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Determination of Photoprotective Capacity of Topical Gel Formulations Containing Bioactive Compound Rutin and Evaluation of Physicochemical Stability

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ABSTRACT

A series of skin damages are mediated by excessive exposure to ultraviolet B (UVB) radiation. Chemical UV filters can cause photodegradation and adverse effects on the skin. Rutin is a natural multifunctional nutraceutical that can prevent damage from sun radiation. This compound is found in fruits, vegetables, and grains. Previous studies have reported rutin as a good antioxidant and photoprotective agent in sunscreen formulations. This work focused on the development of a formulation and evaluation of the protective activity of rutin as a single compound in sunscreen preparations. The Design Expert software was used for formulation optimization. Photoprotection testing was conducted in vitro by observing the parameters of Sun Protection Factor (SPF), percentage of erythema transmission, and pigmentation. Freeze-thaw cycling test and storage at room temperature were performed to evaluate the stability of the formulation. The optimum formula has good characteristics with a pH value of 6.207±0.013, viscosity of 76.5±0.1, spreadability of 10.82±0.440, and adhesiveness of 1.280±0.027. This sunscreen formulation provided a high level of protection with a sun protection factor (SPF) value of 38.11±0.082. The percentage of erythema and pigmentation transmission fell within the sunblock category. Although there were variations in the SPF values when rutin was formulated into the sunscreen, the overall quality of protection was not compromised. The physical and chemical properties of this sunscreen formulation remained stable after stability testing.

Keywords: gel, rutin, sunscreen, sun protection factor

Introduction

Excessive exposure to sunlight serves as an exogenous mediator of skin damage.[1] Compounds with photoprotective activity have great potential to reduce the detrimental effects of ultraviolet radiation on the skin.[1] Organic UV filter agents have negative effects on the skin, such as dermatitis, a burning sensation, increased risk of skin cancer, and allergic reactions. Meanwhile, inorganic substances have aesthetic issues by leaving white marks on the skin.[1] Both organic and inorganic filter compounds are susceptible to photodegradation, resulting in photodegradation products that contribute to changes in the intensity of ultraviolet absorption spectrum.[2]

Rutin (quercetin-3-rutinoside) is a glycoside form of the flavonol quercetin. This compound can be found in fruits (oranges, lemons, grapes, limes, berries, peaches), vegetables, grains and even tea leaves.[3] Geographic conditions and genetic types influence the concentration of rutin in various plants.[4,5] The best source of rutin is found in more than 70 plant species *Ruta graveolens* L. (Rutaceae), *Sophora japonica* L. (Fabaceae), *Strelitzia reginae* Banks ex Aiton (Strelitziaceae), *Maranta leuconeura* (Marantaceae), *Orchidantha maxillarioides* (Lowiaceae), *Eucalyptus* spp. (Myrtaceae), *Canna indica* L. (Cannaceae), and *Canna edulis* Ker Gawl. (Cannaceae). [4,6]

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A concentration 54.09 (µg/mL) of rutin is also found in the leaves of *Dictyandraarborescen* (Welw.).[7] Active polyphenolic compounds such as rutin have activity in common with organic UV filter compounds, with chromophore groups and aromatic rings that provide photoprotective action and have antioxidant properties.[8] This structure can prevent skin damage from UV radiation by absorbing high energy photons of sunlight and releasing them as low energy rays.[9]

The hydroxyl groups present in the structure of rutin contribute to its ability to scavenge free radicals.[10] Rutin is known to possess photoprotective activity against ultraviolet radiation,[2] as well as antioxidant,[11] anti-inflammatory,[12] anticancer,[13] anti-aging,[14] and antimicrobial properties.[15] A concentration of 0.1% rutin exhibits good compatibility with the skin.[3] Furthermore, rutin demonstrates functional and chemical stability in topical preparations,[2] and can enhance the sun protection factor (SPF) value in formulations containing ultraviolet B (UVB) filter compounds. Formulating sunscreen preparations with compounds that have multifunctional activities will result in multifunctional products with broad benefits.

In previous studies, rutin has been used as an ingredient to enhance antioxidant capacity, improve photoprotective properties, and maintain the photostability of synthetic UV filter.[2,3,16,17] Replacing chemical UV filters with bioactive compounds that have broad benefits is an established strategy to develop multifunctional photoprotective products. Topical formulations in the form of gels are chosen due to their biodegradable and biocompatible nature, higher gel retention time compared to other semi-solid forms, and good skin tolerance.[18] Water-based gels make them non-greasy formulations, which are suitable for oily and acne-prone skin conditions.[19] We chose Hydroxypropyl methylcellulose (HPMC) as the gelling agent for our formulation because it can form neutral, colorless, and stable preparations at pH 3-11.[20] Cellulose derivative polymers have been reported to possess good adhesive properties and are resistant to microbes.[20] Due to the poor solubility of rutin in water,[21] we added a substance to improve the solubility of this bioactive compound,

namely glycerin. The addition of glycerin to water has the effect of reducing surface tension, however, due to its predominantly hydrophobic nature, glycerin alone is not an effective stabilizing agent.[22] The addition of propylene glycol to enhance the efficacy of glycerin is the best solution, as propylene glycol can also be used as a co-surfactant that reduces the surface tension between non-polar compounds and water.[23] The stability of propylene glycol also improves when combined with glycerin.[24] Based on the study results,[25] propylene glycol enhances the permeation of active compounds in topical formulations such as creams and gels. It also helps maintain the distribution of compounds in various layers of the skin (stratum corneum, epidermis, dermis) when incorporated into the formulation.

