was filtered using a flannel cloth to obtain the filtrate. Subsequently, it was concentrated using a rotary evaporator at 40°C to preserve the stability of thermolabile compounds, followed by further evaporation on a water bath at 40°C until a thick extract was obtained (Putri et al., 2024). The water content of both the powder and the extract of 2 grams each was determined using a moisture analyzer at 105°C until a constant weight was achieved. This analysis was performed in triplicate (Nurwanto & Suswantinah, 2021)

Product Manufacturing Procedure

The preparation of the lip hydrating containing 96% ethanolic extract of Pegagan herb referred to the study by Sutthiboonyapan et al. (2025) with several modifications. The comparison of the formula compositions for the lip hydrating preparations with varying concentrations of Centella asiatica (L.) herb extract is presented in Table 1.

Table 1 Formulation of Lip Hydrating Preparation Containing Pegagan (*Centella asiatica* L.) extract

Ingredients	F1 (%)	F2 (%)	F3 (%)	Function
96% Ethanol extract of Pegagan herb	3	5	7	Active ingredient
Cetostearyl alcohol	5	5	5	Emulsifier
Xanthan gum	0.6	0.6	0.6	Thickening agent
Glycerin	5	5	5	Humectant
Phenoxyethanol	1	1	1	Preservative
Iron Oxides Red	1	1	1	Colorant
Vanilla Fragrance	0.1	0.1	0.1	Fragance
Distilled Water (Aquadest)	Ad 100	Ad 100	Ad 100	Solvent

Notes :
F1 : Lip hydrating preparation with 3% ethanolic extract of Pegagan herb
F2 : Lip hydrating preparation with 5% ethanolic extract of Pegagan herb
F3 : Lip hydrating preparation with 7% ethanolic extract of Pegagan herb

The preparation of the oil in water (O/W*)* lip hydrating cream was initiated by weighing all ingredients. The oil phase, consisting of cetostearyl alcohol and iron oxide, was melted in a water bath at 70°C. Meanwhile, the aqueous phase was prepared by dispersing xanthan gum into warm aquadest and stirring rapidly until a gel was formed. Afterward, glycerin and phenoxyethanol were then added and stirred until homogeneous. The oil phase was poured into a pre-heated mortar, followed by the addition of the aqueous phase and fragrance and stirred constantly until a cream base was obtained. The 96% ethanol extract of *Centella asiatica* (L.) herb, as the active ingredient, was incorporated into the cream base gradually and triturated until homogeneous. The final lip hydrating product was then packaged into tubes (Setianingrum, 2025).

Qualitative Flavonoid Test using 10% NaOH and H_2SO_4

Samples of 500 mg were dissolved in ethanol and transferred into a test tube. Several drops of 10% NaOH were added to the first tube, followed by shaking (Inayah et al., 2024). The presence of flavonoid compounds was identified by a color change from light green to yellow or brownish.

Samples of 500 mg were dissolved in ethanol and transferred into separate test tubes. Several drops of concentrated H_2SO_4 were added, followed by shaking (Inayah et al., 2024). The presence of flavonoids was confirmed by the development of a brown color.

Quantitative Analysis of Lip Hydrating Formulation
Standard Solution Preparation

Quercetin stock solution of 1000 ppm was diluted to concentrations of 40, 50, 60, 70 and 80 ppm using ethanol p.a. in 5 mL volumetric flasks (Aditiya et al., 2022). Quercetin solution at 60 ppm with a volume of 0.5 mL was transferred into a test tube, followed by the addition of reagents and solvents (1.5 mL ethanol p.a., 2.8 mL aquadest, 0.1 mL 1 M CH_3COONa and 0.1 mL 10% $AlCl_3$). Absorbance of the standard solution was measured at a wavelength range of 400–600 nm (Solikah et al., 2023). Stability measurements were performed at the maximum wavelength over 60 minutes at 1 minute intervals until a stable absorbance value was achieved (Primadevi & Nafi, 2020).

Quercetin solutions at concentrations of 40-80 ppm with a volume of 0.5 mL each were added to reagents and solvents in test tubes and incubated for the determined operating time. Absorbance was measured at the maximum wavelength. A calibration curve was constructed by plotting absorbance as the (Y) coordinate and concentration as the (X) coordinate. The linear regression equation was obtained to determine the extract content.

Sample Solution Preparation and Total Flavonoid Content Assay of Lip Hydrating

Samples of the 4000 ppm lip hydrating preparation with a volume of 0.5 mL were mixed with the reagents and solvents. The mixture was homogenized, incubated at room temperature for the determined operating time and the absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength. Each sample was analyzed in triplicate (Solikah et al., 2023). Flavonoid content was calculated using the linear regression equation derived from the calibration curve.

