

Sun Protection Factor Activity of Black Glutinous Rice Emulgel Extract (*Oryza sativa* var *glutinosa*)

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ABSTRACT

Background: Recently, emulgel has emerged as a potential hydrophobic drug delivery method. Therefore, this study aims to evaluate the phytochemical content of *Oryza sativa* extract and develop an emulgel formulation using Carbapol 940 as a gelling agent.

Materials and Methods: The emulsion was placed in a gel basis after preparation and the formulations were evaluated for their rheology, pH, spreading coefficient, stability, and sun protection factor. Then, phytochemical analysis of *O. sativa* extract was used to determine the presence of alkaloids, flavonoids, polyphenols, quinones, monoterpenoids, and sesquiterpenoids, as well as triterpenoids and steroids. **Results:** The entire formulations of *O. sativa* emulgel extract filled the emulgel formulation criteria. Furthermore, *O. sativa* emulgel protects against UV radiation, as indicated by the SPF value in each formulation, which increases as the dose of *O. sativa* extract increases. Based on the results, formulations 2 and 3 have an SPF of 5.71 ± 0.063 and 16.07 ± 0.072 , respectively. These suggest that they both fill the Indonesian National Standard, which requires a sunscreen preparation with a minimum of four protection. **Conclusion:** Consequently, *O. sativa* emulgel extract can serve as a novel sunscreen agent against UV radiation. However, further study is required to ascertain the mechanism of action of the active chemicals found in *O. sativa* that function as an antioxidant and give protection against UV radiation.

Key words: *Oryza sativa*, Emulgel, Sun Protection Factor, Ultraviolet, Phytochemical compounds.

INTRODUCTION

The skin as the outermost organ of the human body is susceptible to external harmful effects such as UV radiation exposure.¹ Excessive exposure to UV radiation can have a devastating effect on the skin. Furthermore, sunburn, skin cancer, oxidative stress, and photoaging can all result from this sort of damage, depending on the amount and type of UV radiation and the individual exposed.² The electromagnetic spectrum of ultraviolet radiation is divided into three regions: UVA, which lies between 320 and 400 nm; UVB between 290 and 320 nm; and UVC between 200 and 290 nm. The atmosphere tends to filter UVC radiation before it reaches the ground. Meanwhile, UVB radiation is not entirely filtered by

the ozone layer and causes skin damage, however, the UVA radiation penetrates the epidermis and dermis layers, resulting in premature skin aging. Consequently, the majority of products, including lotions, moisturizers, shampoos, creams, and other skin preparations, contain sunscreen, which protects against the damaging effects of UV radiation. The SPF of sunscreen is defined as the ratio of the amount of UV energy required to create a minimum erythema dose (MED) on protected skin and the amount of UV energy required to produce a MED on unprotected skin.³ The MED is the shortest time interval or lowest dosage of UV irradiation that produces the least apparent erythema on exposed skin.⁴ The

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higher the SPF number, the greater the sunscreen's protection against UV rays. However, several studies indicate that using sunscreen to protect the skin from UV radiation is currently not totally.¹ Hence, it is important to investigate novel sunscreen chemicals produced from natural components. Several studies have demonstrated that natural chemicals are possible sources of sunscreen due to their UV absorption and antioxidant activities.^{5,6} The incorporation of antioxidant chemicals into sunscreens enhances their protective properties against UV radiation and enables the treatment and prevention of UV-induced illnesses.⁷ Indonesia is the world's second most biodiverse country, with 28,000 plant species and an estimated 2,500 plant species with therapeutic qualities,⁸ one of which is Black Glutinous Rice (*Oryza sativa*). This plant is widely utilized in the production of food and natural sunscreens as well as a cosmetic component such as emulgel. *O. sativa* extract cannot be applied directly to the skin, therefore, it is transformed into emulgel. One of the benefits of emulgel is that it is a hydrophobic carrier material that cannot be immediately absorbed into the gel foundation. It also aids in the unification of hydrophobic active substances in the oil phase, after which the oil globules are distributed in the water phase (O/W emulsion) and combined in a gel foundation.^{9,10} Therefore, this study aims to determine the activity of the sun protection factor (SPF) of an emulgel extract from *O. sativa*.