The selection of formula composition is crucial to achieve an optimal sunscreen formulation. For formula optimization, we utilized Design Expert software version 13 with D-optimal mixture design method. The advantage of this method is that it generates a non-simplex and non-regular experimental region. Compared to other designs, D-optimal requires fewer runs, thus reducing the cost of experimentation.[26] The photoprotective properties of sunscreens can be determined through various approaches, including in vivo studies using animal models or human volunteers, in vitro methods using spectrophotometry, or in silico approaches using computer simulations to predict the sun protection factor (SPF)values.[9] The current in vivo method involves using human volunteers to determine SPF values. However, this method is complex, time-consuming, and costly.[27]

In the European Union (EU), cosmetic products are regulated by Cosmetic Regulation (EC) No 1223/2009, which has been in effect since July 2013. This regulation is the first of its kind worldwide to impose a ban on the testing of cosmetic products on animals. Sunscreens are considered cosmetic products in the European Union.[9] Dimitrovska Cvetkovska and colleague[28] have stated that there is a correlation between in vitro and in vivo approaches in determining sun protection factor (SPF), thereby eliminating the need for animal testing in research. In vitro methods, besides being relatively inexpensive, are also easy to conduct.[29] The in vitro method proposed by Mansur[30] using spectrophotometric measurements of the absorption characteristics of sunscreen products, can provide accurate values for sun protection factor (SPF). Physicochemical characterization of the final product is necessary to establish its compliance with quality parameters. To obtain an optimum formulation with good stability, stability testing and a series of quality controls such as pH, viscosity, spreadability, and adhesion can be performed.[31]

Materials and Methods

Materials

The main ingredient used was the bioactive compound rutin with a purity level of ≥ 94%, purchased on December 12, 2022, from Nitra Kimia (Indonesia). Hydroxypropyl methylcellulose (HPMC) (pharmaceutical grade), Propylene glycol (pharmaceutical grade), Glycerin, (pharmaceutical grade) and Dimethyloldimethyl hydantoin (pharmaceutical grade) were purchased from PT. Alfa Kimia Biomedikatama (Indonesia). The fragrance (cosmetical grade), ethanol 70% (v/v) (p.a degree), and buffer solutions (analytical grade) were provided by the Pharmaceutical Laboratory, Gadjah Mada University.

Determination of Active Ingredient Concentration

The determination of the sun protection factor (SPF) value of the rutin compound was conducted by in vitro method using a UV-Vis spectrophotometer instrument (Thermo Scientific GENESYS 50, Thermo Fisher Scientific Inc, Waltham). The rutin compound was dissolved in analytical-grade ethanol 70% (v/v), and a series of concentrations were prepared: 0.02%, 0.04%, 0.06%, 0.08%, and 0.1%. The absorbance of the samples was measured every 5 nm in the wavelength range of 290-320 nm with three repetitions. The obtained absorbance values were then converted into sun protection factor (SPF) values using the mathematical equation developed by Mansur in equation 1.[30] The highest sun protection factor (SPF) value from the measured concentration series would be incorporated into the formulation.

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda) \quad (1)$$

Where: EE represents the erythema spectrum; I is the light intensity spectrum; Abs is the absorbance of the sunscreen sample; and CF is the correction factor (=10). The values of EE x I can be seen in (Table 1).[30]

Table 1: Normal value of EE x I for Sun Protection Factor (SPF) Calculating

Wavelength (λ nm)	EE x I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

Design of gel formulation

Three types of materials as independent variables would be optimized with upper and lower limits for each component, namely HPMC (1.5-2.5%), propylene glycol (5.0-8.5%), and glycerin (5.0-8.5%), with response parameters of pH, viscosity, spreadability, and adhesiveness. The variables and responses were designed using Design Expert software version 13 with the D-optimal design method, resulting in a solution for the formulation run, which will be further subjected to formulation, characterization, analysis, and formula optimization.

Preparation of rutin gel

The humectants, propylene glycol and glycerin, were mixed until homogeneous. This mixture would be used to dissolve the rutin compound, and the other half would be mixed with the gel base to obtain a homogeneous preparation. HPMC, which has been developed for 24 hours with hot distilled water, in this case, hot distilled water at 80°C, was stirred to form the gel base. Humectants, dissolved rutin compound, 0.1% dimethyloldimethyl hydantoin as an antimicrobial agent, and finally, a sufficient amount of jasmine fragrance were added.

