

Optimization of 3,4-Dimethoxychalcone and Rutin Containing Gel with *Simplex Lattice Design* and *In Vitro-In Vivo* Test as a Sunscreen

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ABSTRACT

3,4-Dimethoxychalcone and rutin, a flavonoid that contains chromophore groups, can absorb UV light and thus can be developed as a sunscreen. The objective of this study was to determine the optimum formula of 3,4-dimethoxychalcone and rutin containing gel, evaluate its physical stability, and activity of 3,4-dimethoxychalcone and rutin gel as a sunscreen through *in vitro* and *in vivo* tests. HPMC, CMC-Na, and methylcellulose were formulated into a gel base to obtain good adhesion and a clear appearance gel. *Simplex Lattice Design* (SLD) with Design Expert software version 10 was utilized to determine the optimum gel formulation. UVA-PF protection, photostability with transpore method, and acute dermal irritation test were performed to evaluate sunscreen activity of 3,4-dimethoxychalcone and rutin gel. The data were analyzed using SPSS version 25. The results showed that the optimum formula for 3,4-dimethoxychalcone-rutin gel consisted of 1.5% HPMC, 1.8% CMC-Na and 0.6% methylcellulose, which showed a pH of 6.96, viscosity of 89.10 dpa.s, and spreadability of 16.30 cm². The pH, viscosity, and spreadability of base and 3,4-dimethoxychalcone-rutin gel was stable for 4 weeks of storage. The UVA-PF value is 6.48 which according to the FDA is included in the category of a two star (***) sunscreen label. The sunscreen did not exhibit a shift in wavelength after 6 hours of irradiation. Based on the primary irritation test, 3,4-dimethoxychalcone-rutin sunscreen produced zero (0) erythema and edema index. Thus, it did not cause irritation to the skin of experimental animals. Therefore, the gel containing 3,4-dimethoxychalcone and rutin had potential as a sunscreen product based on *in vitro-in vivo* tests and was safe on animal skin.

Keywords: 3,4-dimethoxychalcone; *in vitro*; *in vivo*; rutin; sunscreen

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INTRODUCTION

Indonesia is a tropical country with higher levels of ultraviolet (UV) rays than to other countries. Ultraviolet light can have several negative effects when it penetrates the layers of the skin. The negative effects of UV rays include sunburn, pigmentation, and skin cancer; therefore, to overcome the adverse effects of UV rays, sunscreen is essential. Sunscreen is a photoprotection agent that absorbs or reflects sunlight. The active substances for sunscreens that are widely used in the market are avobenzone and benzophenone. However, both compounds can penetrate the skin and thus might be absorbed into the systemic blood flow (Matta et al., 2020), disrupt the hormonal system (Ghazipura et al., 2017), and reduce testosterone quality (Scinicariello & Buser, 2016). Moreover, most sunscreen products have to be re-applied to ensure their activity lasts longer, which makes it less efficient for consumers. Based on this background, we used 3,4-Dimethoxychalcone as an active substance in this study. 3,4-Dimethoxychalcone is referred to as a broad-spectrum sunscreen that has an electron donor group that extends electron delocalization. Thus, it can provide UV protection in the UVB and UVA

regions. A broad spectrum sunscreen is recommended, due to its activity to protect the skin in the UVB and UVA areas and also reduce the adverse effects caused by sun exposure (Addor et al., 2022). Moreover, the addition of rutin in gel formulation, which is a derivative of flavonoids, increase skin protection against UV due to its ability to absorb light (Oliveira et al., 2016).

The sunscreen in this study was formulated in gel base due to its advantage of providing a cold sensation and preventing acne development due to clogged pores. The gelling agent used in this study was a combination of HPMC, CMC-Na, and methyl cellulose to obtain good adhesion, to minimize repeated application and improve the appearance of sunscreen gel. The weakness of HPMC is that it has a cloudy appearance but has good gelling agent strength even though it is stored for a long time. HPMC has a hydrophilic group in the form of a hydroxy which is able to form hydrogen bonds with CMC-Na and methylcellulose (Perez-Robles et al., 2022). CMC-Na is used because it can produce a clear gel and a good binding power (Nisa et al., 2017), but to produce a strong gelling agent needs a large concentration of CMC-Na. CMC-Na has hydroxyl and carboxyl groups,

which can form hydrogen bonds with other gelling agents to increase its strength. In this study, we also used methylcellulose, because it can produce a gel that flows more easily so that it would be easier for the gel to be applied to the application site. The combination of the three gelling agents is expected to increase the formation of hydrogen bonds in the hydrophilic groups between polymers so that they increase gel strength and minimize repeated gel application to increase the effectiveness of use for consumers.

