ORIGINAL ARTICLE

3,4-dimethoxychalcone novel ultraviolet-A-protection factor in conventional sunscreen cream

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ABSTRACT

UltravioletA (UVA) rays with an intensity of 95% can induce skin cancer due to the activation of reactive oxygen species (ROS). The 3,4-dimethoxychalcone (3,4-DMC) chalcone derivative has a wide wavelength, antioxidant activity, presumed has activity as sunscreen (UVA rays). Topical delivery of water-insoluble 3,4-DMC with log *P* 3.84 required capable, cream formulation was chosen because it was suitable for application this chemical sunscreen. This study aims to obtain the optimal formulation of 3,4-DMC in a sunscreen cream dosage form as a UVA-protection factor (UVA-PF). This study involves experimental design. The cream 3,4-DMC was evaluated physically for 4 weeks by measuring pH, viscosity, spreadability, adhesion, centrifugation, freeze–thaw, photostability, UVA-PF used Tranpore™ tape, and skin irritation test on animals. The result obtained was evaluated statistically using ANOVA (SPSS version 24). The ratio UVA/UVB value of 3,4 DMC sunscreen cream having 5 stars (*****) for all concentrations, shows the product in this study can be used as an anti-UVA agent in sunscreen cream cosmetic products. The stability of the cream has pH 4.0–4.2; spreadability 5–6 cm; viscosity 4.470–5.763; and adhesion <1 s. Freeze-thaw and centrifugation were known did not affect the stability due to the absence of separation. There was no wavelength shift in the photostability test and no skin irritation due to *in vivo* examination using New Zealand rabbits. The 3,4-DMC as a new agent in conventional sunscreen cream dosage form has good properties as a protection against UVA rays.

Key words: 3, 4-dimethoxychalcone, chalcone, cream, sunscreen, ultraviolet-A-protection factor

INTRODUCTION

Around 95% of ultraviolet-A (UVA) rays reaches the earth and able to penetrate into deepest layers of skin. Chemical sunscreen is needed to absorb UVA rays which has a specific wavelength.[1] The development of sunscreen compounds refers to the characteristics of ideal sunscreens, such as UV absorption with a wide wavelength, high photostability, and low toxicity. However, not all sunscreen compounds to provide sufficient UVA protection.[2] Chalcone derivatives are the components that effectively protect the skin from UVA.[3] The 3,4-dimethoxychalcone (3,4-DMC), a chalcone derivative, had a wide wavelength range, insoluble in water (log *P* 3.84) and SPF 18.77.[4,5] It has antioxidant activity, antifungal,[6] and anticancer.[6,7] The novelty of this study investigates the physical stability, photostability, and skin irritation of the 3,4-DMC formulated in cream that

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are often used as sunscreen cosmetics.[8] The potential of UVA-protection factor (UVA-PF) cream 3,4-DMC according to COLIPA[9] was examined using Tranpore ™ tape and transmittance reading using a ultraviolet–visible (UV-Vis) spectrophotometer.

MATERIALS AND METHODS

Materials

The active compound 3,4-dimethoxychalcone (Faculty of Mathematics and Natural Science, Universitas Gadjah Mada), ethanol (Merck). Formulation cream based on pharmaceutical grade like virgin coconut oil, tween 80, span 80, stearic acid, glycerin, DMDM hydantoin, cetyl alcohol, liquid paraffin, triethanolamine, and aquadest.

Determination the wavelength of 3,4-dimethoxychalcone

The 3,4-DMC was prepared at 20 ppm in ethanol. The wavelength was set in 200–400 nm to determination using UV-Vis spectrophotometry (Hitachi, UV-3200, Japan).[10]

Validation method

The validation method was examined based on the international conference on harmonization guidelines.[11]

Linearity

The calibration curve was obtained from series concentrations of 3, 5, 7, 9, and 11 ppm. The absorbance at the maximum wavelength was measured and a linearity equation by comparing r table value at the 95% confidence level was created.