Characterization of rutin gel

pH Determination

pH testing was performed using the pH meter (HANNA H1 5211, Hanna Instruments Inc, Singapore). The pH meter electrode was immersed in the gel sample, which had been previously calibrated using pH 4, 7, and 10 buffer solutions. The pH values was displayed on the pH meter indicator.

Viscosity

Viscosity testing was conducted using the viscometer (Brookfield DV-I prime, AMETEK Brookfield, United States) with spindle number 7 at a rotational speed of 100 rpm. The spindle was immersed in a container filled with the gel, ensuring complete submersion. The viscometer was turned on, and the measurement was taken once the device finished rotating. The viscosity value was displayed on the viscometer indicator.

Spreadability

The spreadability of the formulation was tested using a small sample weighing 0.5 g (OHAUS Analytical Balance, OHAUS Corporation, United States), placed on a round glass plate with a scale. It was covered with another known-weighted round glass plate, and a weight of 50 g was added. The spreadability was determined at intervals of 1 minute until reaching a maximum weight of 150 g by measuring the spread diameter both longitudinally and transversely.

Adhesiveness

The adhesive properties of the gel were determined using the (TA1 Texture Analyzer Ametek LLOYD, AMETEK, United States) in texture profile analysis mode. An analytical probe with a cylindrical shape and a diameter of 35 mm was compressed twice into the sample at a speed of 1 mm/s and a depth of 10 mm. Parameters were determined based on the force-time plot.

Optimization of the formula components

The determination of the optimum formula was based on the significance value obtained from the one-way ANOVA analysis of the response data. To meet the optimization criteria, the p-value of the model was <0.05, indicating significance, and the p-value of the lack of fit was >0.5, indicating insignificance. This meant that the chosen model by the software had an influence on the response. The mathematical model determined by the software represented the phenomenon observed in the research. Meanwhile, the insignificant lack of fit explained that there was no significant difference between the experimental response values and the predicted values. When the analysis results met the criteria, the optimization stage could be continued by setting the target response to obtain the optimal formula solution. The optimal formula was determined based on the desirability value, which approached 1. The software-determined optimal formula was then formulated into a gel preparation, and the response was observed and verified.

Verification of the optimal formula

Verification was conducted by comparing the predicted values with the observed experimental values. The acceptance criteria would be met if the experimental values fall within the range of the predicted values suggested by the software.

Testing the sun protection activity of rutin gel

The sun protection capability of the optimal rutin gel formula was determined by measuring the sun protection factor (SPF), erythema transmission percentage (%TE), and pigmentation transmission percentage (%TP) through in vitro testing. The gel samples were dissolved in analytical-grade ethanol, vortexed (MX-S DLAB, DLAB Scientific Inc, USA), and centrifuged (Hettich EBA-8, Hettich, Germany) at 300 rpm to separate the gel base from the compound. The supernatant was then measured for absorbance using a UV-Vis spectrophotometer with analytical-grade ethanol as a blank. The determination of sun protection factor (SPF) values followed the Mansur method, while transmittance was tested by measuring the sample absorbance at wavelengths ranging from 292.5 nm to 317.5 nm for erythema and from 322.5 nm to 372.5 nm for pigmentation, with a 5 nm interval and three repetitions. The %TE and %TP values were calculated using mathematical equations proposed by Cumpelik in equation 2 and 3.[32]

$$\%TE = \frac{\Sigma(\%T \times Fe)}{\Sigma Fe} \quad (2)$$

Where: T represents transmittance percentage; Fe is the erythema flux, which is a constant value; ΣFe is the total erythema flux; ΣT x Fe is the sum of erythema flux inhibited by the photoprotective product.

$$\%TP = \frac{\Sigma(\%T \times Fp)}{\Sigma Fp} \quad (3)$$

Where: T represents transmittance percentage; Fp is the pigmentation flux, which is a constant value; ΣFp is the total pigmentation flux; ΣT x Fp is the amount of pigmentation flux inhibited by the photoprotective product. Erythema flux and pigmentation were adopted from the book "Cosmetics Science and Technology" by Balsam and colleagues.[33]

The in vitro approach was important to predict the sunscreen's photoprotective ability. Currently, in vivo methods no longer involve animal testing but instead use human volunteers. This method is quite complex, time-consuming, and expensive. However, it is possible to apply this method in future studies.

Stability testing

Physical and chemical stability testing was performed to determine any changes in the formula's physical and chemical properties. The stability of the sunscreen gel was determined using the cycling test method, with exposure to temperatures of 8°C and 45°C for three cycles. Each cycle consisted of 24 hours at cold conditions and 24 hours at hot conditions. This test was conducted to assess any syneresis experienced by the formulation. The percentage of syneresis can be calculated using equation 4.

$$\text{The percentage of syneresis} = \frac{\text{The initial volume (mL)} - \text{The final volume (mL)}}{\text{The initial volume (mL)}} \times 100\% \quad (4)$$

Stability testing related to shelf life under storage conditions was crucially conducted to ensure the quality, safety, and efficacy of the product. This test played a significant role in the development and improvement of the formulation, determining the validity and monitoring the physical and chemical characteristics. Stability testing at room temperature of 28±2°C was performed for a four-week storage period. The evaluated parameters included pH, viscosity, spreadability, adhesiveness, organoleptic properties, and compound content. For the determination of compound content in the formulation, method validation was conducted prior Parameters of the method validation include accuracy, precision, specificity, linearity, limit of detection and limit of quantitation.[34]

Statistical analysis

Statistical analysis was performed using IBM SPSS software version 25 to compare the mean values of each test group. All experimental data in this study were measured with three repetitions, and the means and standard deviations were calculated.