MATERIALS AND METHODS

Plant determination and extraction

A total of 10 kilograms of fresh *O. sativa* were collected in Bungur Gede Village, Subang Regency, West Java, Indonesia, and transported to Central Laboratory, Buana Perjuangan Karawang University for cleaning, drying, grinding, and extraction. The plant was recognized as *O. sativa* by the School of Life Sciences and Technology, Bandung Institute of Technology in West Java. Then, 3 kilograms of *O. sativa* powder were macerated completely in 96% ethanol for over 3x24-hr. Then, the liquid extract was collected and concentrated at a temperature of 50°C using a rotary evaporator (Eyela OSB-2100).

Phytochemical screening

Qualitative screening of *O. sativa* extract was performed to determine the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, phenolic compounds, tannins, saponins, and quinones.¹¹

Emulgel preparation

A variety of emulgel producing agents were used to create the formulation, which begins with the same emulsion preparation. The gel phase was prepared by dispersing HPMC in distilled water and stirred at a speed of 300 rpm with a magnetic heater stirrer (Bioevopeak Co., Ltd., China). Furthermore, carbopol 940 was dissolved in distilled water and then triethanolamine was added to form the aqueous phase of the emulsion (TEA). Afterward, mechanical stirring was used to ensure consistency at a medium pace. The emulsion's oil phase was made by dissolving tween 80 in liquid paraffin. Propylene glycol is dissolved in methylparaben, and then glycerin is added. Then, the oil and water phases were heated separately at 70-80°C, and the oil phase was introduced to the water with constant stirring until it reaches room temperature. *O. sativa* extract was then dissolved in distilled water and combined with a room temperature combination of water and oil phases, then swirled until homogenous and scent was added. The resulting emulsion was combined with the gel phase at a ratio of 1:1 and then agitated to form an emulsion.¹² Table 1 contains the composition of the emulgel formulation utilized in the investigation.

Physical examination

Color, appearance, and the consistency of the produced emulsion compositions were visually evaluated.¹³

Rheological studies

The viscosity of different emulgel compositions was measured using a cone and plate viscometer equipped with a spindle 7 (Lamy Rheology, France). Afterwards, the assembly is linked to a thermostatically regulated water bath and kept at a temperature of 25°C. The viscosity-determining formulation is placed in a glass enclosed in a thermostatic jacket and the spindle was let to freely travel within the emulgel. This test was conducted for 10 min at a speed of 100 rpm.¹⁴

Measurement of pH

The pH of different emulgel formulations was determined using a pH meter (NeoMet, istek inc, Seoul). The formulation whose pH is to be determined was placed in a container, the electrode was then inserted and the results were recorded.¹⁵

Spreading coefficient

The dispersion coefficient was measured by placing 1 g of the emulgel formulation on a clear glass lined with graph paper. Then, it was covered by a glass plate with a specified load (5-30 g), and allowed to stand for

Table 1: Emulgel formulations of *O. sativa* extract.

Ingredient	Concentration (%w/w)			
	Blank	F1	F2	F3
<i>O. sativa</i> extract	-	0.1	0.5	1
HPMC	0.3	0.3	0.3	0.3
Carbopol 940	0.3	0.3	0.3	0.3
Triethylinolamin	0.3	0.3	0.3	0.3
Tween 80	0.82	0.82	0.82	0.82
Propylene glycol	1	1	1	1
Glycerin	3	3	3	3
Methyl parabene	0.1	0.1	0.1	0.1
Parfume	3 drops	3 drops	3 drops	3 drops
Aquadestilata	50 ml	50 ml	50 ml	50 ml

60 sec. The area specified by the emulgel formulation was calculated. Greater dispersion coefficients are indicated by shorter distance intervals.¹⁶

Stability test

Emulgel samples of *O. sativa* extract were stored at cold ($4\pm 2^\circ\text{C}$), room ($27\pm 2^\circ\text{C}$), and hot temperatures ($40\pm 2^\circ\text{C}$) for 90 days, and the physical appearance, pH, and viscosity tests on all formulas and all temperatures were observed.¹⁷

Sun protection factor (SPF) assay

A total of 5 g of the emulgel formulation was dissolved with 50 ml of ethanol p.a and filtered. Afterwards, 4 ml of the solution was placed in the cuvette to determine the SPF value using a UV-Vis spectrophotometer (Shimadzu UV Mini-1240) at wavelengths of 290, 295, 300, 305, 310, 315, and 320 nm. The results were compared using ethanol solution as a blank and was repeated 4 times. Then, the SPF value is determined using the Mansur equation as follows.¹⁸

$$\text{SPF spectrophotometric} = \text{EF} \times \sum_{290}^{320} \text{EE}(\lambda) \times 1(\lambda) \times \text{abs}(\lambda)$$

Where:

CF = 10 (correction factor).