Precision

The absorbance was measured from three series concentrations of 3, 7, and 11 ppm at the maximum wavelength. Precision was determined as standard deviation (SD) or coefficient of variation (KV) by equation:

$$\%\ RSD\ (KV) = \frac{standard\ deviation}{average\ concentration} \times 100\%$$

Accuracy

Absorbance values in the precision test were compared with a standard curve that has been created before used to calculate the percentage recovery by the equation below:

$$Recovery(\%) = \frac{measurable\ concentration}{concentration\ \ (C)} \times 100\%$$

Limits of detection and limit of quantitation

Five concentrations were used in the linearity test. The limit of detection (LOD) and limit of quantitation (LOQ) can be determined by the equation below:

$$LOD = \frac{3{,}3\ x\ Sy\ /\ x}{Sl}$$

$$LOQ = \frac{10\ x\ Sy\ /\ x}{Sl}$$

Sl = slope (b at linear equation y = a + bx)

Sy/x = blank/residual SD (Sb)

Determination of cream base

The O/W cream formulation was prepared as shown in Table 1. The oil phase and the aqua phase were heated separately at 75°C. The oil phase was added to aqua phase and stirred at 1500 rpm to homogeneous cream. Put into a container and evaluated after 24-h.[8]

Formulation cream 3,4-dimethoxychalcone

The 3,4-DMC with various concentrations 50 ppm (F1), 100 ppm (F2), and 150 ppm (F3) each dissolved with 2 mL ethanol then formulated into a cream base stirrer at 1500 rpm to form a cream and do a physical evaluation after 24 h of storage.[8]

Evaluation cream 3,4-dimethoxychalcone

pH determination

The pH measurements were carried out using a pH meter (Hanna).[8,12]

Viscosity

The sample was measuring used a Brookfield viscometer (DV-I, Prime), with spindle 6 rpm 100 and the viscosity value on mPa. S.[8,12]

Spreadability

Approximately 0.5 g of sample was placed between two glass plates, given a load of 50–250 g interval 50 g for 1 min. Diameter measurements were carried out vertically, horizontally, and diagonally.[8,12]

Adhesive

Approximately 0.1 g of sample was applied on a glass object, covered with other glass, and pressed with 1 kg load for 5 min. The object glass was then installed in the equipment, the duration was recorded when both sides of the glass separated.[12]

Table 1: The formulation of cream base

Material	Base I (%)	Basse 2 (%)	Base 3 (%)
Stearic acid	1.25	3.796	8
Cetyl alcohol	1	4	9.71
Glycerin	1.25	10	7
DMDM hydantoin	0.3	0.3	0.3
Paraffin liquid	1.25	-	-
TEA	0.15	-	-
VCO	-	10	29
Tween 80	-	2.204	3.29
Span 80	-	2	1.15
Aquadest	Ad 100	Ad 100	Ad 100

VCO: Virgin coconut oil, DMDM: Dimethyloldimethyl hydantoin, TEA: Trietanolamin

Freeze–thaw

The sample was prepared in a container, kept at 45°C and 5°C for 3 cycles (1 cycle = 24 h), and checked any phase separation.[8,12]

Centrifugation

The sample was centrifuged at 5000 rpm for 10 min and checked any phase separation.[8]

In vitro ultraviolet-A-protection factor

In vitro model to assessed UVA-PF in this research was tested using transpore method. This method uses Transpore™ tape affixed to a quartz glass (4.5 cm^2). All samples were placed on the tape approximately 2 mg/cm^2 and flattened. The transmittance was measured using UV-Vis spectrophotometer (Pharmaspec UV-1700) at the maximum wavelength.[13]

The measurements were limited UVA/UVB ratio and UVAPF because the wavelength of 3,4-DMC was known. Determination of UVA value using method from Diffey and Robson.[14] The UVA/UVB ratio was calculated with the following equation:

$$UVA/UVB ratio = \frac{\int_{320}^{400} lg\left[1/T(\lambda)\right]d\lambda / \int_{320}^{400} d\lambda}{\int_{290}^{320} lg\left[1/T(\lambda)\right]d\lambda / \int_{290}^{320} d\lambda}$$

The UVAPF was suggested by Ferrero *et al*.[14,15] using modification equal below:

$$UVA\text{-}PF = \frac{\sum_{320}^{400}\Delta\lambda}{\sum_{320}^{400} T(\lambda).\Delta\lambda}$$

According to the Food and Drug Administration (FDA), the UVA protection level is based on both *in vivo* UVAPF and an *in vitro* method. UVA protection level was introduced as a UVA star rating by 1–4 stars defined as the UVA/UVB ratio. In the 2004 and 2008 version, five classes based on the ratio values were distinguished; however, since the European Commission has recommended consideration of photostability, a new version was recently delivered,[16] UVA star rating is shown in Table 2.

