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Characteristics, Antioxidant Activity, and Sun Protection Factor of Black Pomegranate Peel Ethanolic Extract Hydrogel

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Abstract.
Solar UV radiation can inactivate the SARS-CoV-2 virus and indirectly accelerate the recovery rate of Covid-19 patients by converting pro-vitamin D3 in the skin into pre-vitamin D. On the other hand, prolonged exposure to UV radiation might be harmful to skin health, making it necessary to use sunscreen. Synthetic chemical sunscreens can have adverse side effects, and alternatives utilizing natural ingredients are needed. The peel of the black pomegranate has strong antioxidant properties and it also has the potential as a sunscreen by making it into a topical hydrogel preparation. The purpose of this study was to examine the characteristics, antioxidant activity, and sun protection factor (SPF) capacity of the ethanol extract of black pomegranate peel hydrogel preparations. Hydrogel was created containing black pomegranate peel ethanolic extract at concentrations of 0.5%, 1.0%, and 1.5%. Then, the characteristic properties were determined, the antioxidant power was examined using DPPH (2,2′-diphenyl-1-picrylhydrazyl radical) assay, and the SPF was investigated by the spectrophotometry method. The hydrogel containing extracts of 0.5%, 1.0%, and 1.5% had characteristics that fulfilled the specified requirements, having an inhibition percentage of 27.39%, 54.90%, and 83.91%, respectively, at the sample concentration of 600 ppm, and the SPF of this hydrogel was 2.67, 4.36, and 6.65, respectively. Therefore, according to the findings, hydrogel of black pomegranate peel ethanolic extract is good as a topical antioxidant but is less effective as SPF sunscreen.

Keywords: antioxidant, hydrogel, SPF, black pomegranate peel ethanolic extarct

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1. Introduction

At late 2019 there was an outbreak of Coronavirus infection (Covid 19) in Wuhan, China [1] which became a pandemic [2]. UV radiation from the sun decreases the growth rate of Covid 19 [3]. Uv radiation aids the body produce Vitamin D [4] that has a role in an innate immune system [5]. The exposure of the arms and legs to sunrays 15-30 minutes to produce adequate vitamin D[6].

The prolonged exposure to sun rays has adverse effects such as sunburn. UV radiation causes free radicals, and damage occurs when the critical balance between

free radical generation and antioxidant defenses is not good, resulting in premature aging and cancer [7] therefore need sunscreen and antioxidants. Synthetic chemical sunscreens have adverse side effects, and alternatives are needed by utilizing natural ingredients.

Pomegranate (*Punica granatum* L.) is a fruit tree that belongs to the *Lythraceae* family. Pomegranate peel is the non-edible part of the pomegranate fruit considered as a waste. The phytochemical content in pomegranate peel is mainly anthocyanins, ellagitannins, and high catechins, which are antioxidants properties, and black pomegranate peel contains the highest total phenolic and flavonoid content than other pomegranate cultivars [8] The are correlation between sun protection factor and antioxidant activity, phenol and flavonoid contents in a plant, Antioxidant activity, phenol and flavonoid content in plant will also affect the capacity of the Sun Protection Factor (SPF). More the phenolic content, the higher the SPF value [9].

Hydrogel has non-sticky properties, provides a cooling sensation, good flexibility, biocompatible, and has an attractive appearance[10][11]. In this study, black pomegranate peel ethanolic extract was made of hydrogel using Hydroxypropyl methylcellulose (HPMC) as a gelling agent, then tested for characteristics and determined the capacity as an antioxidant and sunscreen.

2. Methodology

2.1. Materials

The black pomegranate obtained from Situbondo, East Java which determined by Materia Medika, Batu. Ethanol 96%, Methanol (Merck), Hydroxypropyl methyl cellulose (FRAGRON), Propyleneglycol (CHEMWORLD), Methylparaben (G. AMPHARY LABORATORIES), *aquadest*, sunscreen SPF30 PA***.

2.2. Apparatus

Branson 2510 MT Ultrasonic Bath, Buchi RotorVap R 215 Rotary Evaporator, Analytical Balance BT224S Sartorius, Oven Venticell MMM, Inkubator Memmert GmbH IN 55, Spectrophotometer UV-Vis-1700 Shimadzu, pH MeterBasic 20+ Crison, Brookfield RVT Viscometer, Heidolph Brinkmann Stirrees Overhead Rzr.