Simplex Lattice Design (SLD) method was applied in this study to optimize the gel formula which meet the criteria for the good physical properties of the gel. The optimal sunscreen gel formula was then determined for the value of UVA-PF protection and photostability using the UV-Vis spectrophotometer with the transpore method. An acute irritation test on rabbit skin was then performed as an *in vivo* test.

METHODS

Materials

3,4-Dimethoxychalcone (Faculty of Mathematics and Natural Sciences UGM Yogyakarta, Indonesia), rutin (Sigma Aldrich, USA), HPMC (Changsu Technology, China), CMC-Na (Changsu Technology, China), methylcellulose (Changsu Technology, China), methyl paraben (Anant Pharmaceuticals, India), propyl paraben (Anant Pharmaceuticals, India), propylene glycol (Chemfe Chemicals, India), ethanol 96% (RCI Labscan, Thailand), and distilled water (General Labora Lab Equipment Yogyakarta, Indonesia).

Evaluation of the Maximum Wavelength of 3,4-Dimethoxychalcone and Rutin

3,4-Dimethoxychalcone and rutin were dissolved in pro-analytical ethanol, then maximum wavelength was evaluated at 290-400 nm using a UV-Vis spectrophotometer Shimadzu UV2600i (Shimadzu, Japan).

Gel Formulation

The gel base was optimized using variation of three polymers including 1.5-2.0% HPMC, 1.5-2.0% CMC-Na 1.5-2.0%, and 0.5-1.0% methylcellulose. The variations of gel formulation are described on Table 1. Gelling agents were prepared using hot water. As much as 0.2 gram of methyl paraben and 0.1 gram of propyl paraben were dissolved in 5.0 mL of propylene glycol. The mixture of the gelling agent and preservative was mixed in another beaker glass with 10.0 mL of propylene glycol. Water was then added up to 100 grams. The gel was stirred until a homogenous mixture was reached. The gel was then evaluated for its physical properties, including organoleptic, pH, viscosity, and spreadability.

Evaluation of the Optimum Gel Formula

The optimum formula was chosen using Design Expert software version 10 with the results of One Way ANOVA statistical analysis of the physical properties of the gel product. Determination of the target response maximize, in range, minimize, target, and the degree of importance is carried out on the response target related to the desired value. The 0.5% 3,4-dimethoxychalcone and 0.1% rutin were then added into the optimum gel base to obtain sunscreen gel product.

Evaluation of the Physical Properties and Stability of the 3,4-Dimethoxychalcone-Rutin Gel

Evaluation of the physical properties

Evaluation of the physical properties were carried out for pH, viscosity and spreadability of the sunscreen gel.

Gel syneresis

10 mL of gel was taken and placed in a conical centrifuge tube. The gel was stored for 72 hours at $\pm 10^{\circ}\text{C}$ and a decrease in gel weight was observed at 0, 24, 48 and 72 hours (Lattimer, 2012).

Stability test

This test was evaluated using the freeze-thaw cycling method. This method is carried out by storing the gel samples at -20°C and 25°C alternately for 3 cycles.

Table 1. Optimization of gel formula

Material (%w/w)	Run												
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
HPMC	1.83	1.50	2.00	1.50	1.75	2.00	1.58	1.75	1.50	1.50	1.50	1.58	1.67
CMC-Na	1.58	1.50	1.50	2.00	1.75	1.50	1.83	1.50	2.00	1.75	1.50	1.58	1.67
Methylcellulose	0.58	1.00	0.50	0.50	0.50	0.50	0.58	0.75	0.50	0.75	1.00	0.83	0,67
Propylene glycol	15	15	15	15	15	15	15	15	15	15	15	15	15
Methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propyl paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Distilled water up to	100	100	100	100	100	100	100	100	100	100	100	100	100

One cycle consisted of 20 hours of storage at -20°C and 4 hours at 25°C and changes were observed in each storage condition (Ye et al., 2018).

Activity Evaluation of 3,4-Dimethoxychalcone and Rutin Sunscreen Gels

Evaluation of *in vitro* UVA-PF value

The test was carried out using the transpore method, using a quartz glass (4.5 cm^2) affixed with a transpore tape. The UVA-PF value was determined by measuring the transmittance using a UV-Vis spectrophotometer at a wavelength of 290 – 400 nm (Kamel & Mostafa, 2015).

Evaluation of *in vitro* UVA-PF values after UVA exposure

The test is carried out in the same way as determining the UVA-PF value, but the results are calculated after exposure to UVA light for 90 minutes.

Evaluation of *in vitro* photostability

The sample was flattened on a glass plate, then exposed to UVA lamps with a distance of 10 cm for 0, 1, 2, 3, 4, 5 and 6 hours in a closed cabinet and room. Measurements used a UV-Vis spectrophotometer at a wavelength of 200–400 nm and the graph of the shift in wavelength was observed (Asfour et al., 2019).