Photostability

Approximately 0.25 g apply to glass plate with transpore tape and give exposure UVA lamp (Beckman Coulter, USA) with a distance of 10 cm for 0–6 h interval 1 h in a closed cabinet. Measure absorbance used UV-Vis spectrometer (Genesys 50) at maximum wavelength. Determine of shift wavelength might be happen with ethanol as blank.[13,17]

Skin irritation

Skin irritation test used New Zealand rabbit animals based on "The *In Vivo* Nonclinical Toxicity Test" Guidelines from the FDA.[18] Each female rabbit was shaved back then applied a sample of 0.5 g. Observations were carried out at 24, 48 and 72 h, determine erythema and edema on animal test after 4 h exposure.

Ethics

The certified of ethical clearance for this research is 00067/04/LPPT/I/2020, approved in LPPT ethic committee, Universitas Gadjah Mada.

DISCUSSION

Determine maximum wavelength 3,4-dimethoxychalcone

The maximum wavelength of 3,4-DMC in 260 nm UVB area and 356 nm UVA region is illustrated in Figure 1, then for this research Uv-Vis spectrophotometric method is selective.

Validation method

The curve calibration showed good linearity with the calculated *r* value greater than *r* table ($P < 0.05$) which is equal to $r = 0.9998$ with the regression y = 0.0824x–0.0254, as illustrated in Figure 2. The precision is a parameter of homogeneous samples with minimum concentration that is relative standard deviation ≤2%. The result showed good

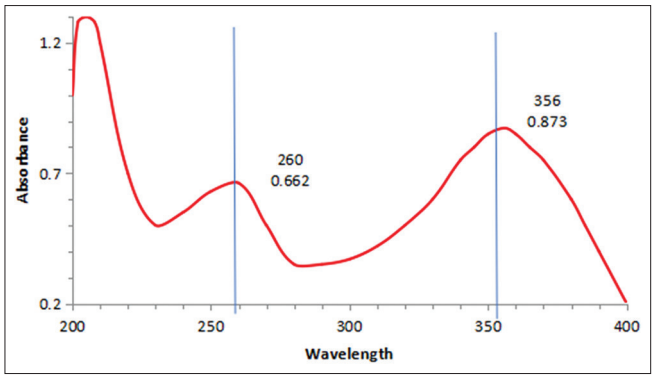

Figure 1: Spectra 3,4-dimethoxycalcone

Table 2: Ratio UVA:UVB (2008 version)

Postexposure mean UVA: UVB ratio	Initial mean UVA: UVB ratio			
	0.0-0.59	0.6-0.79	0.8-0.89	≥0.9
0.0-0.56	No rating	No rating	No rating	No rating
0.57-0.75	No rating	***	***	***
0.76-0.85	No rating	***	****	****
≥0.86	No rating	***	****	*****

UVA: Ultraviolet-A, UVB: Ultraviolet-B

precision (0.475%–0.627%), as shown in Table 3. Accuracy showed the closeness of the analysis results to the actual analyte. Measurement parameters of recovery 98%–102%. The results of the accuracy test showed good results and this method meets the accuracy-test requirements, as shown in Table 3. The calculation result based on the absorbance value 3,4-DMC showed the LOD value is 0.35 ppm and the LOQ value is 1.06 ppm.

Determine cream base and optimal formulation

Optimization base creams were evaluated for 14 days and the results are shown in Table 4. The base 1 had viscosity not readable and spreadability <5 cm. The base 3 had adhesion of <4 s. The selected formula was base 2 to delivered 3,4-DMC as UVA-protection factor (UVA-PF).

Evaluated cream 3,4-dimethoxychalcone

All formula (F1-F3) of sunscreen creams O/W 3,4-DMC was evaluated for 4 weeks. The evaluation results are based on statistical analysis with normally distributed data. The pH test of all formulas showed a significantly different value of 0.000 ($P < 0.05$) each week, as shown in Table 5. The variation concentration of 3,4-DMC lowered the pH value of cream base, but the pH of all formulas is still on the psychological pH of skin 4–6.[19]

The viscosity of all formulas showed a significant value of 0.003–0.000 ($P < 0.05$), it is unstable each week, as shown in Table 6. However, it is still suitable with SNI character 2000–40000 mpas and affects spreadability and adhesion of cream.[20]

The spreadability of all formulas showed not significant ($P > 0.05$) each week, except F2 with 0.03 ($P < 0.05$), s shown in Table 7. The adhesive ability of all formula given value <1 s should be >4 s.[21] Freeze–thaw and centrifugation test used to know the stability of the creams in temperature and mechanical stress. All formulas were not separated that it showed 3,4-DMC stable in a cream formula.