RESULTS AND DISCUSSION

Crude drug material consisted of *Centella asiatica* (L.) herb equipped with a Certificate of Analysis (CoA), which stated a moisture content of 4%, thus meeting the requirement of not more than 10% (Ministry of Health of the Republic of Indonesia, 2017). Identification results confirmed that the plant belonged to the species *Centella asiatica* (L.) Urban from the *Apiaceae* family (Sulistio, 2021). Pulverization was performed using an 80-mesh sieve, as this particle size provides optimal results in terms of yield percentage and density compared to 50-mesh (Ritonga et al., 2022). Maceration was chosen for extraction as it is a cold extraction method that preserves the stability of thermolabile active compounds such as flavonoids (Khasanah & Muslihin, 2025). Ethanol 96% was selected as the universal solvent, as it has been shown to produce the highest total flavonoid content of 11.92 mg QE/g compared to lower concentrations (Solikah et al., 2023).

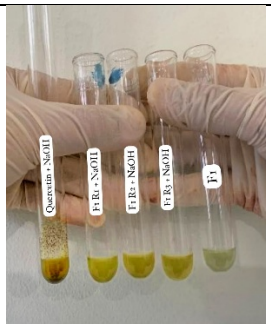
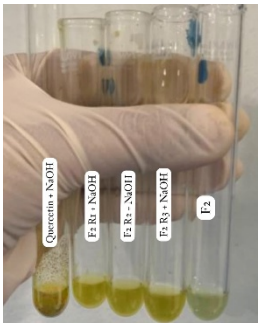
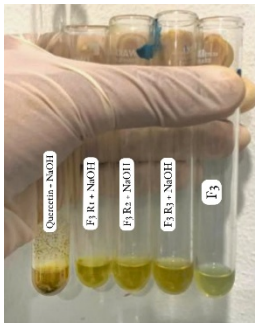
Extraction yield serves as a parameter to measure the effectiveness of the extraction process by comparing the weight of the obtained extract to the initial weight of the dried herb (Putri et al., 2024). The extract yield was 9.98%, which met the minimum standard requirement of 7.3% (Ministry of Health of the Republic of Indonesia, 2017). Organoleptic testing indicated that the thick extract was dark brown, suggesting the presence of polyphenols and flavonoids, with a characteristic aromatic odor due to the presence of total phenols, volatile oils and specific compounds such as caryophyllene, farnesol and elemene (Triastuti & Romalasari, 2022).

Moisture content of the powder and 96% ethanol extract, determined using the gravimetric method, was 7.47% ± 0.361 and 1.78% ± 0.330, respectively. These values demonstrate that the samples met the quality standard requirements of not exceeding the 10% limit (Ministry of Health of the Republic of Indonesia, 2017). Controlling this parameter is crucial, as moisture content above 10% can trigger enzymatic activity and microbial growth, which may compromise the stability of the preparation (Putri et al., 2024)

Organoleptic evaluation was conducted to observe the color, form and odor of the lip hydrating preparation. Results showed that increasing the concentration of Pegagan herb extract from F1 to F3 caused the color of the lip hydrating preparation to become increasingly dark brown (Septiani et al., 2020). Evaluation of odor and consistency indicated a vanilla scent and uniform semi-solid consistency across all formulas, proving that the variation in extract concentration did not affect the structural integrity of the base or the aromatic stability of the preparation (Kresnawati et al., 2024).

Qualitative analysis was performed to confirm the presence of flavonoid compounds in the samples through specific reactions using 10% NaOH and H_2SO_4. The first qualitative test used NaOH reagent, which acts as a basic catalyst to trigger the decomposition of the flavonoid structure into acetophenone molecules (Ferdinan & Rizki, 2021). Results of the qualitative flavonoid test using 10% NaOH are presented in Table 2.

Table 2 Results of the Qualitative Flavonoid Test using 10% NaOH Reagent

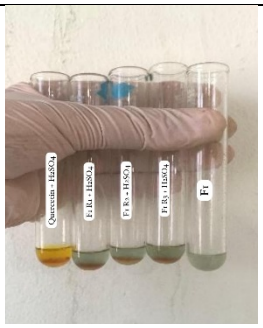
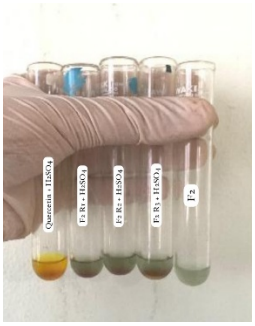
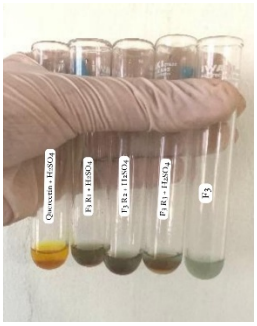
Sample	Reagent	Picture	Initial Color	Final Color	Result	Required Color (+)
F1	NaOH		Green	Yellow	+	Yellow to brownish (Valentine & Yunita, 2023)
F2	NaOH		Green	Yellow	+	
F3	NaOH		Green	Yellow	+	