Evaluation of acute dermal irritation test

The test was performed according to the BPOM 2014 guideline. The total animal test were three male albino rabbits which weight around 2 kg. Before testing started, the rabbits were acclimatized in the room for 5 days and placed in cages. 24 hours before testing, rabbit's hair was shaved on its back approximately $10 \times 15\text{ cm}$ for the place of exposure to sunscreen preparations. Shaving started from the shoulder to the waist bone. The gel used for each area was 0.5 gram. The gel sample was applied to a skin area of $\pm 6 (2 \times 3)\text{ cm}^2$ with three patches for three exposures, then observed for 3 minutes, 1 hour and 4 hours. Two test animals were added and observed as the initial step and the observations were continued for 14 days if there was no erythema (redness on the animal skin) and edema.

RESULTS AND DISCUSSION

This study was approved by Faculty of Veterinary Medicine Ethical Committee, Universitas Gadjah Mada under certification of ethical clearance No. 091/EC-FKH/Eks./2022.

The Maximum Wavelength of 3,4-Dimethoxychalcone and Rutin

As showed in Figure 1, 3,4-dimethoxychalcone, rutin and a mixture of the two had a maximum wavelength in the UV-A region, specifically at 360 nm. 3,4-dimethoxychalcone and rutin did not show maximum

wavelength in the UV-B region but still can provide UV protection when sunscreen is applied to the surface layer of the skin. Mixing the two compounds does not give a shift in maximum wavelength to a higher direction since it is only physical mixture (de Oliveira et al., 2015). Physically mixing chalcone and rutin does not extend the electron delocalization system so that when reading the absorbance with a UV-Vis spectrophotometer, the absorbance of the mixture of the two compounds is not much different from that of each single compound.

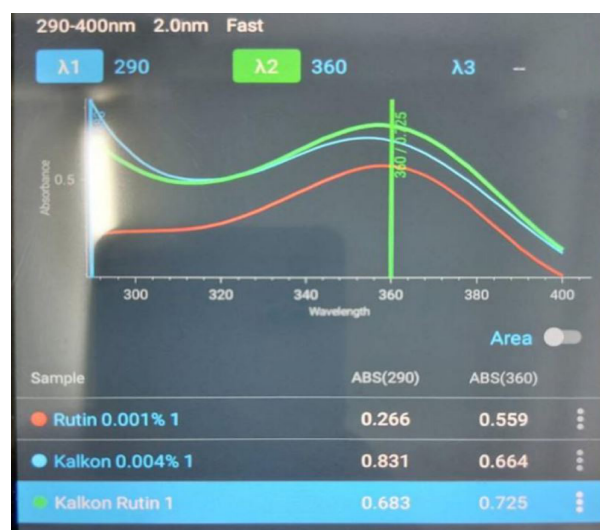


Figure 1. UV-Vis spectra of 3,4-dimethoxychalcone, rutin, and both compounds. Red spectra is rutin, blue spectra is 3,4-dimethoxychalcone, and green spectra is mix of both compounds which evaluated in 290–400 nm

Optimum Gel Formula

The optimization results for 13 run formula gel were explained in Table 2. The target of pH, viscosity, and spreadability were explained in Table 3. The composition of the optimum predictive formula from the results of the Design Expert software version 10 is HPMC 1.5%; CMC-Na 1.8%; and 0.6% methylcellulose. The predicted value obtained from the software were for a pH response of 6.5; viscosity of 90.16 dpa.s; and spreadability of 16.30 cm^2 . Then, the results of the experimental gel base response were a pH of 6.51; viscosity of 89.50 dpa.s; and spreadability of 16.54 cm^2 as explained in Table 4. The predicted result was not significantly different with the experimental results because the data show all above the *p*-value (0.05).

From the Design Expert contour plot data as explained in Figure 2, the component that had the greatest positive effect on the pH response was the concentration of methylcellulose with a coefficient value of +6.69. This is because methylcellulose is alkaline. The higher the concentration of methylcellulose in the gel preparation,