EE = Erythemaous radiation effect spectrum.

I = Sunlight intensity spectrum.

Abs = Absorbance value of sunscreen products.

Data analysis

The experimental results were expressed as the mean \pm standard deviation (SD) of the four replicates and the data were analyzed using SPSS (version 22). For statistical analysis, a one-way analysis of variance (ANOVA) was used.



Figure 1: Black glutinous rice (*Oryza sativa* var. *glutinosa*).

RESULTS AND DISCUSSION

Plant determination and extraction of *O. sativa*

The plant was identified as *O. sativa* (Figure 1) by the School of Life Sciences and Technology, Bandung Institute of Technology, West Java, Indonesia with No. 6721/11.CO2.2/PI./2019. *O. sativa* extraction yielded 151.37g (5.05%) concentrate (fixed weight of extract divided by weight of simplicia multiplied by 100%).

Phytochemical screening

Phytochemical screening of *O. sativa* extract revealed the presence of chemical elements such as alkaloids, flavonoids, polyphenols, quinones, monoterpenoids, sesquiterpenoids, triterpenoids, and steroids (Table 2).

Physical examination

The prepared emulgel formulations are visually inspected for colour, appearance, and consistency. The results are shown in Table 3 and Figure 2.

Table 2: Phytochemical screening of *O. sativa* extract.

Phytochemical compounds	Reagents	Observation	Results
Alkaloids	Dragendorff	(+) Light brown	(+) Alkaloids
	Bouchardat	(+) Dark brown	
	Mayer	(+) Muddy and white sediment	
Flavonoids	Zn + HCl (p)	(+) Red	(+) Flavonoids
	Mg + HCl (p)		
Polyphenols	1% FeCl ₃	(+) Dark blue	(+) Polyphenols
Tannins	1% Gelatin	(-) White sediment	(-) Tannins
Quinones	5% KHO	(+) Yellow	(+) Quinones
Saponins	Hot water + HCl	(-) Bubble	(-) Saponins
Monoterpenoids and Sesquiterpenoids	Vanillin + H ₂ SO ₄ (p)	(+) Pink	(+) Monoterpenoids and Sesquiterpenoids
Triterpenoids and Steroids	Liebermann-Burchard	(+) Purple	(+) Triterpenoids and Steroids

(+) = Contained, (-) = Not contained.

Table 3: Physical parameters of the *O. sativa* extract emulgel formulation.

Formulation	Color	Homogeneity	Consistency
F0	Clear	Excellent	Excellent
F1	Pale brown	Excellent	Excellent
F2	Light brown	Excellent	Excellent
F3	Brown	Excellent	Excellent

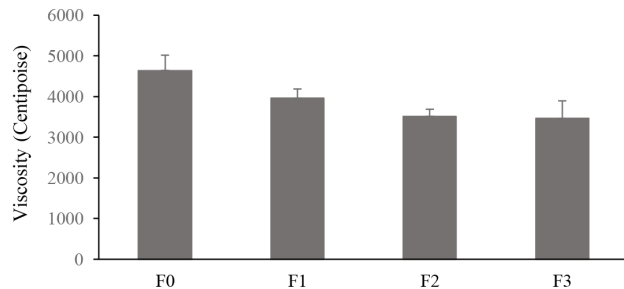
**Figure 2: Emulgel formulations of *O. sativa* extract.**

Rheological studies

The results of viscosity testing on all formulations of *O. sativa* emulgel extract showed a change in viscosity in each formulation (F0 = 4642 cPs, F1 = 3963 cPs, F2 = 3514 cPs, F3 = 3463 cPs), however, the changes were still within the range that filled the requirements. Furthermore, the desired viscosity in the preparation of emulgel is between 2000-4000 cPs. This is due to the ointment-like viscosity possessed by the gel that ensures a longer contact time with the skin.¹⁹ The results of the rheology studies are shown in Figure 3.