Result and Discussion

Concentration of the active ingredient

The concentrations of each measured concentration could be seen in (Figure 1). Among all the measured concentration series, 0.1% rutin exhibited the highest sun protection factor (SPF) value of 49.90±0.001, thus this concentration was selected to be incorporated into the sunscreen formula. This compound could absorb ultraviolet radiation due to the chromophore groups present in its structure. Rutin contains hydroxyl groups that can capture free radicals.[10] According to de Oliveira and colleagues,[2] the highest activity of rutin was attributed to the catechol group in ring B. Ultraviolet B rays were within the wavelength range of 290 – 320 nm, while rutin had a relatively wide UV spectrum coverage.[2] The absorbance of ring A in the structure of rutin was within the range of 250 – 285 nm, while ring B had an absorbance of 320 – 385 nm.[2] Previous findings suggested that, although rutin has a broad absorption range, it was not effective in providing protection, as evaluated based on the critical wavelength and UVA parameters tested using in vitro methods for this flavonoid compound.[2]

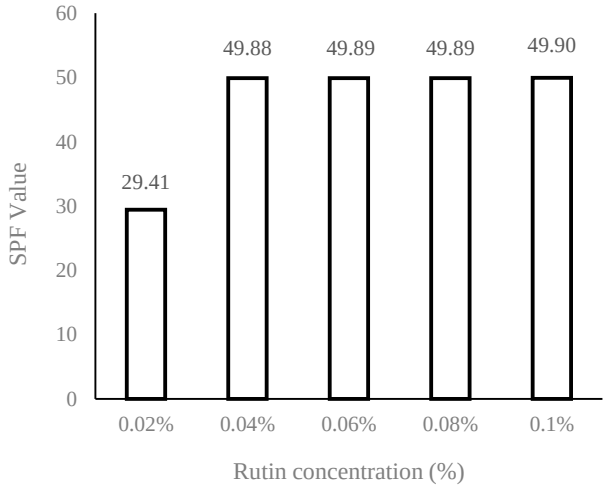


Figure 1: Sun Protection Factor (SPF) values of rutin at various concentrations

Evaluation of formula response characteristics

The analysis of the response to each measured formula run obtained several mathematical equations to explain the relationship between variables and response. The quadratic equation for pH response can be seen in Equation 5.

Y= 5.15411 (A) + 6.24505 (B) + 6.14139 (C) + 0.93976 (AB) + 1.51554 (AC) + 0.0956763 (BC) (5)

Description: Y is the pH response; A is the proportion of HPMC; B is the proportion of propylene glycol; C is the proportion of glycerin.

It was observed that all components had an impact on the pH response. The highest positive influence was found in the propylene glycol component with a coefficient value of +6.24505. This is because propylene glycol tends to have a basic pH, ranging from 6 to 9.[35] The higher the proportion of propylene glycol in the formulation, the higher the resulting pH of the preparation. However, the pH obtained in this experiment still falls within the range of normal skin pH. Normal skin pH ranges from 4.1 to 7.[36]

The equation for the linear model of viscosity response is presented in equation 6.

Y= 467.049 (A) + 68.5674 (B) + 65.2011 (C) (6)

Description: Y is the viscosity response; A is the proportion of HPMC; B is the proportion of propylene glycol; C is the proportion of glycerin.

The highest influence was given by HPMC with a coefficient value of +467.049. This was due to the proportion of HPMC present in the formula. The higher the polymer concentration in the formula, the higher the viscosity of the formulation.[37] This result is consistent with previous studies that showed variations in HPMC concentration increase the consistency and viscosity values of gel formulations. Gel formulations consist of polymers as raw materials.[38] In gel formulations, swelling occurred due to the penetration of solvents, which causes the polymer to stretch. The solvent can modify the hydrogen bonding characteristics between water, solvent, and polymer, thus affecting the swelling behavior of the polymer.[39] An increase in HPMC concentration led to an increase in the number of polymer fibers, resulting in more trapped and bound fluid by the gelling agent and triggering viscosity.[40]

The quadratic model equation for the spreadability response can be seen in equation 7.

Y= 44.0785 (A) + 15.8102 (B) + 16.8494 (C) – 75.4867(AB) – 81.7708 (AC) – 8.0426 (BC) (7)

Description: Y is the spreadability response; A is the proportion of HPMC; B is the proportion of propylene glycol; C is the proportion of glycerin.