In vitro ultraviolet-A-protection factor

The measurement UVA-PF used spectrophotometry Uv-Vis with transpore method is a validated sunscreen measurement UVA protection.[13,14] The 3,4-DMC had 5 stars (*****) for all concentrations as ultra-protection level against UVA-rays with value UVA/UVB ratio 1.0 > 0.96. The results showed 3,4 DMC could be a candidate UVA-protections agents had ability to inhibit exposure UVA rays.

All formulas had value sequentially 1.641 (F1); 1.823 (F2); and 2.191 (F3) which are illustrated in Figure 3, variations concentration affect the amount of protection against UVA exposure. The statistical analysis showed UVA PF value ($P < 0.005$) significantly different of each formula.

Increasing the concentration may increase the UVA-PF value, but the possible toxic effect must be considered. The *in vitro* method UVA-PF simple test from Ferrero *et al*. was good correlation with *in vivo* measure because it gives the closed value.[15,16]

Photostability test

All formulas' result of photostability was not shifting of the maximum absorbance peak (λ max) which remains at 356 nm, as illustrated in Figure 4. Analysis with ANOVA showed that all concentrations had not significantly different, sequentially 0.264; 0.057; and 0.555 ($P > 0.05$). According to these results, 3,4-DMC can be accepted as

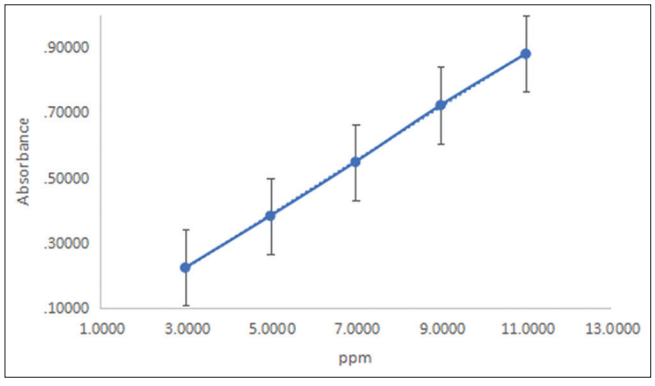

Figure 2: Calibration curve 3,4-dimethoxychalcone

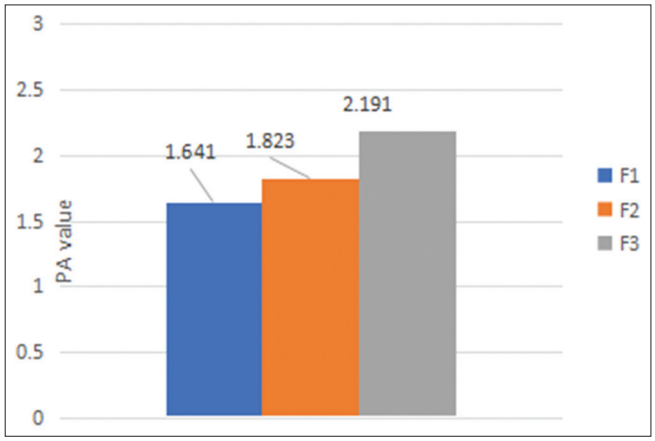

Figure 3: The UVA-Protection factor of cream 3,4 dimethoxychalcone

Table 3: Precision and accuracy analysis

PPM	Average	SD	Percentage RSD	Measurable levels	Percentage recovery
3*	0.2437	0.003	0.627	3.054	101.79
7*	0.8933	0.003	0.475	6.898	98.55
11*	0.5570	0.006	0.617	11.025	100.23

*Data are expressed by as mean±SD (n=3). SD: Standard deviation, RSD: Relative SD, PPM: ppm (concentration)