Measurement of pH

The pH value of a topical preparation must match the skin pH, which is 4.5-6.5. pH values that are too acidic

**Figure 3: Viscosity of the formulations F0-F3 (mean ± SD).**

result in skin irritation, similarly, too much alkaline causes scaly skin.¹⁹ The results of pH measurements in all formulations (F0-F4) using a pH meter, obtained the average value of 4.52, 4.71, 4.80, and 5.03 for F0, F1, F2, and F3, respectively. All formulations fill pH testing requirements and the results are shown in Figure 4.

Spreading coefficient

Good dispersion is an indicator that the gel preparation is easy to apply. Therefore, the dispersion test aims to determine the speed of spread and the softness of the emulgel preparation on the skin. The results showed no significant difference in the dispersion value of each formulation (F0 = 6.0 cm, F1 = 6.2 cm, F2 = 6.7 cm, F3 = 6.8). These indicate that all the formulations filled the dispersion requirements, which are between 5-7 cm in diameter.²⁰ The results are shown in Figure 5.

Stability test

A stability test was carried out to ensure that the emulgel formulation had the same properties after production as well as filled the criteria parameters during storage. It

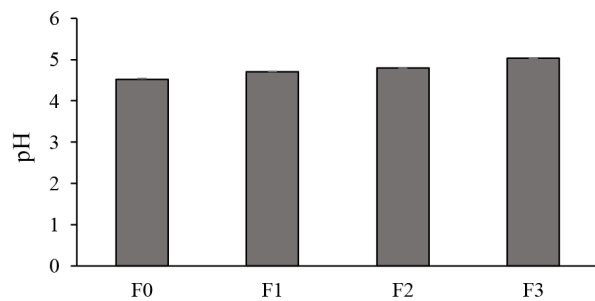


Figure 4: pH value of the formulations F0-F3 (mean ± SD).

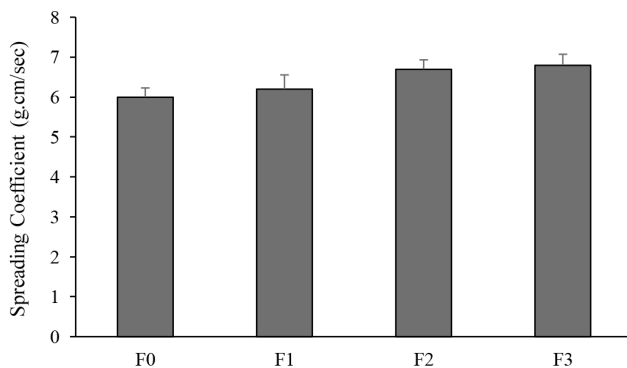


Figure 5: Spreading coefficient of the formulations F0-F3 (mean ± SD).

also aims to obtain the optimum formulation of *O. sativa* emulgel extract in a short period by storing the sample in conditions designed to accelerate changes that usually occur under normal conditions.²¹ The results are shown in Table 4.

Sun protection factor (SPF)

The SPF value is a measure with a higher SPF number indicating greater protection from UV radiation. This SPF rating may be used to determine a sunscreen's efficacy and effectiveness.²² The values are shown in Table 5.

The effectiveness of UV protection from *O. sativa* extract emulgel showed differences in each formulation. This was due to the different dosages of *O. sativa* extract from each formulation. The higher the dose in the emulgel formulation, the higher the SPF value. The results show that formulations 2 and 3 are in accordance with the Indonesian National Standard, which states that a sunscreen preparation must have a minimum protection factor of 4 because most Indonesians have skin types IV and V, as such, the recommended SPF used is 5-15.²³ Furthermore, the protective effect of *O. sativa* emulgel extract against UV is due to its flavonoid compounds, which have protective activity from ultraviolet radiation.¹ Flavonoid compounds are

also known to activate antioxidant enzymes,²⁴⁻²⁶ which protect cells from damage caused by lipid peroxide,^{27,28} reduces alpha-tocopherol radicals,²⁹ catalyzes metal chelation and free-electron transfer,^{30,31} inhibits oxidases,³² and immunomodulators.³³ In addition, *O. sativa* extract was also reported to contain several active compounds including anthocyanins, gamma oryzanol, Vitamin E complex, tocotrienols, and β -sitosterol which can increase the SPF value.³⁴⁻³⁷