For the spreadability response, HPMC also played a crucial role, as indicated by the coefficient value of +44.0785. A lower proportion of polymer in the formulation led to easier flow or increased spreading ability of the product. Spreadability is crucial for the product's ability to deliver active ingredients. The obtained spreadability value was influenced by viscosity, as it related to the strength of the gel matrix formed. A higher viscosity of a product resulted in decreased flowability, thus reducing its ability to spread.[41]

The special cubic model equation for the adhesion response can be seen in equation 8.

Y= 1.74646 (A) + 1.73194 (B) + 1.25462 (C) – 5.5793(AB) + 2.01277 (AC) + 1.2076 (BC) – 19.2855 (ABC) (8)

Description: Y is the adhesion response; A is the proportion of HPMC; B is the proportion of propylene glycol; C is the proportion of glycerin.

Based on the model equation, the adhesion ability was influenced by the interaction between HPMC and glycerin, with a coefficient value of +2.01277. Cellulose derivative polymers such as HPMC are known to possess good adhesion properties.[42] This has also been reported in a study on mucoadhesive within in situ hydrogels, where HPMC polymer at a certain concentration was found to provide high adhesion in the formulation.[43] Furthermore, hydrogel formulations containing glycerin in topical mixtures exhibit texture properties such as high adhesion and cohesion.[44] Glycerin in the formulation triggers cross-linking interactions between the polymer molecules, thereby enhancing the cohesive properties of the formulation,[44] this is supported by studies on adhesiveness, which confirm that glycerin imparts adhesive properties to hydrogels.[45] The glycerin molecule, composed of three hydroxyl groups, strengthens the hydrogen bonding present in the cross-linking. The hydroxyl groups of glycerin can interact with the functional groups between polymer molecules through hydrogen bonding, thereby enhancing the interconnected network in the gel physically.[45]

Table 2: Design and Response of Gel Formulation

Formula code	HPMC	Propylene glycol	Glycerin	pH*	Viscosity* (d.Pas)	Spreadability* (cm^2)	Adhesiveness*
1	1.82	5	8.18	6.189 ± 0.002	94.8 ± 0.057	13.51 ± 0.005	1.7947 ± 0.175
2	1.5	6.32	7.18	6.206 ± 0.005	73.2 ± 0.115	14.17 ± 0.011	1.8093 ± 0.103
3	1.60	8.40	5	6.243 ± 0.001	79.6 ± 0.115	14.85 ± 0.015	1.6335 ± 0.279
4	2.02	6.71	6.27	6.221 ± 0.001	118.4 ± 0.115	9.07 ± 0.01	1.0825 ± 0.141
5	2.5	5.92	6.58	6.143 ± 0.002	165.2 ± 0.057	7.78 ± 0.015	0.7721 ± 0.047
6	1.82	7.72	5.46	6.194 ± 0.004	110.4 ± 0.115	9.89 ± 0.005	1.1522 ± 0.124
7	2.02	6.71	6.27	6.201 ± 0.003	118.8 ± 0.057	8.54 ± 0.011	0.9463 ± 0.032
8	2.5	6.89	5.61	6.166 ± 0.004	190.1 ± 0.057	7.78 ± 0.01	0.5554 ± 0.076
9	2.5	7.5	5	6.124 ± 0.001	189.4 ± 0.115	8.54 ± 0.011	0.5782 ± 0.043
10	2.02	6.71	6.27	6.225 ± 0.004	130.0 ± 0.057	9.89 ± 0.01	0.9264 ± 0.027
11	1.5	5.70	7.80	6.152 ± 0.004	90.1 ± 0.115	14.51 ± 0.005	1.4843 ± 0.184
12	1.5	7.35	6.15	6.256 ± 0.002	50.4 ± 0.115	15.54 ± 0.02	1.7961 ± 0.161
13	1.82	5	8.18	6.184 ± 0.001	92.0 ± 0.115	12.56 ± 0.005	1.1480 ± 0.071

14	2.5	5.17	7.33	6.168 ± 0.001	191.2 ± 0.115	6.83 ± 0.011	1.5985 ± 0.147

* Each value represented Mean ± SD (n=3)

Table 3: Criteria for the Optimum Formula

Variable	Goal	Lower limit	Upper limit
HPMC	In range	1.5	2.5
Propylene glycol	In range	5.0	8.5
Glycerin	In range	5.0	8.5
pH	In range	6.124	6.256
Viscosity (d.Pas)	Target (75 d.Pas)	50	100
Spreadability (cm^2)	In range	8	11
Adhesiveness	In range	0.5554	1.8093