2.3. Methods

2.4. Preparation of black pomegranate ethanolic extract.

The peel black pomegranate washed water, then cut into pieces, dried at 40^0C for three days, then crushed into powder. Black pomegranate peel powder as much as 50 grams (mesh 60) was extracted by ultrasonic macerated for 45 minutes at an amplitude of 20-40 Hz in 96% ethanol using a ratio of 1:10 (black pomegranate peel powder: solvent). The extract was pressed, filtered, and removed its ethanol by a rotary vacuum evaporator. Furthermore, the remaining ethanol is evaporated in the oven for three days at 40^0 C until a thick consistency[12].

2.5. Preparation of Black Pomegranate Peel Ethanolic Extract Hydrogel

First, prepared all ingredients according to the formula in **Table 1**, then HPMC was developed in aqua dest overnight. Black pomegranate peel extracts dissolved in water and nipagin dissolved in propylene glycol. The extract solution and gel base blended until homogeneously. Add the nipagin solution, mixer until homogeneous. Each formula was three times replicated.

Table 1: The Formula of Black Pomegranate Peel Ethanolic Extract Hydrogel.

Materials	Quantity (... g/100g)			
	K(-)	F1	F2	F3
Black pomegranate ethanolic extract	0.0	0.5	1.0	1.5
Propylene glycol	10.0	10.0	10.0	10.0
Hydropropyl methyl celuloce	3.0	3.0	3.0	3.0
Methylparaben	0.2	0.2	0.2	0.2
Aqua destilata	ad 100	ad 100	1d. 100	ad. 100

2.6. Physicochemical Test Hydrogel

The organoleptic, physicochemical, antioxidant, and SPF testing of cosmetic hydrogel carried out for the four different formulations. The determination Physico-chemical included physical appearance, homogeneity, pH, rheological, and spreadability.

2.7. Physical Appearance.

The physical appearance of cosmetic hydrogel should be red and transparent, smooth texture, and be non-greasy through a small sample palpated using the fingers.

2.7.1. Acidity degree (pH) Test

The pH test was determined by using a digital pH meter. Dipper the electrode of the pH meter in the sample and the pH value recorded. The pH of formulation hydrogel should be in the range of 4.1 – 5.8[13] .

2.7.2. Homogeneity

As much as 0.5g hydrogel spread on the transparent glass and observed it. The formulation of hydrogel should be homogenous, nothing lumps or coarse grains.

2.7.3. Rheological Study

The viscosity of formulation was determined by Brookfield Viscometer at 0.6, 1.5, 3.0, 6.0, 12.0, dan 30.0 rpm with spindle no 64. The spindle dipped in hydrogel, press 'on' then reading were taken.

2.7.4. Spreadability Test

The hydrogel was weighed to be as high as 1,0 g and then placed on graph paper coated with glass. Then, we put another glass above the gel mass. The gel diameter was calculated by measuring the diameter length of several sides. Then we added an additional load of 150 g, allowed the mixture to stand for 1 min, and measured the diameter of the gel as before. The spreadability test was carried out by placing 1.0 gram of hydrogel between two horizontal plates (20 x 20 cm) and left for 1 minute. The mass of the upper plate was standardized at 125 g. The requirement for good dispersion is 5-7cm [14].

2.8. Antioxidant Activity Test

The antioxidant activity of the hydrogel by measuring the percent inhibition of each formula at a level of 600μg/mL using the free radical scavenging method of 2,2-diphenyl-1-picrylhydrazyl (DPPH).

2.9. Solar Protection Factor (SPF) Test

The determination of sun protection factor (SPF) of the hydrogel by ultraviolet spectrophotometry, weighing 0.1g of hydrogel, transferred to a 20 mL volumetric flask, diluted to volume with ethanol, followed by ultrasonication for 5 min and then filtered through cotton, rejecting the ten first mL. Determined the absorbance in the range of wavelength of 290-320 nm at 5nm intervals. The blank used was 96% ethanol, control is a hydrogel base, and positive control is a sunscreen product SPF30PA*** contains Ethylhexyl methoxycinnamate, 4-Methylbenzylidene Camphor, and Butyl Methoxydibenzoylmethane. Calculated the absorbance results by application of Mansur equation.