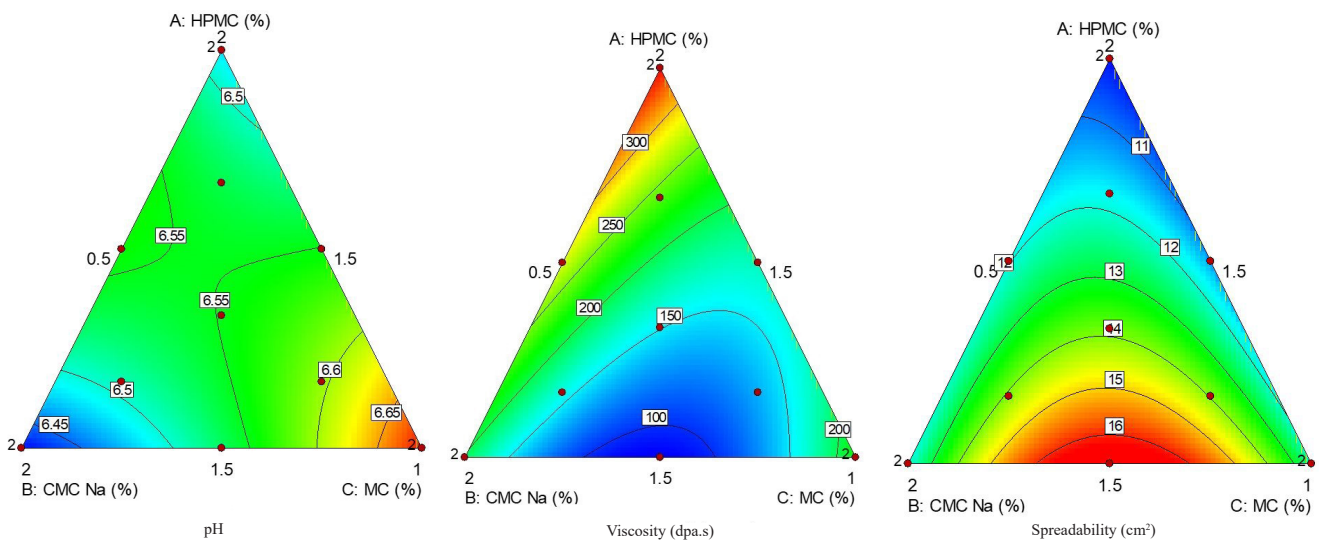


Figure 2. Countour plot of pH, viscosity and spreadability response of gel optimization

Table 2. Physical Properties of gel formula

Response	Run												
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
pH	6.51	6.69	6.47	6.43	6.56	6.49	6.51	6.57	6.42	6.54	12.26	6.58	6.56
Viscosity	243.8	233.8	350.6	190.0	287.3	347.5	149.9	166.8	206.0	86.40	202.5	172.5	120.0
Spreadability	11.81	12.10	10.33	11.20	11.80	10.56	15.05	11.11	12.57	16.27	6.69	13.05	14.70

Table 3. Target response to determine the optimum formula

Response	Goal	Lower	Upper	Importance
pH	<i>In range</i>	6.42	6.50	+++
Viscosity	<i>Minimum</i>	86.40	350.60	+++
Spreadability	<i>Maximum</i>	10.33	16.27	+++

the higher the pH value of the preparation. The component that has the greatest negative effect on the pH response is the interaction between CMC-Na and methylcellulose with a coefficient value of -0.10. This is because CMC-Na is acidic which has a pKa of 4.30 and methylcellulose can provide an alkaline pH. Therefore, the greater the amount of dispersion between CMC-Na and methylcellulose, the lower the pH of the gel preparation.

The component that had the greatest positive effect on the viscosity response is HPMC with a coefficient value of +349.72. HPMC has very strong bonds between particles. The more HPMC added to the formula, the higher the viscosity of the resulting gel. In the study of Afianti and Murrukmihadi (Afianti & Murrukmihadi,

2015), it was stated that the addition of HPMC levels could increase the viscosity of the preparation. The component that has the greatest negative effect on the viscosity response is the interaction between CMC-Na and methylcellulose with a coefficient value of -497.33. The red area shows the highest viscosity response, followed by green and the lowest response is in the blue area. The highest viscosity response was produced in the area with the highest HPMC concentration.

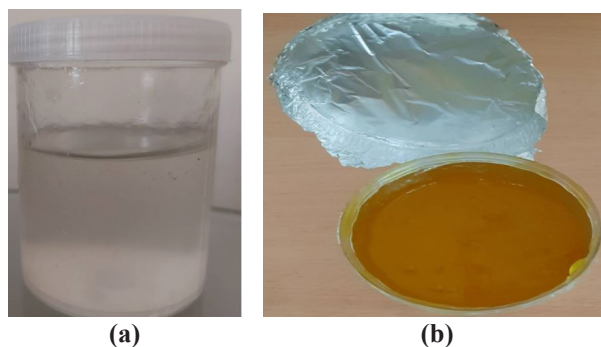
The component that has the greatest positive effect on the spreading response is the interaction between CMC-Na and methylcellulose with a coefficient value of +18.16. This shows that the interaction of 2 factors between CMC-Na and methylcellulose will have the greatest effect on the dispersion of the gel. The component that has