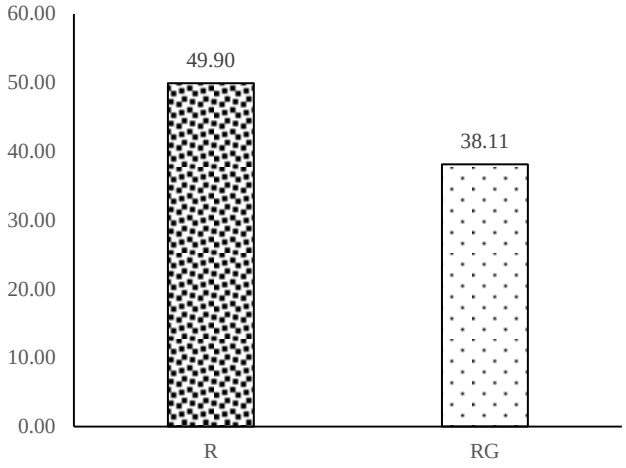


Figure 2: Difference Sun Protection Factor (SPF) values of rutin. Pre-formulation rutin R, and the rutin formulated into a gel RG

Optimum formula and verification

The responses of the 14 observed formulations are presented in (Table 2). To obtain the optimum formulation, it is important to determine the target criteria for the formulation, which will be further optimized using the Design Expert software. The optimization criteria are presented in (Table 3). The optimum formula was obtained with a proportion of HPMC of 1.84317, propylene glycol of 5.92964, and glycerin of 7.22719, resulting in pH values of 6.207±0.013, viscosity of 76.5±0.1 d.Pas, spreadability of 10.81±0.440 cm^2, and adhesiveness of 1.2804±0.027. This formula was recommended by the software with a desirability value of 0.938. The obtained response aspects have been verified and are in accordance with the values suggested by Design Expert. The physical and chemical properties of the optimum formula meet the criteria for a good gel product. The verification of the formula can be seen in (Table 4).

Evaluation of Sunscreen Protection of Rutin Gel

The obtained sun protection factor (SPF) value of rutin gel, calculated using the Mansur equation, was 38.11±0.082, with erythema transmission percentage (%TE) of 0.22±0.09 and pigmentation transmission percentage (%TP) of 0.67±0.08. According to the sun protection factor (SPF) rating system, the protection provided by the formulation falls within the high category,[46] and the low average transmission values indicate that less ultraviolet radiation is transmitted to the skin in the presence of the sunscreen formulation. This study demonstrates that rutin compound significantly provides full protection against radiation without the presence of synthetic UV filters. The photoprotective ability of this compound has also been recognized in previous studies, which combined rutin with benzophenone-3, which increased the sun protection factor (SPF) from 24.3±1.53 to 33.3±2.89 and enhanced antioxidant activity by 40 times.[2] We assume that the protective ability against the erythema spectrum is related to the compound's benefits as an anti-inflammatory.[12] Previous studies have explained the anti-inflammatory effects of rutin by inhibiting the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), suppressing the p38 mitogen-activated protein kinases (p38 MAPKs) and c-Jun N-terminal kinases (JNKs) signaling pathways. Both enzymes play a role in the activation of activator protein-1 (AP-1) and are overexpressed in the epidermis after UVB exposure.[47] Furthermore, we argue that the protective activity against the pigmentation spectrum is generated by its strong antioxidant ability.[48] Sun exposure-induced inflammation increases the production of reactive oxygen species (ROS), and changes in lipid and protein induced by ROS lead to abnormal cell signaling pathways.[49] The disrupted cell activity results in the formation of cyclobutane pyrimidine dimers (CPD) and 6-4 pyrimidine pyrimidone (6-4PP), which are harmful to cells. These molecules enhance the production of melanin and melanocytes, triggering a tanning response.[50] Rutin can protect against the molecular consequences of ultraviolet radiation by reducing proinflammatory responses and the formation of reactive oxygen species (ROS).[51]

However, there is a difference in sun protection factor (SPF) when rutin is formulated into the sunscreen product, as can be observed in (Figure 2). Prior to formulation, rutin had an sun protection factor (SPF) of 49.90±0.001. According to Dutra and colleagues[27] the difference in sun protection factor (SPF) values can be attributed to several factors, such as the use of solvents for solubilizing the formulation, the effects and interactions among carrier components, pH system, and rheological properties of the formulation, which can decrease or even increase the maximum wavelength absorbance of the sunscreen product. Excipients and other ingredients may also produce absorption bands that interfere with the sunscreen's absorption range for UV radiation. The difference in sun protection factor (SPF) values does not diminish the quality of the obtained formulation. Both formulations still provide high protection and can offer maximum protection against UVB radiation. We state that these results are predictive values for the achieved protection, although the in vitro approach to determine sun protection factor (SPF) values correlates with in vivo methods, studies involving human volunteers can be a future solution.[28]