3. Result and Discussion

3.1. Black pomegaranate peel ethanolic extract

The black pomegranate peel ethanolic extract is sour taste, aromatic, purple, thick, has a pH of 4.45 at 1.0% solution, and its water content is 22.5%.

3.2. The characteristic of black pomegarnate peel etanolic extract hydrogel

3.2.1. Physical Appearance of Hydrogel

The physical appearance of black pomegranate peel ethanolic extract hydrogel by observing the color, texture, and smell. All hydrogel peel pomegranate formulas have a soft texture, non-greasy, with red for hydrogels contained an extract of 0.5% and 1.0%, and tend to be purple in hydrogels with an extract content of 1.5%. They have a characteristic aroma of pomegranate peel (**Figure 1**), and all formulas are homogeneous.

Figure 1: Hydrogel containing black pomegranate peel extract at 0.5% (b), 1.0% (c), and 1.5% (d) levels and hydrogel base as control (a) The ethanolic extract of pomegranate peel is acidic, the use of HPMC as a gelling agent is appropriate because HPMC is a semi-synthetic gel base that is stable at pH 3-11[15].

3.2.2. Acidity degree (pH) of Hydrogel

The pH of the black pomegranate peel extract hydrogel was 4.09 to 4.51 (**Figure 2**). The pH of the black pomegranate peel extract hydrogel decreases with increasing extract content, the content of gallic acid and ellagic acid in the extract which causes to be acidic. In the preliminary test it was found that the 1.0% extract solution had a pH of 4.45.

The subcutaneous tissue on human skin had a pH of 4.1 – 5.8 [2]. So, the hydrogel extract content of 0.5%, 1.0%, and 1.5% suitable for skin. This hydrogel kept the acid mantle, it making various microbes included *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Propionibacterium acne*, all will be no growing so that the skin remains healthy and free from acne [16][17].

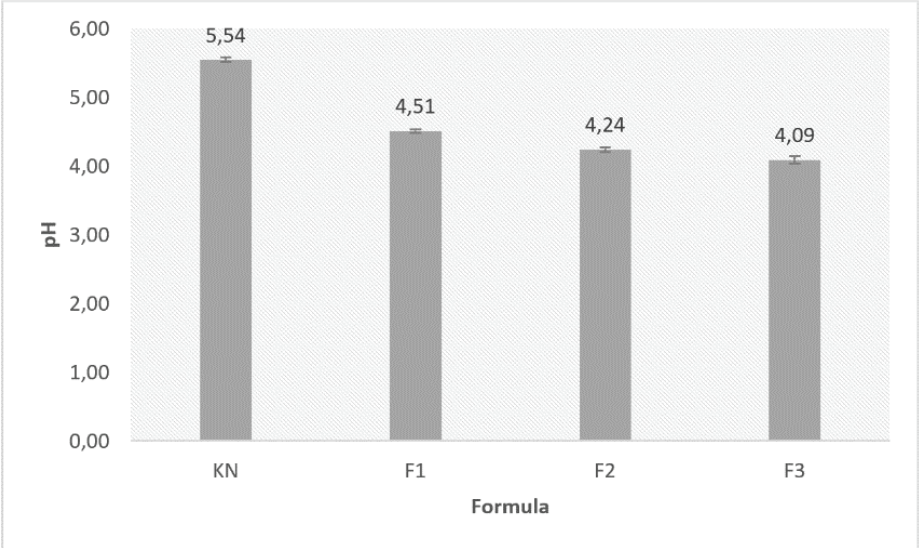

Figure 2: The pH of the black pomegranate peel extract hydrogel decreases with increasing extract content.