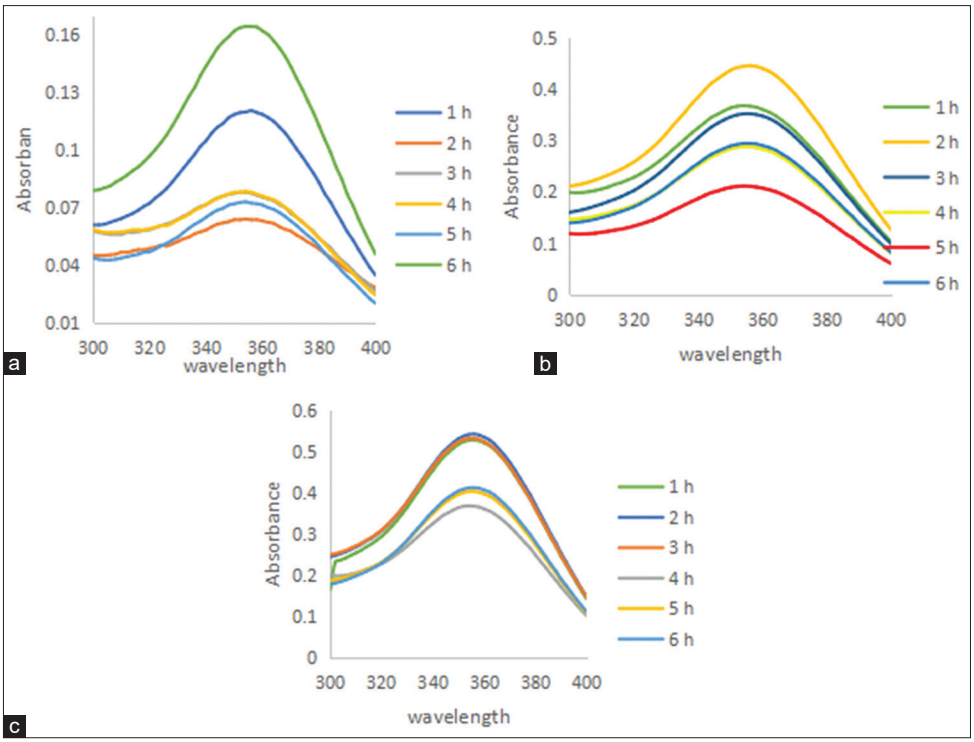

Figure 4: Graph photostability cream 3,4-dimethoxychalcone on (a) Formula 1; (b) Formula 2; (c) Formula 3

Table 4: Evaluated formulation cream base

Parameters	Base I	Base 2	Base 3
Colour	White	White	White
Consistency	Good	Good	Good
Texture	Thick	Thick	Very thick
PH test*	6.16±0.29	5.12±0.23	4.73±0.12
Spreadability*	4.02±0.16	6.22±0.32	5.03±0.23
Adhesion*	17.16±1.21	5.95±0.41	3.44±0.13
Viscosity*	Infinity	19.694±435.83	10.305 ± 137.14

*Data are expressed by as mean±SD (n=3). SD: Standard deviation

Table 5: PH measurement

Weeks	F1*	F2*	F3*
1	4.08±0.01	4.09±0.02	4.11±0.00
2	4.01±0.01	4.14±0.02	4.24±0.02
3	4.14±0.01	4.14±0.02	4.15±0.01
4	4.19±0.01	4.18±0.01	4.23±0.01

*Data are expressed by as mean±SD (n=3). SD: Standard deviation

a candidate for UVA protection agent. The presence of a methoxy group affected stability of the chalcone derivative compounds, as an electron donor group by donating an aromatic ring electron makes an increase the delocalization system which produces the two chalcones more stable when exposed to UV light.[4]

Skin irritation test

The results obtained from skin irritation study were illustrated as sunscreen cream did not raise acute toxicity

Table 6: Viscosity measurement

Weeks	F1*	F2*	F3*
1	5073±126	6750±160	6947±145
2	4340±89	4490±46	6420±85
3	4400±70	5647±91	5267±35
4	4473±312	5760±105	5483±185

*Data are expressed by as mean±SD (n=3). SD: Standard deviation

Table 7: Spreadability measurement

Weeks	F1*	F2*	F3*
1	5.53±0.33	5.72±0.12	5.95±0.20
2	5.90±0.35	6.14±0.17	5.98±0.10
3	6.11±0.17	5.71±0.19	6.00±0.20
4	5.65±0.47	6.29±0.13	6.33±0.19

*Data are expressed by as mean±SD (n=3). SD: Standard deviation

effects such as erythema and edema. Based on the results, all formulas had the best value of 0.33 ± 0.0 and were included in the response category "*negligible*," then the highest concentration (150 ppm) still be tolerated by test animals.

CONCLUSIONS

The 3,4-DMC had an ultra-protection activity against UVA rays then can be used as UVA-PF agent, it has good photostability, and did not irritate the skin of animal test. Based on the intended objectives, cream preparation is a good medium for delivered 3,4-DMC. However, the formulation of cream needs to be re-optimized to get good physical properties. This compound can be developed

by determining the best concentration as UVA-PF with low toxicity and can be combined with a UVB protective compound to become a broad-spectrum sunscreen.

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Conflicts of interest

There are no conflicts of interest.

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