Table 4. Prediction results and experimental of the optimum gel formula

Response	Prediction	Experimental	Sig. (2 tailed)	Conclusion
pH	6.50	6.51	0.134	Not significant different
Viscosity (dPa.S)	90.16	89.50	0.251	Not significant different
Spreadability (cm ²)	16.30	16.54	0.274	Not significant different

Table 5. The results of the optimum formula base gel and 3,4-dimethoxychalcone and rutin gel

Response	Gel Base	Chalcone gel	Sig. (2 tailed)	Conclusion
pH	6.51	6.96	0.002	Significantly different
Viscosity (dPa.S)	89.50	89.10	0.060	Not significant different
Spreadability (cm ²)	16.54	16.30	0.413	Not significant different

**Figure 3. Gel without (a) and with 3,4-dimethoxychalcone and rutin (b)**

the greatest negative effect on the spreadability response is the interaction between HPMC and methylcellulose with a coefficient value of -0.47. This shows that the interaction of the 2 factors has the greatest influence on the decrease in the spreadability of the gel. The red area shows the highest scattering response, followed by green and the lowest response is in the blue area. The highest scattering response was produced around the area between CMC-Na and methylcellulose.

Predictive and experimental response values were verified with a t-test with a 95% level of confidence. The results of the pH response, viscosity, and spreadability did not show a significant difference. The response results of the experimental base gel and 3,4-dimethoxychalcone and rutin were then verified again with the pH, viscosity and spreadability of the 3,4-dimethoxychalcone and rutin gel respectively, namely 6.96; 89.10 dpa.s; and 16.30 cm² as explained in Table 5. The results of the verification were based on the t-test with a 95% confidence level, only the pH value was significantly different because the chalcone and rutin compounds are alkaline so the pH value increases when mixed into the base gel.

Physical Properties of Sunscreen Gel

Organoleptic

The color of the gel without 3,4-dimethoxychalcone and rutin is clear, has a characteristic gel odor and is homogeneous. The color of the 3,4-dimethoxychalcone gel is yellow, has a characteristic odor of propylene glycol and is homogeneous. Organoleptic of basis and 3,4-dimethoxychalcone gel and rutin were unchanged during four weeks of storage as in Figure 3.

pH

The results of base and 3,4-dimethoxychalcone-rutin gel pH for four weeks of storage did not experience a significant difference each week. The pH of the 3,4-dimethoxychalcone-rutin gel was higher than the base, but still within the skin pH range of 6.5-6.7.

Viscosity

The results of the viscosity of the base and 3,4-dimethoxychalcone-rutin gel for four weeks of storage did not differ significantly each week. The base and gel viscosities of 3,4-dimethoxychalcone were in the range of 89.0-92.0 dpa.s.