3.3. Rheological of Hydrogel

At a rate of 0.6rpm, the viscosity of Formula 1 is 18,333cPs, and at a rate of 30rpm, its viscosity is 8.083 cPs; The viscosity of Formula 2 is 15,000 at rate 0.6rpm, and 4,717cPs at rate 60 rpm; and Formula 3 is 4.717cPs at rate 0.6rpm and 2,083 cPs at rate 60rpm as seen in **Table 2**. Overall, the viscosity of all hydrogel formulations in the range of requirements of SNI-16-4399-1996 is 2000-50000 cPs, so it concluded that the viscosity of the hydrogel preparation of black pomegranate peel extract meets the requirements [18]. The higher the shear rate, the lower the hydrogel viscosity, and then stand if not stirred. This is shown hydrogel has pseudoplastic thixotropic property[19]. According to the extracted content, the viscosity decreased with the higher extract content, the viscosity of Formula 1 > Formula 2 > Formula 3 (**Figure 2**). This is due to HPMC molecules are more coiled at lower pH because the ionic strength is increased, resulting in a decrease in viscosity, at the lowest pH, HPMC macromolecules are more, and the possibility of polymer-polymer interactions is reduced [20]

TABLE 2: The Viscosity of the black pomegranate peel extract.

Formula	Viscosity (cPs)						
	0.6 rpm	1.5 rpm	3.0 rpm	6.0 rpm	12.0 rpm	30.0rpm	60.0rpm
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
KN	14,167±1,443	11,389±1,000	4,611±5,874	9,750±750	9,750±500	8,700±656	7,667±325
Formula1	18,333±2,887	15,153±2,646	6,895±7,152	12,583±946	11,833±382	9,900±100	8,083±76
Formula2	15,000±5,000	12,375±1,155	6,177±5,702	7,833±1,155	7,000±661	5,833±473	4,717±412
Formula3	6,667±2,887	5,569±1,732	3,396±1,969	3,333±289	3,250±250	2,500±265	2,083±189

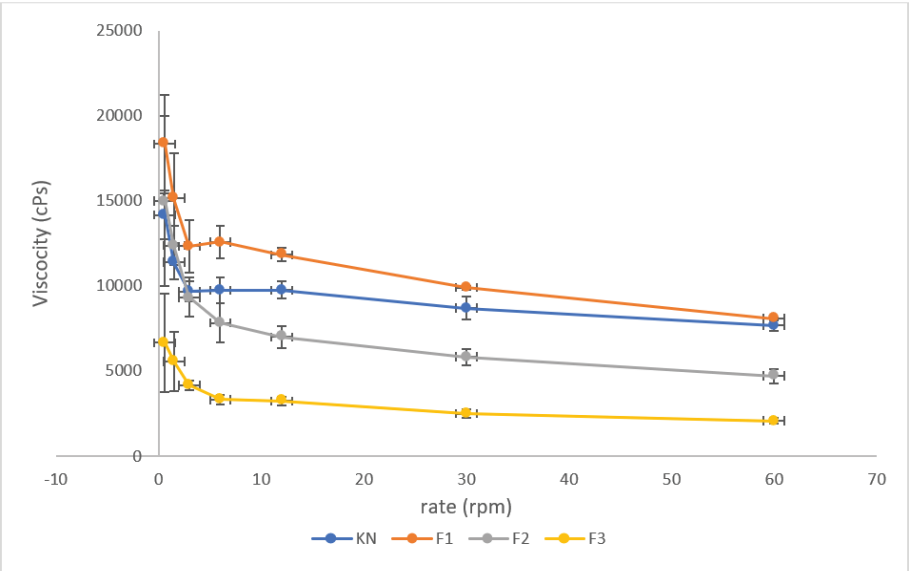

Figure 3: The viscosity of the black pomegranate peel extract hydrogel.

3.3.1. Spreadability of hydrogel

Good dispersion will result in wider contact between the active substance and the skin area which will result in faster drug absorption [21]. The results of the spreadability data in Formula 1 is 6.5cm, Formula 2 is 6.8cm, and Formula 3 is 7.9cm (**Figure 3).** That the dispersion obtained in all formulas is still in the range of 5 – 7 cm, so that all hydrogel formulas have good spreadability[14]. The dispersion of Formula 1 and Formula 2 is not different**,** Formula 2 and Formula 3 are not different, but Formula 1 is lower than Formula 3.

For the rheological dan spreadability, there is an inverse relationship between viscocity and spreadability, that the lower the viscosity, the lower the surface tension and higher the spreadability preparation on the skin.