Polyphenolic active compounds, such as flavonoids, exhibit similar activity to organic UV filters, which possess chromophore groups and aromatic rings that not only provide photoprotective effects but also serve as antioxidants.[8] Continuous exposure to sunlight can generate reactive oxygen species (ROS). Free radicals are not only acquired from external factors but are also produced endogenously by the body during cellular metabolism, which can trigger a series of skin damage. Naturally, the body has antioxidants to prevent the harmful effects of free radicals, but this defence system has limited capacity.[52] The topical application of antioxidants on the skin can increase the concentration of antioxidants in the epidermis and dermis layers. In addition to preventing sunlight from entering the skin, antioxidants can also prevent and reduce the toxicity induced by ultraviolet radiation.[52] Rutin is a compound that exhibits strong antioxidant activity.[48] The potential antioxidant activity in the structure of rutin is attributed to the presence of a conjugated double bond at positions 2,3 and a 4-oxo-function group on the C ring. Furthermore, the functional groups capable of binding transition metal ions and the catechol group on the B ring contribute to its antioxidant activity. The highest antioxidant activity is found in the catechol group on the B ring, which readily donates hydrogen or electrons to stabilize radical species.[53]

Physical and chemical stability of rutin sunscreen

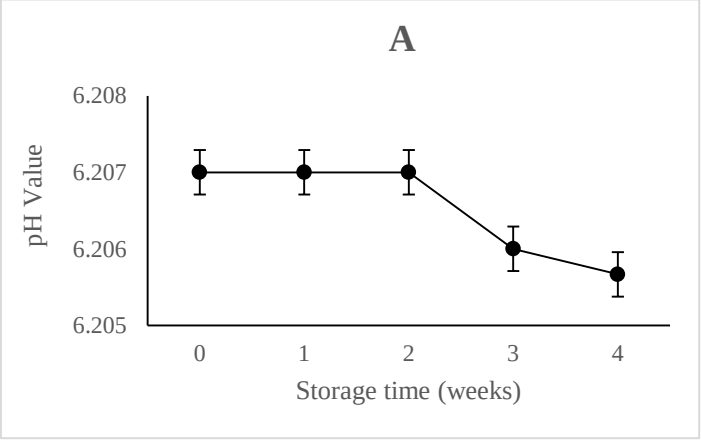
The results obtained after the stability testing of rutin sunscreen samples indicate stability, as evidenced by the obtained percentage of 0 syneresis value. The samples were stored for a period of 4 weeks at room temperature, and the evaluated characteristic parameters showed a decline, as presented in (Figure 3). The pH values of the formulation decreased in the third week and continued to decline until the end of the testing period. Similarly, the viscosity decreased in the first week of storage and maintained its value in the third and fourth weeks. On the other hand, the spreadability response tended to increase due to the decrease in viscosity. In contrast, the gel adhesive property increased until the third week and decreased in the fourth week of storage. The active ingredient content in the samples tended to decrease, with an initial active compound content of 98.80±1.03 before the testing period and a compound content of 98.14±0.82 after the testing period. The difference in the decrease was only 0.66%, which is an acceptable criterion. Although there were reductions in pH, viscosity, adhesive property, and compound content in the samples, the visual aspects of the product remained unchanged. The increased spreadability is associated with the decreased viscosity. The visual aspects, including odor, color, and texture, remained consistent throughout the testing period. Based on the results of one-way ANOVA and post hoc Tukey test, the decrease in each parameter value did not show significant differences, with a significance value >0.05. This stability is supported by each component used in the gel formulation. HPMC polymer can form a neutral, colorless formulation that remains stable at pH 3-11. It exhibits good adhesive properties and is resistant to microbial growth.[20] Propylene glycol, in addition to acting as a humectant, also plays a role in maintaining water loss from the gel formulation, thus improving product stability.[54] Propylene glycol has the advantage of enhancing the permeation of active compounds in cream and gel formulations and maintaining the distribution of compounds across various layers of the skin (stratum corneum, epidermis, dermis).[25] Propylene glycol remains stable at low temperatures, and its stability can be enhanced by the addition of glycerin or water.[55] Additionally, flavonoid compounds have been shown to exhibit photostability in propylene glycol solutions.[56] Glycerin exhibits high resistance to degradation, is capable of absorbing large amounts of water,[57] and imparts good texture and rheological properties.[58] Glycerin has been proven to enhance the oxidative stability of flavonoid derivative compounds such as quercetin.[59]

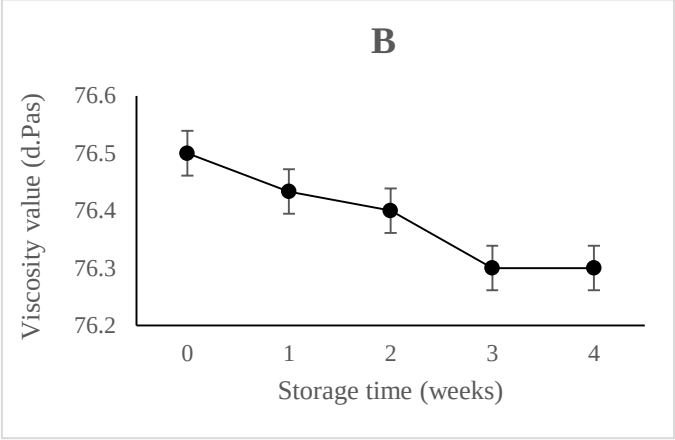
Conclusion

Formulation development and protection evaluation of rutin compounds as single compounds in sunscreen preparations have been carried out. The findings demonstrate that rutin can provide complete protection against ultraviolet B radiation, prevent erythema and pigmentation through in vitro testing. The Sun Protection Factor (SPF) values of rutin appear to differ when formulated into a gel formulation, possibly due to the sample extraction process. However, the provided protection remains in the high category. The optimized formulation can facilitate the incorporation of the active ingredient into a sunscreen product that meets the required criteria. Clinical information and the photostability of the compound are essential considerations, given that the use of this bioactive compound is based on a single formulation.