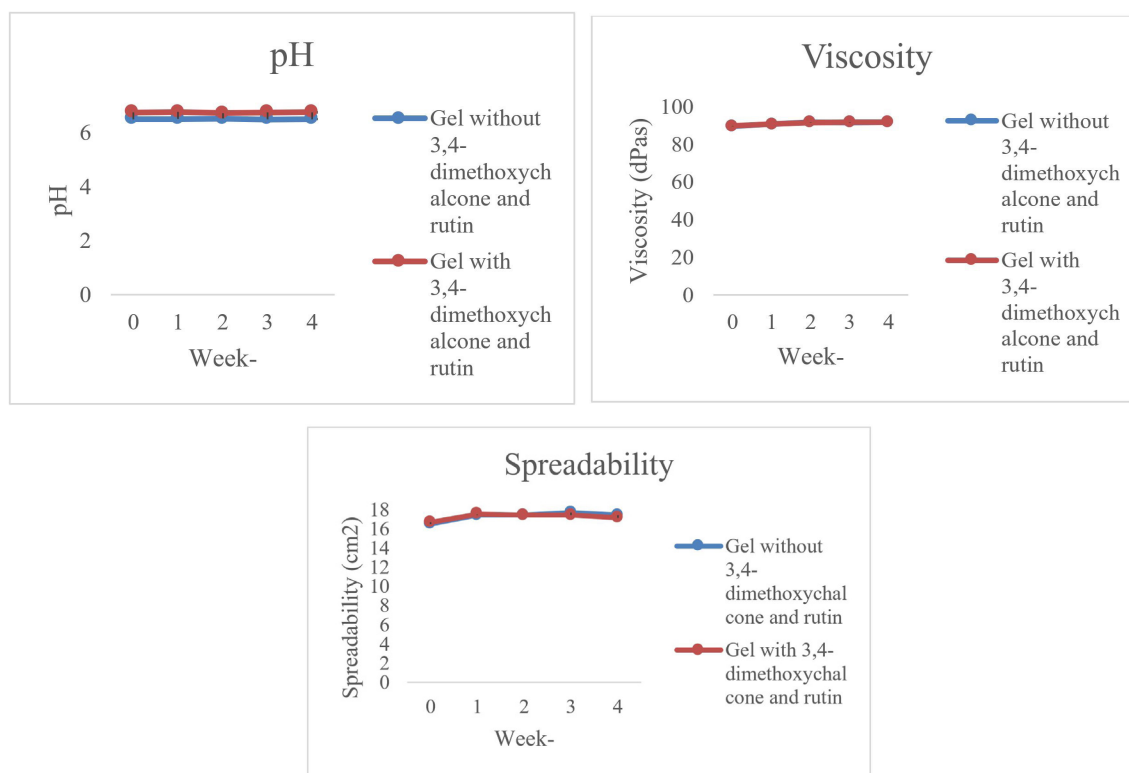


Figure 4. The results of the pH, viscosity and spreadability test for the optimum gel base and 3,4-dimethoxychalcone with 5 samples gel during storage

The viscosity of the 3,4-dimethoxychalcone and rutin gel is formed due to the physical bond between the gelling agents. The gelling agent used is a cellulose derivative in the form of HPMC, CMC-Na, and methylcellulose, each of which has a polar group in the form of a hydroxy group (-OH). The hydroxy groups (-OH) of each gelling agent are able to physically interact with one another and form hydrogen bonds. The existence of this physical interaction makes the mechanism of water absorption into the gelling agent system better than the single component of the gelling agent (Nan et al., 2019).

The formation of H-bond interactions between compatible gelling agent polymer chains results in strong visco-elastic properties. Apart from this, another advantage of this gelling agent mixture is that it has good compatibility when applied to the surface of the skin because there is no chemical crosslink mechanism so that it can reduce the risk of side effects (Parhi, 2017).

The physical interaction between the gelling agents will cause a combination and a mixture of strands from each gelling agent. The combined strands of each gelling agent then form a gel network that is more capable of absorbing water and maintaining its viscosity. The structure of the gel network which resembles a hollow net increases the ability of the gel to absorb

large amounts of water through hydrogen bonds. The gel network resulting from the physical interaction of several gelling agent components resulted in a better gel viscosity compared to the gel network component of a single high-concentration gelling agent. This is caused by the existence of bonds between gelling agents which have characteristics that complement one another (Maitra & Shukla, 2014).

Spreadability

The results of the spreadability of the base and 3,4-dimethoxychalcone-rutin gel during four weeks of storage were not significantly different each week. The spreadability of the 3,4-dimethoxychalcone base and gel was in the range of 16.0-18.0 cm². All of the physical evaluation are explained in Figure 4.

Syneresis

The syneresis test results for the optimum formula base gel and 3,4-dimethoxychalcone-rutin gel at 24, 48 and 72 hours at $\pm 10^{\circ}\text{C}$ were 0%. This shows that there is no syneresis on the optimum formula base gel and 3,4-dimethoxychalcone-rutin gel.

Stability test

The accelerated stability test aims to determine the stability of the preparation under extreme conditions.

The stability test has an F value as a parameter. The F value has a range from 0-1, whereas the closer the F value is to 1, the more stable the preparation is. The accelerated stability test in this study used the freeze-thaw method. This method aims to determine the stability of the preparation in the presence of extreme temperature differences. The results of the stability test using the freeze-thaw method yielded an F value of 1. This indicated that the optimum formula for base gel and 3,4-dimethoxychalcone gel was stable under extreme temperature conditions.

The Activity of 3,4-Dimethoxychalcone and Rutin Sunscreen Gels

UVA-PF value

The UVA-PF value of 0.5% 3,4-dimethoxychalcone gel and 0.1% rutin was 6.48. According to the FDA, the UVA-PF value of 3,4-dimethoxychalcone gel belongs to the category of sunscreen labeled with two stars (**). In this case, research sunscreens with the active ingredients

3,4-dimethoxychalcone and rutin have activity as sunscreens and can protect the skin from UVA exposure.

Measurement of UVA-PF value after exposure to UV light

The UVA-PF value after 90 minutes of exposure was 5.39. The UVA-PF value after exposure to UV rays was smaller than before exposure due to the activity of 3,4-dimethoxychalcone and rutin which had previously been active to absorb UV rays and then experienced slight degradation.

Photostability

The spectral results showed that exposure to UV light from the first to the sixth hour does not experience a shift in wavelength. The maximum wavelength produced is at a value of 360 nm as explained in Figure 5. This suggests that 3,4-dimethoxychalcone and rutin can be candidates for sunscreens with stable UVA protection after exposure to UV light.