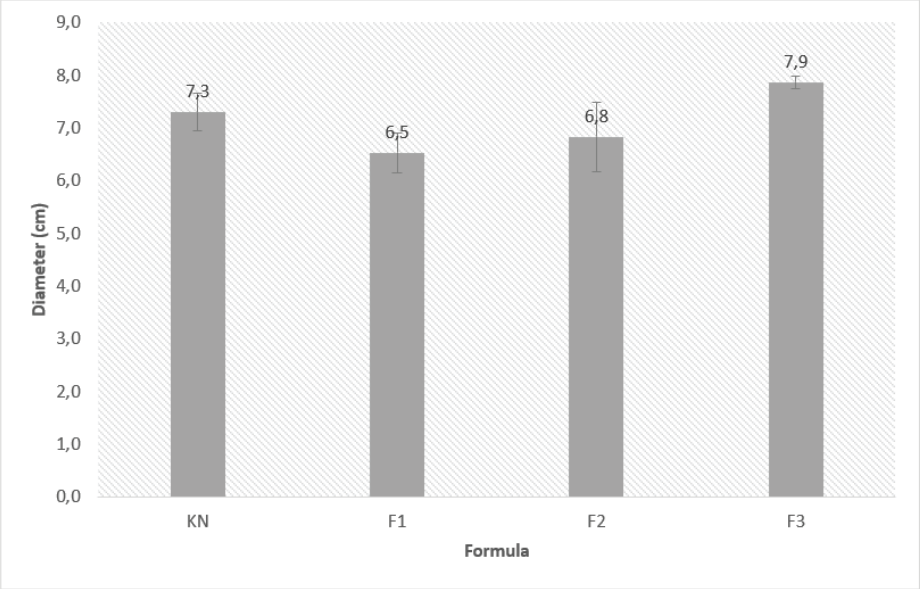

Figure 4: The spreadability of of the black pomegranate peel extract hydrogel.

3.4. Antioxidant activity of Hydrogel

The antioxidant activity of black pomegranate ethanolic extract hydrogel by calculating the percentage of inhibition using the DPPH method of the hydrogel preparation at a concentration of 600 ppm. The result as seen in **Figure 3.** Previously, a preliminary test was carried out on the ethanolic extract of black pomegranate peel and obtained an IC50 value of 7.33 μg/mL with vitamin C as control which had an IC50 of 4.27 μg/mL. The % inhibition of Formula 1 is 27.38%, Formula II is 54.90% and Formula III is 83.91%.

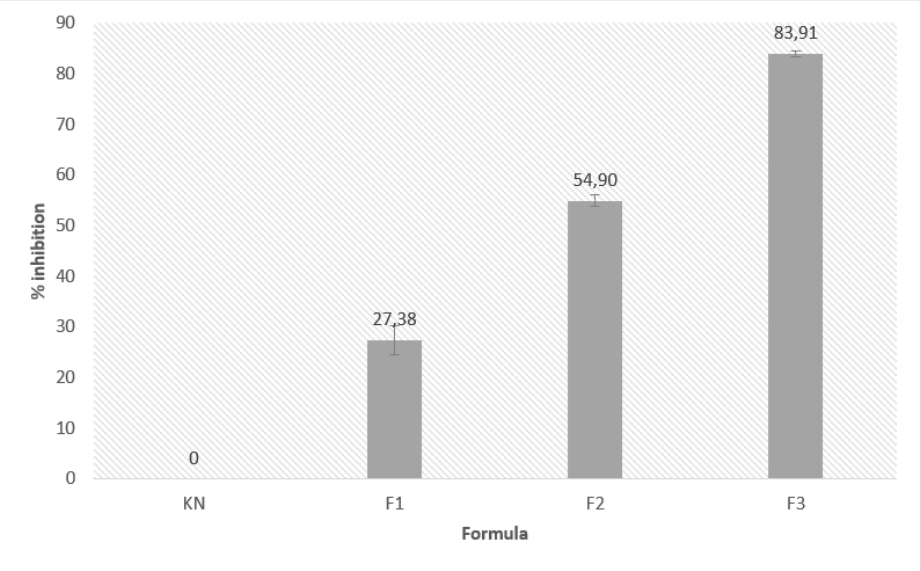

Figure 5: Percent Inhibition of Hydrogel Preparation of Black Pomegranate Peel Extract at concentration of 600 ppm.