The result of an *in vitro* study showed that a cosmetic formulation containing the anthocyanin (Figure 6) of Purple sweet Potatoes (*Ipomoea batatas* L.) TNG73, at a concentration of 0.61 mg/100 g cream absorbs up to 46% of UV radiation exposure. These indicate that the topical use of this type of cosmetic formulation at very low doses helps to prevent UV-induced skin damage by reducing the amount of direct UVB radiation on the epidermis.³⁸ Furthermore, various cellular and animal models were used to elucidate the pharmacological mechanisms through which the anthocyanins prevent UV-induced skin damage. A study reported that it was able to prevent damage to human skin structure (EpiD5erm(TM) FT-200) induced by UVB rays. It also protects the skin's ECM by ameliorating UVB-induced overexpression of various MMPs, such as collagenase (MMP-1), gelatinase (MMP-2, MMP-9), stromelysin (MMP-3), matrilysin (MMP-7), and elastase (MMP-12).³⁹ These MMPs perform an important physiological role in skin regeneration and cell migration (adhesion/dispersion).⁴⁰

Other studies also reported that the anthocyanin C3G inhibits UV-induced translocation of NF- κ B and AP-1 and other inflammatory responses in keratinocytes. Consequently, C3G provides multifaceted protection against skin damage due to NF- κ B and AP-1, which are key modulators of some skin cellular survival programs. These include the synthesis of inflammatory

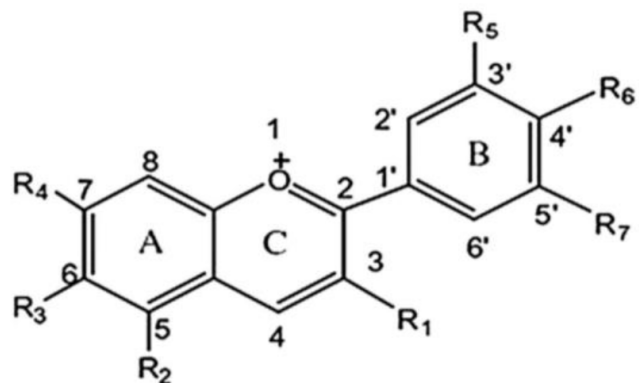


Figure 6: Chemical structure of anthocyanidins.

Table 4: Stability test of *O. sativa* emulgel extract.

Observation Days	Formulations	Physical Observation			
		Color	Homogeneity	Form	Consistency
1	F0	Clear	Excellent	Semi Solid	Excellent
	F1	Light orange	Excellent	Semi Solid	Excellent
	F2	Light brown	Excellent	Semi Solid	Excellent
	F3	Brownish red	Excellent	Semi Solid	Excellent
7	F0	Clear	Excellent	Semi Solid	Excellent
	F1	Light orange	Excellent	Semi Solid	Excellent
	F2	Light brown	Excellent	Semi Solid	Excellent
	F3	Brownish red	Excellent	Semi Solid	Excellent
15	F0	Clear	Excellent	Semi Solid	Excellent
	F1	Light orange	Excellent	Semi Solid	Excellent
	F2	Light brown	Excellent	Semi Solid	Excellent
	F3	Brownish red	Excellent	Semi Solid	Excellent
30	F0	Clear	Excellent	Semi Solid	Excellent
	F1	Light orange	Excellent	Semi Solid	Excellent
	F2	Light brown	Excellent	Semi Solid	Excellent
	F3	Brownish red	Excellent	Semi Solid	Excellent
60	F0	Clear	Excellent	Semi Solid	Excellent
	F1	Light orange	Excellent	Semi Solid	Excellent
	F2	Light brown	Excellent	Semi Solid	Excellent
	F3	Brownish red	Excellent	Semi Solid	Excellent
90	F0	Clear	Excellent	Semi Solid	Excellent
	F1	Light orange	Excellent	Semi Solid	Excellent
	F2	Light brown	Excellent	Semi Solid	Excellent
	F3	Brownish red	Excellent	Semi Solid	Excellent
pH Parameters	Observation Days	pH Test			
		F0	F1	F2	F3
(4.5-6.5)	1	4.55	4.67	4.99	5.03
	7	4.60	4.74	4.95	5.01
	15	4.84	4.79	4.93	5.00
	30	4.88	4.90	4.91	5.01
	60	4.98	4.90	4.92	4.96
	90	4.73	4.89	4.92	4.94
Viscosity Parameters	Observation Days	Viscosity Test			
		F0	F1	F2	F3
(2000-5000 cPs)	1	2974	3963	3514	3463
	7	3824	3914	3484	3423
	15	3145	3844	3401	3384
	30	3512	3534	3013	2976
	60	3341	3213	2978	2836
	90	2431	2980	2890	2785

Table 5: Category of SPF value effectiveness of *O. sativa* emulgel extract.