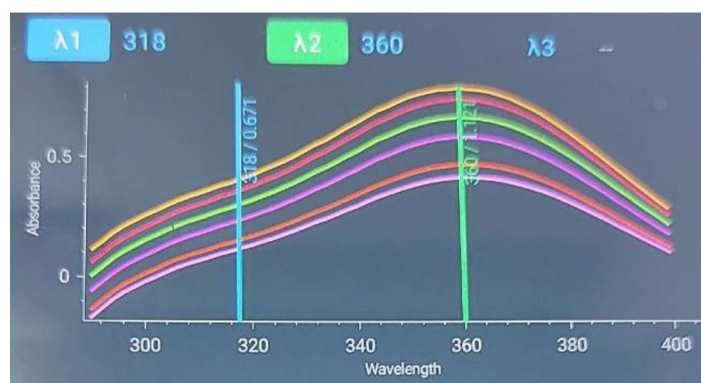


Figure 5. Maximum wavelength after photostability test from 1-6 hours. The highest wavelength (yellow) at 1st hour and the lowest wavelength (light pink) at 6th hour



Rabbit 1 – after 14 days testing



Rabbit 2 – after 14 days testing



Rabbit 3 – after 14 days testing

Figure 6. Irritation test on three rabbit skin

Acute dermal skin irritation test

The first test animals did not experience irritation, either edema or oedema for 3 minutes, 1 hour and 4 hours of observation so the resulting edema and oedema index values were 0. Then, the observations were continued for 14 days to confirm the irritation results shown.

Based on the Prottox[®] predictor software, the active compounds 3,4-dimethoxychalcone and rutin tend to be hydrophilic due to their tendency to enter the aqueous phase compared to the organic phase based on logP studies. This shows that during gel formulation, the active compound will enter into the hydrophilic dispersion system. The advantage of active compounds that enter into the hydrophilic dispersion system is to reduce the risk of irritation or side effects when applied to the skin. The more hydrophobic a preparation is, the easier it is to pass through the skin layers because the skin is more easily penetrated by preparations that are hydrophobic (Lv et al., 2022). Skin irritation can occur when the active compound of a preparation penetrates into the stratum corneum and then irritates the tissues under the epidermal layer of the skin (Ruan et al., 2022). A good sunscreen preparation is when the sunscreen is on the top layer of the skin and is not transported through the stratum corneum which is lipophilic so as to minimize the risk of skin irritation. Based on this, 3,4-dimethoxychalcone and rutin can be categorized as non-irritant to the skin based on the *in vivo* primary irritation test. Based on 14 days of observation on test animals, rabbits did not experience erythema and edema on the surface of the skin that had been smeared with 3,4-dimethoxychalcone gel and rutin as shown in Figure 6.

CONCLUSION

Formulation of HPMC 1.5%, CMC-Na 1.8%, and 0.6% methylcellulose without the addition of active substances can produce the optimum gel formula. The optimum base and gel formula of 3,4-dimethoxychalcone-rutin is stable in pH, viscosity, and spreadability for four weeks of storage. The gel of 3,4-dimethoxychalcone 0.5% and rutin 0.1% produces a UVA-PF value of 6.48 which according to the FDA is included in the category of sunscreen labeled with two stars (**). The sunscreen did not experience a shift in wavelength after 6 hours of irradiation. Based on the primary irritation test, 3,4-Dimethoxychalcone sunscreen produced an erythema and edema index of 0, thus did not cause irritation to the skin of experimental animals. 3,4-dimethoxychalcone and rutin-containing gel has potential as a sunscreen with *in vitro-in vivo* test and was safe on animal skin.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the author(s).

REFERENCES

- Addor, F. A. S., Barcaui, C. B., Gomes, E. E., Lupi, O., Marçon, C. R., & Miot, H. A. (2022). Sunscreen lotions in the dermatological prescription: Review of concepts and controversies. *Anais Brasileiros de Dermatologia*. <https://doi.org/10.1016/j.abd.2021.05.012>
- Afianti, H. P., & Murrukmihadi, M. (2015). Pengaruh variasi kadar gelling agent hpmc terhadap sifat fisik dan aktivitas antibakteri sediaan gel ekstrak etanolik daun kemangi (*Ocimum basilicum* L. forma citratum Back.). *Majalah Farmaseutik*, 11(2), 307-315
- Asfour, M. H., Kassem, A. A., & Salama, A. (2019). Topical nanostructured lipid carriers/inorganic sunscreen combination for alleviation of all-trans retinoic acid-induced photosensitivity: Box-Behnken design optimization, *in vitro* and *in vivo* evaluation. *European Journal of Pharmaceutical Sciences*, 134, 219–232. <https://doi.org/10.1016/j.ejps.2019.04.019>
- de Oliveira, C. A., Peres, D. D., Rugno, C. M., Kojima, M., Pinto, C. A. S. de O., Consiglieri, V. O., Kaneko, T. M., Rosado, C., Mota, J., Velasco, M. V. R., & Baby, A. R. (2015). Functional photostability and cutaneous compatibility of bioactive UVA sun care products. *Journal of Photochemistry and Photobiology B: Biology*, 148, 154–159. <https://doi.org/10.1016/j.jphotobiol.2015.04.007>
- Ghazipura, M., McGowan, R., Arslan, A., & Hossain, T. (2017). Exposure to benzophenone-3 and reproductive toxicity: A systematic review of human and animal studies. *Reproductive Toxicology (Elmsford, N.Y.)*, 73, 175–183. <https://doi.org/10.1016/j.reprotox.2017.08.015>