Increasing the extracted content in the hydrogel increased the antioxidant activity of the hydrogel. Black pomegranate contains polyphenols with high anthocyanins [22] that have long conjugated double bonds capable of absorbing light in the visible light range and have antioxidant activity to scavenge free radicals [23], thus making pomegranate peels have high antioxidant activity [22].

3.5. Sun Protection Factor (SPF) of Hydrogel

In the SPF measurement, as a positive control, one of the SPF 30 PA*** gel products on the market was selected that contains the active ingredients Ethylhexyl methoxycinnamate, 4-Methylbenzylidene Camphor, and Butyl Methoxydibenzoylmethane, three ingredients are known as synthetic chemical sunscreens [24], also enriched with vitamin E and pro-Vitamin B5. This product protects the skin from exposure to UV B and UV A rays.

The results of the measurement of SPF hydrogel three times replication obtained the average for Formula 1 is 2.67, Formula 2 is 4.36, Formula 3 is 6.65 with hydrogel base as a negative control having an SPF value of 0.29, and SPF30PA*** is 33.06 as a positive control. That hydrogel has SPF power due to the anthocyanin content present in the black pomegranate peel extract. The significant role of anthocyanin pigments is to prevent photooxidative damage and function as a defensive UV [25]. This photoprotective power is due to ellagitannins and catechins, phenolic groups in

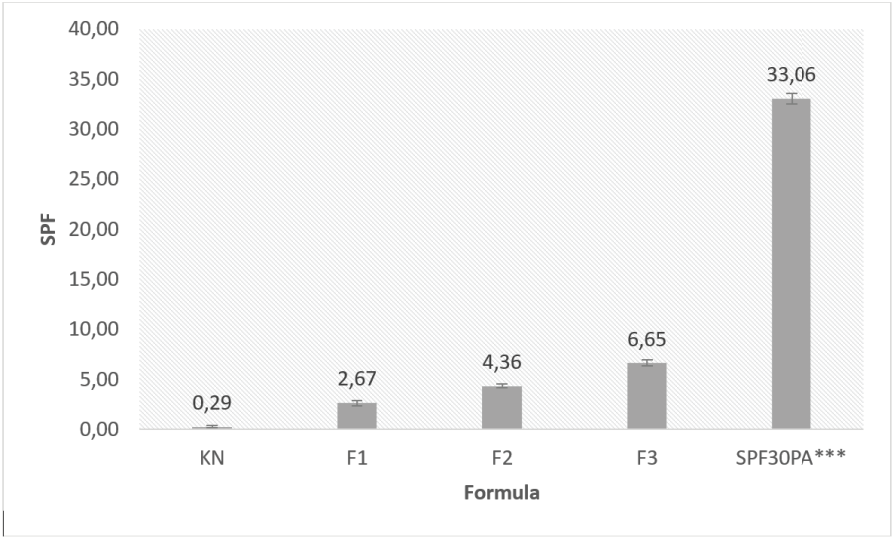

Figure 6: SPF hydrogel ethanol extract of black pomegranate peel with extract content of 0.5% (F1), 1.0% (F2), 1.5% (F3), as a negative control is a hydrogel base (KN), and as positive control is a gel. SPF30PA***.

the ethanolic extract of black pomegranate peel [9]. The SPF of the hydrogel is lower than SPF30 P*** is a positive control due to the chemical SPF. There are Ethyl hexyl methoxycinnamate and 4-Methylbenzylidene Camphor as sunscreens against UVB [26], and Butyl Methoxy dibenzoyl methane, a sunscreen agent that provides proper UVA protection[27]. Formula 3 has an SPF value of 6.65 is the highest, meaning effective as sunscreen for 66.5 minutes. Therefore, it is necessary to combine other active ingredients to obtain a higher SPF value. However, the various bioactive compounds in black pomegranate peel extract have beneficial effects on the skin, as antibacterial, antiviral, and anti-inflammatory [28].

4. CONCLUSION

The hydrogel of black pomegranate peel ethanolic extract has characteristics that meet SNI standards. As an antioxidant, the percentage of inhibition of each formula increases according to the extracted content in it. The SPF of the formula' hydrogels is low. It is necessary to combine it with other sunscreen ingredients.

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