Table 4: Formula Verification

Response	Predicted data	Observed data	95% PI low	95% PI high
pH	6.207	6.207	6.170	6.244
Viscosity (d.Pas)	76.54	76.5	66.91	86.16
Spreadability (cm^2)	11.00	10.82	9.04	12.95
Adhesiveness	1.321	1.280	1.016	1.624





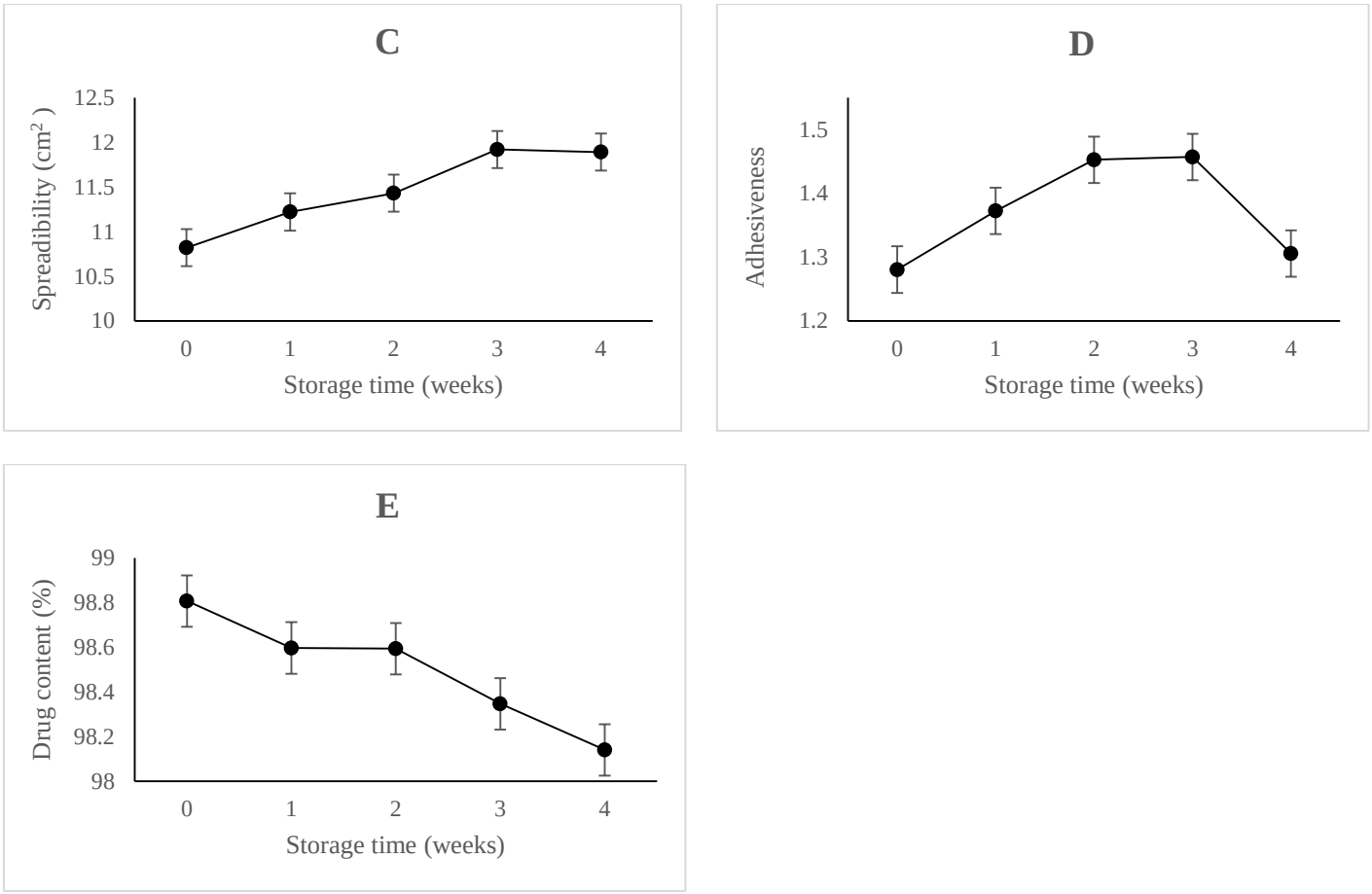


Figure 3: Physical and chemical stability of rutin sunscreen at room temperature. pH values A, viscosity value (d.Pas) B, spreadibility (cm^2) C, adhesiveness D, drug content (%) E

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

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