No	Formulations	SPF value	Effectiveness
1	F0	2.12 ± 0.062	Have not protection
2	F1	3.13 ± 0.035	Minimum protection
3	F2	5.71 ± 0.063	Medium protection
4	F3	16.07 ± 0.072	Very maximum protection

*All the observed values are mean ± SD (n=4).

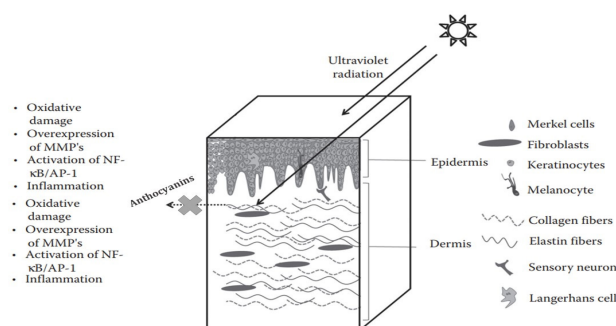


Figure 7: Mechanism of skin protective effect by anthocyanins.

mediators, both innate and adaptive immune effectors. Furthermore, C3G prevents UV-induced overexpression of IL-8, caspase-3 activation, and DNA fragmentation in human keratinocytes. These results suggest that the potential protective role of C3G is not only against skin damage from accumulative UV but also against psoriasis, which is characterized by hyperactivity of NF-κB in keratinocytes.⁴¹ It also suggests that anthocyanins may offer protection against photoaging (Figure 7).⁴⁰

CONCLUSION

This study established that the emulgel formulation of *O. sativa* extracts satisfied the formulation criteria. Furthermore, it exhibits anti-UV activity, as indicated by the SPF value in each formulation, where a higher dose resulted in a higher SPF value. This is also demonstrated by the result, which showed that both formulations 2 and 3 fill the Indonesian National Standard. Consequently, the emulgel of *O. sativa* extract have the potential as a novel sunscreen agent that protects against UV radiation. However, further study is required to ascertain the mechanism of action of the active chemicals found in *O. sativa* that function as an antioxidant and protect against UV radiation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

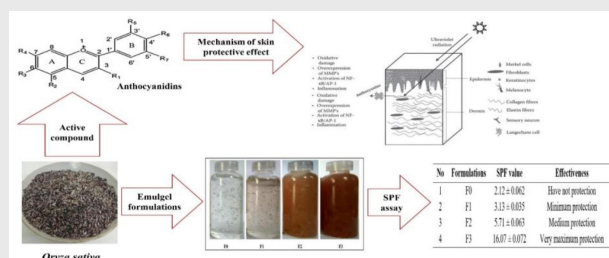
UV: Ultraviolet; **MED:** Minimum erythema dose; **O/W:** Oil/water; **HPMC:** Hydroxypropyl methylcellulose; **TEA:** Triethylolamin; **IL:** Interleukin; **MMP:** Metalloproteinases; **C3G:** Cyanidin-3- O-glucoside; **AP-1:** Activator protein 1; **DNA:** Deoxyribonucleic acid; **ECM:** Extracellular matrix; **NF-κB:** Nuclear factor-kappaB.

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PICTORIAL ABSTRACT



SUMMARY

In this study, we formulated *O. sativa* into an emulgel extract preparation and evaluated the preparation with various parameters including physical examination, rheological studies, measurement of pH, spreading coefficient and stability test. As a result, all formulations of *O. sativa* emulgel extract met the criteria for emulgel formulations. In addition, we also tested the activity of sun protection factor (SPF) for all formulations of *O. sativa* emulgel extract. As a result, the higher the dose of the extract, the higher the SPF value in the formulations of the *O. sativa* emulgel extract.

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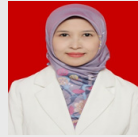
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