Notes :
F1 : Lip hydrating preparation with 3% ethanolic extract of Pegagan herb
F2 : Lip hydrating preparation with 5% ethanolic extract of Pegagan herb
F3 : Lip hydrating preparation with 7% ethanolic extract of Pegagan herb
R1 : Replication 1
R2 : Replication 2
R3 : Replication 3
Quercetin : Positive control
\+ : Contains flavonoids

Table 2 shows that testing on all three lip hydrating samples, performed in triplicate, consistently produced a yellow color upon the addition of 10% NaOH. This color shift signifies the presence of flavonoids, resulting from the decomposition and cleavage of the isoprene structure by the base in flavone or flavonol-derived flavonoids (Ni'ma & Lindawati, 2022).

H_2SO_4 was subsequently used in qualitative testing as an acid catalyst to induce an electrophilic substitution reaction. Results of the qualitative flavonoid test using H_2SO_4 reagent are presented in Table 3.

Table 3 Results of the Qualitative Flavonoid Test using H_2SO_4 Reagent

Sample	Reagent	Picture	Initial Color	Final Color	Result	Required Color (+)
F1	H_2SO_4		Green	Brown	+	
F2	H_2SO_4		Green	Brown	+	Brown (Ulfah et al., 2024)
F3	H_2SO_4		Green	Brown	+	

Notes :
F1 : Lip hydrating preparation with 3% ethanolic extract of Pegagan herb
F2 : Lip hydrating preparation with 5% ethanolic extract of Pegagan herb
F3 : Lip hydrating preparation with 7% ethanolic extract of Pegagan herb
R1 : Replication 1
R2 : Replication 2
R3 : Replication 3
Quercetin : Positive control
\+ : Contains flavonoids

Table 3 shows that qualitative testing of the samples yielded positive results, confirming the presence of flavonoids. Addition of H_2SO_4 facilitates the formation of flavylium salts through an oxidation-reduction reaction, producing complex compounds via an electrophilic substitution reaction where the -OH group positions in the flavonoid structure are substituted by H atoms from H_2SO_4, resulting in a brown color (Ulfah et al., 2024).

Total flavonoid content analysis using colorimetric techniques is based on the color formation reaction between the sample and the $AlCl_3$ reagent, with quercetin serving as the standard. Quercetin is selected as the reference because it is a flavonol capable of forming complexes with $AlCl_3$ due to the presence of a keto group at the C-4 atom and hydroxyl groups at the C-3 or C-5 atoms adjacent to the flavone or flavonol structure (Ni'ma & Lindawati, 2022). Addition of $AlCl_3$ aims to form a stable complex with the keto group at C-4 and the hydroxyl groups at C-3 or C-5, causing a bathochromic shift toward the visible light region. Sodium acetate functions as a shift reagent to detect the 7-OH group while maintaining the stability of the wavelength in the visible region (Aditiya et al., 2022).

Maximum wavelength is defined as the point at which a substance exhibits optimal radiation absorption. Maximum wavelength results for the quercetin standard solution were found at 433 nm, which is consistent with previous studies (432–433 nm) (Aditiya et al., 2022; Primadiamanti et al., 2022). Operating time is determined to identify the constant measurement time, indicating an optimal complex formation reaction between the sample and reagent Operating time results were achieved between 22 and 28 minutes and therefore the midpoint of 25 minutes was selected and this aligns with previous studies of 28 minutes (Ni'ma & Lindawati, 2022).

Calibration curve in this study was used to generate a linear regression equation describing the correlation between standard solution concentrations of 40, 50, 60, 70 and 80 ppm and their absorbance values. Results of the calibration curve are presented in Figure 1.