- Kamel, R., & Mostafa, D. M. (2015). Rutin nanostructured lipid cosmeceutical preparation with sun protective potential. *Journal of Photochemistry and Photobiology B: Biology*, 153, 59–66. <https://doi.org/10.1016/j.jphotobiol.2015.09.002>
- Lattimer. (2012). *Official Methods of Analysis of AOAC International, 19th edition*. AOAC International.
- Lv, X., Wu, Z., & Qi, X. (2022). High skin permeation, deposition and whitening activity achieved by xanthan gum string vitamin c flexible liposomes for external application. *International Journal of Pharmaceutics*, 628, 122290. <https://doi.org/10.1016/j.ijpharm.2022.122290>
- Maitra, J., & Shukla, V. K. (2014). Cross-linking in Hydrogels—A Review. *American Journal of Polymer Science*, 4(2), 25–31.
- Matta, M. K., Florian, J., Zusterzeel, R., Pilli, N. R., Patel, V., Volpe, D. A., Yang, Y., Oh, L., Bashaw, E., Zineh, I., Sanabria, C., Kemp, S., Godfrey, A., Adah, S., Coelho, S., Wang, J., Furlong, L.-A., Ganley, C., Michele, T., & Strauss, D. G. (2020). Effect of sunscreen application on plasma concentration of sunscreen active ingredients: A randomized clinical trial. *JAMA*, 323(3), 256–267. <https://doi.org/10.1001/jama.2019.20747>
- Nan, N. F. C., Zainuddin, N., & Ahmad, M. (2019). Preparation and swelling study of CMC hydrogel as potential superabsorbent. *Pertanika Journal of Science and Technology*, 27(1), 489-498.
- Nisa, O. N. L., Hermadi, A. V. L., Khoiriyah, H., Purwojati, N., & Ashari, N. (2017). Uji stabilitas pada gel ekstrak daun pisang (gelek usang). *The 6th University Research Colloquium 2017*.
- Oliveira, C. A. de, Peres, D. D., Graziola, F., Chacra, N. A. B., Araújo, G. L. B. de, Flório, A. C., Mota, J., Rosado, C., Velasco, M. V. R., Rodrigues, L. M., Fernandes, A. S., & Baby, A. R. (2016). Cutaneous biocompatible rutin-loaded gelatin-based nanoparticles increase the SPF of the association of UVA and UVB filters. *European Journal of Pharmaceutical Sciences*, 81, 1–9. <https://doi.org/10.1016/j.ejps.2015.09.016>
- Parhi, R. (2017). Cross-linked hydrogel for pharmaceutical applications: A Review. *Advanced Pharmaceutical Bulletin*, 7(4), 515–530. <https://doi.org/10.15171/apb.2017.064>
- Perez-Robles, S., Carotenuto, C., & Minale, M. (2022). HPMC hydrogel formation mechanisms unveiled by the evaluation of the activation energy. *Polymers*, 14(3), 635. <https://doi.org/10.3390/polym14030635>
- Ruan, J., Liu, C., Wang, J., Zhong, T., Quan, P., & Fang, L. (2022). Efficacy and safety of permeation enhancers: A kinetic evaluation approach and molecular mechanism study in the skin. *International Journal of Pharmaceutics*, 626, 122155. <https://doi.org/10.1016/j.ijpharm.2022.122155>
- Scinicariello, F., & Buser, M. C. (2016). Serum testosterone concentrations and urinary bisphenol a, benzophenone-3, triclosan, and paraben levels in male and female children and adolescents: NHANES 2011-2012. *Environmental Health Perspectives*, 124(12), 1898–1904. <https://doi.org/10.1289/EHP150>
- Ye, J., Yang, R., Liu, C., Luo, S., Chen, J., Hu, X., & Wu, J. (2018). Improvement in freeze-thaw stability of rice starch gel by inulin and its mechanism. *Food Chemistry*, 268, 324–333. <https://doi.org/10.1016/j.foodchem.2018.06.086>