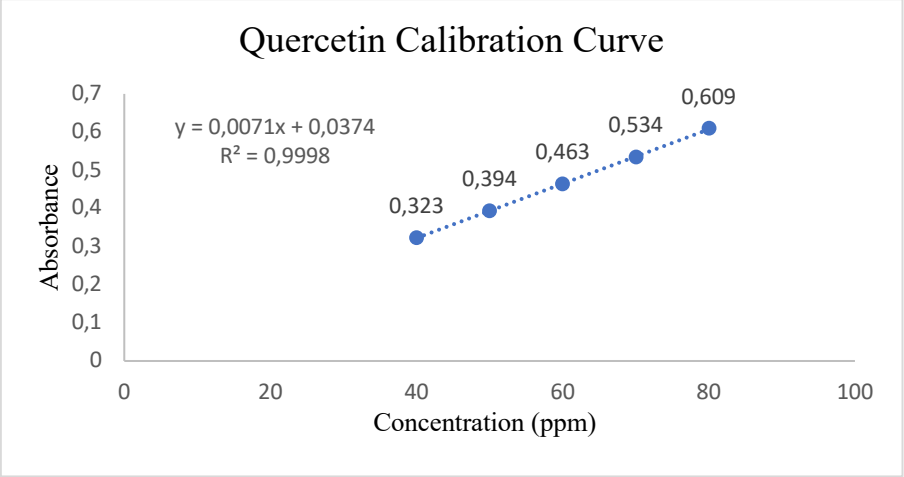


Figure 1 Quercetin Calibration Curve

Figure 1 shows that for quercetin standard solutions of 40, 50, 60, 70 and 80 ppm higher concentrations yield higher absorbance values (Aditiya et al., 2022). Determination of the quercetin calibration curve yielded a linear regression equation of y = 0.0071x + 0.0374 with an R^2 value of 0.9998 and a correlation coefficient (r) of 0.99989. These values satisfy the criteria for a valid calibration curve which requires a linearity value of $r \geq 0.995$ (Mahmud et al., 2024).

Lip hydrating preparations containing 96% ethanolic extract of *Centella asiatica* (L.) herb at varying concentrations of 3%, 5% and 7% were analyzed for total flavonoid content. Results of the total flavonoid content calculations for these preparations are presented in Table 4.

Table 4. Total Flavonoid Content of Pure Extract and Lip Hydrating Preparations

Formula	Replicate	Sample Weight (g)	Absorbance	Concentration (mg/L)	Flavonoid Content (mg QE/g)	Average (mg QE/g) ± SD
Pure Extract	R1	0.01	0.216	25.1549	12.5774	12.5540[a] ± 0.011
	R2	0.01	0.217	25.2958	12.6479	
	R3	0.01	0.214	24.8732	12.4366	
F1	R1	0.04	0.385	48.9577	6.1197	6.2254[b] ± 0.093
	R2	0.04	0.395	50.3662	6.2958	
	R3	0.04	0.393	50.0845	6.2606	
F2	R1	0.04	0.569	74.8732	9.3592	9.4237[c] ± 0.097
	R2	0.04	0.570	75.0141	9.3768	
	R3	0.04	0.579	76.2817	9.5352	
F3	R1	0.04	0.642	85.1549	10.6444	10.6561[d] ± 0.071
	R2	0.04	0.647	85.8592	10.7324	
	R3	0.04	0.639	84.7324	10.5915	

Notes :
F1 : Lip hydrating preparation with 3% ethanolic extract of Pegagan herb
F2 : Lip hydrating preparation with 5% ethanolic extract of Pegagan herb
F3 : Lip hydrating preparation with 7% ethanolic extract of Pegagan herb
mgQE/g : mg *Quercetin Equivalent*/g
Different superscript letters ([a] , [b] , [c] , [d]) in the average column indicate significant differences based on Tukey HSD ($p < 0.05$).

Table 4 shows that the total flavonoid content in the lip hydrating preparation is directly proportional to the concentration of the 96% ethanolic extract of *Centella asiatica* (L.) herb, wherein increasing the extract concentration from 3% to 7% leads to a proportional rise in the total flavonoid content, which is consistent with previous studies (Sherly et al., 2025; Riska et al., 2024). Observed flavonoid levels of the lip hydrating preparations are lower than those of the pure extract, likely due to the extensive formulation process which may induce active ingredient instability, combined with potential physicochemical interactions between the active substance and excipients that lead to degradation (Ermawati et al., 2017; Alfaridz & Musfiroh, 2020). Statistical analysis confirms a significant difference between groups ($p < 0.05$, with F3 (7%) yielding the highest flavonoid content. This proves that variations in the concentration of the *Centella asiatica* (L.) ethanolic extract of 3%, 5% and 7% significantly influence the total flavonoid content of the lip hydrating preparation.

CONCLUSION

Based on the research data regarding the total flavonoid content of the lip hydrating preparations containing 96% ethanolic extract of Pegagan herb, it was concluded that the varying concentrations of 3%, 5% and 7% yielded total flavonoid levels of 6.2254 ± 0.0932 mg QE/g, 9.4237 ± 0.0970 mg QE/g and 10.6561 ± 0.0712 mg QE/g, respectively. Increasing the concentration of Pegagan extract consistently resulted in a higher total flavonoid content. Statistical analysis indicated that variations in the extract concentration caused significant differences in the total flavonoid content of the lip hydrating preparations. Formula F3 with a concentration of 7% Pegagan extract represented the lip hydrating formula that produced the highest flavonoid content compared to the other formulations. Future developments of this research should include further studies using advanced analytical methods such as HPLC and LC-MS to gain a more detailed profile regarding the specific types and precise concentrations of flavonoids contained in the lip hydrating preparations.

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